

# Preserving infrapyloric vein reduces postoperative gastric stasis after laparoscopic pylorus-preserving gastrectomy

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## Abstract

**Purpose** Laparoscopic pylorus-preserving gastrectomy (LPPG) is performed to preserve function in treating early gastric cancer. However, gastric stasis is a potential complication of LPPG that could decrease postoperative quality of life, possibly due to gastric edema of the pyloric cuff caused by venous stasis. We introduced an infrapyloric vein (IPV)-preserving LPPG (iLPPG) procedure to prevent pyloric cuff edema and thus minimize the incidence of gastric stasis and investigated the early clinical outcomes of iLPPG.

**Methods** We reviewed 150 patients with gastric cancer who underwent LPPG between August 2011 and June 2013 at the Cancer Institute Hospital and analyzed postoperative complications, incidence of gastric stasis (requiring starvation longer than 72 h or an invasive treatment), and transient delayed gastric emptying (TDGE).

**Results** Of the 150 patients, 56 underwent iLPPG and 94 underwent conventional LPPG without preservation of the IPV (cLPPG). Morbidity rates were 5.4% in the iLPPG group and 23.4% in the cLPPG group ( $P = 0.003$ ). The incidence of both gastric stasis and TDGE was significantly lower in the iLPPG group than in the cLPPG group (0 vs. 8.5%,  $P = 0.03$  and 0 vs. 7.4%,  $P = 0.046$ , respectively). Median postoperative stay was significantly shorter in the iLPPG group

compared to the cLPPG group (9 vs. 11 days,  $P < 0.001$ , respectively).

**Conclusions** Preservation of the IPV might prevent the incidence of postoperative gastric stasis after LPPG, resulting in a shorter postoperative stay.

**Keywords** Laparoscopic pylorus-preserving gastrectomy · Infrapyloric vein · Gastric stasis · Gastric cancer

## Introduction

Pylorus-preserving gastrectomy (PPG) was first reported by Maki et al. [1] to be effective in the treatment of benign gastric diseases. It is known that the preservation of the hepatic and celiac branches of the vagus nerve in gastrectomy contributed to the recovery of weight loss and help in preventing postoperative cholelithiasis and diarrhea [2]. Recently, PPG with preservation of the hepatic branches of the vagus nerve has been performed as a function-preserving surgical treatment for gastric cancer [3]. PPG has several advantages for treating early gastric cancer over distal gastrectomy that are attributable to the preservation of pyloric function, such as prevention of dumping syndrome, protection against bile mucosal injury of the remnant stomach, and less postoperative body weight loss [4–6]. All these benefits are also maintained in the less invasive procedure of laparoscopic PPG (LPPG) [7, 8], although the early postoperative complication of gastric stasis occurs in 3–8% of patients [7, 9–11].

In LPPG, the distal part of the stomach is resected while retaining a 4-cm pyloric cuff with preservation of the right gastric artery and vein, the infrapyloric artery (IPA) and the pyloric branch of the vagal nerve [7, 9, 12]. The infrapyloric vein (IPV) is usually divided in LPPG because the surgical technique required to preserve the IPV is relatively

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complicated in laparoscopic surgery. However, venous stasis due to division of the IPV can cause pyloric edema, which might cause transitional pyloric dysfunction and subsequent postoperative gastric stasis. Interestingly, there are few reports about the effects of preserving the IPV.

To avoid complete transient edema of the pyloric cuff after LPPG, we introduced preservation of the IPV to our LPPG procedure. In this study, we reviewed the characteristics and surgical outcomes of 150 patients who underwent LPPG with special reference to the preservation of IPV.

## Materials and methods

### Patients

We reviewed 150 patients with gastric cancer who had undergone LPPG between August 2011 and June 2013 at the Cancer Institute Hospital, Tokyo, Japan. An IPV-preserving LPPG (iLPPG) procedure was introduced in August 2012; thus, during the transitional period from August 2012 to December 2012, both operations were performed (conventional LPPG without preservation of the IPV (cLPPG) and iLPPG). From December 2012, all operations were performed using the iLPPG procedure.

Surgical records and videos of all iLPPG cases were reviewed to confirm preservation of the IPVs. Clinicopathological characteristics including age, gender, preoperative comorbidities, American Society of Anesthesiologists (ASA) score, pathological diagnosis, operation data, postoperative stay, complications, and postoperative gastric stasis were obtained from our hospital database and clinical records. If the patients rehospitalized within a month after surgery, number of days of rehospitalization was added to postoperative stay. This study was approved by the Institutional Review Board committee of the Cancer Institute Hospital, Tokyo, Japan.

### Surgical procedure

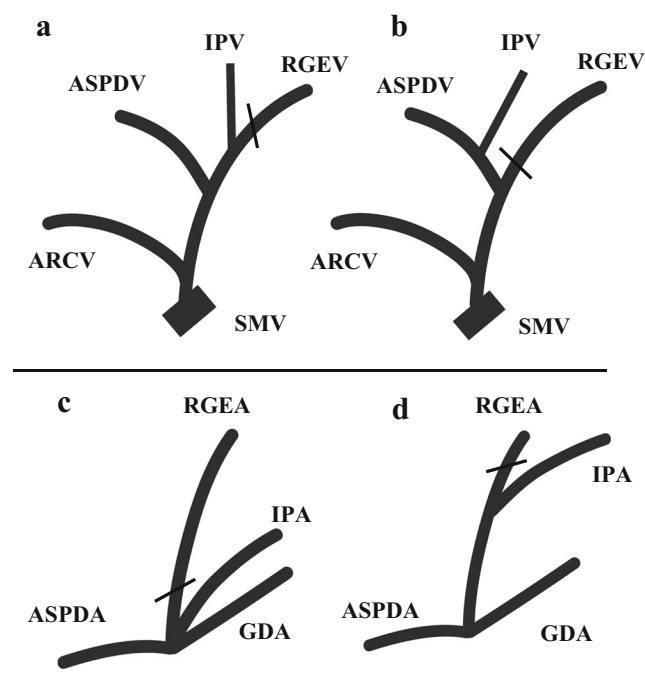
Our indication for PPG was intra-mucosal or submucosal carcinoma without lymph node metastasis (cT1, cN0), located in the middle third of the stomach, with a maximum diameter of 5 cm. Clinical classification of tumor depth (cT) and nodal involvement (cN) was determined by preoperative and intra-operative evaluation including upper gastrointestinal tract endoscopy, CT imaging, or endoscopic ultrasonography.

The detailed surgical procedures of the conventional laparoscopy-assisted PPG (LAPPG) are described in our previous article [7, 12, 13]. LPPG with D1 plus

lymphadenectomy was performed either with hand-sutured anastomosis using a 4-cm skin incision (LAPPG) or by complete intra-corporeal surgery (compLPPG). Lymph nodes of stations 1 (right cardia), 3 a/b (lesser curvature), 4sb (left gastroepiploic artery), 4d (right gastroepiploic artery), 6 (infrapyloric), and 7 (left gastric artery) and stations 8a (anterosuperior group of common hepatic artery) and 9 (celiac artery) were excised [14]. The lymph nodes at station 5 (suprapyloric) were usually left untouched.

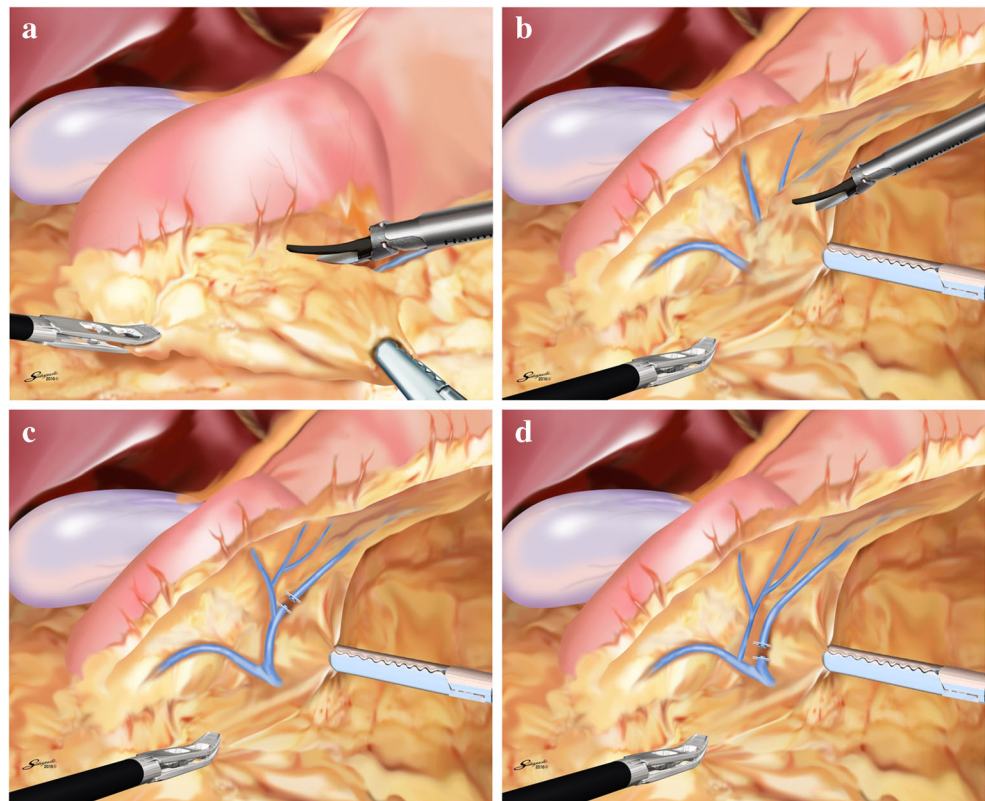
Under laparoscopy, the gastrocolic ligament was incised 4 cm from the gastroepiploic arcades, and the greater omentum was preserved.

The IPV was preserved first, to maintain drainage from the pyloric cuff, and then, the IPA was preserved to maintain sufficient blood supply (Fig. 1). During dissection of the lymph nodes with preservation of the IPV branch, the draining point of this vein was carefully identified (Fig. 2a, b). When the IPV drained to the right gastroepiploic vein (RGEV), the RGEV was divided just proximal to the drainage point of the IPV (Figs. 1a and 2c, Online resource Video 1). When the IPV drained to the anterior superior pancreaticoduodenal vein (ASPDV), the RGEV was divided just proximal to the drainage point of the ASPDV (Figs. 1b and 2d, Online resource Video 2).



**Fig. 1** The variation of the infrapyloric vein (a, b) and artery (c, d). Fine lines indicate the dividing line. The IPAs originating from ASPDA were included in c. ARCV accessory right colic vein, ASPDV anterior superior pancreaticoduodenal vein, ASPDA anterior superior pancreaticoduodenal artery, GDA gastroduodenal artery, IPA infrapyloric artery, IPV infrapyloric vein, RGEA right gastroepiploic artery, RGEV right gastroepiploic vein, SMV superior mesenteric vein

**Fig. 2** Exposure of the IPV at the caudal side of the duodenal bulb (a). Dissection of the station 6 lymph nodes with preserving IPV (b). GEV type: RGEV was divided just proximal to the drainage point of IPV (c). ASPDV type: RGEV was divided just proximal to the drainage point of ASPDV (d). *IPV* infrapyloric vein, *RGEV* right gastroepiploic vein, *ASPDV* anterior superior pancreatoduodenal artery



When the IPA originated from the gastroduodenal artery (GDA) or anterior superior pancreatoduodenal artery (ASPDV), the right gastroepiploic artery (RGEA) was divided at the root (Fig. 1c) [15]. When the IPA originated from the RGEA, the RGEA was divided just distal to bifurcation of the IPA (Fig. 1d). The root of the right gastric artery was preserved and transected just distal to the first branch. The hepatic and pyloric branches of the vagus nerves were routinely preserved, and preservation of the celiac branch of the vagus nerve was the surgeon's decision.

After lymph node dissection, the tumor location was confirmed by palpating the preoperative clipping (LAPPG) or by intra-operative gastroscopy (comLPPG). The distal part of the stomach was resected while retaining a 4-cm pyloric cuff. The proximal portion of the stomach was resected just proximal to the line between the right and left gastroepiploic arteries, and a gastro-gastro anastomosis was performed by hand suture (extracorporeal, layer-to-layer suture) or by using linear staplers (intra-corporeal, delta-shaped [16].)

All resected stomachs were examined immediately after the surgery, and retrieved lymph nodes were classified according to anatomical distribution and numbering of the regional lymph nodes based on the JGCA classification. Retrieved lymph nodes at each nodal station were labeled and counted. Specimens were fixed in formalin and processed for hematoxylin-eosin staining of sections to histologically

determine depth of wall invasion, number of harvested lymph nodes, and presence or absence of lymph node metastasis.

### Postoperative complications

Postoperative complications were classified according to the Clavien-Dindo classification system, which categorizes surgical complications from grade I to grade V based on the invasiveness of the treatment required [17]. Grade I represents any deviation from the normal postoperative course that requires no therapy (with exceptions of analgesic, antipyretic, antiemetic, diuretics, electrolytes drugs, and physiotherapy). Grade II represents complications requiring pharmacological treatment with drugs other than those allowed for grade I complications. Grade III represents complications requiring surgical, endoscopic, or radiological intervention. Grade IV represents life-threatening complications that require intensive care. Grade V represents the patient's death.

Complications classified as grade II or above were recorded. A blood test on postoperative day 7 was routinely performed, and if needed, an additional blood test was subsequently conducted. Intra-abdominal infection is a complication when patients have leukocytosis or prolonged elevated levels of C-reactive protein (CRP), administering antibiotic drug, if other possible complications were excluded. Others categories included urinary infection and whole-body eczema.

## Definition of gastric stasis

Nasogastric tubes were removed immediately after surgery. Generally, patients were given only liquids on the first postoperative day and a soft meal on the third postoperative day.

In this study, feeding disorder was diagnosed when the patient showed poor oral intake requiring treatment, and such a disorder was divided into two groups: gastric stasis and transient delayed gastric emptying (TDGE). Gastric stasis was defined by the patient requiring starvation for longer than 72 h or an invasive treatment such as insertion of a nasogastric (NG) tube or dilation of the pyloric ring with a gastroendoscope. TDGE was defined when the patient required either starvation shorter than 72 h.

## Statistical analysis

Characteristic parameters were analyzed by Student's *t* test. Relationships between categorical variables were analyzed using the chi-square test or Fisher's exact test. Median postoperative days of each group were compared by the Wilcoxon test. The overall survival (OS) rate and disease-specific survival (DSS) rate were calculated by the Kaplan-Meier method.  $P < 0.05$  was considered significant. JMP version 10.0.0 for Windows (SAS Institute, Cary, NC) was used for all statistical analyses.

## Results

### Patient characteristics

Table 1 details the characteristics of patients. Of the total patients reviewed, 56 underwent iLPPG and 94 underwent conventional LPPG procedure without preservation of the infrapyloric vein (cLPPG). In two patients for whom iLPPG was initially planned, bleeding in the infrapyloric region resulted in a failure to preserve the IPV. Thus, they were included in the cLPPG group. The pyloric branch of the vagus nerve was preserved in all patients.

Sex, BMI, and tumor depth were not significantly different between the iLPPG and cLPPG groups. Patients tend to be younger in the cLPPG group than in the iLPPG group, and although all the study patients were preoperatively diagnosed as T1 in tumor depth, two patients in the iLPPG group and four in the cLPPG group were postoperatively diagnosed as at having at least T2 depth. Four patients in the iLPPG group and five in the cLPPG group showed lymph node metastasis, such that occurrence of pathological stage II tended to be higher in the cLPPG patients.

**Table 1** Patient characteristics

Characteristic	Value		
	iLPPG	cLPPG	<i>P</i> value
Number of patients	56	94	
Sex (male/female)	24/32	46/48	0.47
Age (years) [mean (range)]	59 (32–75)	55.7 (32–76)	0.10
BMI	22.5 ± 2.9	22.5 ± 3.3	0.88
ASA score			
Score 1	28	52	0.52
Score 2	26	41	
Score 3	2	1	
pTumor depth <sup>a</sup>			
pT1a	29	46	0.87
pT1b	25	44	
pT2	2	3	
pT3	0	1	
Lymph node metastasis <sup>a</sup>			
pN–	52	89	0.73
pN+	4	5	
Pathological stage <sup>a</sup>			
p Stage I	56	89	0.16
p Stage II	0	5	

The  $\chi^2$  test or Fisher's exact test was used to test the independence between groups.  $P < 0.05$  was considered statistically significant

*iLPPG* infrapyloric vein-preserving laparoscopic pylorus-preserving gastrectomy, *cLPPG* conventional LPPG procedure without preservation of the infrapyloric vein, *BMI* body mass index

<sup>a</sup>Japanese Classification of Gastric Cancer 14th, The 7th edition of the International Union Against Cancer (UICC)-TNM Classification for Gastric Cancer

### Operative data

All patients underwent elective R0 resection surgery, with the right gastric artery and vein, IPA, and the pyloric branch of the vagal nerve preserved.

Although operating time, estimated blood loss, proportion of extracorporeal and intracorporeal anastomosis, and proportion of the celiac branch of the vagus nerve preserved were not significantly different between the groups, postoperative stay was significantly shorter in the iLPPG group (Table 2). Specifically, median postoperative stay was 9 days in the iLPPG group, while 11 days in the cLPPG group ( $P < 0.001$ ). In addition, 18 patients (19.1%) stayed more than 15 days after surgery in the cLPPG group compared to none in the iLPPG group ( $P < 0.001$ ).

### Occurrence of gastric stasis

Feeding disorder occurred in 15 patients (16.0%) of the cLPPG group while 0% in iLPPG group. Gastric stasis

**Table 2** Operative data

Characteristic	Value		
	iLPPG ( <i>n</i> = 56)	cLPPG ( <i>n</i> = 94)	<i>P</i> value
Operating time (min; mean ± SD)	249 ± 50	246 ± 46	0.71
Estimated blood loss (ml; mean ± SD)	34 ± 47	34 ± 43	0.93
Anastomosis			
Extracorporeal (hand suture)	40	62	0.49
Intra-corporeal (linear stapler)	16	32	
Celiac branch of the vagus nerve			
Preserved	28	59	0.13
Not preserved	28	35	
The number of the harvested lymph nodes			
All (mean ± SD)	39 ± 11	40 ± 13	0.77
Station 6 (mean ± SD)	6 ± 2	6 ± 3	0.77
Postoperative stay [median, days (range)]	9 (7–14)	11 (7–40)	<0.001*
Postoperative stay of more than 15 days	0	18 (19.1%)	<0.001*

*iLPPG* infrapyloric vein-preserving laparoscopic pylorus-preserving gastrectomy, *cLPPG* conventional LPPG procedure without preservation of the infrapyloric vein

\* $P < 0.05$  was considered statistically significant

occurred in 8 patients (8.5%) and required administration of a drug to improve gastrointestinal motility. Among them, a NG tube was inserted to 6 patients (6.4%) and 4 patients (4.3%) required both insertion of a NG tube and a treatment with gastroscopy. TDGE occurred in 7 patients (7.4%), and all required administration of a drug to improve gastrointestinal motility.

### Postoperative complications

Postoperative complications classified as grade II or above by the Clavien-Dindo classification system are detailed in Table 3. In-hospital mortality rate was 0%, while the morbidity rate was 5.4% in the iLPPG group and

23.4% in the cLPPG group. The incidence of postoperative complication, gastric stasis, and TDGE was significantly higher in cLPPG than iLPPG patients ( $P = 0.003$ ,  $P = 0.03$ , and  $P = 0.046$ , respectively). Intra-abdominal infection occurred in nine patients in the cLPPG group; one patient was administered with antibiotics owing to leukocytosis, five patients because of prolonged elevated levels of CRP, and three patients for both reasons. The average CRP level that triggered the decision to administer antibiotics was 9.0 mg/dl (range 5–16.6 mg/dl). In the iLPPG group, one patient was administered with antibiotics because of both leukocytosis and prolonged elevated CRP levels (9.8 mg/dl). Intra-abdominal infection showed a higher incidence in the cLPPG group than the iLPPG

**Table 3** Postoperative complications

Postoperative complications	Value		
	iLPPG ( <i>n</i> = 56)	cLPPG ( <i>n</i> = 94)	<i>P</i> value
Morbidity	3 (5.4%)	22 (23.4%)	0.003*
Gastric stasis	0	8 (8.5%)	0.03*
Transient delayed gastric emptying	0	7 (7.4%)	0.046*
Intra-abdominal infection	1 (1.8%)	9 (9.6%)	0.09
Ileus	0	1 (1.1%)	1.00
Others	2 (3.6%)	1 (1.1%)	0.56

The  $\chi^2$  test or Fisher's exact test was used to test the independence between groups

\* $P < 0.05$  was considered statistically significant

**Table 4** Analysis of risk factors for gastric stasis

Category	Value ( <i>n</i> = 8)	<i>P</i> value
Type of operation		
iLPPG	0/56	0.01*
cLPPG	8/94	
Sex		
Male	3/70	0.59
Female	5/80	
Age (years)		
<70	7/127	0.81
≥70	1/23	
BMI		
<25	6/117	0.84
≥25	2/33	
Operating time (min)		
<250	3/79	0.84
≥250	2/33	
Operating time (min)		
<250	3/79	0.38
≥250	5/71	
Estimated blood loss (ml)		
<34	5/107	0.58
≥34	3/43	
Type of anastomosis		
Extracorporeal	4/102	0.28
Intra-corporeal	4/48	
Celiac branch of the vagus nerve		
Preserved	4/87	0.64
Not preserved	4/63	
Intra-abdominal infection		
Present	4/10	<0.001*
Absent	4/140	

The  $\chi^2$  test or Fisher's exact test was used to test the independence between groups

\**P* < 0.05 was considered statistically significant

group, although the difference did not reach significance (*P* = 0.09), and four cLPPG patients experienced both intra-abdominal infection and gastric stasis. Three patients were rehospitalized within a month after surgery, with gastric stasis the reason in two of these cases. One of the two patients who failed to achieve preservation of the IPV developed gastric stasis 16 days after surgery and was therefore rehospitalized.

### Short-term outcomes

The median follow-up time after surgery was 42.2 months (range: 14.5–60.2 months). The 3-year OS was 99.3% and

DSS was 100%. One patient in cLPPG group died of lung cancer.

### Analysis of the factors underlying gastric stasis

Analysis of the potential risk factors for gastric stasis among the study patients (Table 4) showed a significantly higher incidence of gastric stasis in the cLPPG group and in those who developed intra-abdominal infection (*P* = 0.01, *P* < 0.001, respectively), although there was no difference between the groups in sex, age, BMI, operating time, estimated blood loss, type of anastomosis, and preservation of the celiac branch of the vagus nerve.

### Discussion

The postoperative complication of gastric stasis after LPPG necessitates longer hospitalization and thus needs to be prevented. Before introducing the iLPPG technique, we preserved the IPA to maintain blood supply to the remaining pyloric cuff, but not the IPV, resulting in the right gastric vein serving as the only possibly venous drainage route from the pyloric cuff. If such drainage from the right gastric vein is insufficient, edema and dysfunction of the pyloric cuff can occur, possibly leading to gastric stasis. In the present study, the incidence of postoperative gastric stasis and TDGE was significantly decreased in patients who underwent iLPPG procedure compared to those undergoing cLPPG. Maintenance of infrapyloric venous flow might therefore be an important factor in preventing gastric stasis and TDGE.

We defined TDGE as a mild feeding disorder such as occasional skipping of meals or fasting for a few days. Skipping meals sometimes may occur after gastrectomy; however, this is not considered to be due to gastric stasis. We tried to assess those mild symptoms as some kind of feeding disorder but not as gastric stasis. However, TDGE did not occur in the iLPPG group. It is possible that TDGE is a kind of gastric stasis with very mild symptoms, as the incidence of postoperative gastric stasis and TDGE was both decreased by preserving the IPV. Further investigation is required to resolve the mechanism underlying postoperative gastric stasis.

It is also possible that the postoperative gastric stasis was due to a factor other than lack of infrapyloric venous flow. Intra-abdominal inflammation is one such culprit since the frequency of the gastric stasis was significantly higher in the patient with intra-abdominal infection.

Another possible contributor to the gastric stasis could be operating skill, although that was unlikely in this study because six of the eight patients with postoperative gastric stasis were operated on by experts in laparoscopic gastrectomy.

A third possible contributor to decreasing the incidence of gastric stasis is the less-traumatic lymph node dissection

around the pancreas with the iLPPG procedure compared to cLPPG. The resultant prevention of inflammation around the pyloric cuff could thus reduce the risk of pyloric cuff dysfunction. During the IPV-preserving lymph node dissection, the fat tissue including lymph nodes was carefully detached from the pancreas in a procedure that might help to prevent pancreatic minor injury and intra-abdominal inflammation. In addition, the less traumatic procedure might have contributed to the lower incidence of intra-abdominal infection in the iLPPG group, since our definition of intra-abdominal infection could include intra-abdominal inflammation without infection.

The origins of the IPA vary across the reports. Michels described the IPA by using the term “pyloric branch,” and it usually arises at the division point of the GDA and superior pancreaticoduodenal artery or the RGEA [18]. Kuroda et al. reported that most IPAs (78.5%) arise from the GDA, 17% from the RGEA, and 4.5% from the ASPDA [19]. Recently, it was shown that 64.2% of the IPA arises from the ASPDA by using videos and specimens from laparoscopic gastrectomy [20]. Wherever the IPA branches, the dividing point of the RGEA to preserve the IPA is either the root of the RGEA or after branching of the IPA. Careful exposure of the IPA roots may avoid injuring the IPA.

However, there are few reports about the anatomy of the IPV. According to Petré’s description, the IPVs drain the lower parts of the terminal pyloric portion of the stomach and the first part of the duodenum [21, 22]. It starts in a spray of numerous fine veins that unite to form several stems, and then usually, a single vein empties into the RGEV close to the entrance of the ASPDV. Similar to that for the artery, the dividing point of the RGEV to preserve the IPV is either just proximal to the drainage point of the ASPDV or just proximal to the drainage point of the IPV.

Petré also reported that anastomoses between the IPV and suprapyloric vein that run on the upper border of the pyloric region were always small [21, 22]. As in our present report, dividing the IPV does not always cause gastric stasis. The presence of anastomosis may be one of the factors that may prevent venous stasis in some patients and thus prevent gastric stasis.

Although the surgical technique required to preserve the IPV is relatively complicated in laparoscopic surgery, this excellent result prompted all surgeons in our institution to choose iLPPG in relevant cases, and currently, all LPPG is performed in this way. It should be noted that because iLPPG technique can be difficult and complicated, resection of the infrapyloric region should be performed with the utmost care to avoid bleeding from the little venous branch of the IPV. In this study, we failed to preserve IPV in two patients due to intra-operative bleeding in the infrapyloric region.

It may be possible to dissect station 6 lymph nodes with preservation of the RGEA and vein; however, we divided the RGEA and vein after preserving the IPA and IPV because in

some patients, advanced gastric cancer was pathologically diagnosed postoperatively although the preoperative diagnosis was early gastric cancer. For those patients, the risk of insufficient lymph node dissection must be minimized.

Despite the significant difference in the incidence of gastric stasis and TDGE between the iLPPG and cLPPG groups studied herein, the postoperative stays only differed by a median of 2 days. One of the reasons for this small difference is that most gastric cancer patients in Japan choose to remain in hospital for 10 days or more after surgery, even if their postoperative course is favorable and they are medically fit for discharge around 7 days. This custom due to the Japanese culture and health insurance system prolongs the postoperative stays of patients without complication and decreases the difference between groups such as ours. However, no patients in the iLPPG group stayed longer than 15 days (more than 2 weeks) after surgery, compared to 19.1% of the cLPPG group (range 15–40 days), suggesting that the iLPPG procedure could reduce the risk of prolonged hospitalization.

We previously reported that cLPPG with precise dissection of station 6 lymph nodes with division of the IPV resulted in excellent long-term outcomes [10]. In the present study, preserving the IPV possibly resulted in insufficient dissection of station 6 lymph nodes. However, our published database of gastric cancer cases [23] showed that 0.6% of T1 gastric cancers in the middle third of the stomach had lymph node metastases in the station 6 lymph node (unpublished data). If the tumor matches our PPG criteria, iLPPG is less likely to worsen the oncological outcome because the incidence of station 6 lymph nodes metastasis was very low. However, further investigation of the long-term outcomes is required to confirm the curative nature of iLPPG.

## Conclusion

The preservation of IPV might prevent the incidence of postoperative gastric stasis after iLPPG and result in a shorter postoperative stay. Further investigation is required to resolve the mechanism underlying postoperative gastric stasis.

## Compliance with ethical standards

## Authors’ contributions

- Study conception and design: Takashi Kiyokawa, Naoki Hiki, and Souya Nunobe.
- Acquisition of data: Takashi Kiyokawa, Michitaka Honda, and Manabu Ohashi.
- Analysis and interpretation of data: Takashi Kiyokawa, Michitaka Honda, and Naoki Hiki.
- Drafting of manuscript: Takashi Kiyokawa, Naoki Hiki, and Takeshi Sano.
- Critical revision of manuscript: Naoki Hiki and Takeshi Sano.

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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