Analysis of learning curve for laparoscopic pancreaticoduodenectomy

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Background: Laparoscopic pancreaticoduodenectomy (LPD) may have potential minimal invasive advantages for selected patients in limited center. However, few studies analyzed the learning curve. This study aimed to analyze the learning curve of this procedure at a large volume set, and share our experience to surmount it.

Methods: All prospectively maintained data of the consecutive LPDs was reviewed retrospectively. The procedures were performed by single surgeon. Patients were divided into four groups according to staged approach with different focuses: Group A (the first 30 patients), Group B (the second 30 patients), Group C (the third 30 patients), and Group D (the fourth and last 30 patients). And the changes of outcomes during different learning periods were analyzed.

Results: Between September 2012 and July 2015, 120 patients underwent LPD. One hundred and eleven of them underwent totally LPD, and 9 patients underwent laparoscopic assisted pancreaticoduodenectomy (LAPD). The mean operative time (OT), mean blood loss and average length of hospital stay (LOS) was 359.8±57.6 min, 169.7±152.6 mL and 17.0±9.8 d respectively. A total of 42 (35%) patients developed morbidity with no mortality. The mean overall OT tended to decrease from 370.2±52.8 min in Group A to 342.0±73.1 min in Group D with the accumulating experience of the surgeon. Moreover, mean OT of pancreatojejunostomy and choledochojejunostomy also tended to decrease from 55.0±8.7, 39.8±11.7 min in Group A to 43.6±7.6, 27.7±11.8 min in Group D respectively. Meanwhile, the clinical outcomes tended to get better. Mean blood loss, morbidity and LOS decreased from 219.3±147.9 mL, 43.3%, 18.7±10.0 d in Group A to 140.1±73.6 mL, 23.3%, 14.4±6.2 d in Group C respectively except for Group D.

Conclusions: Routine practice of the LPD procedure was feasible and safe. Gained experience can improve clinical outcomes in 30 to 60 operations by overcoming the learning curve.

Keywords: Laparoscopic; pancreaticoduodenectomy; learning curve

Received: 12 July 2016; Accepted: 18 July 2016; Published: 18 August 2016.

doi: 10.21037/jovs.2016.07.25

View this article at: <http://dx.doi.org/10.21037/jovs.2016.07.25>

Introduction

Laparoscopic pancreaticoduodenectomy (LPD) has minimally invasive advantages for selected patients in specialized centers with growing evidence, including smaller

surgical incision, reduced intraoperative blood loss, less postoperative pain and earlier recovery compared with open pancreaticoduodenectomy (OPD) (1-4). Similar advantages in the setting of pancreatic ductal adenocarcinoma (PDAC)

were also confirmed with a longer progression-free survival in patients undergoing LPD than OPD (5). Moreover, the costs associated with LPD can be balanced when reductions in length of hospital stays (LOS) translated into reduced expenses, which make it comparable with open approach (6).

However, LPD has its inherent challenges and demanding skills as a novel technique, for example, the retroperitoneal location, proximity to major vascular structures, technical difficulty of three anastomoses, and risk of high morbidity and mortality. It was still limited to selected high-volume centers. Just the same as the open approach, a learning curve was supposed to exist for LPD, which may promise new attempts to be routine practice with feasibility and safety once identified and surmounted.

This study aims to analyze the learning curve of the single surgeon by evaluating the changes of clinical outcomes out of the first 120 consecutive LPDs via a prospective staged approach, and share our experience to overcome the learning curve.

Materials and methods

All data of patients were reviewed retrospectively from a prospective database. From September 2012 to July 2015, a series of consecutive LPDs performed by the same surgeon (Dr. Yi-Ping Mou) in the Sir Run Run Shaw Hospital (from September 2012 to June 2015) and the Zhejiang Provincial People's Hospital (from June 2015 to July 2015) were identified. All the patients had a backup for its perioperative data and recorded video reviewed afterwards. The surgeon had prior experience with OPD and laparoscopic procedure such as radical gastrectomy, distal or central pancreatectomy. All patients were informed of the potential benefits and risks of LPD preoperatively with written consents. And the protocols were conducted following the approval of the Institutional Review Board.

Patients with superior mesenteric vein (SMV)/portal vein (PV) reconstruction and combined multi-visceral resection were excluded. All the data were retrospectively reviewed, including demographic data, operative time (OT), estimated blood loss (EBL), diameter of main pancreatic duct (MPD) and common bile duct (CBD), 90-day morbidity and mortality, LOS, and pathologic results. Postoperative pancreatic fistula (POPF) was defined and graded according to the criteria of International Study Group of Pancreatic Fistula (ISGPF) (7). POPF graded B and C were considered as clinically relevant. Other major postoperative complications were defined and graded by

the consensus of International Study Group of Pancreatic Surgery (ISGPS) including delayed gastric emptying (DGE) and post-pancreatectomy hemorrhage (8,9). Bile leakage was defined by the definition of the International Study Group of Liver Surgery (ISGLS) (10).

To analyze the changes in different learning periods of this procedure, the identified 120 cases were divided into four groups according to the surgeon's staged approach. In the first period (Group A, the first 30 patients), the surgeon focused on the feasibility and safety of LPD. In the second period (Group B, the second 30 patients), the surgeon performed total mesopancreas excision (TMpE) for cancer, aiming to get better oncological outcomes. In the third period (Group C, the third 30 patients), he paid more attention to surgical details, in terms of the sutures. In the fourth period (Group D, the last 30 patients), former primary assistant were trained to perform some steps of LPD, for example, the resection part.

Operative methods

The operative methods used have been described in detail previously. For patients with resectable lesions, we routinely used the modified approach based on "Five Trocars" (11). However, for patients who had SMV encasement with difficulty creating the retro-pancreatic tunnel, that is, borderline resectable pancreatic cancer (BRPC), we used the "Easy First" strategy to perform LPD (12). TMpE had been performed for cancer since Group B. The definition of mesopancreas indicated the soft connective tissue along celiac axis, superior mesenteric vessels and uncinate process of pancreas, especially the lymphatic and nervous structures of retroperitoneal margin as reported (13,14).

After the specimens were removed from the enlarged umbilical port, a frozen section was sent to confirm the negative margins. Then we performed child's reconstruction in a complete laparoscopic manner following individual construction. Laparoscopic pancreaticojejunostomy (LPJ) was performed using duct-to-mucosa method. If the diameter of MPD was between 2 and 5 mm, LPJ was carried out using interrupted sutures up to 4–6 stitches with stents of proper diameter. As for MPD larger than 5 mm, running sutures were performed without stent. In Group C, we used the non-absorbable sutures instead of absorbable sutures. Laparoscopic choledochojejunostomy (LCJ) was performed with running suture if the CBD was larger than 8 mm, while with interrupted sutures in CBD less than 8 mm. As for the laparoscopic gastrojejunostomy (LGJ), we used an endoscopic

Table 1 Demographic characteristics of the patients who underwent LPD

Variable	Overall	Group A	Group B	Group C	Group D	P value
Gender (male/female)	80/40.0	18/12.0	20/10.0	19/11.0	23/7.0	0.552
Age (years)	59.7±12.1	59.2±11.3	59.2±9.6	55.4±14.7	65.2±11.6	0.002
Aged patients (≥80 yr, n/%)	5/4.2	0/0.0	0/0.0	0/0.0	5/16.7	0.004
BMI (kg/m ²)	23.4±3.5	23.3±3.7	24.2±4.3	22.7±2.7	23.1±3.1	0.403
ASA score (n/%)						
I	23/19.2	6/20.0	8/26.7	8/26.7	1/3.3	0.070
II	84/70.0	20/66.7	18/60	21/70.0	25/83.4	0.248
III	13/10.8	4/13.3	4/13.3	1/3.3	4/13.3	0.507
Prior surgery (n/%)	23/19.2	4/13.3	4/13.3	7/23.3	8/26.7	0.433
Symptoms (n/%)						
Jaundice	54/45.0	15/50.0	15/50.0	11/36.7	13/43.3	0.222
Abdominal pain	61/50.8	13/43.3	17/56.7	16/53.3	15/50.0	0.761

LPD, laparoscopic pancreaticoduodenectomy; BMI, body mass index; ASA, American Society of Anesthesiology.

linear stapler to perform a side-to-side anastomosis with running sutures to close the common opening.

Statistical analysis

Continuous data was summarized as means ± standard deviations, and the categorized data was expressed as frequencies and percentages. Variants of numeric data with normal distributions in different group were analyzed using analysis of variance (ANOVA) while the non-normally variable were analyzed by Kruskal-Wallis test. And categorical data was tested using Scheffé tests. P value <0.05 was considered statistically significant. For all these analyses, SPSS version 20.0 (Chicago, IL, USA) statistical software was used.

Results

Patient demographics and pathologic outcomes

Between September 2012 and June 2015, 120 patients underwent LPD by single surgeon in our institution. Among them, 111 patients underwent totally LPD, 9 patients underwent laparoscopic assisted pancreaticoduodenectomy (LAPD).

Detailed demographic data was summarized in *Table 1*. The mean age of patients was 59.7±12.1 (range, 20–85) years old with 80 male and 40 female patients. Average body mass index was 23.4±3.5 (range, 17.4–36.8) kg/m². A total of 23 (19.2%)

patients had a prior surgery. The most common present symptoms at diagnosis were abdominal pain (61, 50.8%) and jaundice (54, 45%). Compared among groups, all of them showed no statistical significance except for the average ages and aged patients older than 80 years old. The average age at diagnosis of Group D is older than the prior three groups (Group D *vs.* Group A, Group B, Group C; P=0.048, 0.033, 0.006 respectively). There were five aged patients. Of note all of them were in Group D.

Pathological results were listed in *Table 2*, it confirmed malignancy in 85 (70.8%) patients, including PDAC in 36 patients, ampulla of Vater tumor in 32 patients, distal bile duct carcinoma in 14 patients, and other malignancies in 3 patients. Distribution disequilibrium was found only for distal bile duct adenocarcinoma and serous cystic neoplasms. There was no difference of tumor sizes among groups with the cutoff of 5 cm.

Operative data and overall clinical outcomes

The operative data and clinical outcomes were shown in *Tables 3,4*. In general, the mean OT and mean EBL was 359.8±57.6 min, 169.7±152.6 mL respectively. Only 19 (15.8%) patients needed intraoperative blood transfusion. In addition, 42 (35%) patients developed morbidity with no mortality. Clinically relevant pancreatic fistula developed in 12 (10%) cases. Among them there were 9 (7.5%) Grade B POPF, which improved with conservational treatment and 3 (2.5%) Grade C POPF required reoperation. Bile

Table 2 Pathological results of the patients who underwent LPD

Variable	Overall	Group A	Group B	Group C	Group D	P value
Histological diagnosis (n/%)						
PDAC	36/30.0	8/26.7	9/30.0	9/30.0	10/33.3	0.957
Tumor of ampulla of Vater	32/26.7	8/26.7	10/33.3	5/16.7	9/30.0	0.496
Distal bile duct adenocarcinoma	14/11.7	5/16.7	1/3.3	7/23.3	1/3.3	0.033
IPMN	9/7.5	3/10.0	1/3.3	1/3.3	5/16.7	0.187
Serous cystic neoplasm	9/7.5	0/0.0	5/16.7	4/13.3	0/0.0	0.019
Tumor size (n/%)						
<5 cm	99/82.5	24/80.0	26/86.7	25/83.3	24/80.0	0.888
≥5 cm	21/17.5	6/20.0	4/13.3	5/16.7	6/20.0	0.888

LPD, laparoscopic pancreaticoduodenectomy; PDAC, pancreatic ductal adenocarcinoma; IPMN, intraductal papillary mucinous neoplasms.

Table 3 Intraoperative outcomes of patients who underwent LPD

Variable	Overall	Group A	Group B	Group C	Group D	P value
Overall OT	359.8±57.6	370.2±52.8	366.5±51.0	360.6±67.9	342.0±73.1	0.303
Time of resection	170.7±37.7	181.3±38.2	157.0±27.2	157.9±35.5	186.7±46.1	0.002
Time of LPJ	50.1±10.2	55.0±8.7	46.6±6.8	55.2±11.9	43.6±7.6	0.000
Diameter of MPD	0.42±0.28	0.39±0.22	0.35±0.15	0.36±0.18	0.42±0.29	0.591
Time of LCJ	35.2±12.9	39.8±11.7	38.6±14.2	34.7±10.4	27.7±11.8	0.001
Diameter of CBD	1.48±0.73	1.68±0.82	1.36±0.69	1.51±0.70	1.29±0.65	0.086
Blood loss (mL)	169.7±152.6	219.3±147.9	169.6±99.1	140.1±73.6	150.0±88.0	0.022
Transfusion (n/%)	19/15.8	13/43.3	0/0.0	2/6.7	4/13.3	0.000

LPD, laparoscopic pancreaticoduodenectomy; LPJ, laparoscopic pancreaticojejunostomy; LCJ, laparoscopic choledochojejunostomy; CBD, common bile duct; MPD, main pancreatic duct; OT, operative time.

Table 4 Postoperative outcomes of patients who underwent LPD

Variable	Overall	Group A	Group B	Group C	Group D	P value
Morbidity (n/%)	42/35.0	13/43.3	12/40.0	7/23.3	10/33.3	0.380
POPF grade B/C	12/10.0	2/6.7	3/10.0	3/10.0	4/13.3	0.864
Bile leakage	4/3.3	2/6.7	0/0.0	0/0.0	2/6.7	0.247
Hemorrhage	12/10.0	2/3.3	6/20.0	1/3.3	3/10.0	0.158
Reoperation (n/%)	10/8.3	2/6.7	2/6.7	1/3.3	5/16.7	0.269
Mortality (n/%)	0/0.0	0/0.0	0/0.0	0/0.0	0/0.0	1.000
LOS (days)	17.0±9.8	18.7±10.0	18.7±11.8	14.4±6.2	16.3±11.6	0.290

LPD, laparoscopic pancreaticoduodenectomy; POPF, postoperative pancreatic fistula; LOS, length of hospital stay.

leakage occurred in 4 (3.3%) patients. Twelve patients developed postpancreatectomy hemorrhage, with one managed conservatively, three controlled by embolism with digital subtraction angiography (DSA), eight managed by surgery (including five combined with anastomosis leakage,

three Grade C POPF, one bile leakage, one leakage of gastrojejunostomy). Wound infection occurred in 6 (5%) patients, with 3 occurred after reoperation. The average LOS was 17.0±9.8 d.

The mean overall OT tends to decrease continuously

from 370.17 ± 52.83 min in Group A to 342 ± 73.10 min in Group D without statistical significance ($P=0.092$). Resection time of Group A decreased significantly compared with that of Group B and Group C (Group A *vs.* Group B, Group C; $P=0.0062$, 0.017 respectively). And it elevated with significance in Group D (Group D *vs.* Group C; $P=0.0088$). As for the time of the each anastomosis, mean OT of LPJ had a significant decrease from 55 ± 8.72 min in Group A to 43.57 ± 7.62 min in Group D ($P=0.0001$), however, it jumped to 55.2 ± 11.9 min ($P=0.0017$) in Group C with certain reason. The mean time of LCJ decreased continuously from 39.8 ± 11.68 min in Group A to 27.67 ± 11.81 min in Group D ($P=0.0036$). The diameter of MPD and CBD were comparable ($P=0.591$, 0.086 respectively). Meanwhile, the EBL decreased from 219.3 ± 147.9 mL in Group A to 140.1 ± 73.6 mL in Group D significantly ($P=0.011$), at the same time, significant decrease of the transfusion rate was identified ($P=0.020$). All the postoperative parameters including morbidity, reoperation and LOS remained comparable among groups ($P=0.380$, 0.269 , 0.290 respectively). However, consistent trend of decrease was observed including EBL, morbidity and LOS from 219.33 ± 147.88 mL, 43.3% , 18.67 ± 10.03 d in Group A to 140 ± 73.60 mL, 23.3% , 14.38 ± 6.23 d in Group C respectively with reversed increase in Group D.

Discussion

In this study, we presented our experiences with 120 consecutive LPDs of routine practice by staged approach. Such staged approach was carried out with the expectation of better outcomes or the development of our team. Of note all the LPDs were performed by the same surgeon, and with prospectively established database it may be more objective to analyze the learning curve. To the best of our knowledge, no study analyzed the learning curve by separating OT into resection part and each anastomosis before. The stratification of LPD is essential for individualized treatment, for different operative indications had varied focuses and difficulties over the learning curve. We showed the feasibility and safety of routine practice by surmounting the learning curve with staged approach and the continuous improvements in the clinical outcomes with gained experience.

The overall clinical outcomes of our study were comparable with studies published before. From the view of latitudinal comparison with LPD, Gumbs *et al.* (2) reviewed the 285 LPDs performed between 1994 and 2010 and conducted a weighted average OT of 371 min with

morbidity of 48% and mortality of 2%. The combined POPF and bile leakage rate was 15%. Tran *et al.* (6) analyzed nationwide outcomes of LPD in United States and concluded a morbidity of 39.4% with an LOS of 11 d. Moreover, hospitals of high volume were associated with decreased complications of 36.7% and reduced LOS of 9 d. Similarly, our experience indicated a mean OT of 359.8 ± 57.6 min with morbidity of 35% and no mortality. Clinically relevant POPF developed in 12 (10%) patients and bile leakage in 4 (3.3%) patients with a mean LOS of 17.0 ± 9.8 d. Increased LOS with comparable morbidity may due to the different discharge criteria based on not only the morbidity but the medical insurance system as well. While compared longitudinally with OPD, Cameron *et al.* (15) reported 2,000 consecutive OPDs with a morbidity and mortality of 45% and 1.6% respectively, and POPF accounted for 15% of morbidity. Ahmad *et al.* (16) reported 1,302 patients underwent OPD with a mean OT, intraoperative EBL and overall complications rate of 328.5 min, 626 mL and 44% respectively. Slightly elevated OT with reduced EBL and comparable morbidity were identified. In general, we can conclude that routine practice with staged approach of LPD was feasible and safe.

The learning curve of LPD was identified in several studies published before (1,17-19). Kendrick *et al.* (1) reported a reduction from a mean time of 7.7 h for the first ten patients to 5.3 h for the latest ten patients. Moreover, Kim *et al.* (17) reported a decreased OT from 9.8 h for the first 33 patients to 6.6 h for the last 40 patients. And complication rate decreased from 33.3% to 17.6% with shortened LOS from 20.4 to 11.5 d. Song *et al.* (18) conducted a matched cohort analysis comparing LPD with OPD and concluded that their late LPDs had significantly shorter OTs (399.4 *vs.* 566.5 min, $P<0.001$), less EBL (503 *vs.* 685 mL, $P=0.018$) and shorter LOS (11.2 *vs.* 17.3 d, $P<0.001$) than their early practice. Finally, Speicher *et al.* (19) also indicated decreased OT and EBL during learning curve, and proposed a staged learning process to surmount learning curve. They believed that learning curve for LPD consisted of a slow and difficult beginning phase, a steep acceleration phase, and finally a plateau phase with slower but continuous improvements.

Similarly, our study confirmed the stead improvements in the clinical outcomes during learning curve as above. Generally, continuous improvements with significant decrease were identified in EBL, transfusion rate and reconstruction time, especially for LPJ and LCJ. But time of LPJ elevated in Group C with significance. Reduced

Group	Operative time (min)	SD	Pancreaticojejunostomy time (min)	Choledochojejunostomy time (min)	Comlication rate (%)	Comlication rate (%)	Postoperative hospital stay (days)			
Group A	370.17	52.83	55.0	8.7	39.8	11.7	43.3 (13.0)	0.43	18.7	10.0
Group B	366.46	50.96	46.6	6.8	38.6	14.2	40.0 (12.0)	0.40	18.7	11.8
Group C	360.6	67.88	55.2	11.9	34.7	10.4	23.33 (7.0)	0.23	14.4	6.2
Group D	342.0	73.1	43.6	7.6	27.7	11.8	33.33 (10.0)	0.33	16.3	11.6

Figure 1 Changes in operative time (OT) and clinical outcomes with the accumulating of experience in performing laparoscopic pancreaticoduodenectomy (LPD) consecutively. All parameters tended to improve continuously except for Group D, which started a trainee program for the assistants. LPJ, laparoscopic pancreaticojejunostomy; LCJ, laparoscopic choledochojejunostomy; LOS, length of hospital stay.

EBL and transfusion may result from the familiarity of “laparoscopic anatomy” and the command of the skills of hemostasis with more precise operation as gained experience, which was of great importance for LPD, especially for TMpE. The laparoscopic reconstruction was critical to perform totally LPD, especially for LPJ and LCJ. Our finding showed clear reduction of time with continuous improvements over the learning curve, especially after the initial 30 patients for LPJ in Group A and initial 60 patients for LCJ. However, this is not to say that LCJ needs a longer learning period than LPJ given the prior experience and insignificant differences in diameter of the ducts. As for the reversed trend of LPJ’s time in Group C, we believed that the switch using from the Vicryl sutures to the Prolene sutures may partly account for that, because it was more difficult to tie the Prolene into knots and it also required more knots (usually 5–6 knots) than Vicryl (Usually 3 knots). We switched Prolene to PDSII in Group D, and a significant reduction was observed.

However, the reduction of reconstruction time was not achieved at the cost of operative quality. No significant difference was found in terms of morbidity (including POPE, bile leakage, hemorrhage), mortality and LOS. Moreover, consistent trend of decrease was observed including EBL, morbidity and LOS from Group A to Group C except for Group D (Figure 1). And the overall OT tended to decrease continuously from 370.17 min (6.2 h) in Group A to 342 min

(5.7 h) in Group D. As mentioned above, former primary assistant started to perform several steps of LPD in Group D, such as the resection part, and this can be confirmed by the observed significantly elevated resection time. Moreover, patients in Group D were significantly older than the prior groups, and all aged patients older than 80 years old were in Group D. Reasons above partly explained the slightly reversed trend of clinical outcomes comparable to Group C.

In general, the reconstruction time and clinical outcomes improved mostly after 30 to 60 cases by routine practice, and we concluded that such number was essential to get experienced rather than ten cases as supposed (20), which we believed to represent the difficult beginning phase. As shown, we successfully performed LPDs with staged approach with slower but continuous improvements, just as the plateau phase. The underlying explanation may be that the surgeon followed a prior staged approach before heading for LPD (Figure 2), and gradually extended the indications step by step before routine practice. And he had previous experience on performing laparoscopic radical gastrectomy for lymph node (LN) dissection and LGJ, laparoscopic central pancreatectomy (LCP) for LPJ and laparoscopic operation on congenital biliary cyst for LCJ, which also helped to overcome the initial learning curve. That may explain why Kim *et al.* (17) and Kendrick *et al.* (1) reported a steep acceleration phase of rapid learning while we not.

This study has some limitations needed to be pointed

Figure 2 Our prior staged approach prepared for laparoscopic pancreaticoduodenectomy (LPD). OPD, open pancreaticoduodenectomy; LDG, laparoscopic distal gastrectomy; LTG, laparoscopic total gastrectomy; LCP, laparoscopic central pancreatectomy; LBB, laparoscopic biliary bypass; LRCCC, laparoscopic resection of congenital choledochal cyst; LN, lymph node; LGJ, laparoscopic gastrojejunostomy; LPJ, laparoscopic pancreaticojejunostomy; LCJ, laparoscopic choledochojejunostomy; IPMN, intraductal papillary mucinous neoplasms; PDAC, pancreatic ductal adenocarcinoma; MPD, main pancreatic duct; CBD, common bile duct; BRPC, borderline resectable pancreatic cancer.

out. Firstly, we did not conclude the definite number needed to overcome the learning curve. The main reason lies in the varied indications and the specificity of our previous experience. As mentioned, different operative indications had varied difficulties and focuses in performing LPDs. For example, for ampulla of Vater tumors it was convenient to get the biopsy preoperatively, and the dilation of both ducts makes the LPJ and LCJ much easier. For benign pancreatic head lesion, it's easier to dissect the lesions with less emphasis on LN dissection, but most patients have smaller MPD and CBD with a fragile pancreatic parenchyma which makes reconstruction challenging. As for pancreatic cancer, it's technically demanding to resect the lesions en-bloc and get complete lymphadenectomy. And special emphasis on acquiring negative retroperitoneal margin along superior mesenteric vessels was required. Learning curves of this kind can never be assessed only by OT and morbidity, but the oncological outcomes as well. Moreover, given the surgeon's

staged exploration, the learning curve for new adopters may be longer. Secondly, we did not show the oncological outcomes of long-term follow-ups, which needed to be taken into consideration. We plan to analyze and report it in the near future. Last but not least, prospective studies of high quality and comparisons between LPD and OPD are awaited to bring about more objective evaluations.

Conclusions

In conclusion, routine practice of the LPD procedure was feasible and safe in specialized center. Gained experience can improve clinical outcomes in 30 to 60 operations by overcoming the learning curve.

Acknowledgements

Funding: This study was supported by Zhejiang Provincial

Key Subject of Medical Science Foundations (Grant No. 11-CX-21) and Zhejiang Provincial Key Project of Research Foundations (Grant No. 2015C03G1360047).

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The study was approved by the Institutional Review Board in the Sir Run Run Shaw Hospital and Zhejiang Provincial People's Hospital (No. 20160133) and written informed consent was obtained from all patients.

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doi: 10.21037/jovs.2016.07.25

Cite this article as: Lu C, Jin W, Mou YP, Zhou J, Xu X, Xia T, Zhang R, Zhou Y, Yan J, Huang C, Zhang B, Wang J. Analysis of learning curve for laparoscopic pancreaticoduodenectomy. *J Vis Surg* 2016;2:145.