MICROPARTICULATE ICE SLURRY FOR RENAL HYPOTHERMIA : LAPAROSCOPIC PARTIAL NEPHRECTOMY IN A PORCINE MODEL

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INTRODUCTION

Ischemia-reperfusion (IR) injury due to warm ischemia (WI) occurring during laparoscopic partial nephrectomy (LPN) remains an obstacle for wide-spread adoption of laparoscopic approach to nephron-sparing surgery. Renal hypothermia during open partial nephrectomy is easily achieved with ice and provides adequate protection against IR injury [1]. In the recent years several systems for laparoscopic ice slush delivery [2, 3] and cooling devices [4, 5] have been tested but none of them gained clinical acceptance. Other approaches for renal cooling such as perfusion of renal collecting system [6] or vasculature [7] with cold saline are either ineffective or cumbersome.

External surface irrigation with near-frozen saline is a simple method of renal cooling; however it requires an extensive volume of coolant in order to induce sufficient renal hypothermia and is associated with undesirable systemic hypothermia [8].

In our previous animal studies we have evaluated highly fluidic microparticulate ice slurry for renal hypothermia in laparoscopic porcine model [9, 10] . The ice slurry was developed in the Argonne National Laboratory as a coolant for industrial purposes and currently is evaluated for multiple medical applications. It is produced from sterile hypertonic saline and ice and comprises microscopic (<100μm) globular particles compared to ice slush consisting of dendritic crystals. This structural difference accounts for high fluidity of the ice slurry allowing effective delivery even through 100 cm long 3 Fr catheters.

A potential coolant for LPN should be easily prepared, quickly delivered and effective. It should allow extending ischemia time without compromising renal function, avoid systemic hypothermia, should not compromise visualization and pneumoperitoneum pressure or interrupt surgery.

The laparoscopic porcine model is suitable for assessing such properties but not without caveats [11]. Porcine kidneys are much more resilient to IR injury compared to humans. We have previously established that in porcine kidneys an irreversible damage occurs with WI time above 90 minutes [12] but a time threshold for cold ischemia is unknown; moreover, it may vary with the level of renal hypothermia and the amount of resected tissue.

The objectives of the current study were to compare the efficacy and safety of the ice slurry versus near-frozen saline for renal hypothermia and its ability to protect renal function in the LPN porcine model.

MATERIALS AND METHODS

The study was approved by the Institutional Animal Care and Use Committee. Thirty female pigs weighing 45 kg were used. The animals recovered for three days from the stress of transportation prior to surgery. The surgery was performed under general anesthesia with endotracheal intubation and isoflurane and sterile conditions. A warming blanket (Bair-Hugger) was used during all procedures. Neither Mannitol nor nephrotoxic agents were administered. All the animals received Penicillin-based prophylactic antibiotics for 48 hours and Fentanyl patch for postoperative analgesia.

Surgery

Right laparoscopic nephrectomy was performed via a three-port transperitoneal approach with pneumoperitoneum at 12 mm Hg. Renal vessels were clamped with Hem-o-lock clips (Weck Closure Systems, Research Triangle Park, NC) and transected following by division of the ureter and kidney removal through an extended port incision. After re-positioning the animal and ports placement, the left hilum was dissected and the kidney released circumferentially. The renal artery and vein were clamped separately with laparoscopic bulldog clamps (Aesculap, Center Valley, PA) one clamp on each vessel. Next, we sharply excised the lower pole of the kidney approximately 5 mm below the renal hilum perpendicular to the longitudinal kidney axis and repaired the collecting system with 3-0 Polyglactin (Vicryl) running suture and LapraTy™ clips (Ethicon, Picataway, NJ). Parenchyma was approximated in the same manner with 2-0 suture. The excised tissue was removed through an extended port incision. The excised tissue was weighed to assure that similar amount was removed in each animal. In all animals the partial nephrectomy was started 10 minutes after vessel clamping.

Study design

Phase I. Eighteen pigs were divided in 3 equal groups. The renal hilum was clamped for 90 min in all the animals. In Slurry group1 renal hypothermia was induced with the ice slurry and maintained for 90 min. In Saline group 1 renal hypothermia was induced with near-frozen saline irrigation and maintained for 90 min. In both these groups cooling started immediately following hilum clamping. No cooling was applied in WI group.

Phase II. Twelve pigs were divided in 2 equal groups. The renal hilum was clamped for 120 min in these animals. In Slurry 2 and Saline 2 groups renal hypothermia was induced for 10 minutes only with slurry or near-frozen saline, respectively and the kidneys were allowed to re-warm during the rest of the clamping time.

Cooling technique

The ice slurry was prepared from sterile hypertonic saline and ice in a blender as previously described [8,9] and pumped via a tube connected to a laparoscopic suction tip. The equipment was gas-sterilized with ethylene oxide prior to surgery. Immediately following hilar clamping a standard 4mm laparoscopic suction tip connected straight to a silicone tube of the same inner diameter is introduced through a 5-mm port and the slurry infusion started at 200cc/min. Initially, a half-inch layer was created below the kidney, next the rest of kidney surface was covered with the slurry. In Slurry group 1 the slurry was applied in order to maintain renal temperature between 5-15 ^{o}C during the surgery and melted slurry was suctioned out. In Slurry group 2 the slurry was applied only during the first 10 minutes of clamping allowing for renal subsequent re-warming, melted slurry was not suctioned.

Near-frozen saline was prepared using 1 liter bags of 0.9% saline placed in a freezer at $0^{o}C$ for 72 hours. This regimen produces a near-frozen solution with 10-20% of ice allowing free flow. In order to assure an adequate irrigation rate we used infusion pressure-bags and a laparoscopic irrigation Y-set. In both Saline groups 1 and 2 five liters of saline were used during initial 10 minutes of cooling. We applied the technique of intermittent irrigation and suctioning of saline runoff accumulating around the kidney as described by Weld et al [8] . In Saline group1 we aimed to achieve same kidney temperature range as in the Slurry group 1 (5-15 ^{o}C) however not at the expense of core temperature decline. As such the irrigation was stopped when core temperature dropped below 35 ^{o}C and restarted the irrigation when core temperature rose or when the kidney temperature was above 25 ^{o}C, whichever occurred first.

Endpoint measurements

The renal temperature was measured with 2 insulated thermocouples introduced through the abdominal wall and inserted in the kidney to 1 cm depth in the upper pole and mid-kidney. Core temperature was measured with a transnasal thermocouple. The thermocouples were connected to an electronic thermometer (Fluke, Everett, WA). We recorded time to recovery from anesthesia, defined as an animal's ability to stay on its feet and core temperature above $37^{o}C$. Renal function was assessed with serum creatinine levels at baseline and at day 1, 3, 7 and 14 after the surgery. We evaluated slurry sterility with aerobic and anaerobic cultures obtained before the slurry was infused into abdominal cavity. We also measured serum osmolarity levels in Slurry1 group animals at baseline and immediately after the surgery to evaluate possible free water shift due to exposure of peritoneum to hypertonic slurry. Histological evaluation of renal tissue was

performed following animal sacrifice at day 14 after the surgery. The specimen was fixed in 10% buffered Formaldehyde and stained with Periodic Acid-Schiff (PAS) stain. The slides were evaluated by a renal pathologist blinded to the study details who semi-quantitatively graded the extent of glomerulosclerosis, acute tubular injury, interstitial fibrosis and tubular atrophy, and interstitial inflammation on a 0-4 scale [13] .

Statistical analysis

Continuous variables were compared using one-way ANOVA and categorical ones with Chi-square test. P-values <0.05 were considered statistically significant. Power analysis for group size was done based on our previous experience in warm ischemia time studies in solitary kidney porcine model [12](12). The analysis was performed with STATA 10 SE software (StataCorp, College Station, TX)

RESULTS

The comparisons of renal and core temperatures as well as renal function outcomes between Slurry1 and Saline1 and between Slurry 2 and Saline 2 groups are summarized in Table 1.

Temperature recordings

The renal temperature (Mean±SD) in WI group remained stable at 37.6±0.4^{o}C during 90 min of clamping. Renal units in Saline group 1 cooled down on average to 25±4 ^{o}C versus 10±4 ^{o}C in Slurry group 1 ($p<0.0001$) .Mean core temperature declined in both Saline 1 and Slurry 1 groups and by the end of surgery (90 min clamping) it was 35±0.7 ^{o}C in the Saline group 1versus 37±0.5 ^{o}C in Slurry group 2 ($p<0.0001$).

Nadir renal temperature in Saline group 2 (10 min cooling) was 22±2°C compared to 9±1°C in Slurry group 2 ($p<0.0001$) (Figure 1). Mean core temperature dropped to 35 ^{o}C in Saline group 2 vs 37 ^{o}C in Slurry group 2 ($p<0.0001$)

(Figure 2).

Renal function

Mean serum creatinine was significantly lower in Saline 1 and Slurry 1 groups (90 min cooling) compared to WI group ($p<0.0001$) at day 1, 3 and 7 after surgery (Figure 3). However, no difference in serum creatinine was noted between Saline group 1 and Slurry group 1 or Saline group 2 compared to Slurry group 2 at any time point (Table 1).

Renal histopathology

There was a spectrum of acute tubular injury in all treatment groups ranged from minimal to severe. The WI kidneys (average = 1.6, range: 0-3) showed the most acute tubular injury of the three treatment groups with slightly less injury in the slurry1 (average = 1.3,

range: 0-2) and the least in saline 1 (average = 1.1, range: 0-3), but the difference was not statistically significant.

Other measurements

We did not observe a change in serum osmolarity in animals from Slurry group 1 with both baseline and postoperative mean values of 271±12 mOsm/L (p=0.9). The animals in Saline group 1 and 2 recovered from anesthesia on average 40 minutes longer (Table 1) compared to the animals from Slurry group 1 and 2. All the slurry aerobic and anaerobic cultures (n=12) returned sterile after 7 days of incubation.

Adverse events

One animal in Saline group 1 died on day 3 after the surgery. Necropsy revealed a completed obstruction of the collecting system by a suture and a large urinoma. Another animal in Slurry group 2 died immediately after the surgery. On necropsy we found severe unilateral pulmonary edema and a clot in the right ventricle suggesting massive pulmonary embolism. These animals were replaced and their data excluded from the analyses.

DISCUSSION

The current study demonstrates that the ice slurry has superior cooling ability compared to near-frozen saline. These findings are in concordance with our previous experiments [9, 10] and other studies evaluating renal cooling with ice slush [2, 14, 15] . In these reports renal hypothermia in the range of 5-15°C was achieved which is considered optimal for renal protection based on the classic Ward experiment [16]. Weld et al reported that external irrigation with large amounts (20 L) of near frozen saline may cool a kidney to 17-20 ^{o}C, however concomitant systemic hypothermia (below 34 ^{o}C) occurred. In our study we found a similar effect of near-frozen saline on systemic temperature with even smaller volume (less than 10 L) [8]. Notably, core hypothermia was associated with longer recovery from anesthesia. Although the implication for post-surgical recovery in humans should be determined, this is a worrisome finding given that the convalescence after LPN is expected to be fast. A significantly smaller volumes of slurry as compared to near-frozen saline provide an additional advantage. Finally, a single 10-minutes slurry application provided twice longer hypothermia than 10 minutes cooling with near-frozen saline. Although it is possible to deliver additional boosts of near-frozen saline in order to maintain renal cooling, it interferes with surgery, particularly at the phase of collecting system and parenchimal suturing and added need for suctioning can actually extend ischemia time.

Alternative approaches to renal cooling such as collecting system perfusion with saline[6] and renal vasculature perfusion[7] have also been reported. The efficacy of the former is inferior to the ice (renal temperature 22-27 ^{o}C) while the latter requires central venous access, fluoroscopy and contrast injection.

Cooling with the slurry and saline in the Phase I of the experiment (90 min clamping, 90 min cooling) lead to seven-fold lower serum creatinine on day 3 compared to warm ischemia. However, all the groups reached the same creatinine levels at two weeks post-surgery. Additionally, the PAS staining of renal tissue at the time of necropsy did not show any difference in the extent of tissue damage. Furthermore, there was no difference in serum creatinine in Phase II (120 min clamping, 10 min cooling) between slurry and saline groups at any time point. These findings point to extreme resilience of porcine renal tissue to ischemia which is further amplified by even moderate hypothermia. The accuracy of creatinine for the assessment of acute kidney injury is also a concern. In a single kidney model used in this experiment serum creatinine changes reflect the extent of kidney injury without the compensatory effect of a contralateral renal unit. However, it still may not be sensitive enough to discern more subtle differences in renal function. More accurate way of GFR measurement by Iohexol clearance may be used. In our experience this method is difficult to implement in animals and may lead to serious complications due to central line infections and plugging. There are novel accurate markers of GFR and tubular injury such as Cystatin-C and NGAL [(17)] but they cannot be measured in a swine model due to lack of porcine commercially available antibodies for ELISA.

None of the renal hypothermia animal studies, with the exception of Ward experiment [(16)], compared renal function outcomes at different cooling temperatures. Moreover, there are no human studies comparing different approaches for renal hypothermia in terms of renal function preservation.

We have previously established that porcine kidneys sustain irreversible renal damage after 90 minutes of warm ischemia [(12)]. The threshold for irreversible renal damage under cold ischemia following heminephrectomy is unknown in a porcine model.

Our experience with cooling by both slurry and saline is also limited. In Phase I of the experiment we set clamping and cooling time to 90 minutes in order to prevent irreversible renal failure and subsequent animals' death. Here we learned the properties of both cooling techniques and established the safe limits of the model. Next we proceeded to a prolonged 120 minutes ischemia time and short 10 minutes cooling time in Phase II attempting to approach a limit for irreversible renal damage in porcine model under cold ischemia. Based on our findings it appears that with cooling this threshold is far beyond the two-hour limit. This design also allowed us to study the dynamics of kidney temperature changes in conditions maximally simulating human renal surgery.

A rare complication of pulmonary embolism leading to animal death should be noted. We don't have an explanation for this event, however it's unlikely that is was associated with cooling given that usually hypothermia causes coagulopathy rather than a hypercoagulable state in humans and pigs [18,19]

Laparoscopic partial nephrectomy is largely underused [20] , in part due to a concern for exposing a kidney to prolonged warm ischemia. There is growing evidence that warm ischemia time beyond 30 min may translate into decline of the long-term renal function [21] especially in patients with diminished renal reserve. As such, a simple and effective method of renal cooling during laparoscopy is demanded. In this work we have shown that the ice slurry is easily prepared and delivered, cools better compared to near-frozen saline, is safe microbiologically and has minimal influence on core temperature.

The main limitation of this study is our inability to define whether deeper hypothermia confers better renal function preservation, apparently because we did not approach the threshold for irreversible renal damage under cold ischemia in porcine model.

CONCLUSION

Microparticulate ice slurry is more effective and safe than near-frozen saline for renal cooling during LPN in a porcine model. A prospective human study is needed to determine whether deeper cooling translates into a better preservation of renal function.

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FIGURE LEGENDS

Figure 1. Renal temperatures* during Phase II (120 min clamping and 10 min cooling) **

* Mean values , error bars represent standard deviations

** The plot shows the first 90 minutes (out of 120).

Figure 2. Core temperatures during Phase II (120 min clamping and 10 min cooling)*

*Mean values, error bars represent standard deviations

Figure 3. Serum Creatinine during Phase I (90 min clamping and 90 min cooling)*

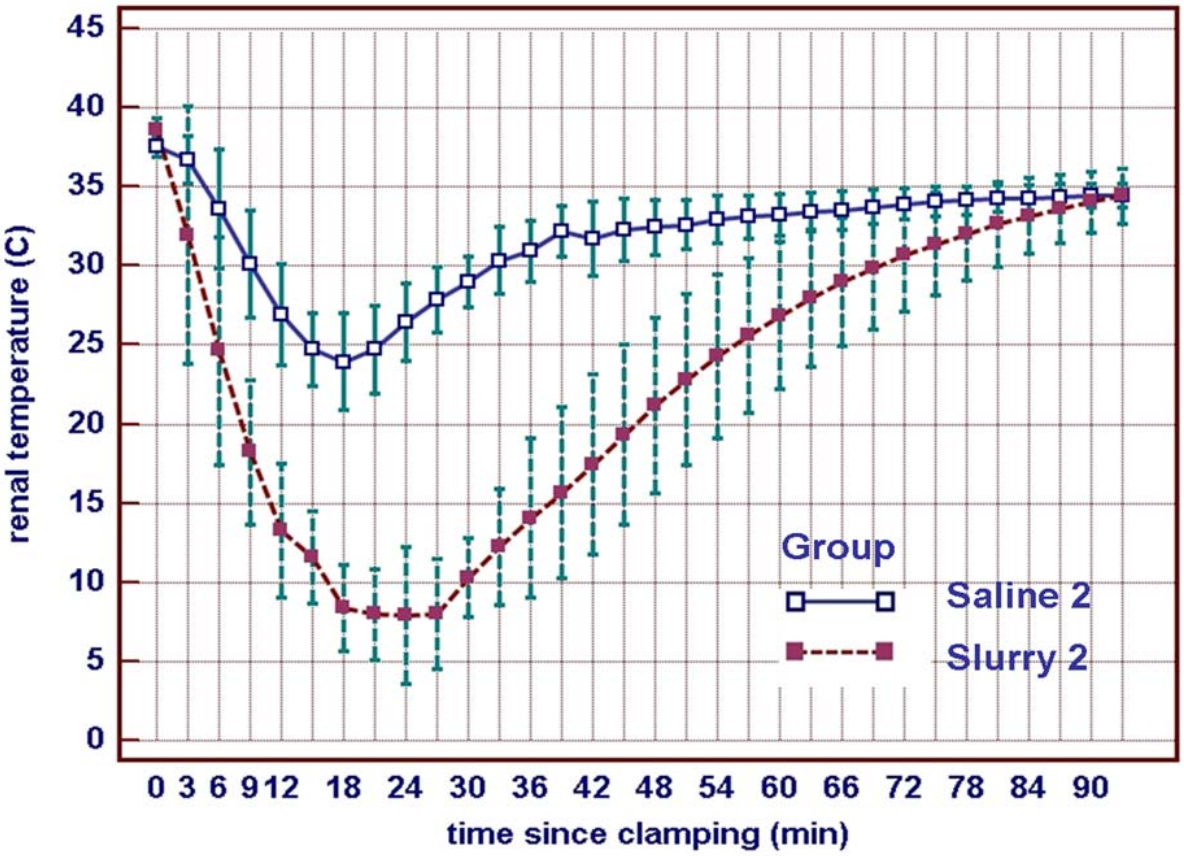
* Mean values, error bars represent standard deviations

Table 1: Comparison of renal temperature and serum Creatinine in Saline and Slurry groups*

Parameter	*Phase I (90 min clamp and 90 min cooling)*			*Phase II (120 clamp and, 10 min cooling)*		
	Slurry group 1	Saline group 1	p-value	Slurry group 2	Saline group 2	p-value
Number of animals	6	6	-	6	6	-
Weight of resected renal tissue, gr	25 ± 6	25 ± 6	0.9	25 ± 6	25 ± 6	0.9
Amount of coolant, ml	1900 ± 200	8700 ± 200	<0.0001	1200 ± 100	5000 ± 0	<0.0001
Nadir renal temperature, ^{o}C	8 ± 4	23 ± 3	<0.0001	9 ± 1	22 ± 2	<0.0001
Nadir core temperature, ^{o}C	37 ± 0.5	35 ± 0.7	0.0001	37 ± 0.7	35 ± 0.5	0.0001
Time to re-warm a kidney to 30°C, min	-	-	-	61 ± 7	24 ± 6	<0.0001
Time to recover from anesthesia, min	50 ± 8	91 ± 6	0.003	28 ± 7	69 ± 15	<0.0001
Serum Creatinine, mg/dl						
Baseline	1.4 ± 0.1	1.3 ± 0.1	0.3	1.4 ± 0.1	1.4 ± 0.2	0.8
POD1	4 ± 0.7	4 ± 0.8	0.9	5.9 ± 1.1	5.9 ± 1.3	0.9
POD3	3.0 ± 0.5	2.9 ± 0.4	0.5	5.3 ± 2.3	5.4 ± 2.5	0.9
POD7	2.5 ± 0.4	2.3 ± 0.3	0.4	2.9 ± 0.5	2.7 ± 0.5	0.4
POD14	2.3 ± 0.4	2.2 ± 0.1	0.6	2.5 ± 0.1	2.4 ± 0.2	0.9

*Continuous variables presented as Means and standard deviations

Figure 1.


45
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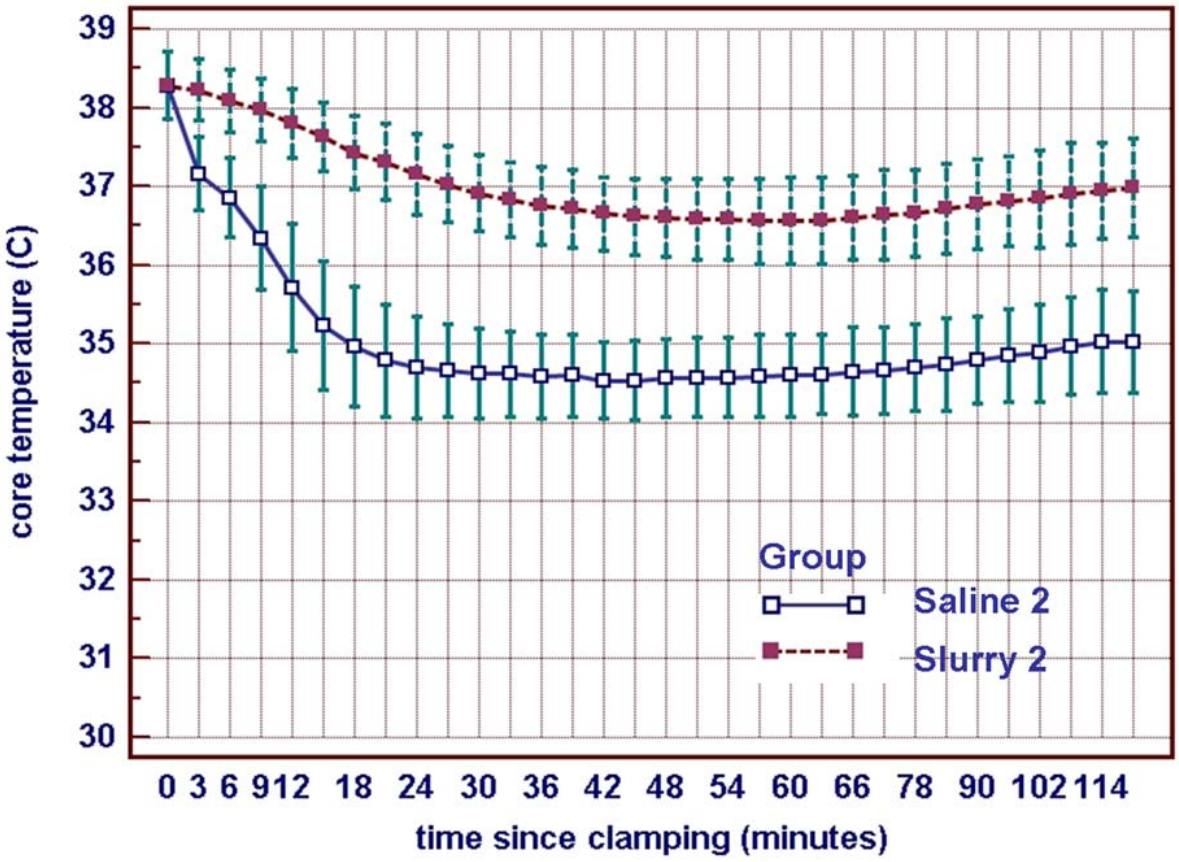
renal temperature (C)
0 3 6 9 12 18 24 30 36 42 48 54 60 66 72 78 84 90
time since clamping (min)
Group
Saline 2
Slurry 2

Figure 2


core temperature (C)
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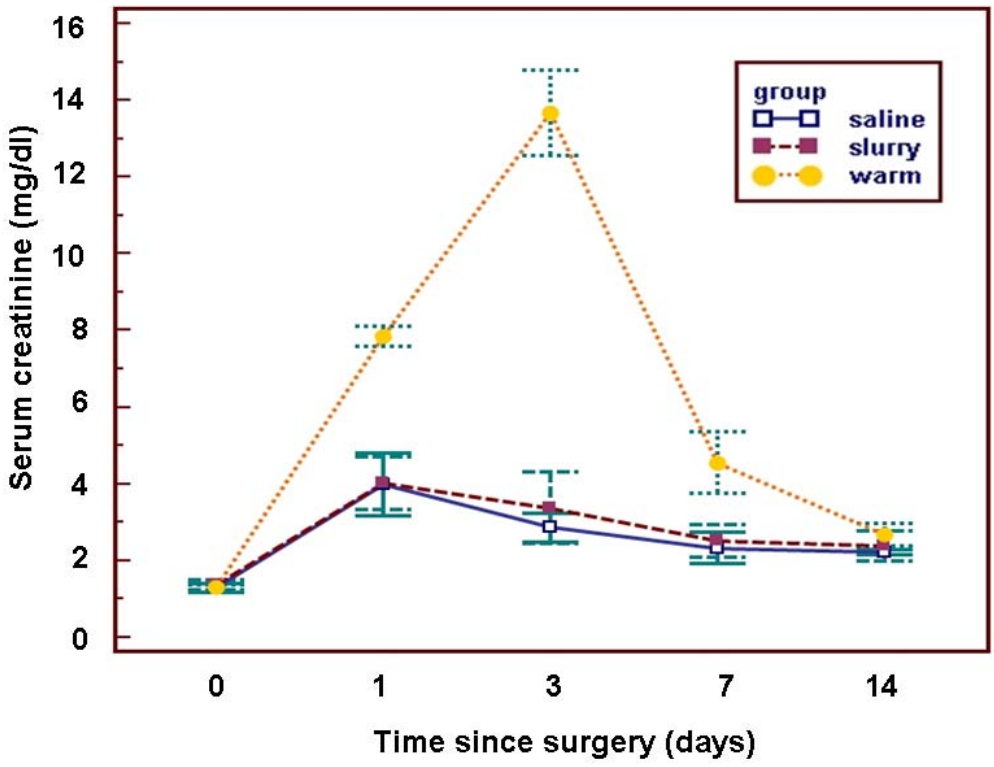
time since clamping (minutes)
Group
Saline 2
Slurry 2

Figure 3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Serum creatinine (mg/dl)
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Time since surgery (days)