

THE ROBOTIC LIVING DONOR KIDNEY DONATION: TECHNICAL ASPECTS AND RESULTS

Stefano Di Sandro^{1*}, Barbara Catellani^{1,2*}, Daniela Caracciolo¹, Giuseppe Esposito^{1,2}, Roberta Odorizzi¹, Tiziana Olivieri¹, Giacomo Assirati¹, Cristiano Guidetti¹, Paolo Magistri¹, Giacomo Mori³, Gabriele Donati³, Gian Piero Guerrini¹, Fabrizio Di Benedetto¹

¹ Hepato-pancreato-biliary Surgery and Liver Transplantation Unit, University of Modena and Reggio Emilia, Modena, Italy; Department of Surgical, Medical, Dental and Morphological Sciences with Interest transplant, Oncological and Regenerative Medicine, University of Modena and Reggio Emilia, Modena, Italy; ² Clinical and Experimental Medicine PhD Program, University of Modena and Reggio Emilia, Modena, Italy; ³ Nephrology, Dialysis and Transplant Unit, University Hospital of Modena and Reggio Emilia, Modena, Italy. Department of Surgical, Medical, Dental and Morphological Sciences, Section of Nephrology, University of Modena and Reggio Emilia, Modena, Italy

*Authors contributed equally to the manuscript.

Summary

Background. Living donor kidney transplantation is the best option for patients with end-stage renal disease, in terms of both patient and graft survival. Minimally invasive donor nephrectomy represents the standard of care for living donors and robotic technique appears to be safe and increasingly widespread. The aim of this study was to report technical aspects and results of full Robotic Living Donor Nephrectomy (RLDN) at our center, with particular focus on surgical complications and outcomes.

Materials and methods. In November 2019, a RLDN program has been established at our institution. A multidisciplinary board evaluated all potential donors and the nondominant kidney was selected. All cases were performed with daVinci[®] Surgical System in a three-arm configuration. Full RLDN was adopted with a Kustner fascial incision for extraction of the graft in an Endo-Catch[®].

Results. Thirty-eight consecutive RLDN were performed at our center: 11 right and 27 left. Median donor age was 54.7 years and median donor body mass index was 24.7 kg/m². In all cases, RLDN was carried out without need of open conversion and intraoperative complications and with minimal blood loss. Median surgical time was 240 min and median warm ischemia and cold ischemia times were 3.38 min and 79 min, respectively. The median hospital stay was three days; four cases of Clavien-Dindo complication grade 2 occurred. None of the recipients experienced delayed graft function.

Conclusions. RLDN can achieve optimal functional outcomes for both donor and recipient. Full RLDN is a feasible, safe and effective technique in experienced hands and in highly specialized transplant centers, able to ensure optimal perioperative and functional outcomes.

Key words: living donation, living donor nephrectomy, full robotic approach, donor outcomes

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Correspondence

Stefano Di Sandro
E-mail: sdisandro@unimore.it

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List of abbreviations

ASA: American Society of Anesthesiologists
 BMI: body mass index
 CT-scan: computed tomography angiography
 ESKD: end-stage kidney disease
 KT: kidney transplant
 GFR: glomerular filtration rate
 KDIGO: kidney disease improving global outcomes
 LDKT: living donor kidney transplantation
 LDN: living donor nephrectomy
 LLDN: laparoscopic living donor nephrectomy
 MRI: magnetic resonance imaging
 NRS: numerical rating scale
 POD: postoperative day
 RLDN: robotic living donor nephrectomy
 SRM: small renal mass
 UNOS: united network for organ sharing

INTRODUCTION

Kidney transplant (KT) is the treatment of choice for patients with end-stage kidney disease (ESKD) since it provides significant survival benefit and better quality of life than long-term dialysis ¹. Moreover, waiting time on dialysis is associated to worse outcomes after living or cadaveric transplant ².

Currently, in the United States more than 100,000 patients are listed for KT ³ and 27,332 kidney transplants were performed in 2023 (6,290 from living donors) ^{3,4}. In the last ten years, the number of candidates for kidney transplant in the United States has almost doubled ⁵. In Europe, about 90,000 people are waiting for a KT, and it is estimated that 6,000 patients die every year while on the waiting list ⁶. In Italy, about 50,000 patients receive a dialysis regimen and in 2023, unfortunately only 1,898 deceased donor kidney transplants (84.6%) and 346 living donor kidney transplants (15.4%) were performed ⁷.

Living donor kidney transplantation (LDKT) is the best treatment option for patients with ESKD, in terms of both patient and graft survival ¹. Shorter cold ischemia time, lower rejection rate, immediate allograft function, superior long-term patient and graft survival and easier access to pre-emptive KT are just a few of the several advantages ^{1,8}. Kidney Disease Improving Global Outcomes (KDIGO) Clinical Practice Guidelines recommend pre-emptive transplantation with a living kidney donor as the preferred treatment for transplant-eligible patients with ESKD ⁹. LDKT offers substantially superior graft function and survival and better quality of life compared with deceased kidney transplantation. In addition, living donor nephrectomy (LDN) is considered a safe and well-tolerated procedure, which allows a quick full recovery of donors ¹⁰.

Therefore, in the last decade, the United Network for Organ Sharing (UNOS) has reported a continuous increase in the numbers of LDKT performed per year ¹¹. Since 2009, over 27,000 living donor nephrectomies were performed yearly worldwide, with the majority of countries reporting a 50% increase in the past decade ¹¹.

The introduction of minimally invasive surgery represented a significant innovation in the development of surgical technique for living kidney donation ¹² with the aim to improve donors' perioperative results while ensuring optimal grafts for KT. Less postoperative pain, shorter hospital stay, faster recovery, rapid patient rehabilitation, better cosmetic results and lower incisional hernia rate compared with the traditional open approach are some of the advantages of minimally invasive surgery ¹³. Thanks to these, a significant increase of living kidney donations occurred in the United States and around the world ¹⁴. In 2001, the University of Illinois, Chicago reported the first series of robotic-assisted laparoscopic donor nephrectomies ¹⁵. Since that experience, the use of robotic approaches increased around the world with several technical variations and improvement, although higher cost and still scarce reports ¹⁶.

The aim of this study was to report the results of full robotic living donor nephrectomy (RLDN) at our center, with particular focus on surgical complications and short- and mid-term outcomes.

MATERIALS AND METHODS

In November 2019, a RLDN program has been established at Modena University's HPB and Transplantation Center. All cases were performed with daVinci Si[®] (until November 2021) and daVinci Xi[®] surgical system (Intuitive Surgical, Sunnyvale, CA) in a three-arm configuration. The full RLDN was adopted in all the consecutive cases performed. Data were prospectively collected in dedicated database after obtainment of a signed informed consent. Variables such as donor's characteristics, intraoperative donor data (kidney laterality, operating time, warm and cold ischemia time, blood loss and rate of conversion), peri and postoperative donor data (time of resumption of oral intake, hospital stay, complications), donor kidney function and recipient and graft survival were analysed. Intraoperative and postoperative complications were graded according to the Clavien-Dindo classification ¹⁷. After discharge, all the patients underwent regular follow-up, including renal function tests and blood pressure monitoring at least annually.

Donor selection criteria

Donors were selected according to the principles of KDIGO clinical practice guidelines ¹⁸. Donor candidates were

at least 18 years old, with either kinship or unfamiliar relationship. At the first screening, the demographic data of every potential donor and recipient (including sex, age, ethnicity, height, weight, body mass index, comorbidities, biological relationship between the donor and the recipient, work, and education level) were recorded. After that, all potential donors were evaluated by a multidisciplinary transplantation board and underwent a pre-operative evaluation, including physical examination, laboratory tests and psychological assessment. After the donation statement, candidates underwent legal evaluation to unveil coercion to donation.

Donors' vascular anatomy and split renal function were respectively evaluated by a computed tomography angiography (CT-scan) with 3D vessels reconstruction and a sequential renal scintigraphy with Tc-99m-labeled mag3 (mercaptoacetyltriglycine-3). No contraindication was set ab initio for use of right-sided kidneys. The choice between right or left RLDN was based in the first place on split renal function (if differential function was more than 10%, the nondominant kidney was selected) and secondarily on vascular anatomy (single vs multiple vessels) or other features (kidney malformation and/or anomalies, urolithiasis, renal cysts). The left kidney was usually selected in case of functional equivalence, because of the longer renal vein. Multiple renal arteries did not represent a criterion for kidney selection. In cases involving two or more renal arteries, vascular reconstruction was carried out before implantation to the recipient vessels, with bovine pericardium patch at the back table.

Surgical technique

All the procedures were performed with a transperitoneal access, using the daVinci Si^o Xi^o platform in a three-arm configuration. Patients were placed in the right lateral $\approx 60^\circ$ decubitus for left nephrectomy or in left lateral $\approx 60^\circ$ decubitus for right nephrectomy with a cushioned bean-bag between the legs. The robotic operating table is flexed at flank level and placed in minimal Trendelenburg position, to expose the space between the anterior-superior iliac crest and the costal margin.

The first surgical step is to perform a cutaneous Pfannenstiel incision around 7-8 cm, followed by the detachment of the subcutaneous layer toward the umbilicus and a Kustner fascial incision. A 8 mm robotic paraumbilical trocar is placed that will serve as camera port. A 15 mm Endo-Catch^o specimen retrieval bag (Covidien Fridley, Minnesota, USA) is placed through the mini-laparotomy that is closed with a running suture. A 10-12 mmHg pneumoperitoneum is established. Under camera vision, two 8 mm robotic trocars are placed into the ipsilateral anterior axillary line, in the subcostal and flank region, respectively and a 12 mm AirSeal^o trocar for assistant (ConMed Largo Tampa Bay, FL, USA) is placed on the

ipsilateral extreme side of the prepared Kustner incision. In right donor nephrectomy, an additional 5 mm trocar is placed in epigastrium under the xiphoid for assistant for liver retraction. At this time, robotic cart is docked coming from the ipsilateral side of the patient (Fig. 1). During the procedure, we usually use monopolar hook for dissection, bipolar forceps, medium-large and large Hem-o-lok^o clip (Weck Closure Systems, Research Triangle Park, NC), metallic titanium clips, round-tip scissors, and, when necessary, a needle holder and/or a second bipolar forceps. The assistant generally uses forceps and suction and irrigation system.

The procedure begins with the medialization of the colon along the line of Toldt to expose the renal region. Then, the usual retroperitoneal landmarks are identified (psoas muscle, ureter and gonadal vein). The ureter is isolated from all surrounding tissues in caudo-cranial direction, marked with elastic vessel-loop and pulled medially in relation to the axis of gonadal vein. Following the gonadal vein, the renal hilum is identified. The renal vein and artery are carefully isolated; on the left side, the adrenal and gonadal veins are freed and dissected between Hem-o-lok^o (on the right side, the gonadal vein is preserved). Moreover, possible lumbar veins are identified, isolated and transected between Hem-o-lok^o or titanium clips. After hilar dissection, the kidney is detached from the

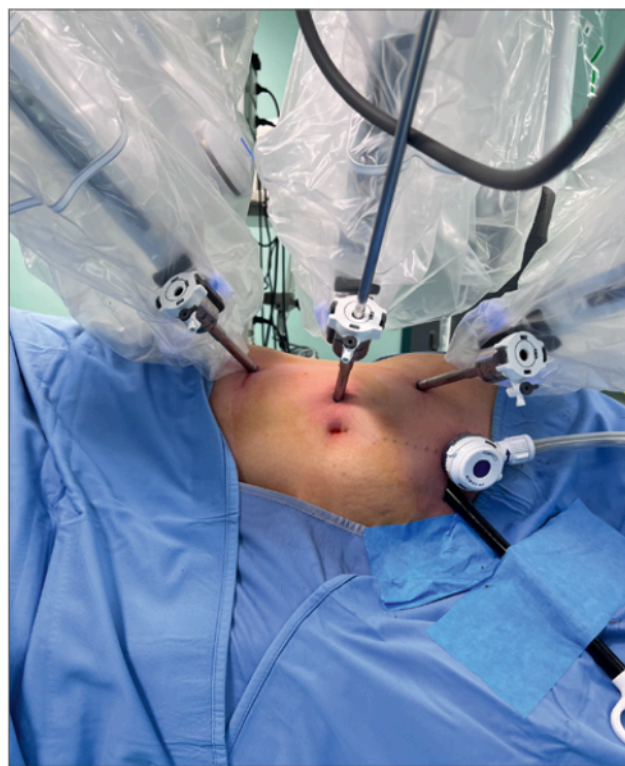


Figure 1. Patient position, trocars placement and surgical incision for left RLDN.

adrenal gland and the anterior and posterior kidney surfaces are completely freed from peri-renal fat. Particular attention should be paid to avoid extensive dissection of the "golden triangle" to preserve the vascularization of the ureter.

After complete mobilization of the kidney, intravenous bolus of furosemide (20 mg) is administered and the ureter is cut approximately at the level of its crossing of the iliac vessels. Distal ureter is closed by single or double Hem-o-lok® with the proximal end left open. The kidney is allocated into the Endo-Catch® and intravenous heparin (60-80 U/kg) is administered. The renal artery is divided by 35 Endo-GIA® vascular stapler (Ethicon INC, Somerville, USA) at its origin from the aorta and, immediately after, with a second Endo-GIA® vascular stapler, the renal vein is cut. The Endo-Catch® is closed and the running suture of the fascia is opened to quickly and accurately remove the kidney from the mini-laparotomy to be taken to the back-table. Intravenous protamine is administered (ratio 1:1 with heparin). Reinduction of the pneumoperitoneum is done for hemostasis check; an abdominal drain is placed when necessary. Control of trocar access holes is performed; the mini-laparotomy and trocar access holes are closed.

Data analysis

A comprehensive analysis was conducted on the demographic and anamnestic data of the donors, as well as on the intraoperative, perioperative, and postoperative variables of the donors themselves, including the kidney-graft function and the survival rates of both the recipients and the grafts. Continuous data are presented as median and interquartile range. Dichotomous data are presented as proportion and percentage. The statistical descriptive analysis was conducted using the SPSS version 22.0 software. The overall patient and graft survival rates of the studied population are also reported.

RESULTS

Donor and graft characteristics

From November 2019, 38 consecutive RLDN were performed at our center. Pre-operative clinical characteristics of the donors are shown in Table I. The median age was 54.7 years (interquartile range - IQR 32-77); 27 donors were females (71.1%) and 11 were males (28.9%). The median BMI was 24.7 kg/m² (IQR 19-35); three cases had obesity grade I and one case grade II. Twenty-eight patients were classified as American Society of Anesthesiologists (ASA) 1 and ten patients as ASA 2. The risk factors reported were smoking, with eight active smokers at surgery (21.1%) and three former smokers (7.9%), hypertension

in five cases (13.2%), dyslipidaemia in 11 cases (28.9%), hypercholesterolemia in seven cases (18.4%) and overweight in 15 case (39.5%). Nineteen patients (50%) had previous abdominal surgery. One patient had a history of malignant disease with a negative oncologic follow-up: in 2016 the patient underwent radical prostatectomy for prostate cancer (T2a, R0, pN0, Gleason 3+4). At surgery, the median pre-operative creatinine was 0.8 mg/dl (IQR e 0.5-1.4) and the median pre-operative glomerular filtration rate (GFR), calculated using the Cockcroft-Gault equation, was 100.0 ml/min/1.73 m² (IQR 49.3-172.5). In six cases, donor and recipient were ABO blood group incompatible. There was one case of RLDN for a National cross-over LDKT program.

Eleven right RLDN (29%) and 27 left RLDN (71%) were consecutively performed. Vascular anomalies were reported only in seven cases of procured grafts (18.4%); in one case the artery bifurcation was very proximal to the aorta, in the other cases there were venous vascular anomalies. At back-table, the two branches of the artery were reconstructed using bovine pericardium patch (Fig. 2), while in the cases of double renal veins were sutured together to create a single ostium. In all cases, we obtained a common patch for single anastomosis. The preoperative study with CT-scan encountered an 'incidental' small renal mass (SRM) in three donors. Magnetic resonance imaging (MRI) was performed for characterization of the lesions: we diagnosed an angiomyolipoma at the left inferior renal pole in one case and a Bosniak cyst type II-F in two cases (one of 19 mm at the right upper renal pole and one of 15 mm at the left inferior renal pole). After a multidisciplinary evaluation and according with KDIGO clinical practice guidelines¹⁷, we decided for procurement and transplantation of living donor kidneys with SRM after complete excision of the lesions before implantation. Detailed informed consent of the donor and recipient was carried out, as well as understanding and acceptance of these risks by the donor and recipient were performed. All graft's characteristics are shown in Table II.

Perioperative outcomes

The median overall operative time was 240 minutes (IQR 160-420); 225 minutes for right side and 240 minutes for left side. In only one case, we performed an associated procedure of cholecystectomy and the operative time was 305 minutes. The median warm ischemia time was 3.38 min (IQR 2.05-6.30) and the cold ischemia time, excluding cross-over LDKT, was 79 minutes (IQR 25-201). Intra-operative blood loss was irrelevant, less than 100 cc. In all cases, RLDN was completed without need of open conversion and no intraoperative adverse events were recorded. In the last 11 consecutive cases (28.9%), the drain was not placed. Of those who required enucleoresection, at the histological examination, the excised lesion was in the

Table I. Donors' clinical characteristics and pre-operative data (n. 38).

Age (years) (median, IQR)	54.7 (32-77)
Sex female-male (ratio)	27:11
BMI (kg/m ²) (median, IQR)	24.7 (19-35)
Pre-operative creatinine (mg/dL) (median, IQR)	0.8 (0.5-1.4)
Pre-operative GFR (ml/min/1.73 m ²) (median, IQR)	100.0 (49.3-172.5)
Previous abdominal surgery (n, %)	19 (50)
Previous malignancy (n, %)	1 (2.6)
Ethnicity (number, %)	
Caucasian	35 (92.1)
African	1 (2.6)
Asian	2 (5.3)
Relation to the recipient (n, %)	
Sibling	8 (21.1)
Parent	10 (26.3)
Spouse	16 (42.1)
Other kinship	3 (7.9)
No relative to the recipient	1 (2.6)
Risk factors (active smoker/ex-smoker, arterial hypertension, dyslipidemia, hypercholesterolemia, previous deep vein thrombosis, overweight) (n, %)	
No	9 (23.7)
One or More	29 (76.3)

BMI: body mass index; GFR: glomerular filtration rate

first case an angiomyolipoma and in the second and third case a clear cell carcinoma (diameter 2.2 cm, G1 and diameter 2 cm, G2 respectively). At a mean follow-up of 33.2 months, both donors and recipients have no recurrence. The median hospital stay was three days (IQR 2-10) without any re-admission. In the evening of the day of surgery, the donors were allowed to drink clear fluids. When the postoperative course was regular, urinary catheters and surgical drain were removed within the first and the second postoperative day (POD). In one case, urinary catheter was replaced and removed in 3rd POD for bladder globe due to a known prostatic hypertrophy. Four patients (10.5%) experienced postoperative complications: 2 cases of chyle fistula, 1 case of urinary infection and 1 case of postoperative paralytic ileus. All cases were treated conservatively (Clavien-Dindo complication grade

**Figure 2.** Reconstruction of double artery on a bovine pericardium patch.

2). No major post-operative complications (Clavien-Dindo grade 3-5) were recorded during the first 90 days after surgery or later (> 3 months). Two donors reported moderate pain (score 4-6 according to numerical rating scale, NRS). None of the donors developed significant renal failure or needed dialysis. The mean creatinine registered at two years of post-operative follow-up was 1.06 mg/ml (IQR 0.9-1.38). No vascular complications occurred in our experience. The overall donor survival rate was 100%. Peri-operative clinical characteristics are summarized in Table III.

In all cases, we observed an immediate allograft function. LDKTs were pre-emptive in 19 cases (50%). The median follow-up period was 25.4 months (IQR 0.5-55). The survival rates of kidney transplant recipients at six months, one year, and two years were 97.1% (33/34), 93.3% (28/30), and 90.9% (20/22), respectively. The overall survival rate was 94.7% (36/38 patients). Two recipients died, both with normal graft function. One recipient died of heart failure secondary to acute myocardial infarction 48 days after LDKT; he was a patient with cardiovascular and respiratory comorbidities, whose suitability had been assessed by the multidisciplinary board. The other recipient died of severe COVID-19 disease 10 months after LDKT. No cases of graft loss occurred among the recipients.

DISCUSSION

Minimally invasive donor nephrectomy represents the standard of care for living donors with an effect on development and implementation of living kidney donation¹⁴. In recent years, the robotic approach has gained increasing success around the world and, presently, it is widely used, with results that are comparable with those

Table II. Kidney's characteristics (n. 38).

Left RLDN (n, %)	27 (71)
Right RLDN (n, %)	11 (29)
Total vascular anomalies found (n, %)	19 (50)
Vascular anomalies of procured grafts (n, %)	7 (18.4)
Very early artery bifurcation (proximal to aorta)	1 (2.6)
Retroaortic left renal vein	2 (5.3)
Double renal vein	4 (10.5)
Kidney malformation and/or anomalies (urolithiasis, renal cysts and/or lesions) (n, %)	9 (23.7)
Split renal function of procured grafts (%) (median, IQR)	49 (41.5-55)
Pre-emptive LDKT (n, %)	19 (50)

RLDN: robotic living donor nephrectomy; LDKT: living donor kidney transplantation.

of laparoscopic LDN (LLDN) ^{16,19}. Thanks to the stable 3D vision system, the high magnification and EndoWrist technology, as well as the optimal ergonomics for the surgeon, RLDN seems to improve the technical finesse of LLDN ¹⁹. Moreover, RLDN shows a faster learning curve to achieve standard results compared with LLDN ^{20,21}. However, current EAU Guidelines strongly recommend that RLDN should be performed only in highly specialized centers, stressing the importance of centralization of care to maximize post-operative outcomes. In accordance with previous studies, our results confirm the safety profile and efficacy of RLDN with low open conversion rate and intraoperative adverse events, low morbidity rates and short postoperative length of stay ²⁰⁻²². Some authors attributed the short length of hospital stay in the RLDN to the reduction of manipulation of the peritoneum, thanks to a better identification of dissection planes, and limited energy use for cauterization ²³.

A meta-analysis published in 2018 suggested longer vessels when using clips or Hem-o-lok[®] compared to staplers, with the disadvantage of increased warm ischemia time and additional blood loss ²⁴. However, there was a disclaimer of the manufacturer and there were strong recommendations in the literature for transfixion technique after several avoidable donor death occurred linked to clip malfunction. For these reasons, we prefer to use Endo-GIA[®] vascular staplers after careful preparation of the vessels at the origin. The robotic approach seems to be a useful tool to improve vascular dissection and management of multiple vessels ^{20,21}; surgeon could accurately prepare the renal vessels (especially on the left side for the presence of adrenal and gonadal vein and possible accessory vessels) and preserve an adequate length of renal vessels.

Table III. Perioperative donors' outcomes (n. 38).

Intra-operative data	
Operative time (min) (median, IQR)	240 (160-420)
Right RLDN	225 (160-420)
Left RLDN	240 (190-405)
Conversion rate (n, %)	0
Intraoperative complications (n, %)	0
Blood loss (cc) (median, IQR)	10 (0-100)
Warm ischemia time (min) (median, IQR)	3.38 (2.05-6.30)
Cold ischemia time (min) (median, IQR)	79 (25-201)
Post-operative data	
Hospital stay (days) (median, IQR)	3 (2 - 10)
Pain Score NRS (n, %)	
No Pain (0 NRS)	16 (42.1)
Middle Pain (1-3 NRS)	20 (52.6)
Moderate Pain (4-6 NRS)	2 (5.3)
Severe Pain (7-10 NRS)	0
Clavien I-II (n, %)	
Chyle fistula	2 (5.3)
Postoperative paralytic ileus	1 (2.6)
Urinary infection	1 (2.6)
Clavien III-IV (n, %)	
	0
Readmission (n, %)	
	0
90 days mortality (n, %)	
	0

RLDN: robotic living donor nephrectomy; NRS: numerical rating scale.

In our experience multiples arteries or veins did not represent a contraindication for kidney selection and were not associated with a higher rate of surgical complications. No vascular complications and bleedings occurred. To facilitate the subsequent extraction of the graft and to avoid the need to temporarily stop the procedure, we usually placed at the beginning an Endo-Catch[®] retrieval bag through the Kustner incision, ready for the time of procurement completion. This strategy allowed to reduce the warm ischemia time and makes it similar to the hand-assisted experience or the use of the GelPORT[®] device (Applied Medical Rancho Santa Margarita, CA, USA).

RLDN appears to be equivalent to the LLDN technique in terms of warm ischemia duration ²⁵. These findings give additional evidence of the feasibility of the RLDN technique. In our experience, a full robotic technique was adopted in all cases with a short warm ischemia time; these results reflect the possibility to remove the kidney immediately after transection of the vascular pedicle through the Kustner incision and confirm the accuracy and safety of this approach. In a previous systematic

review, Creata et al. affirmed that warm ischemia times reported during RLDN resulted not significantly different from LLDN²¹. Despite encouraging reports, literature comparing RLDN to other minimally invasive techniques remains scarce and mostly retrospective.

RLDN was associated with longer operative time due to docking and undocking phases, compared to LLDN²⁰. Our median operative time for RLDN was 240 min and it was similar to that reported by other groups^{19,20,24}. Thanks to a shorter learning curve, we observed a decrease of operating time in the last cases.

Robotic approach seems to be useful in terms of complex dissection. Indeed, it offers high microsurgical precision, easier manoeuvrability of the instruments, as well as better identification of dissection planes and control of potentially problematic bleeding. The stable 3D vision system and increased degrees of freedom enhance the dissection and avoid any traction on the vascular pedicle. For these reasons, hand-assistance was not necessary. Moreover, the assistant's hand can cause more of a hindrance than help, limiting the range of robotic action and the freedom of movement and possibly obstructing visualization²⁶. However, the choice of full robotic technique versus hand-assist approach is still controversial.

A Pfannenstiel cutaneous incision (7-8 cm incision along the suprapubic line) associated to Kustner fascial incision appears to be more acceptable for the donors because of better cosmetic results, less post-operative pain and lower incisional hernia rate than standard Pfannenstiel incision. The Kustner fascial incision seems also to reduce damage to muscular tissue.

The limitations of our study are the retrospective nature of the analysis, the single-center basis and the relatively small sample size.

CONCLUSIONS

In conclusion, full RLDN is a feasible, safe and effective technique in experienced hands and in highly referral transplant centers, able to ensure optimal perioperative and midterm functional outcomes. Our experience confirms that RLDN provides optimal functional outcomes, both in donors and recipients. On the bases of our results, compared with the results of the major LLDN series reported in the literature, we believe that robotic approach appears not inferior to laparoscopy in terms of surgical technique, perioperative outcomes, and midterm results.

Comparative studies with larger cohorts are needed to point out further advantages of robotic approach.

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Conflict of interest statement

The authors declare no conflict of interest.

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Author contributions

SDS: study design, manuscript drafting, data collection and analysis and critical review; BC: study design, manuscript drafting, data collection and analysis and critical review; DC, GE: data collection; RO, GA, TO, CG, PM, GM, GD, GPG: critical review; FDB: study design and critical review.

Ethical consideration

This study was approved by the Institutional Ethics Committee of the AOU - Azienda Ospedaliera-Universitaria - Policlinico, Modena, Italy (rotocol of living donor kidney transplantation with full robotic procurement n° Rev. 1/2023).

The research was conducted ethically, with all study procedures being performed in accordance with the requirements of the World Medical Association's Declaration of Helsinki.

Written informed consent was obtained from each participant/patient for study participation and data publication.

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