



The relationship between renal warm ischemia time and glomerular loss. An experimental study in a pig model¹

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Abstract

Purpose: To investigate the glomerular number after different warm ischemia times.

Methods: Thirty two pigs were assigned into four groups. Three groups (G10, G20, and G30) were treated with 10, 20, and 30 minutes of left renal warm ischemia. The sham group underwent the same surgery without renal ischemia. The animals were euthanized after 3 weeks, and the kidneys were collected. Right kidneys were used as controls. The kidney weight, volume, cortical-medullary ratio, glomerular volumetric density, volume-weighted mean glomerular volume, and the total number of glomeruli per kidney were obtained. Serum creatinine levels were assessed pre and postoperatively.

Results: Serum creatinine levels did not differ among the groups. All parameters were similar for the sham, G10, and G20 groups upon comparison of the right and left organs. The G30 group pigs' left kidneys had lower weight, volume, and cortical-medullary ratio and 24.6% less glomeruli compared to the right kidney. A negative correlation was found between warm ischemia time and glomerular number.

Conclusions: About one quarter of glomeruli was lost after 30 minutes of renal warm ischemia. No glomeruli loss was detected before 20 minutes of warm ischemia. However, progressive glomerular loss was associated with increasing warm ischemia time.

Key words: Kidney. Nephrons. Nephrectomy. Warm Ischemia. Swine.

■ Introduction

Partial nephrectomy provides oncological outcomes comparable to those of radical surgery of small renal cell masses as well as better preservation of renal function¹. Thus, partial nephrectomy is considered the gold standard for treating localized renal tumors^{2,3}. Warm renal ischemia is commonly performed during partial nephrectomy to achieve a bloodless surgical field, however renal ischemia has been associated with renal function impairment⁴.

Warm ischemia during laparoscopic partial nephrectomy is considered the most negative modifiable factor for this kind of surgery⁵⁻⁷. Traditionally, 30 minutes is considered the maximum safe time for renal warm ischemia. However, recent literature recommends that efforts should be made to reduce warm ischemia time to the shortest time possible time⁸. Some authors suggest that the time should be preferably kept below 20 or 25 minutes in order to avoid postoperative acute renal failure, because every minute counts when the renal hilum is clamped^{8,9}.

Most studies assessing the impact of warm ischemia on the kidney were based on functional tests such as serum creatinine level and glomerular filtration rate¹⁰⁻¹². Few researchers have used quantitative morphological methods to investigate the effects of renal warm ischemia in experimental models. They found that warm ischemia reduced the number and density of glomeruli^{13,14}. However, they did not investigate glomerular loss relative to warm ischemia time. The hypothesis of the present study was that the longer the renal warm ischemia time, the higher the glomerular loss. Thus, the objective of this study was to investigate the number of glomeruli after different renal warm ischemia times in a

pig model, using an unbiased stereological method.

■ Methods

All experiments were performed according to the Brazilian law for scientific use of animals, and this project was formally approved by the local Ethics Committee for animal experimentation (CEUA 048/2011).

Thirty-two male domestic pigs (commercial crossbreed strain) with a mean weight of 25 kg (about three months old) were used in this study. The animals were randomly assigned into four experimental groups of eight animals. The sham group was subjected to kidney and hilar dissection, but not to renal ischemia. Three other groups (G10, G20, and G30) were respectively subjected to 10, 20, or 30 minutes of renal warm ischemia.

Animals were premedicated with acepromazine (0.05 mg/Kg, IM), midazolam (0.5 mg/Kg, IM), and cetamine (5.0 mg/Kg, IM). General anesthesia was induced with propofol (3mg/kg, IV) and maintained with isoflurane (2% in oxygen, given by an endotracheal tube). Under general anesthesia and by using aseptic technique, the surgical procedure involved transperitoneal laparoscopic access, as previously used in pigs^{15,16}. The left kidney was completely exposed by dissection. Renal vessels were clamped en bloc in all groups with a laparoscopic Satinsky clamp (G10, G20, and G30), except in the sham group. After the predetermined time of ischemia, the vascular clamp was removed and the normal color of the kidney was verified. In the sham group, after dissecting the renal pedicle, the animals were maintained under anesthesia for 20 minutes without renal ischemia. The right kidneys were not manipulated during the experiment and were used as controls. The animals received analgesia for 48 hours after surgery, and food and water ad libitum six hours after surgery.

It took up to four hours after the procedure for recovery to normal ambulation. Serum creatinine levels were determined before surgery and on postoperative days 10 and 21 in order to assess renal function.

The animals were evaluated daily for 21 days after surgery, and after this period they were euthanized by anesthetic overdose (sodium thiopental 200 mg/kg IV). The kidneys were harvested, weighed, and their volumes measured by the Scherle's method^{17,18}; then, the organs were fixed by immersion in 4% buffered formaldehyde according to our laboratory routine. Samples were collected from the cortical region of these 64 kidneys and were processed by routine histological methods for stereological analysis. Each kidney was sectioned longitudinally and from the created cut surface, samples were collected randomly, in the same fashion as in previous studies^{14,18}. The specimens were paraffin embedded, sectioned at 5- μ m thickness, and stained by hematoxylin & eosin. The cortical-medullar ratio was estimated by using the point-counting-method according the Cavalieri principle^{18,19}. The absolute cortical volume (CV) was calculated by multiplying the cortical-medullar ratio by the renal volume.

From each kidney, 25 histological fields obtained from different sections of the renal cortex were photographed under 200X magnification with a digital camera (DP70, Olympus, Tokyo, Japan) coupled to a microscope (BX51, Olympus). Glomerular volumetric density

($V_v[\text{glom}]$), was estimated by the point-counting technique with a M42 test-system^{14,20,21}. The test-system was digitally superimposed over each image by using the ImageJ software (version 1.37v, NIH, Bethesda, Maryland, USA)

The volume weighted mean glomerular volume (VWGV), was estimated by using the point-sampled intercepts method^{14,16,18,22,23}, analyzing 50 glomeruli per kidney.

The estimation of the total number of glomeruli per kidney was calculated by multiplying the CV by the $V_v[\text{glom}]$ and dividing the result by the VWGV.

For all stereological parameter, left kidneys were compared to right organs of each group by using the Student's unpaired t-test. In addition, linear regression was used to examine the relationship between the number of glomeruli per kidney and the warm ischemia time.

Mean creatinine serum levels were compared using one way ANOVA. For all comparisons $p < 0.05$ was considered significant. Data was expressed as mean \pm standard deviation. Analyses were performed using GraphPad Prism 5.0 (GraphPad Software, San Diego, USA).

■ Results

All animals recovered well from surgeries. Serum creatinine levels were not different among the studied groups (Table 1).

Table 1 - Serum creatinine levels drawn before surgery and on postoperative days 10 and 21 of pigs submitted to sham surgery or to renal ischemia for 10 (G10), 20 (G20) or 30 minutes (G30).

	Preoperative	10 days Postoperative	21 days Postoperative	p value
Sham	1.52 \pm 0.38	1.10 \pm 0.10	1.39 \pm 0.69	0.62
G10	1.24 \pm 0.29	1.19 \pm 0.87	1.03 \pm 0.16	0.75
G20	1.15 \pm 0.24	0.97 \pm 0.23	1.10 \pm 0.66	0.83
G30	1.13 \pm 0.32	1.13 \pm 0.19	1.20 \pm 0.28	0.85
p value	0.21	0.94	0.57	

Data expressed as mean \pm S.D.

The weight and volume of left kidney of group G30 decreased by 6.2% and 6.3%, respectively, in comparison to the right kidney. In the sham, G10, and G20 groups, no difference was observed between the weight and volume of the kidneys.

The cortical-medullary ratio and absolute cortical volume was different among left and right kidneys of G30 group alone, with the left kidney having a 2.3% and 3.7% decrease in these parameters, respectively. For the other groups, no difference was noted in these parameters. Regarding Vv[glom] and VWGV, no difference was found among all

groups.

Finally, the total number of glomeruli in left kidneys of G30 group decreased by 24.6% in comparison to right kidneys. This represented a loss of approximately 290.000 glomeruli caused by warm ischemia for 30 minutes (Figure 1). Although no difference was observed in the total number of glomeruli in kidneys treated with warm ischemia for 10 and 20 minutes, a negative correlation was found between the warm ischemia time and the number of glomeruli (Figure 2). All stereological data is presented in Table 2 and histological examples of all groups are presented in Figure 3.

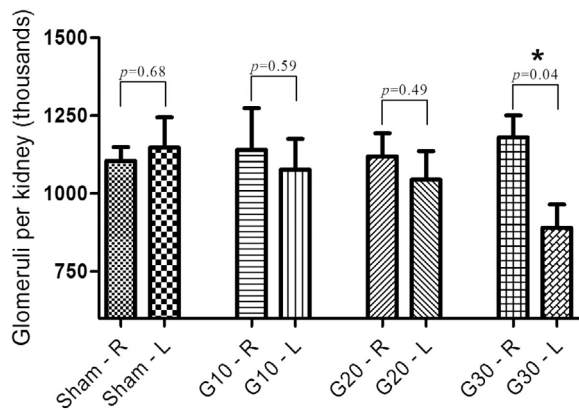


Figure 1 - Number of glomeruli in the right and left kidneys of pigs submitted to sham surgery or to left renal ischemia for 10 (G10), 20 (G20) or 30 minutes (G30). *different ($p < 0.05$) from contralateral kidney.

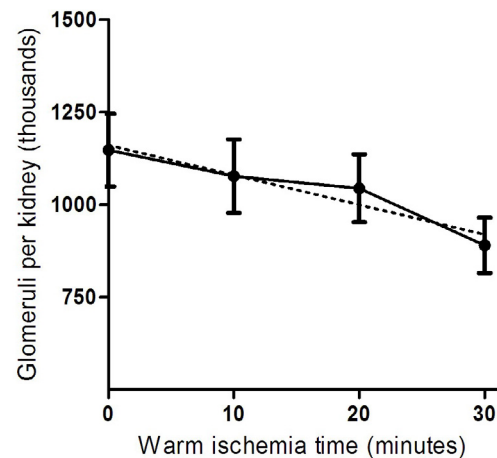


Figure 2 - Graphic showing the number of glomeruli in kidneys submitted to sham surgery or warm ischemia for 10, 20 or 30 minutes (solid line); and linear regression line (dashed line) showing the negative correlation between warm ischemia time and number of glomeruli ($r^2 = 0.9147$).

Table 2 - Stereological data of right and left kidneys of pigs submitted to sham surgery or to left renal ischemia for 10 (G10), 20 (G20) or 30 minutes (G30).

	Sham			G10			G20			G30		
	Right	Left	p value	Right	Left	p value	Right	Left	p value	Right	Left	p value
Kidney weight (g)	56.8 ± 4.9	58.2 ± 8.5	0.52	58.0 ± 7.1	55.9 ± 7.5	0.47	64.5 ± 11.1	64.0 ± 11.5	0.51	59.2 ± 10.9	55.5 ± 11.0*	0.008
Kidney volume (ml)	54.4 ± 4.1	55.2 ± 7.7	0.70	55.4 ± 6.4	53.1 ± 6.5	0.37	56.7 ± 7.3	56.8 ± 8.2	0.93	56.6 ± 9.8	53.0 ± 10.5*	0.007
Cortical-medullary ratio (%)	71.6 ± 2.3	70.4 ± 4.1	0.32	71.8 ± 1.8	72.1 ± 2.1	0.48	72.3 ± 5.5	71.6 ± 3.9	0.39	71.8 ± 2.4	70.1 ± 2.2*	0.04
Cortical volume (ml)	38.9 ± 3.3	38.9 ± 6.5	0.98	39.8 ± 4.8	38.3 ± 4.6	0.38	41.0 ± 5.9	40.7 ± 6.5	0.82	40.6 ± 7.4	37.2 ± 7.9*	0.002
Vv[glom] (%)	3.79 ± 0.5	3.72 ± 0.5	0.68	3.95 ± 1.0	3.82 ± 0.7	0.61	3.83 ± 0.6	3.57 ± 0.6	0.30	3.59 ± 0.4	3.08 ± 0.9	0.16
VWGV (105 μm ³)	13.4 ± 1.6	12.7 ± 1.1	0.25	14.1 ± 2.6	13.9 ± 2.5	0.76	14.1 ± 2.4	14.1 ± 2.1	0.97	12.5 ± 2.4	12.4 ± 1.2	0.94
Glomeruli (millions)	1.10 ± 0.1	1.14 ± 0.2	0.68	1.14 ± 0.3	1.07 ± 0.3	0.59	1.12 ± 0.2	1.04 ± 0.2	0.49	1.18 ± 0.2	0.89 ± 0.2*	0.04

Data expressed as mean ± S.D. * different ($p < 0.05$) from contralateral kidney.

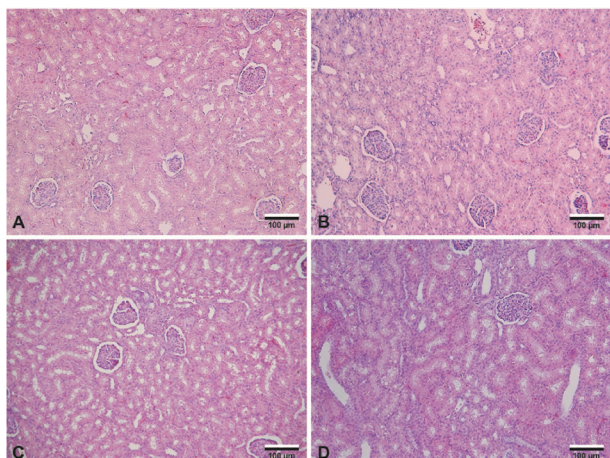


Figure 3 - Photomicrographs from renal cortex of pigs submitted to sham surgery (A), 10 minutes (B), 20 minutes (C), and 30 minutes of warm ischemia (D). Hematoxylin and eosin, x200.

■ Discussion

Warm ischemia was recently mentioned as the ultimate enemy for partial nephrectomy⁶. There are conflicting opinions in the literature on the suggested maximum safe time for renal warm ischemia. The optimal time has been stated as 20, 25 or 30 minutes or even longer than 30 minutes by some authors^{24,25}. Although the maximum time for a safe warm ischemia is controversial, the consensus is that it is an important aspect during partial nephrectomy. The results of the present experiment showed that a 30-minute period of renal warm ischemia led to a significant decrease in the number of glomeruli. Therefore, the use of warm ischemia for 30 minutes or more is discouraged, and this is in accordance with the authors who recommend the time for safe warm ischemia to be lower than 30 minutes^{24,25}.

Animals subjected to 10 or 20 minutes of warm ischemia did not show a statistically significant alteration of the glomerular number. This suggests that 20 minutes of warm ischemia could be considered as a “statistically safe” period for renal warm ischemia in the pig model. However, one may note that the kidneys

subjected to 20 minutes of warm ischemia had 75.000 less glomeruli than the contralateral organs not subjected to ischemia. In addition, kidneys exposed to only 10 minutes of warm ischemia lost 64.000 glomeruli. Although the glomerular loss after 10 and 20 minutes of renal warm ischemia was not statistically significant, linear regression demonstrated a progressive decrease in functional nephrons the longer the kidney remains under warm ischemia. It supports the statement that “every minute counts/matters” when the renal hilum is clamped during partial nephrectomy^{8,25}.

However, there is no published information about how much a minute counts for a kidney under warm ischemia. We have used stereology to quantify the number of glomeruli in the kidney, and correlated this number to warm ischemia time. Linear regression indicated that 8.000 glomeruli were lost per minute during warm ischemia.

Stereological quantification was used to precisely quantify the number of glomeruli. Stereological methods have been used to determine the glomerular number in kidneys affected by pneumoperitoneum, diabetes, and chronic stress^{19,22,26}. Stereological glomeruli quantification has also been used to study renal damage after partial nephrectomy¹⁴ or for comparing arteriovenous with arterial renal ischemia¹³. As the goal of partial nephrectomy is to remove renal tumors sparing as much nephrons as possible, it is reasonable that an unbiased determination of the glomerular number is important for studying the impact on the kidney. To our knowledge, this study is the first of its kind to show that warm ischemia time reduces the number of glomeruli and consequently the number of nephrons in the kidney.

The serum creatinine levels remained unaffected even though there was a significant reduction in glomeruli in kidneys subjected to 30 minutes of warm ischemia. This suggests that using serum creatinine levels as single method for studying the impact of ischemia is

flawed. Quantitative morphological methods are preferable. However, as indicated by another study, the serum creatinine levels may be normal because the right kidney was unaffected²⁷. Hence, a single kidney model should be used for studies measuring global renal function after warm ischemia²⁸.

Several methods have been proposed for increasing the ischemia time without affecting the kidney, some of which are commonly used in clinical practice. These include arterial or selective clamping instead of arteriovenous clamping, use of renal protective drugs, and cold renal ischemia. However, most of these methods are recommended based on functional tests only, with little quantitative morphological evidence.

A limitation of this study is that the kidney damage due to warm ischemia cannot be directly transposed to the damage that may occur in humans. Although the pig model is the most similar to human anatomy and physiology^{15,29}, this is still an experimental setting and different from the clinical setting. These animals were healthy, without renal tumors or any other medical condition. The pigs were not subjected to partial nephrectomy, but only to warm ischemia. However, we can imagine that in a clinical scenario the glomerular loss would be even higher after partial nephrectomy than what was observed in this study.

■ Conclusions

About one quarter of the glomeruli is lost after 30 minutes of renal warm ischemia in a pig model. No significant glomerular loss occurs at 10 or 20 minutes of warm ischemia. There is a progressive loss of kidney glomeruli relative to warm ischemia time. This confirms that every minute counts when dealing with a kidney under warm ischemia.

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