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Neutrophil gelatinase-associated lipocalin (NGAL) levels in response to unilateral renal ischaemia in a novel pilot two-kidney porcine model

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Abstract

- To test a novel porcine two-kidney model for evaluating the effect of controlled acute kidney injury (AKI) related to induced unilateral ischaemia on both renal units (RUs)
- To use neutrophil gelatinase-associated lipocalin (NGAL) and physiological serum and urinary markers to assess AKI and renal function.
- Twelve female Yorkshire pigs had bilateral cutaneous ureterostomies placed laparoscopically with identical duration of pneumoperitoneum for all cases.
- An experimental group ($n=9$) underwent induced unilateral renal ischaemia with left hilar clamping of timed duration (15, 30, 60 min) and a control group ($n=3$) had no induced renal ischaemia.
- Urine was collected and analysed from each RU to assess creatinine and NGAL concentration preoperatively and at multiple postoperative time points. Serum was collected and analysed daily for creatinine and NGAL levels.
- Statistical comparisons were made using the rank-sum and sign-rank tests.
- Three pigs were excluded because of intra-operative and postoperative complications.
- In the RUs that experienced renal ischaemia ($n=7$), the median urine volume was lower ($P=0.04$) at 6, 12, 24 and 48 h and the median NGAL concentration was higher ($P=0.04$)

at 12 and 48 h compared with the RUs of control pigs that experienced no renal ischaemia ($n=2$).

- When comparing the ischaemic (left) RU of the pigs in the experimental group with their contralateral non-ischaemic (right) RU, ischaemic RUs had a lower median cumulative urine volume at 6, 12, 24 and 48 h ($P = 0.05$) and a higher median NGAL concentration at 12, 24 and 48 h ($P<0.05$).
- At 48 h, no significant increase was found in serum NGAL in pigs in the experimental group compared with controls ($P=0.2$).
- Creatinine clearance (CC) was lower in ischaemic RUs compared with non-ischaemic RUs 1 day after surgery ($P=0.04$) with decreasing CC as the duration of ischaemia increased.
- We have developed a promising novel small-scale pilot surgical model that allowed the evaluation of bilateral RU function separately during and after unilateral renal ischaemia.
- The induction of unilateral renal ischaemia corresponds with physiological changes in both the ischaemic and contralateral RU.
- AKI as measured by increases in NGAL and decreased renal function as measured by decreases in CC, are specific to the RU exposed to ischaemia.

Keywords

human NGAL protein; ischaemia; acute kidney injury; models; animal; biomarkers

INTRODUCTION

Iatrogenic human renal ischaemia in the setting of partial nephrectomy (PN) occurs when a clamp is applied to the renal artery or renal hilum to reduce the bleeding that may occur when resecting a portion of one renal unit (RU). To investigate the impact of such ischaemia on renal function, experiments have been performed in solitary kidney models to reduce the impact from the contralateral RU [1–4]. The results of these experiments are informative but fail to adequately account for the compensatory mechanisms of the contralateral RU, and hence may not adequately reproduce endogenous physiological reactions to ischaemia.

Acute kidney injury (AKI) is defined as a rapid decline in renal function. The markers most commonly used to measure AKI in the setting of PN are serum creatinine (SCr) alone or its derived formula, estimated GFR (eGFR). Changes in SCr, and thus eGFR, are delayed by 24–48 h from the time of AKI [5–7] and SCr level or eGFR may also be influenced by a variety of clinical factors such as hydration status or changes in filtration by the contralateral RU [8, 9]. Additionally, eGFR shows how much renal function remains after an insult, and shows only indirectly the amount of renal damage incurred. An improved marker of AKI should allow a more rapid detection of injury and, in turn, the initiation of interventions that can mitigate renal damage and the subsequent loss of renal function.

Elevated neutrophil gelatinase-associated lipocalin (NGAL) has been shown to correlate with AKI and to precede a rise in SCr level in a variety of clinical settings, such as cardiac

surgery [7, 10, 11], receipt of i.v. contrast [10, 12], renal transplant [10, 13, 14] and in the emergency room [15]. Because urine and serum NGAL levels are independent of SCr, they may be less influenced by clinical factors that affect eGFR. In addition, recent studies have shown that NGAL may be more sensitive to AKI and may be a better predictor of morbidity than SCr [16]. Large animal models using pigs have evaluated the role of NGAL to detect renal injury in renal transplant models [17, 18, 19], but no-one has yet evaluated NGAL levels in response to induced unilateral renal ischaemia in an animal with intact bilateral kidneys. Furthermore, there is currently no documented evidence in the literature that NGAL production is specific to the side of injury or if there is a dose–response relationship between unilateral NGAL levels and the duration of renal ischaemia. We developed a novel two-kidney porcine model of AKI to evaluate independently the effects of unilateral renal ischaemia on each individual RU as well as to elucidate the utility of NGAL as a marker of AKI in a kidney surgery model.

METHODS

Study design

After approval from our Institutional Animal Care and Use Committee, 12 female Yorkshire farm pigs (Archer Farms, Darlington, MD, USA) underwent bilateral laparoscopic ureteric dissection and cutaneous ureterostomy placement. The pigs were sequentially randomized to receive left laparoscopic clamping of the renal hilum for 15, 30 or 60 min, with three pigs in each group. The control group comprised three pigs without clamping. The pigs had free access to fluids and had to survive for a minimum of 48 h after surgery to be included in the analysis. Necropsy was performed after the pigs were killed to evaluate the integrity of the urinary drainage system, and to harvest kidneys for pathological interpretation.

Serum creatinine and NGAL levels were determined before surgery and then daily. Urine was collected from each RU at ureterostomy placement, hilar unclamping, and 3, 6, 9, 12, 18, 24, 36, 48, 60, 72, 84 and 96 h after removal of the left hilar clamp. For each urine collection time point, urine volume was recorded and aliquots from each kidney were tested for SCr and NGAL. Both urinary and serum porcine NGAL levels were determined using a commercially available ELISA assay (Bioport Diagnostics, Gentofte, Denmark) that was internally validated in a CLIA-certified clinical laboratory.

Surgical technique

All pigs were intubated and maintained on isoflurane (1.5–2.5%) during surgery. They were sedated with tiletamine/zolazepam (4.4 mg/kg) and glycopyrrolate (0.007 mg/kg) and injected with a s.c. local infiltration of bupivacaine (0.25%) at the laparoscopic port site before port placement. A three-port, purely laparoscopic technique was used on each side. After sedation and intubation, the pigs were placed in the left lateral decubitus position and a Veress needle was used to establish pneumoperitoneum to 15 mmHg. An upper midline 10-mm trocar was placed and a laparoscopic camera introduced. Under direct visual guidance, a spinal needle was passed percutaneously into the bladder to extract a baseline urine specimen. A second midline trocar was placed under direct vision; then a third lateral port was placed, which ultimately became the cutaneous ureterostomy. The ureter was dissected

below the crossing of the gonadal vessels, ligated and transected. The ligated distal ureter remained *in situ* and the most distal portion of the proximal ureter was brought to the skin through the lateral port. A Mac-Loc multipurpose 8.5 F drainage catheter (Cook Medical, Bloomington, IN, USA) was introduced into the lumen of the ureter; a curl was visualized in the renal pelvis under direct laparoscopic vision and locked into place. The catheter was secured in the ureter using a free tie and the most distal portion of the ureter affixed to the skin with six interrupted sutures. The drainage catheter was attached to a drainage bag and urine collection was initiated.

The pig was then repositioned in the right lateral decubitus position maintaining two midline trocars *in situ*. A new lateral trocar was introduced on the left side and the same procedure was repeated. The left renal hilum was then skeletonized with great care to ensure that all vascular sources to the kidney were identified. For nine pigs, a vascular clamp was applied across the hilum incorporating the artery and the vein for varying times (15, 30 or 60 min). In the three control pigs, we performed an identical dissection, but the clamp was not applied. In all pigs, insufflation was maintained for 160 min and no ice slush was used, so the kidney remained at the ambient temperature of the pigs. Before the pigs emerged from anaesthesia, the ureterostomy sites were covered and secured in a sterile fashion and the pigs placed in hand-sewn custom-designed vests (Lomir Biomedical Inc., Notre-Dame-de-l'île-Perrot, QC, Canada) with pockets housing urine collection bags attached to the intra-operatively placed ureterostomy catheters (Fig. 1). The pigs received i.v. fluid until emergence from anaesthesia. They were then monitored and evaluated by trained veterinary staff every 6 h for the first 24 h after surgery then twice daily for the duration of the study.

Cutaneous ureterostomies rather than percutaneous nephrostomies were used based on previous experience with limited complication-free placement of percutaneous nephrostomies in pigs. The patency of the ureterostomies was confirmed at the time of urine collection by observation of flowing urine, or the tube was flushed. At the time of necropsy ureterostomy patency was again verified.

Statistical analysis

Statistical comparisons were made between urine volume, NGAL levels, and the normalized NGAL (ratio of urine NGAL to urine creatinine) from the RUs of experimental animals (those with any amount of renal hilar clamping) and control animals (those with no hilar clamping) using the rank-sum test. We evaluated normalized NGAL to account for some of the differential renal function between the ischaemic and non-ischaemic kidneys. Serum NGAL and SCr levels were also compared between the control and experimental groups. Urine volume and NGAL levels are shown in Figs 2 and 3 for each of the ischaemic times (0, 15, 30 and 60 min). Owing to small sample sizes within each of the ischaemic time groups, statistical comparisons were made between the pigs that experienced ischaemia and those that did not. For each pig, we also compared levels of these markers from the ischaemic RU with levels in the contralateral non-ischaemic RU using the sign-rank test. Creatinine clearance (CC) was calculated on days 1 and 2, using the first and second 24 h of urine collection and the corresponding SCr drawn on days 1 and 2. All statistical analyses were performed using Stata 11.0 (StataCorp, College Station, TX, USA).

RESULTS

Of the 12 pigs that underwent surgery, nine (seven experimental and two control) were included in the final analysis. Three animals were excluded because of intra-operative or postoperative complications, including intra-operative bowel injury, postoperative pyelonephritis, and unilateral complete ureteric obstruction. The nine pigs in the final analysis included two with 0-min clamp time, one with 15-min clamp time, three with 30-min clamp time, and three with 60-min clamp time.

There were several significant differences between the experimental and control pigs when comparing the left ischaemic RUs (Table 1A–C). At 6, 12, 24 and 48 h the median cumulative urine volume was lower for the ischaemic (left) RU of experimental pigs compared with the same RU in control pigs (experimental vs control at 6 h, 10 vs 96 mL; at 12 h, 18 vs 268 mL; at 24 h, 223 vs 838 mL; and at 48 h, 757 vs 2203 mL, respectively; $P = 0.04$ [Table 1A]). In addition, the median urine NGAL concentration was greater in the ischaemic RU of experimental pigs than in the same RU in control pigs at 12 and 48 h (experimental vs control at 12 h, 226 vs 30 mg/dL; and at 48 h, 608 vs 94 mg/dL, respectively; $P = 0.04$ [Table 1B]); a twenty-sevenfold higher median NGAL concentration was apparent in the experimental pigs' ischaemic kidney at 24 h, but this was not significant (1022 vs 38 pg/mL; $P = 0.14$). Importantly, when comparing the non-ischaemic (right) RUs in experimental vs control pigs there were no significant differences in urine NGAL concentration, normalized NGAL, or cumulative urine volume at any time point (all $P = 0.14$), although there was a discernible trend towards higher urine output (more than double at all time points within the first 24 h) from the contralateral non-ischaemic (right) RU compared with control animals. By 72 h cumulative urine volume was equivalent in the contralateral non-ischaemic (right) RU compared with control animals (data not shown).

When comparing contralateral RUs (left vs right) in the same pigs, there were several important differences for the experimental group who underwent unilateral ischaemia (Table 2A–C). In experimental pigs, the ischaemic (left) RU had a lower median cumulative urine output than the contralateral non-ischaemic (right) RU at 6, 12, 24 and 48 h (left vs right at 6 h, 10 vs 220 mL; at 12 h, 18 vs 520 mL; at 24 h, 223 vs 1285 mL; and at 48 h, 757 vs 1965 mL, respectively; $P < 0.05$ [Table 2A, Fig. 2A]). Meanwhile, median NGAL concentrations at 12, 24 and 48 h (left vs right at 12 h, 226 vs 23 mg/dL; at 24 h, 1022 vs 81 mg/dL; and at 48 h, 608 vs 213 mg/dL, respectively; $P < 0.05$ [Table 2B, Fig. 3A]) and median normalized NGAL concentrations at 12 and 24 h (left vs right at 12 h, 3.31 vs 0.20; and at 24 h 7.23 vs 1.06, respectively, $P = 0.04$ [Table 2C]) were greater in the ischaemic (left) RU than in the non-ischaemic (right) RU. Control pigs showed no significant differences between left and right RU with respect to cumulative urine output (Table 2A, Fig. 2B), urine NGAL concentration (Table 2B, Fig. 3B), or normalized NGAL (Table 2C).

Serum creatinine and NGAL levels for the experimental and control animals are shown in Table 3A and B. No significant differences were observed between ischaemic pigs and non-ischaemic pigs (all $P = 0.14$). Evaluation of CC (Table 3C) by RU and ischaemia time had significantly higher CC in control left RUs than in the experimental ischaemic RUs on day 1 (357 vs 23 mL/min, $P = 0.04$), but not on day 2 (64 vs 30 mL/min, $P = 0.14$). We observed that

the trend of increasing duration of ischaemia was associated with a lower CC on the ischaemic side within the first 24 h, but this was less pronounced on day 2 (Table 4). CC was similar between the ischaemic and non-ischaemic RUs on the second day, regardless of duration of ischaemia.

Histopathological analysis of RUs exposed to ischaemia showed tubules with cytoplasmic basophilia, mild nuclear enlargement, and occasional mitoses (Fig. 4), changes that were consistent with the mild tubular necrosis and tubular regeneration associated with ischaemic injury. No such changes were observed in RUs not exposed to ischaemia.

DISCUSSION

We describe a novel two-kidney porcine surgery model that was designed to monitor the effects of unilateral renal ischaemia on both the ischaemic and non-ischaemic kidney using the urinary and serum biomarkers NGAL and SCr. This model was developed to more closely approximate the natural physiological state during renal surgery than do models found in the current literature, which includes iatrogenically induced unilateral renal ischaemia in surgically created solitary kidney models [1–4], or kidney transplant models in pigs.[17–19] These previous studies increased our understanding of renal physiology, but the removal of an entire RU is an aberration from the endogenous two-kidney state, and fails to reveal the potentially important physiological changes in the non-ischaemic RU during a unilateral ischaemia-inducing renal procedure.

In addition to CC from each individual kidney, we evaluated the volume of urine output after ischaemic insult. Cumulative urine output from the non-ischaemic right RU was more than double that of controls in the first 24 h after injury, although this lacked significance, possibly because of the small sample size. The present study suggests that the reductions in renal function that accompany acute temporary occlusion of the renal hilum may last several days and appear to be associated with a compensatory (but not significant in the present study) increase in urine output and, potentially, renal function from the contralateral kidney. CC calculations clearly show much higher function in the non-ischaemic RU at 24 h after ischaemic insult. Although speculative by design as it is a pilot trial, such changes may be critically important in assessing the impact of renal ischaemia in the setting of PN, as these shifts in function may mask the damage incurred by the ischaemic RU. The concept that one RU may have a significant impact on the contralateral RU has long been proposed [24], but animal models demonstrating these physiological changes have been lacking to date.

The mechanism by which a change in function in the non-ischaemic RU might occur are purely speculative in this instance, but vasoconstriction and increased systemic pressure-mediated increases in renal plasma flow and GFR in the non-ischaemic kidney have been reported in other settings [25]. Such compensatory changes may be critically important in assessing the impact of renal ischaemia in the setting of PN, as these mechanisms may mask the damage incurred by the ischaemic RU.

Serum creatinine is the most widely used marker of renal function, but it has several widely accepted limitations, including the influence of non-renal related factors such as age, race

and muscle mass. In addition, SCr may not accurately reflect changes in renal function in the acute setting because of the time delay needed to establish a new equilibrium between production and excretion of creatinine. Urine biomarkers for renal ischaemic damage are able to detect AKI immediately after insult, 24–48 h before a rise in SCr is evident [5–7].

To better understand the impact of acute unilateral ischaemia in our two-kidney model, we used the early biomarker of renal ischaemia NGAL, which has very recently been used in pig models as a marker of kidney injury in the setting of kidney transplantation [17–19]. NGAL is a 25-kilodalton protein, found in neutrophils and expressed at very low levels in epithelial cells in a variety of tissues, where it can be markedly induced in response to damage to those tissues [8, 26, 27]. NGAL has been most intensely investigated as a marker of renal ischaemia in a variety of clinical settings as an early marker for impending AKI [7, 10, 12, 13, 15]. We found that NGAL concentration increased dramatically in the RU exposed to ischaemia while there was little change in the NGAL concentration in the contralateral RU that did not receive ischaemia. Significant increases in NGAL were also absent in control pigs that did not receive ischaemia. Our data, though preliminary, suggest that the increasing amounts of ischaemia may correspond with increased peak concentration of urine NGAL (Fig. 3A). While NGAL levels have been associated retrospectively with post-insult AKI based on SCr levels in mice [8] and cardiopulmonary bypass patients [11], these novel findings suggest that NGAL is specific to the side of injury and duration of ischaemia.

Serum NGAL, also a marker of AKI, increased in pigs exposed to ischaemia. The rise, however, was not significantly greater than in the pigs that did not receive any ischaemia. Despite our small sample size, these results suggest urine NGAL concentration, when extracted directly from the ischaemic RU, may be a more sensitive marker of renal ischaemia than serum NGAL concentration. While the reason for this finding is unknown, it may be attributable to the location of NGAL production in the kidney, and urine levels may be more sensitive than serum levels for mild to moderate AKI. Selective kidney urine sampling may not be practical in all clinical settings, but is valuable in controlled experimental settings attempting to measure responses to controlled insults to the kidney. While NGAL remains a promising biomarker that appears to reflect the severity of AKI, its predictive performance may be confounded by a variety of clinical characteristics including age, pre-existing kidney disease, chronic illness, diabetes or malignancy [28, 29] or the presence of kidney infection.

Interestingly, SCr was elevated in one of the control pigs at 48 h. Urine volume from this pig's right kidney decreased significantly at 24 h and remained low, while NGAL was also elevated from the right RU compared with the left at 24, 36, 48 and 72 h in this pig (data not shown). We do not know the cause of the apparent decreased function of this kidney; it could be related to extended time in the right lateral decubitus position with a pneumoperitoneum of 15mm Hg, partial or intermittent obstruction of the ureter, or dehydration related to poor oral intake. A pneumoperitoneum of 15mmHg may be too high for pigs; evaluations in pigs with variable pneumoperitoneum pressures found changes in renal haemodynamics (renal blood flow) and urine output at 12mmHg [30, 31]. While intermittent or partial obstruction of the ureterostomy tube is a possibility, this was not

apparent at the time of necropsy for this animal; in fact, the only pig that had evidence of obstruction was excluded from analysis.

There were also several other significant limitations in the present pilot trial, the most obvious of which was the very small number of pigs included; however, despite these small numbers this novel model allowed unique investigation into the function of each RU and further study is warranted. Additional limitations included an inability to measure fluid intake in the postoperative setting. The pigs had free and unencumbered access to water but we did not have the capacity to measure each pig's individual fluid consumption. Differences in fluid intake may have influenced urine output, renal functional measurements such as SCr and potentially NGAL values. The findings in our control group show that even those animals without induced experimental ischaemic injury had changes in their renal functional parameters over time, possibly because of the small number of controls or perhaps because the surgical intervention itself was not benign.

We believe the present model is sound and can help identify many of the bilateral renal physiological factors at play during unilateral renal ischaemia. Possible improvements in our methodology for future studies would include: decreased pneumoperitoneum pressure to 10 mmHg, controlled access to water to measure fluid intake, and increased duration of survival to measure return to baseline in serum and urine markers. Porcine models are often limited to a small sample size given the cost and effort required to maintain the pigs. Future application of this model may require transition to a different animal species after, or for, validation.

Overall, this porcine model provided us with detailed information regarding renal function, urine output, and NGAL concentration in each individual RU. While the generalizability of our findings may be limited by a small sample size, we fulfilled our study objective to develop an animal model for evaluating bilateral renal function in response to unilateral kidney surgical interventions.

In conclusion, induced unilateral renal ischaemia in this novel two-kidney model revealed physiological changes in both the ischaemic and the corresponding non-ischaemic RU as early as 6 h after insult and persisting for at least 24 h. Additionally, urine NGAL identified kidney injury as a result of controlled renal ischaemia that is specific to the RU exposed to ischaemia.

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Abbreviations

AKI	acute kidney injury
NGAL	neutrophil gelatinase-associated lipocalin
RU	renal unit
CC	creatinine clearance
PN	partial nephrectomy
SCr	serum creatinine
eGFR	estimated GFR

Fig. 1. Surgery schematic. **A**, (top left panel) nephro-ureterostomy tube coiled in renal pelvis and traversing cutaneous ureterostomy site. **B**, (central panel) bilateral laparoscopic ureteric dissection and cutaneous ureterostomy placement. **C**, (bottom right panel) custom-designed vests housing the ureteric catheters and attached drainage bags to collect urine separately from each RU.

Fig. 2.

Mean cumulative urine volume collected over 48 h after surgery from the nine pigs in the final analysis. Controls, $n=2$; 15-min clamp time, $n=1$; 30-min clamp time, $n=3$; and 60-min clamp time, $n=3$. **A**, Left experimental kidney; **B**, right non-experimental kidney.

Fig. 3. Mean NGAL concentration of experimental RUs (left) and the corresponding non-experimental RUs (right) in pigs exposed to varying amounts of ischaemia: 0, 15, 30 or 60 min.

Fig. 4.
Representative histopathological analysis of renal parenchyma in RUs exposed to 60 min of warm ischaemia (left) vs non-ischaemic RUs (right). Scale bar 20 μ m.

Table 1Urine analysis for experimental vs control pigs for **A**, volume, **B**, NGAL and **C**, normalized NGAL

Group	Time	Experimental pigs: ischaemia in left RU, <i>n</i> =7	Control pigs: no ischaemia [*] , <i>n</i> =2	<i>P</i> [†]
A, Median (IQR) cumulative volume, mL				
Left RU	6h	10 (0, 36)	96 (72, 119)	0.04
Right RU		220 (159, 493)	77 (49, 105)	0.08
Left RU	12h	18 (14, 113)	268 (192, 344)	0.04
Right RU		520 (414, 1072)	230 (169, 290)	0.08
Left RU	24h	223 (55, 318)	838 (577, 1099)	0.04
Right RU		1285 (840, 1500)	578 (276, 880)	0.14
Left RU	48h	757 (361, 828)	2203 (1769, 2637)	0.04
Right RU		1965 (1696, 2226)	1045 (379, 1710)	0.14

B, Median (IQR) urine NGAL concentration, mg/dL				
Left RU	6h	12 (0, 69)	40 (11, 69)	0.6
Right RU		23 (8, 31)	36 (24, 48)	0.2
Left RU	12h	226 (165, 496)	30 (21, 39)	0.04
Right RU		23 (15, 34)	31 (28, 34)	0.4
Left RU	24h	1022 (339, 1842)	38 (21, 56)	0.14
Right RU		81 (13, 258)	113 (54, 172)	0.8
Left RU	48h	608 (285, 1220)	94 (51, 137)	0.04
Right RU		213 (114, 395)	253 (82, 424)	1

C, Median (IQR) normalized NGAL				
Left RU	6h	1.20 (0.00, 2.25)	0.17 (0.12, 0.22)	0.4
Right RU		0.13 (0.12, 0.20)	0.15 (0.15, 0.15)	0.8
Left RU	12h	3.31 (2.70, 12.51)	0.20 (0.18, 0.22)	0.04
Right RU		0.20 (0.13, 0.38)	0.22 (0.19, 0.26)	1
Left RU	24h	7.23 (2.80, 14.59)	0.49 (0.48, 0.49)	0.14
Right RU		1.06 (0.19, 2.64)	0.65 (0.43, 0.88)	0.8
Left RU	48h	9.54 (9.20, 15.90)	1.42 (0.59, 2.26)	0.04
Right RU		1.90 (0.79, 5.20)	2.00 (0.98, 3.02)	1

* Control group values are listed for each animal as well as the median (*n*=2).[†] Rank-sum test.

Table 2

Urine analyses of left and right RUs at multiple time points evaluating **A**, volume, **B**, NGAL and **C**, normalized NGAL

Group	Time	Left RU: ischaemia in experimental group	Right RU: no ischaemia *	$P^{\dagger}$
A, Median (IQR) cumulative volume, mL				
Experimental	6h	10 (0, 36)	220 (159, 493)	0.02
Control		96 (72, 119)	77 (49, 105)	0.18
Experimental	12h	18 (14, 113)	520 (414, 1072)	0.02
Control		268 (192, 344)	230 (169, 290)	0.18
Experimental	24h	223 (55, 318)	1285 (840, 1500)	0.02
Control		838 (577, 1099)	578 (276, 880)	0.18
Experimental	48h	757 (361, 828)	1965 (1696, 2226)	0.03
Control		2203 (1769, 2637)	1045 (379, 1710)	0.18

B, Median (IQR) NGAL, mg/dL				
Experimental	6h	12 (0, 69)	23 (8, 31)	0.7
Control		40 (11, 69)	36 (24, 48)	0.7
Experimental	12h	226 (165, 496)	23 (15, 34)	0.02
Control		30 (21, 39)	31 (28, 34)	0.7
Experimental	24h	1022 (339, 1842)	81 (13, 258)	0.03
Control		38 (21, 56)	113 (54, 172)	0.18
Experimental	48h	608 (285, 1220)	213 (114, 395)	0.04
Control		94 (51, 137)	253 (82, 424)	0.18

C, Median (IQR) normalized NGAL				
Experimental	6h	1.20 (0.00, 2.25)	0.13 (0.12, 0.20)	0.13
Control		0.17 (0.12, 0.22)	0.15 (0.15, 0.15)	0.7
Experimental	12h	3.31 (2.70, 12.51)	0.20 (0.13, 0.38)	0.02
Control		0.20 (0.18, 0.22)	0.22 (0.19, 0.26)	0.7
Experimental	24h	7.23 (2.80, 14.59)	1.06 (0.19, 2.64)	0.04
Control		0.48 (0.48, 0.49)	0.66 (0.43, 0.88)	0.7
Experimental	48h	9.54 (9.20, 15.90)	1.90 (0.79, 5.20)	0.06
Control		1.42 (0.59, 2.26)	2.00 (0.98, 3.02)	0.2

* Control group values are listed for each animal as well as the median ($n=2$).

† Sign-Rank test.

Table 3

Serum analysis (A, B) and CC (C) for experimental vs control pigs

	Experimental subjects (ischaemia in left RU) (n=7)	Control subjects (no ischaemia) (n=2)	<i>P</i> *
A, Median (IQR) SCr, mg/dL			
Baseline (preoperative)	1.1 (1.0, 1.2)	1.3 (1.2, 1.3)	0.14
24 h after surgery	1.9 (1.6, 2.9)	1.4 (1.2, 1.5)	0.14
48 h after surgery	2.1 (2.0, 3.9)	2.7 (1.4, 4.0)	0.8
B, Median (IQR) serum NGAL, ng/mL			
Baseline (preoperative)	140 (136, 180)	127 (122, 132)	0.14
24 h after surgery	212 (165, 269)	182 (126, 238)	0.6
48 h after surgery	311 (197, 408)	196 (188, 204)	0.2
C, Median (IQR) CC, mL/min			
Day 1: left RU	23 (2,138) [†]	357 (342,372)	0.04
Day 2: left RU	30 (7, 53) [†]	64 (60,68)	0.14
Day 1: right RU	359 (257, 475)	265 (189,340)	0.4
Day 2: right RU	70 (8, 105)	47 (6, 89)	0.8

* Rank-sum test.

[†] RU experienced ischaemia.

Table 4

Mean CC for left and right RUs at 24 and 48 h after induced left renal ischaemia stratified by duration of ischaemia

Ischaemia duration	Mean CC, mL/min			
	Left RU		Right RU	
	Day 1	Day 2	Day 1	Day 2
0 min (control)	357	64	265	47
15 min	138*	50*	695	83
30 min	95*	51*	409	79
60 min	17*	22*	236	38

* RU experienced ischaemia.