

Robot-assisted retroperitoneal laparoscopic partial nephrectomy without hilar occlusion VS classic robot-assisted retroperitoneal laparoscopic partial nephrectomy

A retrospective comparative study

Ju Guo, MD, PhD, Cheng Zhang, MD, PhD, Xiaochen Zhou, MD, PhD, Gongxian Wang, MD, PhD, Bin Fu, MD, PhD*

Abstract

To discuss the feasibility, safety, and effectiveness of off-clamp robotic partial nephrectomy via retroperitoneal approach and provide data for evidence based medicine in the surgical treatment of renal tumor.

The clinical data was documented and compared between robotic retroperitoneal partial nephrectomy with and without hilar occlusion (clamp group and off-clamp group) performed between January 1, 2015 and December 31, 2017.

Six-months post-operative renal function was superior in the off-clamp group compared with clamp group, while long-term results remained to be elucidated. No significant difference in post-operative hospital stay was found between the 2 groups. Estimated blood loss in off-clamp group was significantly higher than clamp group, while no significant difference was found in transfusion rate.

Off-clamp robotic partial nephrectomy via retroperitoneal approach is a safe and effective technique for the removal of renal tumor while the indication of surgery is strictly limited to small (<4 cm) and exophytic renal tumor.

Abbreviations: AJCC = American Joint Committee on Cancer, CT = computed tomography, GFR = glomerular filtration rate test, MRI = Magnetic Resonance Imaging, NCCN = The National Comprehensive Cancer Network, RCC = renal cell carcinoma RN radical nephrectomy, RLPN = robot-assisted laparoscopic partial nephrectomy, RRLPN = robot-assisted retroperitoneal laparoscopic partial nephrectomy, WIT = warm ischemia time.

Keywords: off-clamp technique, partial nephrectomy, renal tumor, retroperitoneal approach, robotic surgery

1. Introduction

Renal cell carcinoma (RCC) is the most common urological malignancy, accounting for 90% of renal malignancies in adults.^[1–3] Radical nephrectomy (RN) is the standard treatment for renal tumor; however, it may significantly impair the patient

renal function and expose the patient to higher cardiovascular risks.^[4] According to 2015 NCCN guidelines, partial nephrectomy (PN) can be applied to T1a renal tumor (AJCC TNM staging). Conventionally, hilar occlusion may significantly reduce intraoperative blood loss, allowing tumor resection and parenchyma reconstruction to be performed in a comparatively

Editor: Giuseppe Lucarelli.

The Ethics Committee of our Hospital approved this study (IRB No.: 201904292c). Patients' informed consent was obtained verbally over telephone and was recorded during phone calls (verbal and recorded consent was approved by the ethics committee of our institution); the patients received an information letter prior to the informed consent; informed consent to participate in the study was recorded on the phone calls; patient's identity, answers and information on medical records were kept confidential.

The authors declare that they have obtained consent to publish the identifying information (individual details, images or videos) contained in this manuscript.

The datasets generated and/or analyzed during the current study are not publicly available due [Follow-up research needs protection] but are available from the corresponding author on reasonable request.

This study was funded by National Natural Science Foundation of China (grant number 81860454), and Jiangxi Provincial Science and Technology Program (grant number 20181BAB205053), and Jiangxi Provincial key Science and Technology Program (grant number 20161ACG70013). The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

The authors declare that they have no competing interests.

Department of Urology, the First Affiliated Hospital of Nanchang University, Nanchang, Jiangxi, PR China.

* Correspondence: Bin Fu, Department of Urology, The First Affiliated Hospital of Nanchang University, 17 Yongwai Zheng Road, Nanchang, Jiangxi 330006, PR China (e-mail: urodocnc@126.com).

Copyright © 2019 the Author(s). Published by Wolters Kluwer Health, Inc.

This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial License 4.0 (CCBY-NC), where it is permissible to download, share, remix, transform, and buildup the work provided it is properly cited. The work cannot be used commercially without permission from the journal.

How to cite this article: Guo J, Zhang C, Zhou X, Wang G, Fu B. Robot-assisted retroperitoneal laparoscopic partial nephrectomy without hilar occlusion VS classic robot-assisted retroperitoneal laparoscopic partial nephrectomy. *Medicine* 2019;98:38(e17263).

Received: 4 June 2019 / Received in final form: 22 August 2019 / Accepted: 27 August 2019

<http://dx.doi.org/10.1097/MD.00000000000017263>

bloodless surgical field.^[5] Nevertheless, ischemic reperfusion injury of the affected kidney is inevitable if hilar occlusion technique is used during the surgery,^[6] which may even extend beyond the occlusion period.^[7] The longer warm ischemia time is associated with the short- and long-term renal consequences.^[8] Recent literature suggested that every minute of ischemia increases the risk of renal function impairment postoperatively.^[9]

Zero-ischemia means the tumor resection and parenchyma reconstruction are performed without occlusion of renal artery.^[10] With the da Vinci robotic system, the Trifecta of partial nephrectomy, negative margin, renal preservation, and minimal complication,^[11] can be achieved thanks to the superior dexterity of robotic instruments and the help of a bedside assistant.^[12]

In this study, we described our technique and experience of robot-assisted retroperitoneal laparoscopic partial nephrectomy without hilar occlusion and compared with conventional hilar-clamping technique in terms of perioperative parameters.

2. Patients and methods

2.1. Clinical data

Ninety three consecutive cases of robotic partial nephrectomy (PN) performed by a single surgical team between January 1, 2015 and December 31, 2017 were selected. All tumors were confirmed by CT or MRI to be exophytic with a diameter <4 cm. 48 cases were performed with off-clamp technique and 45 cases were performed with hilar-clamping. The clinical data and RENAL scores of the 2 groups were documented and compared. The results showed that there were no significant differences between 2 groups (Table 1).

2.2. Surgical techniques

All surgeries were performed using da Vinci Si Surgical System.

Patient position and Trocar configuration (right robotic PN was used as an example) (Fig. 1).

Table 1

Patient data.

Parameters	Off-clamp (n=48)	Hilar-clamping (n=45)	P value
Gender			.946
Male	27	25	
Female	21	20	.568
Age (y, mean±SD)	53.29±13.91	54.39±11.82	.673
Height (cm, mean ± SD)	166.48±6.85	165.09±7.49	.754
Weight (kg, mean ± SD)	72.35±12.46	68.32±13.01	.507
BMI (kg/cm ² , mean ± SD)	24.63±3.44	24.03±4.21	.143
Tumor size (mm, mean ± SD)	32.89±6.37	35.03±7.55	.623
Intra-parenchyma tumor (mm, mean ± SD)	15.76±4.73	16.17±5.11	
Laterality			.936
Left	26	24	
Right	22	21	
Tumor location			.719
Upper-pole	9	11	
Mid-pole	25	20	
Lower-pole	14	14	
Preoperative ipsilateral GFR (ml/min, mean ± SD)	41.25±4.79	42.84±5.03	.760
R.E.N.A.L. scores	8.4±1.7	8.6±1.8	.174

GFR=The renal glomerular filtration rate (GFR) was measured with 99mTc-DTPA.

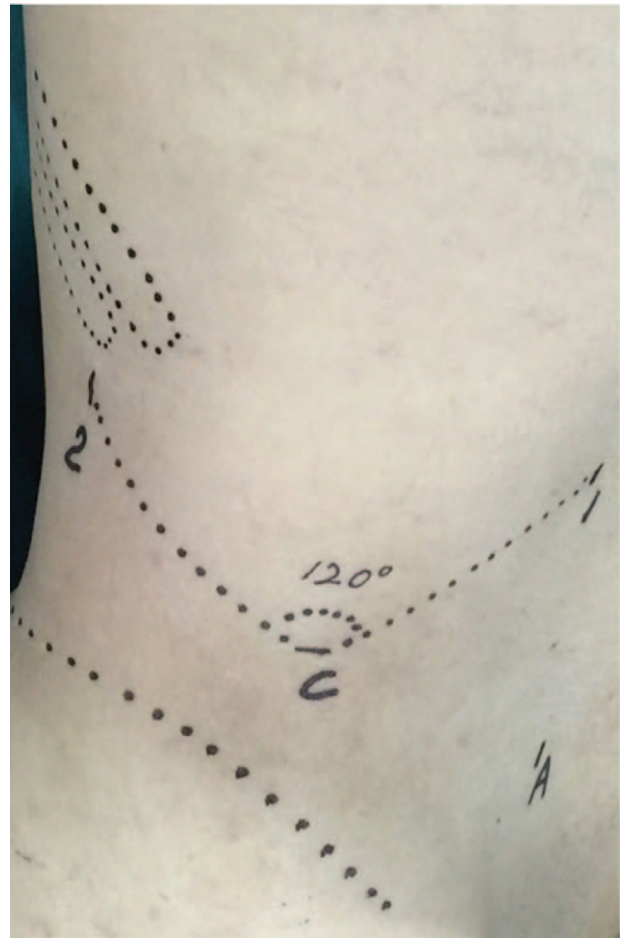


Figure 1. Patient position and trocar configuration. The patient was placed at 90° full flank position with the operating side facing upwards. Using right side surgery as an example, the camera port (c) was placed 2 cm above the anterior superior iliac spine. The angle of 1st arm port(1)-camera port and 2nd arm port (2)-camera port was between 120 and 135. The assistant port (A) was placed medial to the 1st arm port.

All 93 cases of robotic retroperitoneal partial nephrectomy were performed with 90-degree flank position. Four trocars were used (1 camera port, 2 robotic ports, and 1 assistant port).

Surgical steps (right robotic PN was used as an example) (Fig. 2):

Off-clamp robotic partial nephrectomy via retroperitoneal approach surgical procedure 1, after induction of general anesthesia, the patient was intubated and catheterized. Patient was placed in 90-degree full flank position with table flexed. After routine sterilization, retroperitoneal access was developed as following:

1. Trocar configuration was marked with the handle of scalpel. The position of the 2nd robotic trocar (2) was determined 2 cm below the crossing point of right posterior axillary line and lateral margin of the psoas muscle.
2. Camera port (C) was then located 8 cm away from the 2nd robotic trocar and 2 cm above the ipsilateral iliac crest, where a 2-cm initial incision was made at, through which the retroperitoneal space was developed with a combination of finger dissection, forceps dissection and balloon dissection (900 ml air). It is imperative that the peritoneum was pushed medially sufficiently.

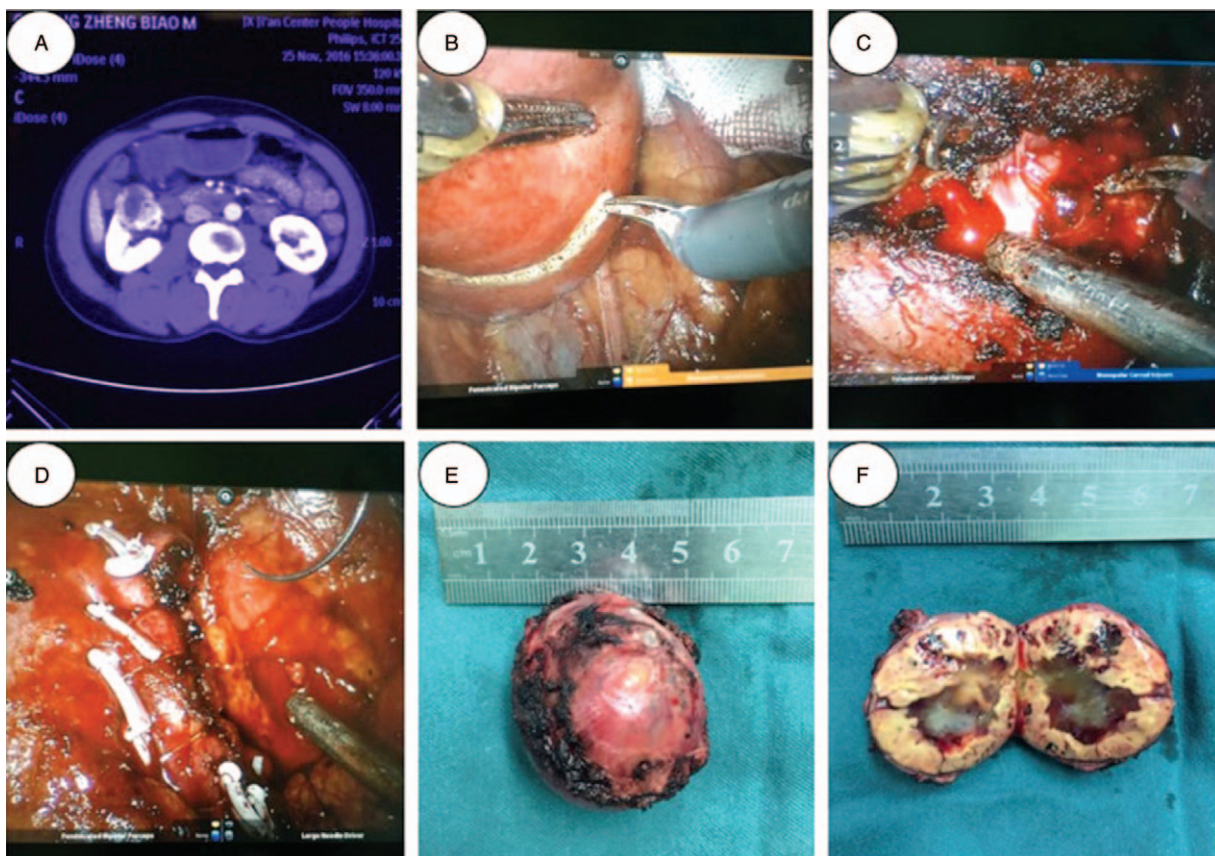


Figure 2. Surgical techniques of robotic partial nephrectomy via retroperitoneal approach and Tumor specimen. (A) Tumor was located in the mid-pole of right kidney, pressing against ipsilateral renal hilum. (B) Demarcation of tumor margin. (C) Tumor resection with a combination of blunt and sharp dissection. (D) Renal reconstruction with barbed suture and hem-o-locls. (E) Resected tumor. (F) <2mm normal parenchyma was resected along with the tumor, for maximized preservation of renal function. (The consent for publication was obtained for the identifying images in identifiable details of patients in Fig. 2 A).

3. A da Vinci 8 mm trocar was placed under finger guidance at previously determined 2nd robotic trocar position.
4. 1st robotic trocar (1) was determined 8cm away from the camera port, below the costal margin and at the same level of 2nd robotic trocar. A 1-cm incision was made here to allow for the placement for a da Vinci 8 mm trocar under finger guidance.
5. A 12-mm assistant trocar (A) was then placed 8 cm away from camera port, forming an equilateral triangle with (C) and (1).
6. The angle between (1) to (C) and (2) to (C) was approximately 120-degree. A 12-mm long trocar was lastly placed at the camera port site and secured with a silk suture.

2, The robotic cart was docked at the patient head and robotic arms was connected with trocars. Camera, monopolar scissors and fenestrated bipolar was introduced under direct vision. Pneumoperitoneum was maintained at 15 mm Hg.

3, Retroperitoneum fat was dissected. Gerota fascia was incised and perinephretic fat was exposed. Renal artery was identified and bulldog clamp was introduced as a precaution. Kidney was sufficiently mobilized and tumor was located. Pneumoperitoneum was raised to 18 mm Hg right before tumor resection.

1. tumor margin was cauterized with monopolar at 0.5 cm away from the tumor
2. For on-clamping patients, clamping of renal artery (s) was achieved using bulldog clamp (s) before resection of tumor.

With the assistant cooperation, the tumor was resected with a combination of blunt and sharp dissection to achieve a “super-thin parenchyma” around the tumor, which is a key technique in partial nephrectomy. Bleeding was controlled by suction, compression, and bipolar when necessary, until the tumor was fully resected.

4, the inner layer and collecting system was closed by 3–0 barbed suture on RB-1 needle in a running fashion, followed by outer layer and parenchyma closure by 2–0 barbed suture on CT-1 needle in a running fashion. Hem-O-Locs was placed on sutures where it came out of the renal parenchyma intermittently.

5, Tumor was packed in specimen bag and removed through an incision elongated from the assistant trocar incision afterwards. Bulldog clamp was removed and surgical field was irrigated with sterile distilled water. After a full inspection and hemostasis, a drain was left in place and robotic cart was undocked. Gauze and instruments were counted and confirmed. All incisions were closed in standard maneuver.

The kidney needs to be sufficiently mobilized for tumor resection. Intracorporeal ultrasound may assist in determination of renal tumor depth and demarcation. Before tumor resection, the renal artery needs to be fully dissected and ready to be clamped. A Hem-o-loc on sutures at exit points can greatly reduce the risk of parenchyma laceration due to excessive tension on sutures. A temporal elevation of pneumoperitoneum pressure to 18 mm Hg

may greatly facilitate tumor resection by compressive hemostatic effect.

On-clamp robotic partial nephrectomy via retroperitoneal approach surgical procedure.

2.3. Clinical data

Main relevant parameter data were assessed, including estimated blood loss operative time, resection time, postoperative hospital stay, Preoperative ipsilateral GFR, postoperative 6-months ipsilateral GFR, drainage, complications, recurrence after the operation, and etc.

2.4. Statistical analysis

All of the data were analyzed using SPSS20.0 software (SPSS Inc., Chicago, IL). The mean \pm standard deviation was used for the expression of the data conforming to a normal distribution, and the median (range) was used to express the data not conforming to a normal distribution. The group differences were analyzed using the Student *t* test. A *P* value of $<.05$ indicated statistical significance.

3. Results

No positive surgical margin was noted in all 93 cases Table 2. No conversion to open was noted in all cases. No conversion to hilar clamping was noted in off-clamp cases. Estimated blood loss in off-clamp group and hilar-clamping group were 120 ± 51.46 ml and 78.84 ± 42.79 ml, respectively ($P < .001$). No significant

difference was noted in tumor resection time, operative time, postoperative drainage, hospital stay, and complications between the 2 groups.

Postoperative pathology confirmed 70 cases of clear cell carcinoma (off clamp 37 cases, hilar-clamping 33 cases), 20 cases of angiomyolipoma (off clamp 10 cases, hilar-clamping 10 cases), and 3 cases of oncocytic papillary renal cell carcinoma (off clamp 1 cases, hilar-clamping 2 cases). There were no statistical differences between the 2 groups.

The postoperative 6-months ipsilateral GFR in off-clamp group were significantly higher than in hilar-clamping group ($P < .05$). The postoperative 6-months ipsilateral GFR change in the off-clamp group was $(8.36 \pm 3.27)\%$, while the hilar-clamping group was $(14.71 \pm 4.68)\%$. The ipsilateral GFR change in the former was significantly lower than the latter ($P < .001$). During a mean follow-up of 12 (6–19) months, there were no complications of postoperative delayed bleeding and leakage of urine in both groups. Local recurrence was noted in one patient from the off-clamp group 13 months after surgery, which did not yield a significant difference in recurrence rate between the 2 groups.

4. Discussion

Robotic retroperitoneal partial nephrectomy has been a favorable choice for patients suffering from localized renal tumor, thanks to the anatomical advantages and preservation of renal function.^[13] Yet, we continue to advance our techniques for better surgical outcomes, especially regarding the postoperative renal function. Many techniques have been proposed in the aim of minimizing the impairment of renal function, including selective clamping, parenchyma clamping and hypothermic technique, etc,^[14,15] but with limited success.^[16] Cold ischemia could theoretically benefit the preservation of renal function, while controversial opinions still exist due to extended ischemic time.^[17] Selective clamping of branches of the main renal artery may benefit the preservation of renal parenchyma.^[18]

However, it still causes some ischemia to the whole kidney. In 2003, Guillonnet et al first reported a partial nephrectomy without clamping of the renal artery, and the true zero ischemia of the partial nephrectomy was initially achieved.^[19] The technology has made great progress. At early stages, many medical centers have reported robot-assisted partial nephrectomy without hilar occlusion.^[20] The feasibility of using off-clamping technique in robotic partial nephrectomy for complex renal tumors was also reported.^[21] Although prospective randomized data are available to refute conclusion that no benefit in the preservation of renal function with the on-clamp technique,^[22] given that renal metabolism is singularly aerobic, most would agree that minimizing renal ischemia duration is a laudable goal.^[23]

Regarding the robot-assisted laparoscopic partial nephrectomy without hilar occlusion, they have been reported that most of them were via the laparoscopic approach,^[10,20] while there were few reports of the retroperitoneal approach. With our extensive experience in laparoscopic retroperitoneal approach, we therefore proposed zero-ischemic robotic retroperitoneal partial nephrectomy and reported our initial experience.

In our patient series, off-clamp robotic retroperitoneal partial nephrectomy has proven to be a safe technique for small

Table 2
Postoperative assessment.

Parameters	Off-clamp (n = 48)	Hilar-clamping (n = 45)	<i>P</i> value
Pathology			.792
Clear cell carcinoma	37	33	
Angiomyolipoma	10	10	
Oncocytic papillary renal cell carcinoma	1	2	
Others	0	0	
Tumor resection time (min, mean \pm SD)	5.74 ± 1.95	5.05 ± 2.18	.192
Reconstruction time (min, mean \pm SD)	17.56 ± 3.39	15.38 ± 3.91	.135
Warm ischemic time (min, mean \pm SD)	0	20.43 ± 5.23	$<.001$
Estimated blood loss (ml, mean \pm SD)	120 ± 51.46	78.84 ± 42.79	$<.001$
Operative time (min, mean \pm SD)	77 ± 19.82	81 ± 19.63	.138
Drainage (ml, mean \pm SD)	203 ± 98.45	178 ± 80.26	.213
Transfusion	1	0	.330
Hospital stay (d, mean \pm SD)	5.47 ± 1.31	5.19 ± 1.32	.625
Postoperative ipsilateral GFR ₆ (ml/min, mean \pm SD)	35.49 ± 4.56	29.18 ± 3.77	.019
Δ GFR ₆ (% , mean \pm SD)	8.36 ± 3.27	14.71 ± 4.68	$<.001$
Positive surgical margin	0	0	1.000
Delayed bleeding	0	0	1.000
Urinary leakage	0	0	1.000
Postoperative recurrence	1 (2.1%)	0 (0%)	.261

GFR = The renal glomerular filtration rate (GFR) was measured with 99mTc-DTPA . Δ GFR₆ = $(1 - \text{GFR}_{\text{pre}} / \text{GFR}_{\text{post}}) \times 100\%$.

exophytic renal tumor, and may better preserve renal function despite of increased intraoperative blood loss. A temporal elevation of pneumoperitoneum pressure to 18 mm Hg may greatly facilitate tumor resection by compressive hemostatic effect.^[24] It seems that postoperative drainage in off-clamp group was slightly more than hilar-clamping group, but not statically significant.

During tumor resection, we used a combination of sharp and blunt dissection technique for better visualization and maintenance of the correct resection plane, minimizing the risk of positive surgical margin. The advantages of the off-clamp technique include unrestricted resection and reconstruction time, while the downside of the technique obviously includes risks of excessive bleeding which may hinder the correct resection plane. It is important to have an experienced bedside assistant to assist during the resection process. Hemostasis can be tricky during tumor resection. Normally, bleeding from a small artery can be effectively controlled by bipolar coagulation, while a large venous bleeding may require suturing.

The outcome of off-clamp technique is largely dependent on the experience and techniques of console surgeon, as well as other factors including tumor size, depth, and relationship with hilum. Yet, it may only be indicated when tumor is small and comparatively exophytic. Wheat reported that every centimeter increase in tumor sides brings up the surgical risk by 33% in partial nephrectomy, especially urinary leakage and excessive bleeding.^[25]

Many techniques that reduce or eliminate warm ischemia time (WIT) have been studied. Gill reported a novel technique of zero ischemia RAPN that transient, pharmacologically induced reduction of blood pressure, timed to precisely coincide with excision of the deep part of the tumor.^[10] Rizkala proposed their novel zero-ischemic technique, called sequential preplaced suture renorrhaphy, in robotic partial nephrectomy,^[26] for better visualization and less bleeding. We will incorporate these techniques in our future cases.

One limitation of our technique lies in the indication for surgery: the technique may not be suitable for endogenous or large renal tumor. Another limitation is that we only collected limited cases. External validation of the results of this research requires more medical centers to participate, larger number of samples and longer follow-up.

5. Conclusions

Off-clamp robotic retroperitoneal partial nephrectomy is a safe and effective technique for the removal of small (<4 cm) and exophytic renal mass. A definitive conclusion on the long term results requires further follow-up.

Author contributions

Conceptualization: Ju Guo, Bin Fu.

Data curation: Cheng Zhang.

Formal analysis: Gongxian Wang.

Funding acquisition: Ju Guo, Gongxian Wang.

Investigation: Xiaochen Zhou, Bin Fu.

Supervision: Bin Fu.

Writing – original draft: Ju Guo.

Writing – review & editing: Xiaochen Zhou.

Bin Fu orcid: 0000-0002-0315-6305.

References

- [1] Eble JN, Sauter G, Epstein JI, et al. World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of the Urinary System and Male Genital Organs. Lyon: IARC Press; 2004.
- [2] Motzer RJ, Agarwal N, Beard C, et al. Kidney cancer. *J Natl Compr Canc Netw* 2011;9:960–77.
- [3] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. *CA Cancer J Clin* 2015;65:5–29.
- [4] Kambara T, Tanimoto R, Araki M, et al. Renal function after nephrectomy influences the risk of cardiovascular events. *Acta Med Okayama* 2018;72:241–7.
- [5] Gill IS, Desai MM, Kaouk JH, et al. Laparoscopic partial nephrectomy for renal tumor:duplicating open surgical techniques. *J Urol* 2002;167:469–77.
- [6] Choi JD, Park JW, Lee SY, et al. Does prolonged warm ischemia after partial nephrectomy under pneumoperitoneum cause irreversible damage to the affected kidney? *J Urol* 2012;187:802–6.
- [7] Andonian S, Coulthard T, Smith AD, et al. Real-time quantitation of renal ischemia using targeted microbubbles:in-vivo measurement of P-selectin expression. *J Endourol* 2009;23:373–8.
- [8] Thompson RH, Lane BR, Lohse CM, et al. Every minute counts when the renal hilum is clamped during partial nephrectomy. *Eur Urol* 2010;58:340–5.
- [9] Patel AR, Eggner SE. Warm ischemia less than 30 minutes is not necessarily safe during partial nephrectomy:every minute matters. *Urol Oncol* 2011;29:826–8.
- [10] Gill IS, Eisenberg MS, Aron M, et al. “Zero ischemia” partial nephrectomy:novel laparoscopic and robotic technique. *Eur Urol* 2011;59:128–34.
- [11] Hung AJ, Cai J, Simmons MN, et al. “Trifecta” in partial nephrectomy. *J Urol* 2013;189:36–42.
- [12] Shin TY, Choi KH, Lim SK, et al. Simplified zero ischemia in robot assisted partial nephrectomy: initial Yonsei experience. *Korean J Urol* 2013;54:78–84.
- [13] Hu JC, Treat E, Filson CP, et al. Technique and outcomes of robot-assisted retroperitoneoscopic partial nephrectomy: a multicenter study. *Eur Urol* 2014;54:2–9.
- [14] Alenezi S, Motiwala S, Eves S, et al. Robotic assisted laparoscopic partial nephrectomy using contrast-enhanced ultrasound scan to map renal blood flow. *Int J Med Robot* 2017;3:e1738.
- [15] IBauza JL, Murthy P, Sagalovich D, et al. Intraoperative renal hypothermia with ice slush for robot-assisted partial nephrectomy in a highly complex renal mass. *Int Braz J Urol* 2019;22:45doi: 10.1590/S1677-5538.
- [16] Hou W, Ji Z. Achieving zero ischemia in minimally invasive partial nephrectomy surgery. *Int J Surg* 2015;18:48–54.
- [17] Volpe A, Blute ML, Ficarra V, et al. Renal ischemia and function after partial nephrectomy:a collaborative review of the literature. *Eur Urol* 2015;68:61–74.
- [18] Shao P, Qin C, Yin C, et al. Laparoscopic partial nephrectomy with segmental renal artery clamping: technique and clinical outcomes. *Eur Urol* 2011;59:849–55.
- [19] Guillonneau B, Bermúdez H, Gholami S, et al. Laparoscopic partial nephrectomy for renal tumor: single center experience comparing clamping and no clamping techniques of the renal vasculature. *J Urol* 2003;169:483–6.
- [20] Gill IS, Patil MB, Abreu AL, et al. Zero ischemia anatomical partial nephrectomy: a novel approach. *J Urol* 2012;187:807–14.
- [21] Kim EH, Tanagho YS, Sandhu GS, et al. Off-clamp robot-assisted partial nephrectomy for complex renal tumors. *J Endourol* 2012;26:1177–82.
- [22] Anderson BG, Potretzke AM, Du K, et al. Comparing off-clamp and on-clamp robot-assisted partial nephrectomy: a prospective randomized trial. *Urology* 126:102–9.
- [23] Cacciamani GE, Medina LG, Gill TS, et al. Impact of renal hilar control on outcomes of robotic partial nephrectomy: systematic review and cumulative meta-analysis. *Eur Urol Focus* 2018;30013–20. pii: S2405-4569(18).
- [24] Wang HK, Qin XJ, Ma CG, et al. Nephrometry score-guided off-clamp laparoscopic partial nephrectomy:patient selection and short-time functional results. *World J Surg Oncol* 2016;14:163.
- [25] Wheat JC, Roherts WW, Hollenbeck BK, et al. Complications of laparoscopic partial nephrectomy. *Urol Oncol* 2013;31:57–62.
- [26] Rizkala ER, Khalifeh A, Autorino R, et al. Zero ischemia robotic partial nephrectomy:sequential preplaced suture renorrhaphy technique. *Urology* 2013;82:100–4.