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Intra-Abdominal Cooling System Limits Ischemia–Reperfusion Injury During Robot-Assisted Renal Transplantation

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Robot-assisted kidney transplantation is feasible; however, concerns have been raised about possible increases in warm ischemia times. We describe a novel intra-abdominal cooling system to continuously cool the kidney during the procedure. Porcine kidneys were procured by standard open technique. Groups were as follows: Robotic renal transplantation with (n = 11) and without (n = 6) continuous intra-abdominal cooling and conventional open technique with intermittent 4°C saline cooling (n = 6). Renal cortex temperature, magnetic resonance imaging, and histology were analyzed. Robotic renal transplantation required a longer anastomosis time, either with or without the cooling system, compared to the open approach (70.4 ± 17.7 min and 74.0 ± 21.5 min vs. 48.7 ± 11.2 min, p-values < 0.05). The temperature was lower in the robotic group with cooling system compared to the open approach group (6.5 ± 3.1°C vs. 22.5 ± 6.5°C; p = 0.001) or compared to the robotic group without the cooling system (28.7 ± 3.3°C; p < 0.001). Magnetic resonance imaging parenchymal heterogeneities and histologic ischemia–reperfusion lesions were more severe in the robotic group without cooling than in the cooled (open and robotic) groups. Robot-assisted kidney transplantation prolongs the warm ischemia time of the donor kidney. We developed a novel intra-abdominal cooling system that suppresses the noncontrolled rewarming of donor kidneys during the transplant procedure and prevents ischemia–reperfusion injuries.

Abbreviations: DGF, delayed graft function; MRI, magnetic resonance imaging

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Introduction

Kidney transplantation is the "gold standard" therapy for patients with end-stage renal disease and offers the best quality of life and life expectancy for these patients. In the last 30 years, advances in the field of renal transplantation have mainly consisted of the fine-tuning of immunosuppressive strategies. Advances in surgical techniques have been limited, and the operation has not changed significantly since it was first performed, more than 60 years ago (1). In contrast, since 1995, living donor nephrectomy has evolved from an open to a laparoscopic procedure (2), followed by the development of a robot-assisted procedure (3). The laparoscopic procedure is now the "gold standard" technique in most transplant centers. Robotic surgery is now being presented as a new tool that enhances safety, allows remote operation, and enables operations to be performed in a limited space (4). When compared to laparoscopic surgery, robotic surgery was found to be equivalent in selected urologic, gynecologic, and visceral operations (5–8). Initial attempts to use robotic assistance in kidney transplantation were successful. However, the technique is currently used only in selected cases and specialized centers (9–11). Multiple groups have demonstrated the feasibility, safety, and reproducibility of robot-assisted kidney transplantation. Partial regional hypothermia (20°C) has been proposed clinically to limit rewarming of the kidney during implantation; however, no control group was presented in this study to demonstrate the impact of the procedure (11). A fully robotic donor and recipient kidney operation, using a combination of transvaginal and robotic surgery, has now been reported (12). The rationale for expanding the use of

robot-assisted kidney transplantation was a decreased rate of surgical site infections in obese patients (mean BMI: 45 kg/m^2) as compared to open surgery (10). Six-month patient- and graft-survival were similar when compared to open surgery. However, other differences were observed, including creatinine level at discharge, which was almost one-and-a-half times higher in patients transplanted with robotic assistance. We hypothesized that secondary warm ischemia during the anastomosis time might have played a role in the observed differences and sought to develop a novel intra-abdominal cooling system that minimizes warm ischemia during kidney implantation.

Here we aimed to compare ischemia–reperfusion injuries after open or robot-assisted kidney transplantation in a porcine model. We also describe a novel intraoperative cooling device aimed at reducing kidney rewarming during implantation.

Materials and Methods

Animals

This animal study was approved by the animal ethics committee of the Geneva Veterinarian Office and the University of Geneva, Geneva, Switzerland (protocol number GE/53/14/22826). Five-month-old male large white pigs (n = 23) with an average weight of 50.5 ± 5.9 kg were obtained from the animal facility from Arare, Switzerland. All pigs were maintained under standard conditions, and water and food were provided *ad libitum*. Animals were allocated into three groups: Group 1 (n = 6), conventionally performed open autotransplant, the grafts manually rinsed with 4°C saline every 3 min during implantation; Group 2 (n = 6), robotically assisted autotransplants without cooling system; and Group 3 (n = 11), robotically assisted autotransplants with cooling system.

Surgery

Anesthesia was performed using the following protocol: premedication, azaperone (2.2 mg/kg IM), midazolam (1.6 mg/kg IM), and atropine (0.02 mg/kg IM); anesthesia, ketamine (2–6 mg/kg/h), fentanyl (4–6 μg/kg/h), midazolam (0.2–0.4 mg/kg/h), and atracurium (1 mg/kg/h). The animals were intubated and ventilated. An arterial line was placed in the internal carotid artery. A nasogastric tube was placed. Heart rate, systemic blood pressure, atrial pressure, pulse oximetry, and end-tidal CO_2 were monitored. Pig temperature was monitored and maintained between 37.5°C and 38.5°C by using a heating pad. The surgery was performed by LB, MH, JB, NB, and RM. LB, JB, and RM are transplant surgeons and MH and NB are bariatric/upper gastrointestinal surgeons who routinely use robotic assistance. Briefly, the peritoneal cavity was accessed through a midline incision in the three groups, and the large and small bowel were reclined. The retroperitoneum was accessed, and both kidney hila were exposed. The aorta and vena cava were prepared. The pigs received 300 IU/kg IV heparin. A thermal probe was placed and secured with 5:0 Prolene sutures into the renal cortex prior to the procurement. A measure was recorded every 10 s. The left kidney was explanted and 5:0 nonresorbable running sutures were placed on the aorta and vena cava. The left kidney was immediately flushed with 4°C Institut Georges Lopez-1 preservation solution (IGL-1) and placed in cold IGL-1 and ice until it was put back in the operative field for anastomoses; this step was performed similarly among the three groups. The left kidney was transplanted onto the subrenal aorta and vena cava. In Group 1, end-to-side vascular venous and arterial anastomosis were performed manually using 5:0 and 6:0 running sutures and the kidney was rinsed manually with 4°C saline. In Groups 2 and 3, vascular sutures were performed end-to-side using the da Vinci Standard Surgical System (Intuitive Surgical Inc., Sunnyvale, CA) (Figure 1A, B). In Group 2, no cooling method was applied from the moment the kidney was removed from cold IGL-1 and ice to be placed into the operative field to perform the anastomoses. In Group 3, the cooling system was placed around the kidney from the back table until the anastomoses were finished. In Groups 2 and 3, the surgical field was reduced to the space required for the kidney and robotic arms placement. One assistant was allowed close to the surgical field to perform suction and retraction. In all three groups, a catheter was placed in the ureter of the transplanted kidney to monitor urine output. A midline incision was closed, and pigs were maintained under general anesthesia for 6 h. At that time point, animals underwent magnetic resonance imaging (MRI) of the kidney with gadolinium contrast. Kidney biopsies were performed, and pigs were sacrificed by intravenous injection of 100 mEq of potassium chloride (KCl) 7 h after midline closure.

Cooling system

The cooling system consisted of a watertight double sheath in silicone surrounding the kidney and continuously perfused by a tubing system with ethanol and methylene blue at 4°C (Figure 1C). The liquid was pumped in a closed circuit into the double sheath through two silicone tubes of 7 mm each. The external thickness was 5 mm, and the internal thickness was 0.8 mm.

MRI

MRI analyses were performed using a 3T scanner PRISMA (Siemens Healthcare, Erlangen, Germany). The MR sequence used was a T1-weighted 3D gradient echo sequence yielding a 1.7-mm isotropic spatial resolution obtained in 1 min, a T2-weighted 2D turbo spin-echo with 4-mm slice thickness and 1.2-mm in-plane resolution obtained in 1 min 5 s. Kidney perfusion was assessed using a dynamic fast gradient echo sequence (TR 376 ms, TE 1.4 ms, TI 240 ms, flip angle 12°) repeated 100 times for an acquisition time of 3 min 46 s. After 10 baseline acquisitions, a bolus of gadoteric acid contrast medium (Dotarem®, Guerbet, France) at a dose of 0.5 mmol/kg animal weight was injected using a power injector (3 mL/s; Medrad® Spectris, Bayer, Leverkusen, Germany) in a carotid artery followed by a 20-mL flush with 0.9% NaCl solution. During all image acquisitions the mechanical ventilation was held for 60 s. Kidney parenchyma were analyzed blinded from group assignments and were classified as homogeneous or heterogeneous on T2-weighted sequences.

Histopathological analysis of biopsies

Wedge kidney biopsies (3 × 4 cm, middle part) were formalin fixed and embedded in paraffin. Sections of 3-μm thickness were prepared and stained with silver Jones and periodic acid–Schiff (PAS) for light microscopy to analyze glomerular injury and proximal tubular lesions, respectively. Images were acquired using the Axiocam color camera (Zeiss, Oberkochen, Germany). The score used for histopathological analysis was performed based on an adaptation of those described in Goujon et al (13), Dittrich et al (14), and Hauet et al (15). Four morphological criteria were assessed to evaluate the impact of ischemia on grafts: (1) percentage of glomerular flocculus retraction in Bowman's space; (2) percentage of brush border loss; (3) percentage of lumina of tubules with cellular debris; and (4) tubular dilatation. Lesion severity was graded 0 to 5 according to the following criteria: no abnormality (0), mild lesions affecting 1–10% (1), 10–25% (2), 25–50% (3), 50–75% (4), and >75% (5) of the sample surface, respectively. Measurements were performed on four different representative fields (Jones: 100× and 40×; PAS: 400×) and blinded to group assignment. The final score for each biopsy ranges from

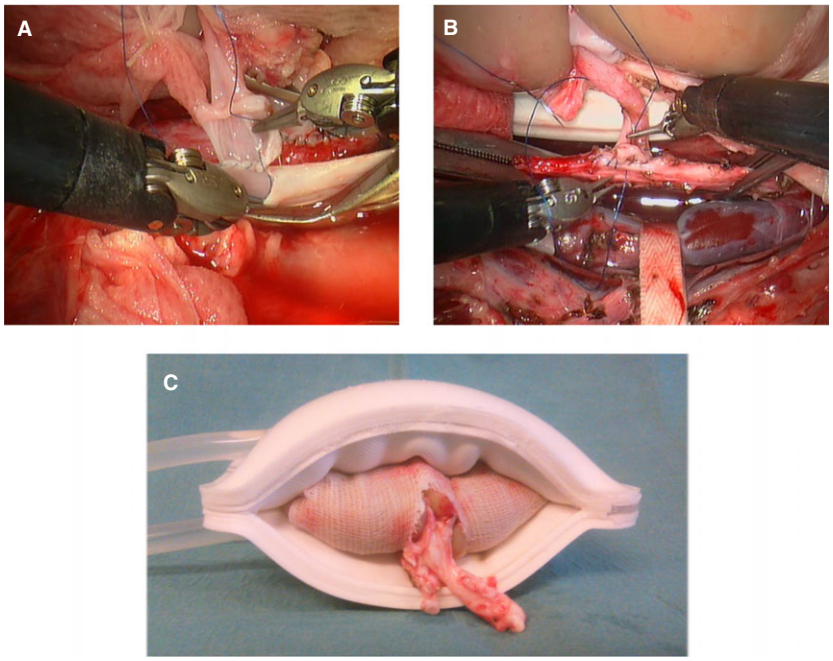

Figure 1: Surgical technique: (A) venous anastomosis, (B) arterial anastomosis, (C) intra-abdominal cooling system.

0 to 20 (addition of the four morphological criteria), a low/high score being the evidence of less/more severe ischemia, respectively. Morphometric analysis of lesions was performed using Osirix software (Bernex, Switzerland).

Statistical analysis

Results were expressed as mean values ± standard deviation. Differences between groups were analyzed using the Student t-test or Mann–Whitney *U* test (two groups) and one-way analysis of variance with Bonferroni multiple testing corrections (more than two groups). $p < 0.05$ was considered statistically significant. Computations were performed using Excel 2016 (Microsoft, Redmond, WA), Prism 7 (Graphpad, La Jolla, CA), and SPSS 22.0 software (IBM, Armonk, NY).

Results

Surgery, temperatures, and immediate function

The 23 pigs were divided into three groups. Six pigs were allocated to open surgery (Group 1), 6 pigs were allocated to robot-assisted surgery (Group 2), and 11 pigs were allocated to robot-assisted surgery with cooling system (Group 3). Pig weights were not statistically different between the three groups. However, pigs in Group 2 tended to be 6–7 kg heavier on average (Table 1). Overall operative time was similar between the three groups, and overall surgery lasted 276 ± 50 min. The kidney explantation was performed in a similar manner in the three groups and the average time between vessel clamping and flush with 4°C IGL-1 was 108 ± 102 s (defined as warm ischemia time). The cold ischemia time was 126 min in groups 1 and 2 and 135 min in group 3. In group 3, 2 to 4 min were required to put in place/remove the cooling system before/after the anastomosis time, respectively. Anastomosis time was significantly longer in the two robot-assisted groups (with and without cooling system) compared to the open surgery group, 70.4 ± 17.7 min ($p = 0.024$) and 74.0 ± 21.5 min ($p = 0.038$) versus 48.7 ± 11.2 min, respectively. Robotic anastomosis times were constant throughout the study (Figure S1A). The cumulative sum method revealed a significant decreasing trend (Figure S1B), consistent with a standard learning curve. In the open surgery group, the "classic" manual administration of topical 4°C saline (NaCl 0.9%) on the kidney allowed a poor control of the temperature, which reached 22.5 ± 6.5°C before reperfusion (Figure 2A). In the absence of a cooling method, kidneys in Group 2 rewarmed to 28.7 ± 3.3°C before reperfusion (Figure 2B). The cooling system used in Group 3 was effective in maintaining the temperature at reperfusion at 6.5 ± 3.1°C ($p = 0.001$ and < 0.001 compared to Groups 1 and 2, respectively) (Figure 2C). Overall, the temperature drop during the flushing phase was similar between the three groups and differed from the beginning of implantation, with poor temperature control in Group 1, no temperature control in Group 2, and good temperature control in Group 3 (Figure 2D). Additional experiments showed that the temperature differential between the kidney cortex and the medulla was marginal (maximum difference: 1.4°C) (Figures S2 and S3). Urine output was lower in the robot-assisted group without cooling system compared to the two other groups (125.0 ± 136.9 mL/h in Group 2 vs. 210.5 ± 230.2 mL/h in Group 1 and 225.9 ± 201.3 mL/h in Group 3) (Table 1), but these differences did not reach statistical significance.

Table 1: Robotic kidney transplant and control animal per- and intraoperative outcomes

Variable	Group 1 Open surgery (n = 6)	Group 2 Robotic-assisted without cooling system (n = 6)	Group 3 Robotic-assisted with cooling system (n = 11)	P value* (Gr 1 vs. Gr 2)	P value* (Gr 1 vs. Gr 3)
Weight, kg	49.6 ± 6.1	55.8 ± 7.3	48.0 ± 2.7	0.052	0.999
Operative time, min	258 ± 22	263 ± 49	288 ± 55	0.548	0.291
Warm ischemia time, s	120 ± 120	110 ± 70	104 ± 120	0.905	0.769
Cold ischemia time, min	126 ± 45	126 ± 37	135 ± 38	0.714	0.555
Anastomotic time, min	48.7 ± 11.2	74.0 ± 21.5	70.4 ± 17.7	0.024	0.038
Temperature at reperfusion, °C	22.5 ± 6.5	28.7 ± 3.3	6.5 ± 3.1	0.114	0.001
Urine output, mL/h	210.5 ± 230.2	125.0 ± 136.9	225.9 ± 201.3	0.662	0.827

Values are expressed as mean ± standard deviation.
*p-values were calculated using Mann–Whitney *U* test.

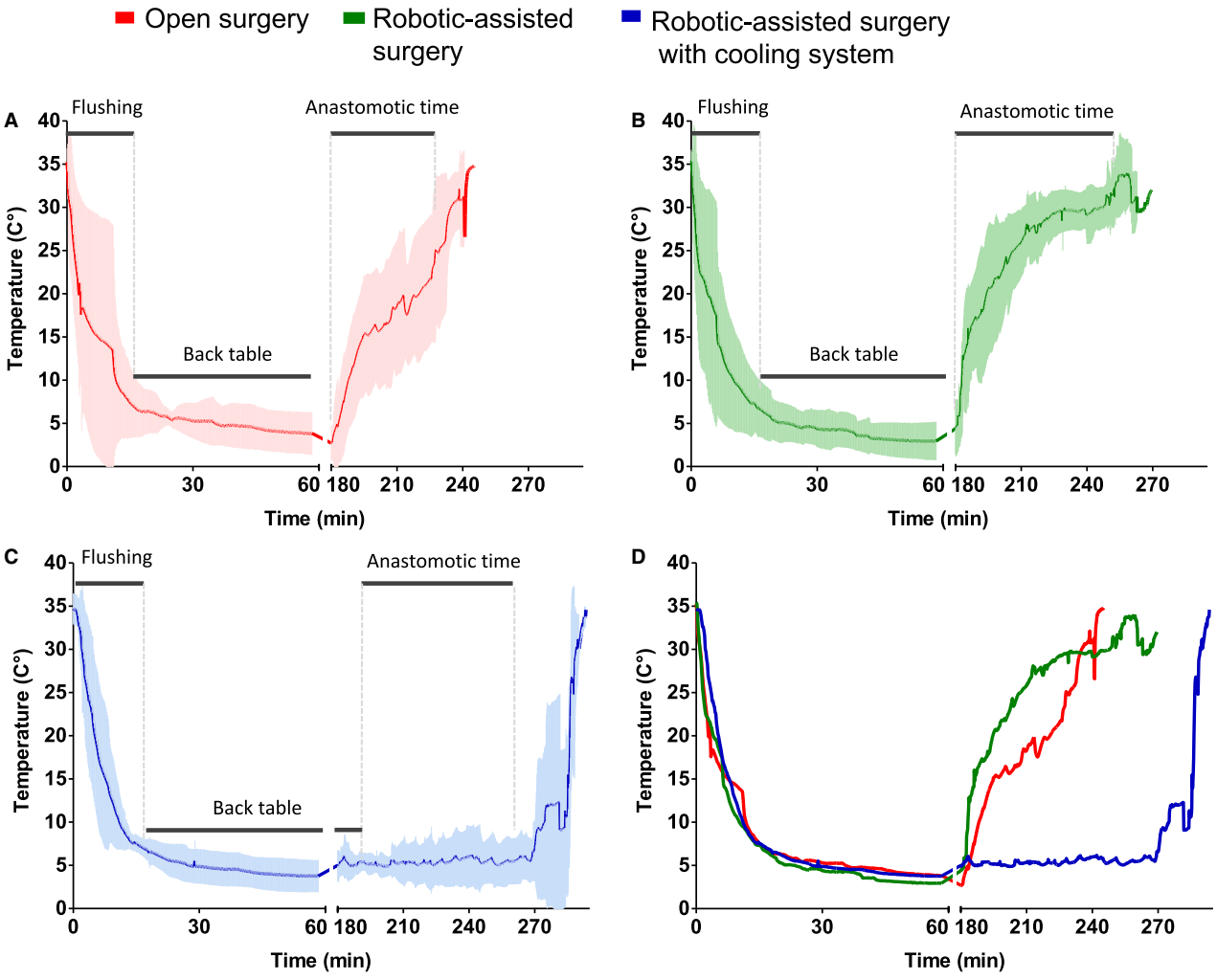

Figure 2: Temperature curves from kidney explantation to kidney revascularization. (A) Open surgery, (B) robot-assisted surgery, (C) robot-assisted surgery with the cooling system, (D) merge. Dashed area indicates the standard deviation.

MRI studies

T2-weighting sequence analyses showed that the two groups with a perioperative cooling strategy had less parenchymal heterogeneities compared to a kidney transplanted robotically without any perioperative cooling. Most (81.8%, 9 out of 11) of the kidneys transplanted

robotically with the use of the continuous cooling system and transplanted with topical cold saline administration (75.0%, 3 out of 4, 2 MRI were nonanalyzable) had a homogeneous parenchyma versus 67% in the group with kidneys transplanted without a cooling system (4 out of 6) (Figure 3A). Images showing parenchymal homogeneity and heterogeneity are shown in Figure 3B.

Ischemia–reperfusion injuries

Ischemia–reperfusion injuries were significantly more severe in the robot-assisted group without cooling than in the open surgery group and the robot-assisted group with cooling system (Table 2 and Figure 4A, B). In the group without a cooling system, the renal tubules had more cellular debris, more brush border loss, and were more dilated compared to the open surgery group. The percentage of Bowman's space with retraction of the flocculus was also higher in Group 2. When these changes were analyzed independently, the only difference identified was that the percentage of renal tubules with brush border loss between Group 1 and Group 2 reached statistical significance ($p = 0.004$). The Goujon score, which combines all the previous histopathological observations into a score, was significantly higher in the robot-assisted group without a cooling system compared to the open surgery group and the robot-assisted group with a cooling system ($p = 0.026$ and 0.011, respectively), indicating that more severe ischemia–reperfusion injuries occurred in Group 2 (Figure 4B).

Discussion

The present study was designed to analyze ischemia–reperfusion injuries during robot-assisted renal transplantation. We first observed that robot-assisted kidney implantation was associated with a longer anastomosis time and higher kidney temperatures. Those changes were associated with more kidney ischemia–reperfusion lesions. Therefore, we developed a temperature control system that efficiently kept the temperature at 4–6°C and allowed the reversal of ischemia–reperfusion lesions.

The first objective of our study was to establish a large-animal model of robot-assisted kidney transplantation. To our knowledge, this is the first reported attempt to analyze key factors of this procedure in a preclinical setting. Groups who first performed this procedure in patients did not report previous *in vivo* experimental attempts (9,10,16). A first important difference that we observed was the increase of the anastomosis time in the robot-assisted transplantation group. We needed 70–74 min to complete both venous and artery anastomoses in the robotic groups and 49 min in the open surgery group. This is different from what was observed by Oberholzer et al, who reported 48 and 49 min of anastomosis time in robot-assisted and open kidney transplantation, respectively, the main difference being that no cooling technique was used/described during implantation. Another group with greater experience could reduce anastomosis time below 30 min (11). Differences such as the learning curve, hand assistance, type of da Vinci Surgical System, surgical times definition, and technique may explain these timing differences. Interestingly, in the Chicago group's study (10), the cold ischemia time was 47 min longer in the robotic group than the open surgery group. Based on our experience and the results of large meta-analyses and series, the use of robotic assistance is likely responsible for an increase in operative time, as compared to open or laparoscopic surgery (17–21). We thus believe that a longer anastomosis time and uncontrolled kidney rewarming are valid concerns in robot-assisted kidney transplantation. The closed environment created by minimally invasive surgery, the time required in case of conversion, unexpected events requiring changing or manipulation of robotic arms, and the presence of a significant learning curve are likely to increase anastomosis time and kidney rewarming, justifying our view that an efficient intracorporeal cooling device should be utilized.

The abovementioned considerations prompted us to develop a new tool to keep the temperature close to 4°C during reimplantation. We developed a novel intra-abdominal cooling system based on the circulation of cold ethanol to continuously refrigerate the kidney. Ethanol is cooled to 0°C in the pump to obtain 4°C in the circuit; the use of ethanol avoids freezing the system. The efficacy of the device was confirmed by the kidney temperature, which was maintained at 6.5°C at the clamp removal. A few minutes were needed to place the cooling system before and remove it after vascular anastomoses; nevertheless, these procedures did not prolong anastomosis time nor significantly increase cold ischemia times in Group 3. We thus obtained three distinct profiles: a group with a short anastomosis time and poor temperature control (Group 1) and two groups with a longer anastomosis time and either no temperature control (Group 2) or good temperature control (Group 3). Of note, the temperature at reperfusion in Group 2 (i.e. 22.5°C) was consistent with other studies reporting kidney temperature using topical ice/cold saline administration during anastomosis time, i.e. 19.0°C (22) and 20.3°C (11). A first observation was that urinary output tended to be lower in group 2 than in Groups 1 and 3. This is consistent with previous reports demonstrating that longer vascular anastomosis time has a negative impact on early kidney transplant function and graft survival (23,24).

We first sought to analyze kidney injuries in the three groups by MRI. MRI image analysis showed marginal differences between the three groups. This observation may be related to the fact that MRI was performed early after transplantation (after 6 h). This time frame was guided by the 3R principles at our institution (Replace, Reduce, Refine) that did not allow us to awaken the pigs

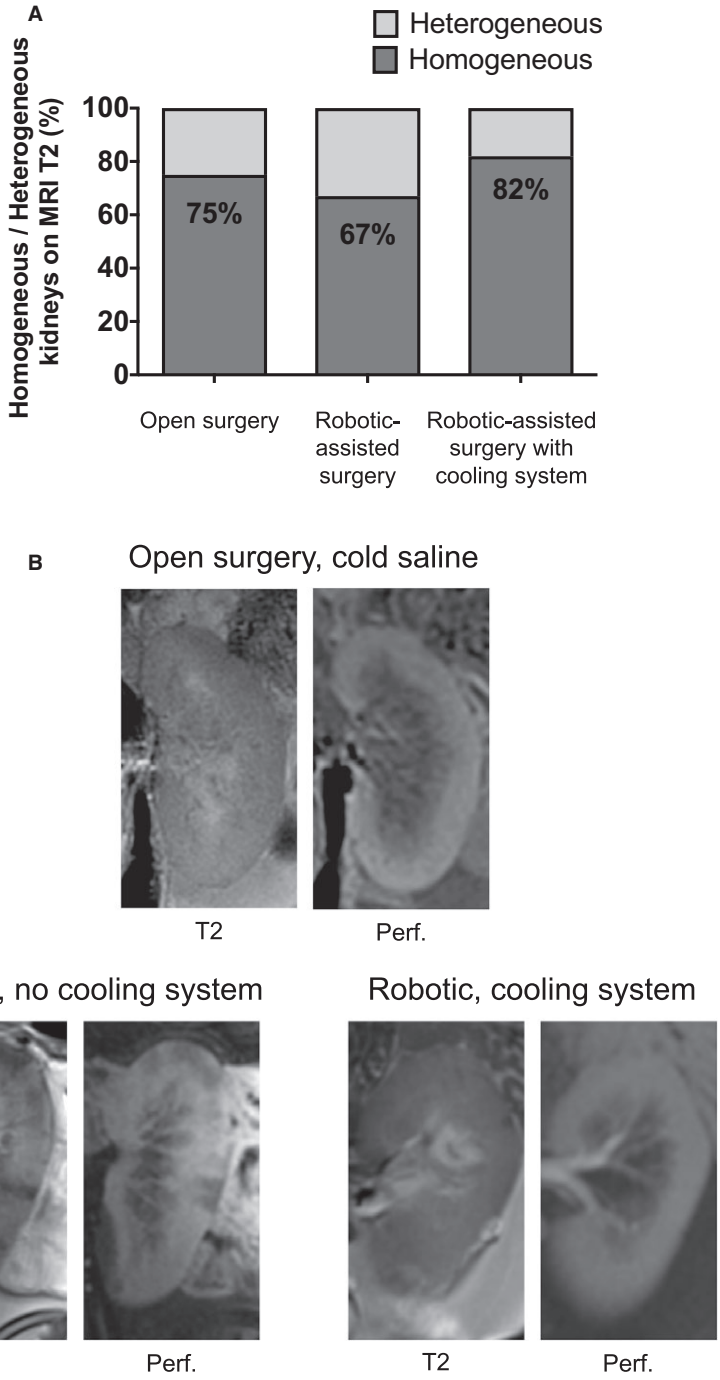

Figure 3: MRI studies. (A) Percentage of homogeneous/heterogeneous renal parenchyma on T2-weighting sequences in the three groups. (B) Image showing an example of parenchymal homogeneity in Group 1 (open surgery, administration of topical cold saline) and Group 3 (robotic surgery, with cooling system) and heterogeneity in Group 2 (robotic surgery, without cooling system).

after such major surgery, to reduce pain and suffering. The results showed that Group 2 (without any cooling) was slightly different from the two other groups. Indeed, parenchymal heterogeneities were more frequent in Group 2, and this was consistent with the histological findings showing more severe ischemia–reperfusion lesions in this group.

Kidney histology, collected 7 h after completion of the surgery, consistently showed that ischemia–reperfusion

Table 2: Robotic kidney transplant and control animal kidney histopathological ischemia-reperfusion lesion analysis and quantification

Variable	Group 1 Open surgery (n = 6)	Group 2 Robotic-assisted without cooling system (n = 6)	Group 3 Robotic-assisted with cooling system (n = 11)	p value* (Gr 1 vs. Gr 2)	p value* (Gr 1 vs. Gr 3)
Renal tubules with cellular debris, %	40.0 ± 32.9	66.7 ± 35.0	41.0 ± 26.9	0.240	0.875
Renal tubules with brush border loss, %	15.2 ± 6.6	53.0 ± 20.1	11.1 ± 6.5	0.004	0.368
Dilated renal tubules, %	34.2 ± 3.8	38.3 ± 8.9	34.4 ± 4.1	0.485	0.875
Bowman's space with retraction of the flocculus, %	31.8 ± 6.8	32.1 ± 6.0	29.8 ± 8.0	0.818	0.792
Modified Goujon score	10.3 ± 2.0	13.5 ± 2.4	10.1 ± 1.5	0.026	0.875

Values are expressed as mean ± standard deviation.
*p-values were calculated using Mann–Whitney *U* test.

lesions were significantly more pronounced in Group 2 (long anastomosis time and no cooling system), suggesting the inferiority of this technique compared to the standard open procedure. Postulating that these lesions were solely due to the prolonged secondary ischemia time, we demonstrated that temperature control counteracted this issue. Indeed, Group 3 had a similar lesion profile to Group 1, suggesting that the lesions could be avoided by controlling the temperature up to reperfusion. The most significant factor was brush border loss, which was present in 15% and 11% of the biopsies respectively in Groups 1 and 3, compared to 53% in Group 2. The modified Goujon score confirmed that Groups 1 and 3 outperform Group 2.

The strengths of our study include the following: (1) the analysis of key factors of robot-assisted kidney transplantation in a preclinical setting; (2) the implementation of a novel intra-abdominal cooling system designed to intraoperatively cool the donor kidney; and (3) a detailed and real-time analysis of kidney temperature starting before kidney retrieval and pursued up to complete reperfusion. Several factors indicate that temperature control is of crucial importance in robot-assisted kidney transplantation: in the study by Oberholtzer et al, the robotic group had 1.4-times higher creatinine levels at discharge ($p < 0.05$), more delayed graft function (DGF) ($p > 0.05$, not statistically significant), and more rejection episodes ($p > 0.05$, not statistically significant) (10). Menon et al used regional hypothermia to lower kidney graft temperature during clinical robot-assisted transplantation (11). The allograft was cooled by repetitively delivering ice slush through a large trocar (GelPOINT) device and this technique allowed the temperature of the graft to reach 20°C before unclamping the vessels. Postoperative kidney function was as follows: creatinine was 1.3 ± 0.6 mg/dL at discharge and 1.1 ± 0.2 at 6 months. In comparison, Oberholzer et al reported slightly higher creatinine levels in the robot-assisted transplant cohort without use of a cooling system (2.0 ± 1.4 mg/dL at time of discharge and 1.5 ± 0.4 at 6 months) (10). The regional hypothermia strategy used by Menon et al is similar to the standard administration of topical cold saline during open surgery, controlling kidney temperature only partially. Our system of continuous cooling allows a more precise and lower temperature control of the graft until reperfusion (i.e. 6.5°C).

We must acknowledge some limitations of our study. First, it would have been of interest to add other groups to the study, such as an open surgery group with the cooling system, an open surgery group without any cooling, or a robot-assisted group with topical cold saline administration. Applying the cooling system to the open surgery group is attractive and will be performed in our next experiments. Nevertheless, our assumption was that the histological lesions observed in the open surgery group were already minimal and no further improvement would be possible. Applying the "classic" topical cold saline administration to robotic surgery appeared to be an intermediate solution that would not fully prevent ischemic reperfusion lesion. It is worth noting that this strategy was used in some cases, as previously reported (12). Potential limitations of this study are the limited number of large animals available for experimentation and short-term endpoints, as guided by the 3R principles. Therefore, an important point to determine is whether these short-term endpoints represent meaningful clinical outcomes. We believe that the short-term but consistent changes we observed could translate into mid- and long-term changes in terms of DGF rates, acute/chronic rejection, and ultimately, graft survival. Tangible evidence previously demonstrated that transplanted kidneys subjected to longer anastomosis times were more prone to DGF, acute/chronic rejection, and shorter graft survival (23,24). Moreover, kidneys with poorer temperature control at reperfusion (>15°C vs. <15°C) were significantly more likely to suffer DGF (22). Of note, the negative effects of longer anastomosis times and poor temperature control can potentiate one another. Another aspect not analyzed in our study was the ureteral anastomosis. We did not include this aspect because of the limited follow-up allowed by our experimental protocol. Finally, we acknowledge the fact that we used a semi-open procedure in both robotic groups. We were limited

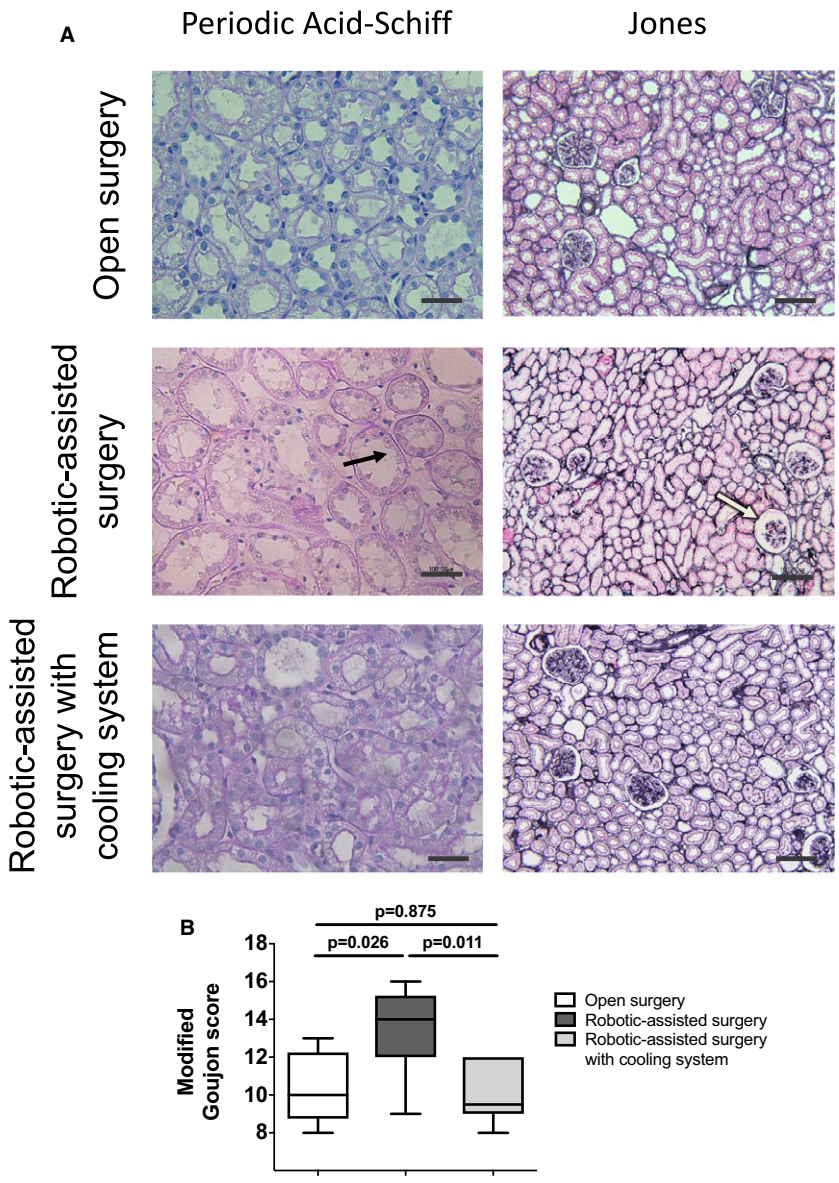

Figure 4: Histology analyses. (A) Kidney histological sections stained with periodic Acid–Schiff (PAS) (proximal tubular lesions) and Jones (glomerular injury). The black arrow indicates brush border loss. The white arrow indicates flocculus retractation. Magnification: PAS 400×; Jones 100×. Scale bars: 100 μm. (B) Modified Goujon score indicating ischemia–reperfusion injury severity for the three groups. *p*-values were calculated using Mann–Whitney *U* test.

by the availability of a safe laparoscopic surgical vessel clamping system, sophisticated robotic device, and the lack of an operating table with a full range of motion in our animal operating room.

Our future aim is to reproduce the clinical experience of the Chicago (10) and the Indian group (11) and implement a clinical program for robot-assisted transplantation in obese patients who did not achieve a sufficient weight loss compatible with an open kidney transplantation (25). It is worth noting that in Europe, morbidly obese patients with end-stage kidney disease are less frequent than in the United States; to date, our policy is to achieve weight loss by way of bariatric surgery, mainly sleeve gastrectomy. Nevertheless, expansion of robotic surgery is under way, and research in this field may benefit the transplant field through the development of new robotic devices, as well as advanced organ preservation techniques. Moreover, robotic surgery brings advantages in minimally invasive surgery such as three-dimensional views, precision, a live assessment of anatomy with augmented reality, and functional monitoring. These advantages render

this advanced surgical tool in transplant settings worthy of assessment.

In conclusion, we demonstrated that prolonged anastomosis time with poorly controlled kidney temperature cause increased ischemia–reperfusion injuries following robot-assisted kidney transplantation. The implementation of a novel intraoperative cooling system successfully prevented ischemia–reperfusion injuries.

Acknowledgments

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Author Contributions

LB designed the study. RM, VP, MH, CJ, NB, JB, AN, RR, FL, and LB performed the experiments. RM, VP, MH, CJ, NB, JB, AN, RR, JPV, SM, FL, PM, and LB collected the data. RM analyzed the data. RM performed statistical analysis. RM, VP, and LB interpreted the data and wrote the manuscript. RM and LB had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Disclosure

The authors of this manuscript have conflicts of interest to disclose as described by the *American Journal of Transplantation*. Monika E. Hagen received personal fees and nonfinancial support from Intuitive Surgical Inc., outside this project. Monika E. Hagen received personal fees from Ethicon Endosurgery Inc., outside this project. The other authors have no conflicts of interest to disclose.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Figure S1: Learning curve. (A) Operative time in minutes plotted against case number. (B) Cumulative sum for operative time plotted against case number. p-value calculation is based on Pearson's correlation.

Figure S2: Placement of the temperature probes into the kidney flushed with IGL-1 (n = 3). Cortical probe was placed 0.6 cm under the capsule surface. Medulla probe was placed 2.3 cm under the capsule surface. Probes were secured with 5:0 Prolene sutures. One additional temperature probe was placed on a heating pad (set at 37.5°C) that simulate the pig (not shown).

Figure S3: Kidney cortex and medulla temperature curves using the cooling system. (A) Heating pad temperature (set a 37.5 °C). (B) Renal cortex temperature. (C) Renal medulla temperature. (D) Merge. The initial phase shows a slow rewarming of the kidney, during this phase, the cortex was slightly warmer than the medulla (maximum difference: 1.4°C). Once the cooling device was activated the temperature began to drop and the two probe curves intersect and progressively merge (maximum difference: 1.3°C). Lines represent mean of three independent experiments and dashed area indicates the standard deviation.