

Total robotic surgical volume influences outcomes of low-volume robotic-assisted partial nephrectomy over an extended duration

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Abstract

Introduction: The objective of this study was to examine the surgeon's experience of low-volume robotic-assisted partial nephrectomy (RAPN) over an extended duration, and whether a high-volume fellowship training influenced the outcomes.

Methods: Data on all RAPN at a tertiary center performed by a uro-oncologist were retrospectively collected. The surgeon experience was assessed by examining perioperative outcomes among three groups of consecutive patients (first=14, second=14, third=15 patients, respectively).

Results: Between February 2014 and February 2020, 45 RAPNs were performed out of a total of 200 robotic procedures. The median tumor size was 3 cm, and 28 (65%) patients had a R.E.N.A.L nephrometry score (RNS) ≥ 7 . The median operative time and warm ischemia time (WIT) were 190 and 16 minutes, respectively. The median estimated blood loss (EBL) was 100 mL. Two (4%) patients had a positive surgical margin (PSM). Overall, five (12%) complications were recorded. All except one were minor (Clavien I-II). The median followup was 26.2 months. Trifecta and pentafecta were achieved in 40 (93%) and 27 (81.8%) patients, respectively. Increased surgeon experience was significantly associated with a shorter operative time and less EBL. Furthermore, there was an independent association between surgeon experience and operative time and EBL, and between RNS and operative time and WIT.

Conclusions: With fellowship training and subsequent adequate total number of robotic procedures during practice, it is possible to perform RAPN with favorable perioperative outcomes in the setting of low-volume of cases over an extended duration.

Introduction

Partial nephrectomy is the standard of care for the treatment of localized T1 renal tumors when feasible.¹ Compared to radical nephrectomy, it provides improved morbidity and

mortality, lower incidence of chronic kidney disease (CKD), and similar oncological outcomes.¹⁻³ Although the open approach has long been considered the gold standard, minimally invasive approaches (laparoscopic and robotic) have increasingly been replacing the open approach.⁴ Robotic-assisted partial nephrectomy (RAPN) also provides advantages over the laparoscopic approach, such as a shorter learning curve, easier suturing, lower conversion rates, and a shorter warm ischemia time (WIT).⁵⁻⁹

Most reports about the surgeon's experience with RAPN include a large number of cases performed consecutively over a short period of time with involvement of multiple surgeons at varying levels of experience.¹⁰⁻¹⁷ The plateau in learning curve of RAPN after fellowship training in high-volume centers has been shown to be around 44 cases.¹⁸ There is a lack of reporting on the outcomes of such a learning curve when performed over an extended duration.

Since the introduction of robotic surgery in Kuwait six years ago, the total number of procedures performed (urological and non-urological) were less than 450 cases. Currently, there are two robots available with only one used exclusively by urologists.¹⁹ Since the start of the robotic program in 2014 up to the time of writing this paper, a certified uro-oncologist (SA) with Society of Urologic Oncology (SUO) fellowship training has performed 200 robotic urological procedures. The objective of this study was to describe the outcomes of RAPN performed by a single surgeon (SA) using the da Vinci[®] Si surgical system (Intuitive Surgical, Inc., Sunnyvale, CA, U.S.) over six years, and whether the total robotic surgical volume can overcome the problem of low-volume RAPN. This study largely analyzed the experience of robotic surgery in a country where robotic surgery is not widely diffused.

Methods

Study population and design

This was a retrospective analysis of all RAPN procedures performed between February 2014 and February 2020 by a

uro-oncologist at Sabah Al-Ahmad Urology Center (SAUC), a tertiary urology referral center in Kuwait. Ethical approval was obtained from the Kuwait Ministry of Health and Kuwait University.

Surgeon background

After completing an SUO fellowship, the surgeon (SA) performed Kuwait's first RAPN on February 14, 2014. Several robotic workshops took place involving international robotic proctors.

As part of extensive training in advanced urologic oncology during fellowship, the surgeon was actively trained in open, laparoscopic, and robotic surgery. The robotic surgical practice included robot-assisted radical prostatectomy (RARP), robot-assisted radical cystectomy (RARC), robot-assisted retroperitoneal node dissection, and RAPN. The institution where the surgeon completed the fellowship has a robotic volume per year averaging 650 cases. Fellow involvement in cases were at least 50% hands-on with independence in decision-making. Furthermore, there were multiple robotic teaching modules involving simulation training, and wet and dry labs using live porcine modules.

RAPN procedure

RARP was performed using the da Vinci[®] Si surgical system. Three robotic arms were used. For right- and left-sided RAPN procedures, five and four ports were used, respectively (Ethicon, Cincinnati, OH, U.S.). A 5 mm port was added on the right side for liver retraction. For the camera and the assistant ports, a 12 mm port was used. The procedure was standardized in all cases.

Patients were positioned in a 45-degree lateral position. The robot was docked towards the back of the patient, perpendicular to the operating table. All cases were done transperitoneally, with pneumoperitoneum pressure set at 12–14 mm Hg. A zero-degree lens was used for most procedures except for posteriorly located tumors, where a 30-degree (down) lens was used. Hilar dissection was performed in all cases. A first assistant sparing technique (FAST) was used whereby all sutures and bulldogs were placed inside the peritoneal cavity prior to clamping.²⁰

After exposure of the tumor, the renal arteries (main and accessory if present) were clamped using Scanlon robotic bulldog clamp[®] (Scanlon International, St. Paul, MN, U.S.) to start WIT. All cases in this study were subjected to global warm ischemia partial nephrectomy. Tumor excision was performed sharply using the robotic scissors. The tumor bed was oversewn using barbed polyglycolic acid/polycaprolactone (PGA/PLC) Stratafix suture size 2–0 on an SH (26 mm, 1/2c) needle (Ethicon, Cincinnati, OH, U.S.) with a Weck Hem-O-Lock clip (Teleflex, Research Triangle Park,

NC, U.S.) at the tail.²¹ Early unclamping was performed in all cases.²² Sliding clip renorrhaphy was performed using polyglactin suture size 0 on a CT (40 mm, 1/2c) needle (Ethicon, Cincinnati, OH, U.S.).²³ No drains or stents were inserted unless urine leak was suspected.

For few initial RAPNs, mannitol and furosemide were administered. However, this practice was discontinued.²⁴ In most procedures, a synthetic biodegradable cyanoacrylate basis glue was sprayed over the tumor bed after completion of renorrhaphy using a laparoscopic spray device (GEM SRL, Viareggio, LU, Italy). The specimen was extracted from the 12 mm camera port site after placing it in a surgical bag. The camera and assistant ports were always closed with polyglactin suture size 1 using a laparoscopic suture passer.

Clinical data and outcome

Data was obtained from the medical records of all patients. This included demographic details, comorbidities, clinicopathological characteristics (tumor stage, histopathology, and positive surgical margin [PSM] status), and R.E.N.A.L nephrometry score (RNS). The latter was based on the preoperative characteristics of the tumors on imaging.²⁵ The surgeon experience was evaluated by examining the operative time (the time spent from insertion of the first port until removal of the last port); WIT (the time spent from the application of the Scanlon robotic bulldog clamp[®] on the renal arteries until its removal); estimated blood loss (EBL), and intra- and postoperative complications (graded according to the Clavien-Dindo classification system and grouped as minor (grades I and II) or major (grades III–V), and early (0–30 days postoperatively) or late (31–365 days postoperatively)).²⁶ Patients were divided into three consecutive tertiles (first tertile [n=14], second tertile [n=14], third tertile [n=15]).

Other variables recorded included hospital stay in days, trifecta, and pentafecta. The latter two parameters were used to assess short-term and long-term success of RAPN, respectively.²⁷ Trifecta was achieved when WIT was ≤ 25 minutes, surgical margins were negative, and there were no perioperative complications \geq grade III. Pentafecta was achieved when the trifecta was achieved in addition to $>90\%$ preservation of estimated glomerular filtration rate (eGFR) and no upstaging of CKD at 12 months postoperatively. Renal functional assessment was performed by measuring serum creatinine level ($\mu\text{mol/L}$) and measuring the change in eGFR (mL/min/1.73m^2), which was calculated using Chronic Kidney Disease-Epidemiology formula.²⁸ These tests were performed preoperatively, as well as one month and three months postoperatively.

Only patients with at least three months of followup postoperatively were included in the analysis. All the patients were followed up at four weeks postoperatively to review pathology reports. Subsequent followup of patients with

malignant renal cell carcinoma (RCC) was according to the Canadian Urological Association (CUA) 2018 guidelines for followup after treatment of non-metastatic RCC.²⁹ Low-risk (pT1) patients were seen annually with history and physical examination, serum creatinine, and a chest x-ray. Enhanced computed tomography (CT) scan of the abdomen was performed at six and 24 months postoperatively. Intermediate-risk (pT2) patients were seen every six months for the first three years and annually thereafter with history and physical examination, serum creatinine, and a chest x-ray. Enhanced CT scan of the abdomen was performed at six and 12 months postoperatively and annually thereafter.

Analysis

Medians and interquartile ranges (IQR) were used for continuous variables' description, whereas number and percentages were used for categorical variables' description. Wilcoxon signed-rank test was used to compare serum creatinine and eGFR before and after the procedure. Pearson Chi-squared test or Fisher exact test were used to compare categorical variables as appropriate, whereas Kruskal-Wallis test was used to compare continuous variables among the three tertile groups.

Univariable linear regression analysis was used to evaluate the association between some operative outcomes (operative time, WIT, and EBL) and certain patients/tumors/surgeon factors (RNS, surgeon experience tertile groups, tumor size, body mass index [BMI], and side of the tumor). Subsequently, a backward, stepwise, multivariable linear regression analysis was used to assess independent risk factors for the abovementioned outcomes and risk factors with a p-value exit criteria of 0.05. Statistical significance was chosen as a p-value of 0.05. The STATA statistical software package (STATA 12, STATA Corporation, College Station, TX, U.S.) was used for all analyses.

Results

Demographic and tumor characteristics

The baseline demographic and clinical characteristics of all patients are shown in Table 1. Between February 2014 and February 2020, a total of 200 robotic urological procedures were performed; 43 (22%) were RAPNs. All RAPNs were performed over six years.

Tumor characteristics are shown in Table 2. Thirty-five (82%) patients had cT1a tumors, and the median RNS was seven [IQR 6, 8]. Twenty-eight (65%) patients had medium to high tumor complexity (RNS \geq 7). The median tumor size was 3 cm [IQR 2.3, 4], and eight (18%) patients had tumors $>$ 4 cm in size.

Table 1. Baseline (preoperative) demographics and clinical characteristics of 43 subjects underwent robot-assisted partial nephrectomy

Variable	n (%) or median (IQR)	
Age (years)	50 (40, 62)	
Gender		
Male	27 (63)	
Female	16 (37)	
Nationality		
Kuwaiti	34 (79)	
Non-Kuwaiti	9 (21)	
Family history of cancer	15 (35)	
Smoking		
No	27 (63)	
Yes	11 (25)	
Ex-smoking	5 (12)	
Comorbidities		
DM	14 (33)	
Hypertension	21 (49)	
CVD	2 (5)	
History of abdominal surgery	18 (42)	
BMI (kg/m ²)	28.7 (25, 32)	
ASA	ASA	
1	1	
2	2	
3	3	
Cases by year	RAPN=43	Total=200
2014	2 (5)	18 (9)
2015	7 (16)	37 (19)
2016	4 (9)	35 (16)
2017	9 (21)	37 (19)
2018	9 (21)	23 (12)
2019	9 (21)	36 (18)
2020	3 (7)	14 (7)

ASA: American society of anesthesiology; BMI: body mass index; CVD: cardiovascular disease; DM: diabetes mellitus; IQR: interquartile range; RAPN: robotic-assisted partial nephrectomy.

Table 2. Tumor characteristics of 43 subjects underwent robot-assisted partial nephrectomy

Variable	n (%) or median (IQR)
Tumor side	
Right	22 (51)
Left	21 (49)
Clinical T stage (cT)	
1a	35 (82)
1b	8 (18)
RNS	7 (6, 8)
4	1 (2)
5	4 (9)
6	10 (23)
7	15 (35)
8	9 (21)
9	3 (7)
10	1 (2)
Tumor size (cm)	3 (2.3, 4)

IQR: interquartile range; RNS: R.E.N.A.L. nephrometry score.

Perioperative and pathological data

There were no conversions to open (Table 3). The median operative time, WIT, and EBL were 190 minutes (IQR 170, 200), 16 minutes (IQR 14, 19), and 100 mL (50, 250), respectively. Two (5%) patients had a PSM and four (11%) were upstaged to pT3a as a result of microscopic fat invasion. The median hospital stay was two days (IQR 2, 3).

Complications

Five (12%) complications occurred in four patients (Table 3). Only one was early and major (Clavien grade IIIa) and required percutaneous drainage of a perinephric urinoma. The four other complications ranged from Clavien grade I–II and included fever due to atelectasis, positioning-related superficial thrombophlebitis, and incisional hernia. None of the patients required angioembolization or ureteric stenting. Only one patient required a blood transfusion, and all patients recovered fully from all complications.

Renal function data

Data on renal function parameters and CKD staging pre- and postoperatively are shown in Table 4. The median preoperative and one month postoperative eGFR values were 95 mL/min/1.73m² (IQR 86, 109.1] vs. 96.9 mL/min/1.73m² (IQR: 82.2, 108.3) (p=0.04), respectively. The median eGFR change one month postoperatively from preoperatively was -2.1 mL/min/1.73m² (-5, 3.6). Only one patient had upstaging of CKD and none of the patients required dialysis during the study period. No followup nuclear renal scans were performed.

Clinicopathological associations

The median followup duration was 26.2 months (IQR 9.8, 45.5), and none of the patients developed recurrence. Trifecta was achieved in 40 (93%) patients. Those who did not achieve it included two with a PSM and one with an early major complication (Clavien IIIa). Because eGFR at one year was not available for 10 patients, pentafecta analysis included 33 patients and was achieved in 27 (82%) of them. Those who did not achieve it included three who did not achieve the trifecta, and three others who had their eGFR at one year from surgery <90% from baseline. There were no significant changes in trifecta and pentafecta achievements over time (Fig. 1).

Increased surgeon experience was significantly associated with a shorter operative time and less EBL (p=0.002, p=0.025, respectively) (Fig. 1). However, no significant changes were observed with respect to WIT, complications, RNS, or postoperative eGFR. Univariate and multivariate linear regression analyses were performed to identify factors associated with operative times, WITs, and EBL (Table 5). A lower RNS was significantly associated with a shorter operative time and WIT, even when adjusting for other factors. In addition, increased surgeon experience was significantly associated with a shorter operative time and less EBL.

Discussion

RAPN is becoming widely adopted as a treatment approach for small renal masses.¹ A number of studies have demonstrated its safety and feasibility.^{4,7,9} With that came the need to improve the efficacy of this procedure through a consistent evaluation of outcomes, and to develop predictive models to know preoperatively which patients influence favorable or unfavorable outcomes.

There are two da Vinci® surgical robots in Kuwait. The first was installed in 2014 and the second in 2017.¹⁹ RAPN was the first procedure to be performed in 2014. The volume of cases was building up, albeit very slowly. Not surprisingly, the number of robotic procedures was less compared to high-volume centers.

Table 3. Perioperative details, complications, and outcomes of 43 subjects underwent robot-assisted partial nephrectomy

Variable	n (%) or median (IQR)
Operative time (min)	190 (170, 200)
WIT (min)	16 (14, 19)
EBL (mL)	100 (50, 250)
Blood transfusion	
No	42 (98)
Yes	1 (2)
Hospital stay (days)	2 (2, 3)
Diagnosis	
Renal cell carcinoma	37 (82)
Chromophobe	6 (13)
Clear-cell	23 (51)
Papillary type 2	6 (13)
Unclassified	2 (5)
Angiomyolipoma	4 (11)
Oncocytoma	2 (5)
Margin	
Negative	41 (95)
Positive	2 (5)
Pathological T stage (pT)	
1a	28 (76)
1b	5 (13)
3a	4 (11)
Complications	5 (12)
Clavien I	2 (5)
Clavien II	2(5)
Clavien IIIa	1 (2)
Trifecta achievement	40 (93)
Pentafecta achievement*	27 (82)

*Total number of subjects with available creatinine one-year post-surgery is 33. EBL: estimated blood loss; IQR: interquartile range; WIT: warm ischemia time.

Table 4. Kidney function data of 43 subjects who underwent robot-assisted partial nephrectomy

Variable	Preoperative (baseline, n=43)	One month post-surgery (n=43)		One-year post-surgery (n=33)*
		n (%) or median (IQR)		
Creatinine (µmol/L)	71 (60, 83)	72.8 (60, 86.5)**		75 (63.3, 82.4)
eGFR (mL/min/1.73 m ²)	95 (86, 109.1)	96.9 (82.2, 108.3)**		94.8 (85.4, 107.5)
CKD 1 (eGFR>90)	26 (60)	27 (63)		22 (67)
CKD 2 (eGFR≥60–90)	14 (33)	13 (30)		9 (27)
CKD 3 (eGFR≥30–60)	2 (5)	2 (5)		2 (6)
CKD 4 (eGFR≥15–30)	0 (0)	0 (0)		0 (0)
CKD 5 (eGFR<15)	1 (2)	1 (2)		0 (0)

*n=33 (number of subjects with available creatinine one-year post surgery). **p is statistically significant (<0.05) in comparison to preoperative (baseline) data using paired t-test. CKD: chronic kidney disease; eGFR: estimated glomerular filtration rate; IQR: interquartile range.

The literature is abundant with studies from centers with vast experience in performing RAPN. However, many regions and institutions in the Middle East lack the high volume of cases despite referral-based practice.^{10-17,19} In the present study, we sought to examine whether high-volume fellowship training in robotic surgery can overcome the problem of low-volume RAPN and whether this is influenced by the total volume of robotic procedures performed.

Achievement of trifecta and pentafecta has been used as a benchmark for RAPN efficacy.²⁷ Our results compare favorably with a study published by Kahn et al.²⁷ They showed trifecta and pentafecta rates of 84.8% and 25.8% compared to 93% and 82% in the present study, respectively. CKD upstaging 12 months postoperatively occurred in 48.3% of their patients, which could have explained the low rate of pentafecta achievement. They found that a lower RNS was associated with an increased odds of trifecta and pentafecta,

a finding we were not able to show, likely due to small sample size.

In a prospective, multicenter study involving 708 patients who underwent partial nephrectomy where 47.3% underwent open, 36.6% underwent laparoscopic, and 16.1% underwent RAPN, Antonelli and colleagues found that the open and laparoscopic approaches were independent predictors of renal functional deterioration compared to the robotic approach.³⁰ These were modifiable factors compared to non-modifiable predictors of renal functional deterioration, such as female gender, baseline eGFR, and age. This supports findings from the present study, where the median eGFR at one month postoperatively was similar to the preoperative median eGFR ($p<0.05$) (Table 4).

PSM rate in the present study was 5%. This is similar to multiple published reports reaching 9.9%.¹⁷ Other perioperative outcomes in the present study compared favorably

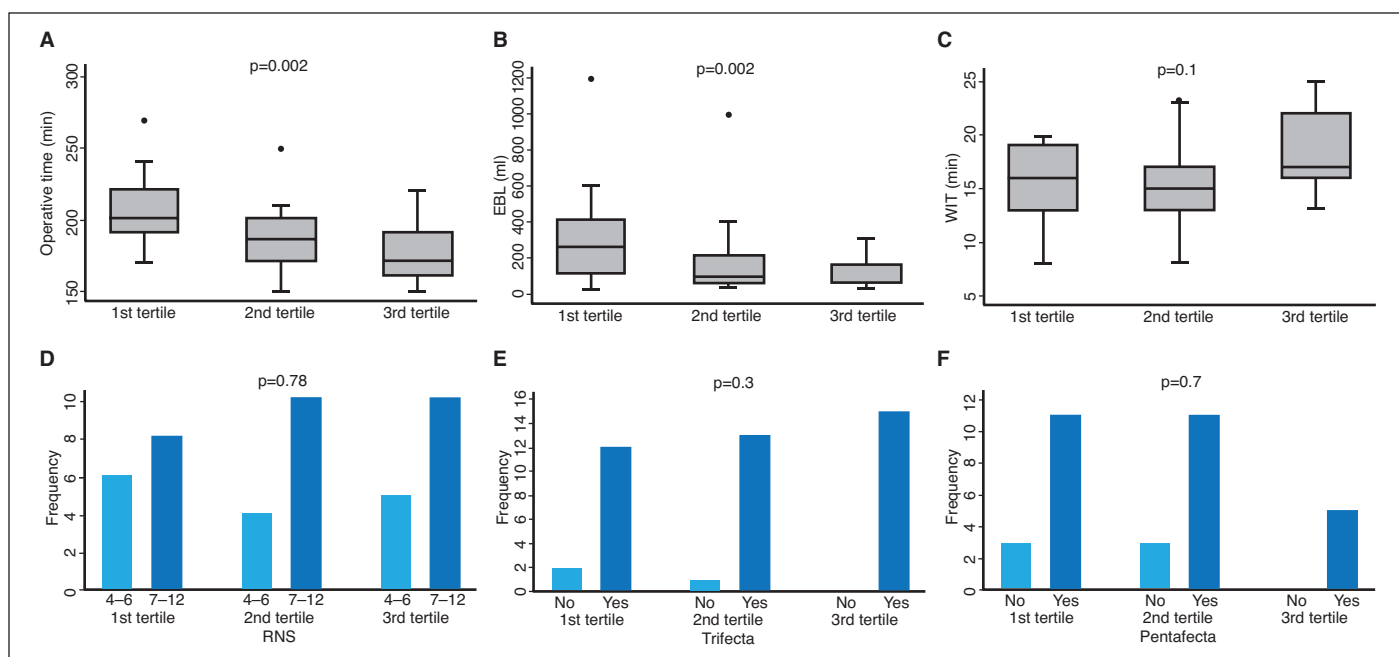


Fig. 1. Effect of surgeon experience on perioperative outcomes. Demonstrated are changes in perioperative parameters over three consecutive tertile groups (first tertile [n=14], second tertile [n=14], third tertile [n=15]). **(A)** Operative time; **(B)** estimated blood loss (EBL); **(C)** warm ischemia time (WIT); **(D)** R.N.S.A.L. nephrometry score (RNS); **(E)** trifecta; **(F)** pentafecta

Table 5. Factors associated with operative time, WIT, and EBL

Variable	Operative time (min)		WIT (min)		EBL (mL)	
	Univariable linear regression	Multivariable linear regression	Univariable linear regression	Multivariable linear regression	Univariable linear regression	Multivariable linear regression
	Coeff (95% CI) p	Coeff (95% CI) p	Coeff (95% CI) p	Coeff (95% CI) p	Coeff (95% CI) p	Coeff (95% CI) p
RNS	7.7 (1.3,14) 0.018	10.0 (4.9,15.2) <0.001	1.5 (0.6, 2.5) 0.002	1.4 (0.4, 2.3) 0.004	33.7 (-28.3, 95.7) 0.28	48.89 (-10.4, 108) 0.103
Surgeon experience*						
2nd tertile (14 patients)	-21.1 (-39.1, -3.1) 0.023	-23.9 (-39.4, -8.4) 0.003	0.2 (-2.8, 3.2) 0.889	-0.2 (-2.9, 2.6) 0.89	-140 (-320.7, 40.7) 0.125	-153.9 (-331.7, 23.8) 0.088
3rd tertile (15 patients)	-32.5 (-50.2, -14.8) 0.001	-38.1 (-53.5, -22.7) <0.001	2.9 (-0.04, 5.9) 0.053	2.2 (-0.6, 4.9) 0.12	-215 (-392.7, -37.3) 0.019	-242.2 (-419.3, 65.1) 0.009
Size of the tumor (cm)	1.9 (-5.6, 9.5) 0.605	1.03 (-4.9, 6.9) 0.72	0.3 (-0.8, 1.5) 0.544		-46.4 (-115.1, 22.1) 0.18	
BMI (kg/m ²)	0.4 (-0.9, 1.7) 0.564		0.2 (-0.04, 0.3) 0.130		4.89 (-7.3, 16.9) 0.429	
Side of the tumor*	2.5 (-14.1, 19.2)		1.2 (-1.3, 3.7)		51.4 (-102.3, 205.1)	
Left	0.76		0.338		0.503	

*Reference category: surgeon experience (1st tertile [14 patients]); side of the tumor (right side). BMI: body mass index; coeff: coefficient; CI: confidence interval; EBL: estimated blood loss; RNS: R.E.N.A.L. nephrometry score; WIT: warm ischemia time.

with multiple published reports, including the operative time, WIT, EBL, hospital stay, and overall complications, despite the low volume.^{14,31,32} Mottrie et al found that WIT and console times for RAPN were optimized after 30 and 20 procedures, respectively.¹³ Their study included 62 RAPNs performed over a three-year period by a single surgeon with extensive prior robotic experience. In the present study perioperative outcomes (operative time and EBL) were optimized after the first tertile group of 14 patients, with only one major complication occurring throughout the series (Clavien IIIa) and thus reflecting an early learning curve (Fig. 1).

Several studies have shown surgical experience improves WIT.^{14,15} We did not find that in the present study. This can be explained by two reasons: first, a median WIT of 16 minutes (IQR: 14, 19) was optimized since the beginning of the study, probably due to the adoption of early unclamping and the FAST techniques in all cases, both of which have been shown to significantly reduce WIT;^{20,22} second, a higher RNS was shown to be an independent predictor of longer WIT.³³ On multivariate analyses, our study showed that a higher RNS was an independent predictor of longer WIT, in addition to longer operative time (Table 5). Since our median RNS did not significantly change over each tertile group, this could explain why WIT did not change over time.

To allow assessment of the surgeon experience of RAPN in a country where robotic surgery was not widely diffused, patients were divided into tertile groups of consecutive patients. This is similar to a recently published, single-surgeon experience of five consecutive groups of 13 patients. It showed significant improvement in perioperative outcomes.¹⁵ Similarly, Mottrie and colleagues reported a 20-case learning curve concerning console time.¹³ In the present study, increasing surgeon experience over the ter-

tile groups was an independent predictor of shorter operative time and less EBL. Motoyama et al found that WIT, but not console time, was independently affected by surgeon experience.¹⁵ The findings in their study and the present study support the significant association between surgeon experience and perioperative outcomes. We believe that acceptable perioperative outcomes can be achieved in a low-volume setting as early as 14 cases performed over an extended duration, provided there was extensive fellowship training. In addition, the influence of other robotic procedures has been shown to improve RAPN outcomes despite low volume.¹³ In our study, 43 RAPN cases were part of a total of 200 robotic procedures, which included RARP, radical nephrectomy, pyeloplasty, adrenalectomy, radical nephroureterectomy, and colo-vesical fistula repair. This likely positively influences the RAPN learning curve.³⁴

We acknowledge several limitations of our study, including the retrospective design and its potential biases. The low volume of RAPN over six years was another major limitation. During the beginning of the robotic program in Kuwait, there was only one certified uro-oncologist with fellowship training in robotic surgery and only one robot. However, more surgeons with fellowship training in robotic surgery are joining the program, which ultimately will increase case volume. The third limitation was the single-surgeon nature of the data, which we feel was an advantage, as it eliminated surgical technique variability from the analysis, albeit not making it possible to extrapolate the results to a broader cohort of surgeons. The last limitation was the low/moderate RNS for most patients, which could have biased the outcomes.

Conclusions

With extensive fellowship training and subsequent adequate total number of robotic procedures in practice, it is possible to perform RAPN using the da Vinci® Si surgical system with favorable perioperative outcomes in a low-volume setting over an extended duration. RNS is an independent predictor of WIT and operative time, and surgeon experience is an independent predictor of operative time and EBL.

Competing interests: The authors do not report any competing personal or financial interests related to this work.

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