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HOW-I-DO-IT ARTICLES

Non-flap hand-sewn esophagogastrostomy as a simple anti-reflux procedure in laparoscopic proximal gastrectomy for gastric cancer

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Abstract

Aims No ideal and generally accepted method of reconstruction for laparoscopic proximal gastrectomy (LPG) has been established because of a high incidence of postoperative reflux and anastomotic stenosis. The aim of this study was to evaluate the short-term outcomes of LPG with a non-flap hand-sewn technique as a simple anti-reflux procedure for the upper part of clinical Stage I gastric cancer.

Methods Between November 2016 and June 2019, 23 consecutive gastric cancer patients, who underwent curative LPG with lymphadenectomy, were enrolled in the study. In this study, we devised a simple hand-sewn technique for esophagogastrostomy, which comprises a 5-cm pseudo-fornix as a fundoplication, the posterior pressure mechanism by the remnant stomach and bilateral crus, and a flat-shaped anastomotic hole as a valvuloplasty.

Results The median operation time and hospital stay was 325 min and 10 days, respectively. There was no patient with anastomotic leakage and delayed gastric empting. No patient had symptoms of gastroesophageal reflux, but two patients (8.6% (2/23): Grade M and Grade A) had endoscopic findings during a follow-up period of more than 6 months. There was no patient with Grade B or more severe reflux esophagitis. One patient (4.3%, 1/23) developed anastomotic stenosis, which was resolved with endoscopic dilatation. The mean body weight loss at 6 months after surgery was 7.5% in comparison with the preoperative body weight.

Conclusion Our non-flap hand-sewn technique for esophagogastrostomy had favorable outcomes and might be one of reliable techniques as an anti-reflux procedure in LPG for gastric cancer.

Keywords Laparoscopic proximal gastrectomy · Esophagogastrostomy · Complication · Gastric cancer

Introduction

Laparoscopic gastrectomy for gastric cancer has recently grown in popularity [1–4] due to not only less invasiveness but also various other merits [5]. Because of the recent increase in upper gastric cancer and carcinoma of the esophago-gastric junction in Western and Eastern countries [6–8], there is a trend toward an increasing number of patients undergoing laparoscopic total gastrectomy (LTG) or proximal gastrectomy (LPG). LPG has been reported to have significant advantages over total gastrectomy, such as preserving gastric capacity and entailing fewer hormonal and nutritional deficiencies [9–11]. These results reflect the preservation of the gastric fundic gland region, gastric-acid secretion, and production of Castle's intrinsic factor and ghrelin [12–14], resulting in less iron and vitamin B12 loss, increased appetite postoperatively [15], and better quality of life. Consequently, there is a trend toward an increased number of patients undergoing LPG than LTG. However, LPG has still not gained widespread acceptance due to its technical demands and high morbidity rate, especially because of gastroesophageal reflux and anastomotic stenosis [16, 17]. Therefore, the standardization

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of surgical procedures for LPG has been an important clinical issue.

Esophagogastrostomy is a classical and simple reconstruction method in LPG. The incidence of early postoperative complications seemed to be lower than in the jejunal interposition, the double tract, and other methods [18–21]. However, esophagogastrostomy is challenging for most surgeons, even for skilled surgeons. To simplify the technique of esophagogastrostomy, and to improve its safety, many surgeons have devised various techniques using a linear stapler [22–24], a circular stapler [14, 25, 26], a hand-sewn technique with a linear stapler [27, 28], a 180-degree wrap technique using the remnant stomach [20, 29], or a hand-sewn double-flap technique [11, 30–32]. Regarding the hand-sewn technique, in 2001, Kamikawa et al. devised an excellent method of esophagogastrostomy with a double-flap technique, in order to prevent reflux through an open approach [33]. This double-flap technique in LPG has been successfully performed at some institutions in Japan, and the gastroesophageal reflux has been reported to be nearly non-existent even through a laparoscopic approach [11, 30–32]. However, this technique may be technically challenging for most surgeons, even for skilled surgeons, because it may have various pitfalls and high technical demands make it considerably time-consuming.

In this study, we devised a simple and easy-to-use non-flap hand-sewn technique in LPG and presented its short-term outcomes retrospectively. Our procedure of esophagogastrostomy comprises a 5-cm pseudo-fornix as a fundoplication, the posterior pressure mechanism by the remnant stomach and bilateral crus, and a flat-shaped anastomotic hole as a valvuloplasty. Our results may provide evidence that an esophagogastrostomy using our simple non-flap technique as an anti-reflux procedure is one of the feasible techniques for most surgeons in LPG.

Methods

Patients

The study was approved by each patient by signed informed consent and was not needed to be approved by the Institutional Review Board of both Kyoto Prefectural University of Medicine and Kyoto First Red Cross Hospital. Between November 2016 and June 2019, 23 consecutive gastric cancer patients underwent curative LPG with lymphadenectomy. LPG was performed by mainly qualified surgeons, who are certified by the Japanese Society of Endoscopic Surgery [34] at Kyoto Prefectural University of Medicine, Kyoto, Japan, and an affiliated high-volume hospital, Kyoto First Red Cross Hospital, Kyoto, Japan.

Patients underwent preoperative assessments including gastric endoscopy, endoscopic ultrasonography, computed tomography (CT) scans, and laboratory tests. The patients enrolled in this study had histologically confirmed gastric cancer and were diagnosed as clinical Stage I (T1N0, T2N0, or T1N1) [35] for the upper part of gastric cancer, according to the Japanese guidelines for the treatment of gastric cancer. There were no patients with esophageal invasion of tumor. The exclusion criteria included carcinoma in the presence of another primary malignancy and a history of chemotherapy or chemo-radiotherapy.

In this study, we performed LPG for the upper part of gastric cancer in patients with more than 5 cm of the distance between distal edge of tumor and gastric angle in preoperative imaging study with gastrografin because the appropriate size of remnant stomach is needed for esophagogastrostomy. The resection lines were fixed. Namely, the proximal resection line was almost on the esophago-gastric junction. While, the distal resection line of the stomach was on the line which starts from the distal edge point of station No.3a lymph node at the lesser curvature to the distal edge point of station No.4sb lymph node at the greater curvature.

The follow-up program after gastrectomy comprised a regular physical examination and laboratory blood tests, chest X-rays or ultrasonography every 3 months, an endoscopy and computer tomography (CT) every 6 or 12 months for the first 5 years, and yearly endoscopy thereafter if possible.

Surgical procedures of proximal gastrectomy and esophagogastrostomy

Each patient was positioned with legs open. A 12-mm umbilical camera port was inserted. The abdominal cavity was insufflated with carbon dioxide to maintain an intra-abdominal pressure of 10 mmHg. A 10-mm flexible fiber optic laparoscope (Olympus Optical Co., Ltd., Tokyo, Japan) was inserted through this port. Trocars of size 12 mm were placed in the upper left side and lower right side of the abdomen, and 5-mm trocars were placed in the lower left side and upper right side of the abdomen.

The extent of lymph node dissection was determined according to the 2014 Japanese gastric cancer treatment guidelines [36]. The lymph nodes along the greater and lesser curvatures of the stomach, such as the right pericardial lymph nodes, the left pericardial lymph nodes, the lymph nodes along the lesser curvature, the lymph nodes along the short gastric vessels, and the lymph nodes along the left gastroepiploic vessels, were dissected. Additionally, the lymph nodes along the left gastric artery (No. 7), along the common hepatic artery (No. 8a), around the celiac artery (No. 9), and along the proximal splenic artery (No. 11p), were dissected to complete D1+ dissection. After completing lymph node dissection around the abdominal esophagus, the vagal nerve was divided in order to stretch the abdominal esophagus and obtain sufficient distance of the abdominal

esophagus. Then, the esophagus was transected near the esophago-gastric junction with a linear stapler in order to preserve the abdominal esophagus for as long as possible (Fig. 1a). The distal side of the stomach was transected with a linear stapler. A 4-cm mini-laparotomy was performed in the umbilicus, and the resected stomach was removed.

Surgical procedure for non-flap hand-sewn esophagogastrostomy with a flat-shaped anastomotic hole

After the dorsal side of the abdominal esophagus was exposed by more than 5 cm (Fig. 1b), the stump edge of the remnant stomach was tightly fixed by 3-0 PROLENE (Ethicon Japan Co., Ltd., Tokyo, Japan) suturing to the bilateral crus of the diaphragm and the dorsal side of the abdominal esophagus by 3-0 PDS® II (Ethicon Japan Co., Ltd., Tokyo, Japan) (Figs. 1c and 2a). These fixations enable the stomach to press the abdominal esophagus from the dorsal side and prevent any reflux (Fig. 2b; gray arrows). In addition, the bilateral sides at 1 cm from the stump edge of the abdominal esophagus were fixed by 3-0 PDS® II (Ethicon Japan Co., Ltd., Tokyo, Japan) suturing at the anterior wall of the remnant stomach (Figs. 1e and 2b), located at 5 cm from the stump edge of the stomach (Fig. 1d). These fixations keep the anastomotic hole flat-shaped and prevent reflux (Fig. 2b; red arrows), even more so after eating by gastric dilatation (Fig. 2b; black arrows). These procedures could play crucial roles in reflux prevention (Fig. 2c).

After the fixation, the stump line of the abdominal esophagus was removed, and an incision was made at the anterior gastric wall (Fig. 1f). The continuous all layer suturing was performed between the posterior wall of the esophagus and the superior wall of the opening hole on the remnant stomach using 3-0 V-Loc™ 180 (Covidien Japan Co., Ltd., Tokyo, Japan) [37] (Fig. 1g). The all layer suturing was added using 3-0 PDS® II (Ethicon Japan Co., Ltd., Tokyo, Japan) in order to avoid loss of the barbed suture. Then, continuous layer-to-layer suturing was performed between the anterior esophageal wall and the inferior wall of the opening hole on the remnant stomach using 4-0 V-Loc™ 180 (Covidien Japan Co., Ltd., Tokyo, Japan) (Fig. 1h). The avoidance of tightening the anterior suturing is crucially important to avoid anastomotic stenosis. Finally, the all layer suturing was added using 3-0 PDS® II (Ethicon Japan Co., Ltd., Tokyo, Japan) to avoid loss of the barbed suture on the anterior and both sides of the anastomosis. Pyloroplasty was not added (Figs. 1i and 2c).

Definitions of postoperative morbidity and mortality

Postoperative morbidity and mortality were defined as complications or death within 30 days of surgery or during hospitalization. Complications were classified according to the Clavien-Dindo classification system reported by Dindo et al. [38]. All patients were scheduled to start oral intake of the soft rice gruel on postoperative day 3, and the solidity of the rice gruel is gradually upgraded day-by-day. Most patients reach the normal rice gruel or solid diet on postoperative day 8. If a patient feels stasis and abdominal X-ray picture indicates the continuous dilatation of remnant stomach due to the food residue, the upgrade of the solidity of the rice gruel would be delayed. We defined the delayed gastric empting (DGE) if patients need more than 8 days to eat the normal rice gruel or solid diet postoperatively because of the gastric stasis. Proton pump inhibitor (PPI) administration was routinely performed during 3 months after surgery. Reflux esophagitis was evaluated by endoscopic findings based on the Los Angeles classification during a follow-up period and on 6 months after surgery in all patients even without any symptoms.

Results

Baseline patient characteristics

Table 1 summarizes the characteristics of 23 LPG patients. There were 18 males and 5 females, with a mean age of 67.0 years. The mean BMI was 23 kg/m^2 (Table 1). Preoperative diagnosis was T1bN0M0 clinical Stage I in all enrolled patients. The numbers of patients in each postoperative pathological stage were as follows: 16 in Stage IA, 4 in Stage IB, 2 in Stage IIA, and 1 in Stage IIB. Two patients with more than Stage II were followed by 1 year postoperative adjuvant chemotherapy using S-1. The median follow-up period is 34 months (range 10–41).

Short-term surgical outcomes

Table 1 provides details of the short-term surgical outcomes. The median operation time was 325 min (range, 210–421). Mean estimated blood loss was 64 ml (range, 0–260). There were no perioperative complications, defined as a Clavien-Dindo classification Grade II or more complications during hospital stay. The median length of postoperative hospital stay was 10 days (range, 7–14). There was no patient with anastomotic leakage and delayed gastric empting during postoperative hospital stay. PPI administration was performed during 3 months after surgery. No patient had symptoms or endoscopic findings of gastroesophageal reflux during a follow-up period of more than 6 months. One patient (4.3%, 1/23) developed anastomotic stenosis, which was resolved with endoscopic dilatation.

A postoperative contrast media swallow test showed the lower esophageal peristalsis. Also, no regurgitation into the esophagus was observed when the patients were lying down or placed in the Trendelenburg position. A flat-shaped

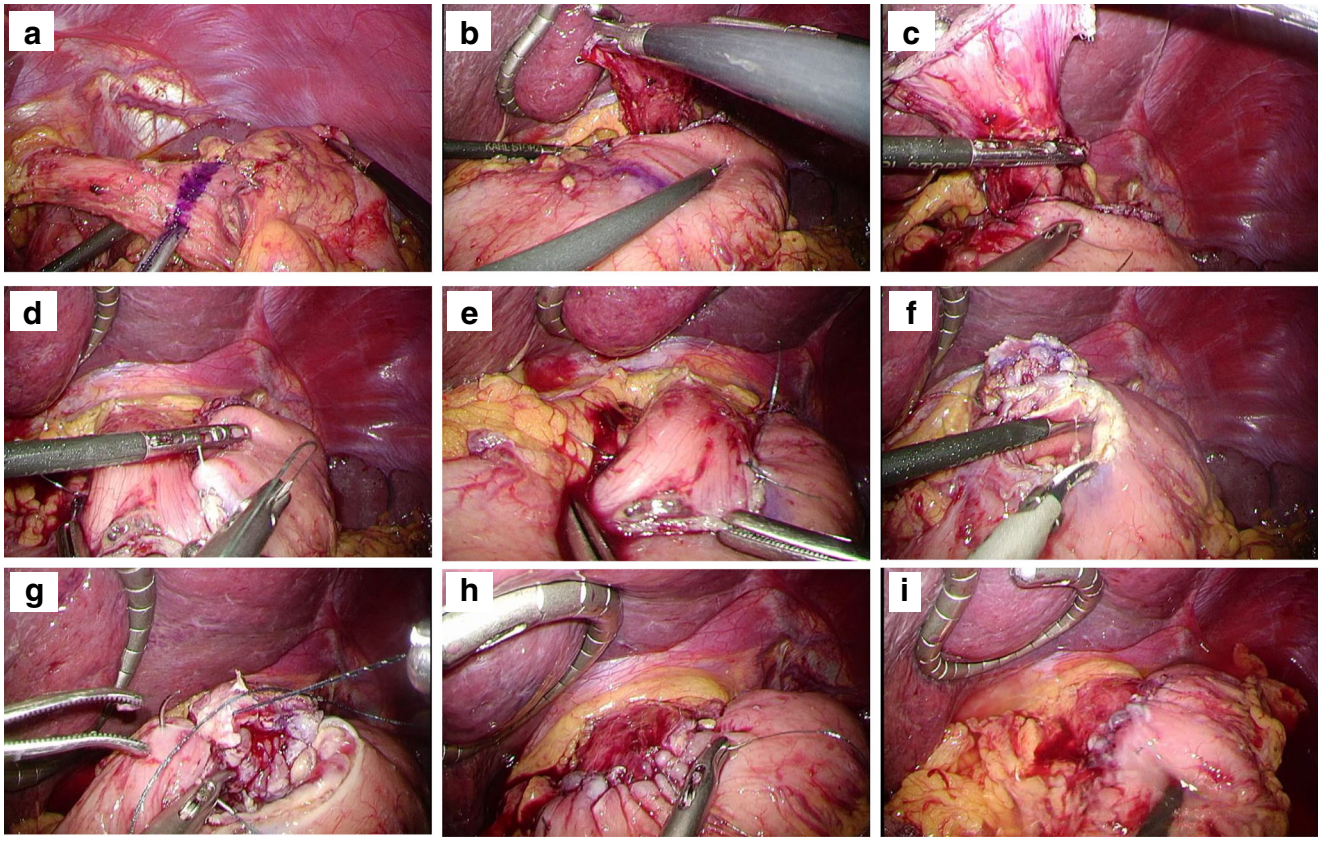

Fig. 1 Surgical procedure for hand-sewn esophagogastrostomy. The abdominal esophagus was transected near the esophago-gastric junction with a linear stapler (**a**). The distal side of the stomach was transected with a linear stapler. After the dorsal side of the abdominal esophagus was exposed by more than 5 cm (**b**), the stump edge of the remnant stomach was fixed by suturing to the bilateral crus of the diaphragm and the dorsal side of the abdominal esophagus (**c**). In addition, the bilateral sides at 1 cm from the stump edge of the abdominal esophagus were fixed by suturing at the anterior wall of the remnant stomach (**d**), located at 5 cm from the stump edge of the stomach (**e**). After the fixation, the stump stapler of the abdominal esophagus was removed, and an incision was made in the anterior gastric wall (**f**). The continuous all layer suturing was performed between the posterior wall of the esophagus and the superior wall of the opening hole on the remnant stomach (**g**). Then, continuous layer-to-layer suturing was performed between the anterior esophageal wall and the inferior wall of the opening hole on the remnant stomach (**h**). A completed hand-sewn esophagogastrostomy with a 5-cm pseudo-fornix as a fundoplication and a flat-shape anastomotic hole as a valvuloplasty (**i**)

anastomotic hole as a valvuloplasty and a pseudo-fornix were found after the operation via gastric endoscopy (Fig. 3a). The shutter mechanism was endoscopically confirmed near the bilateral crus, which is located in 5 cm proximal site of esophagogastrostomy. No patient had symptoms of gastroesophageal reflux. However, based on the Los Angeles classification, two patients (8.6% (2/23): Grade M and Grade A) had endoscopic findings during a follow-up period of more than 6 months. There was no patient with Grade B or more severe reflux esophagitis (Fig. 3b). We performed a 24-h impedance-pH monitoring after surgery. The data of the 24-h impedance- pH monitoring was collected since April 2019. This monitoring was performed at postoperative day 7 after gastrectomy. There was no heartburn and reflux of gastric juice, suggesting the utility of our procedure to prevent a reflux esophagitis. A representative waveform of a patient is shown in Fig. 4**.** The mean percent body weight loss at an initial 6 months after surgery was 7.5% in comparison with the preoperative body weight of each patient (Table 1).

Discussion

Despite clinical issues regarding reconstruction-related complications such as gastroesophageal reflux and anastomotic stenosis in esophagogastrostomy [16, 17], LPG has become a popular treatment option in gastric cancer because of significant advantages regarding body weight loss over total gastrectomy. Because of our familiarity with the circular stapler technique in open surgery, we preferred a circular stapling device in esophagogastrostomy for LPG [26]. However, so far, about 15% of patients have suffered from anastomotic stenosis. Therefore, we have changed from the circular technique to a hand-sewn technique since November 2016. In this study, we presented a simple non-flap hand-sewn technique

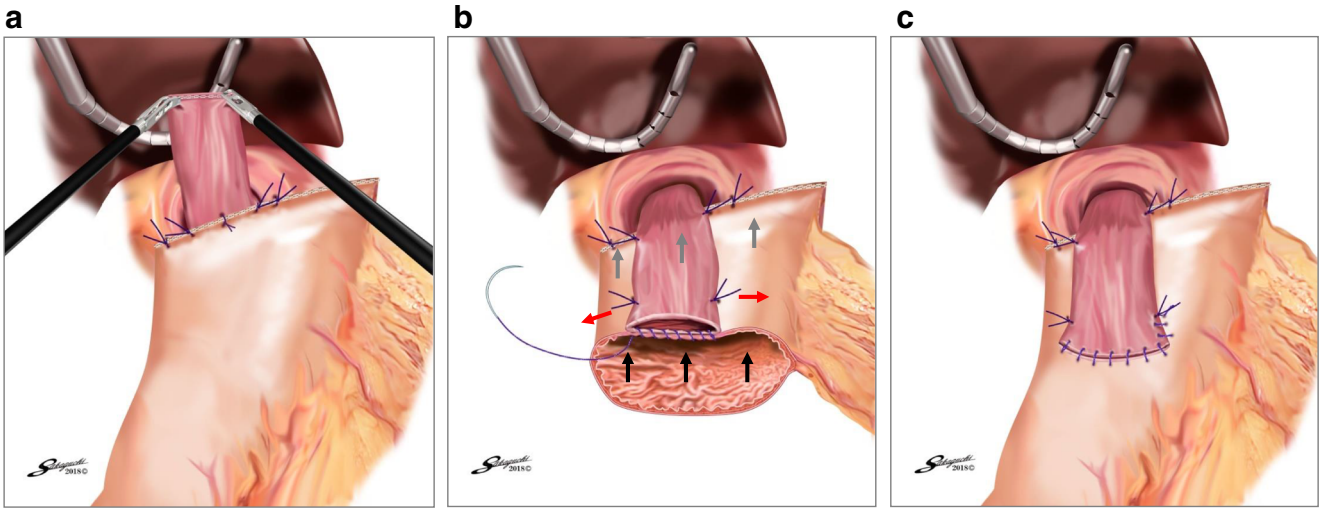

Fig. 2 Schema and concept of our surgical procedure using a hand-sewn esophagogastrostomy. After the dorsal side of the abdominal esophagus was exposed by more than 5 cm, the central stump of the remnant stomach was fixed by suturing to the bilateral crus of the diaphragm and the dorsal side of the abdominal esophagus (**a**). These fixations enable the stomach to press the abdominal esophagus from the dorsal side (**b;** gray arrows). In addition, the lateral side at 1 cm from the stump of the abdominal esophagus was fixed by suturing at the anterior wall of the remnant stomach, located at 5 cm from the stump edge of the stomach (b). These fixations keep the anastomotic hole in a flat shape (b; red arrows), even more so after eating by gastric dilatation (b; black arrows). These procedures could play crucial roles in reflux prevention

with a 5-cm pseudo-fornix as a fundoplication, the posterior pressure and shutter mechanism by the remnant stomach and bilateral crus, and a flat-shaped anastomotic hole as a valvuloplasty.

In our study, the incidence of anastomotic stenosis was reduced to 4.3%, which was better than that of esophagogastrostomy, jejunal interposition, and double tract reconstruction (15.4%, 9.6%, and 11.6%) in recent systematic review [21]. Regarding the reflux esophagitis, two patients (8.6% (2/23): Grade M and Grade A) had endoscopic findings. However, until now, there was no patient (0%) with symptoms and the endoscopic Grade B or more severe reflux esophagitis, which was better than that of esophagogastrostomy, jejunal interposition, and double tract reconstruction (28.6%, 4.5%, and 4.7%) [21]. Our results suggested favorable short-term outcomes and may provide evidence that the esophagogastrostomy using the non-flap hand-sewn technique as an anti-reflux procedure is one of the feasible techniques in LPG.

In our procedure, the stump edge of the remnant stomach was tightly fixed by non-absorbable sutures suturing to the bilateral crus of the diaphragm. These fixations enable the stomach to press the abdominal esophagus from the dorsal side and prevent any reflux (Fig. 2b; gray arrows). Since Professor Kamikawa devised an excellent hand-sewn esophagogastrostomy with a double-flap technique to prevent reflux in open proximal gastrectomy [33], this double-flap technique in LPG has been successfully performed at some institutions in Japan [11, 30–32]. This double-flap technique ensures continuous anterior pressure on the lower esophagus, the so-called shutter mechanism. However, we are afraid that an excessive flap closure by non-expert surgeons may give rise to stenosis and impair the spontaneous dilatation function of the lower esophageal sphincter. In our procedure, this shutter mechanism was also endoscopically confirmed near the bilateral crus, which is located in 5 cm proximal site of esophagogastrostomy (Fig. 3b). We believe that our non-flap shutter mechanism by the posterior pressure of the stump edge of the remnant stomach and bilateral crus may also be effective to prevent the reflux esophagitis and preserve the function of the lower esophageal sphincter.

Regarding the prevention system for gastroesophageal reflux, Professor Kamikawa also suggested the importance of the length of abdominal esophagus fixed on the anterior of the remnant stomach. Namely, Professor Kamikawa insisted on the use of at least 5 cm of the esophagus as a fundoplication. Previously, including our own, reported procedures of esophagogastrostomy in LPG have not suggested the importance of a 5-cm length of esophagus so far. Therefore, we believe that this length is crucially important to prevent the gastroesophageal reflux. The long length of esophagus on the anterior of the remnant stomach allows the stomach to press the abdominal esophagus into a flat shape (Fig. 2b), which results in reflux prevention. Previous other unique reports, which the anastomosis between the esophagus and the posterior wall of stomach was carried out with sufficient distant from the apex of the remnant, also suggested the importance of this length [39] and wrap fundoplication [40]. We have not performed our method in patients with relevant hiatal hernia, short esophagus, and a higher esophageal resection for oncologic

Table 1 Surgical outcomes of non-flap hand-sewn esophagogastrostomy in LPG

No.	Age (years)	Sex	cStage	pStage	Operation time (min)	Blood loss (ml)	Hospital stay (day)	Delayed gastric empting	Reflux esophagitis	Anastomotic leakage	Anastomotic stenos is	6 months postoperative body weight loss rate (%)
1	71	Male	T1bN0M0StageIA	T1bN0M0StageIA	405	0	9	None	None	None	None	− 5.1
2	70	Male	T1bN0M0StageIA	T1bN2M0StageIIA	331	20	10	None	None	None	None	− 14.8
3	76	Male	T1bN0M0StageIA	T2N2M0StageIIB	346	0	14	None	None	None	None	− 1.1
4	70	Male	T1bN0M0StageIA	T1bN0M0StageIA	421	225	8	None	None	None	None	− 5.6
5	56	Male	T1bN0M0StageIA	T1bN0M0StageIA	317	0	10	None	None	None	None	− 12.3
6	47	Male	T1bN0M0StageIA	T1bN1M0StageIB	268	50	9	None	None	None	None	− 11.7
7	62	Male	T1bN0M0StageIA	T1bN0M0StageIA	411	115	11	None	None	None	Present	− 7.1
8	73	Male	T1bN0M0StageIA	T1bN1M0StageIB	210	70	9	None	None	None	None	− 12.4
9	67	Female	T1bN0M0StageIA	T1bN0M0StageIA	214	0	10	None	None	None	None	− 2.4
10	63	Male	T1bN0M0StageIA	T1bN1M0StageIB	277	260	11	None	None	None	None	− 17.1
11	82	Male	T1bN0M0StageIA	T1bN0M0StageIA	303	0	9	None	None	None	None	− 7.1
12	74	Male	T1bN0M0StageIA	T1bN0M0StageIA	280	50	12	None	None	None	None	− 1.8
13	64	Male	T1bN0M0StageIA	T1bN0M0StageIA	319	50	10	None	None	None	None	− 5.3
14	67	Male	T1bN0M0StageIA	T1bN1M0StageIB	420	100	12	None	None	None	None	− 9.5
15	62	Female	T1bN0M0StageIA	T1aN0M0StageIA	341	0	11	None	None	None	None	− 9.6
16	72	Male	T1bN0M0StageIA	T1bN0M0StageIA	353	100	10	None	None	None	None	+ 0.2
17	60	Male	T1bN0M0StageIA	T1bN0M0StageIA	358	200	12	None	None	None	None	− 4.8
18	80	Female	T1bN0M0StageIA	T1bN0M0StageIA	318	0	10	None	None	None	None	− 3.3
19	80	Female	T1bN0M0StageIA	T3N0M0StageIIA	321	0	9	None	None	None	None	− 1.5
20	49	Female	T1bN0M0StageIA	T1aN0M0StageIA	333	0	7	None	None	None	None	− 4.4
21	67	Male	T1bN0M0StageIA	T1aN0M0StageIA	305	0	7	None	None	None	None	− 5.4
22	49	Male	T1bN0M0StageIA	T1aN0M0StageIA	286	0	12	None	None	None	None	− 15.8
23	80	Male	T1bN0M0StageIA	T1aN0M0StageIA	330	250	13	None	None	None	None	− 14.8
					*325**	*64***	*10**	*0%***	*0%***	*0%***	*4.3%***	*− 7.5%***

* madian

** mean

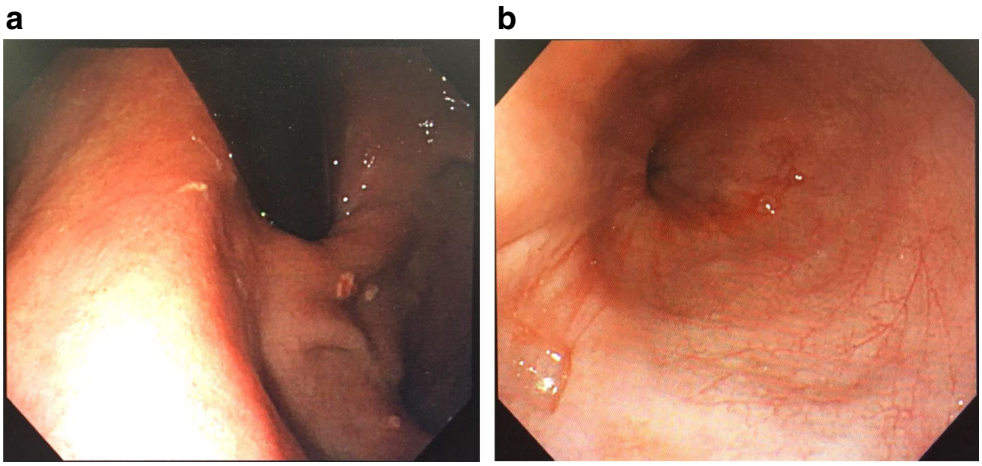

Fig. 3 Postoperative endoscopic findings following LPG. A flat-shaped anastomotic hole as a valvuloplasty and a pseudo-fornix were found via gastric endoscopy (**a**). The shutter mechanism was endoscopically confirmed near the bilateral crus, which is located in 5 cm proximal site of esophagogastrostomy. There was no patient with more than grade A reflux esophagitis based on the Los Angeles classification at 6 months (**b**)

reasons in Siewert Type I and II, because the stump edge of the remnant stomach could not be fixed to the bilateral crus of the diaphragm. For these patients, we have selected to perform the 3-cm short overlap esophagogastrostomy with a sufficient 5-cm pseudo-fornix in the lower mediastinum or other procedure considering the anastomotic safety.

Concerning the valvuloplasty, we fixed by suturing the bilateral sides of the abdominal esophagus to the anterior remnant stomach wall (Fig. 1d). We believe that these bilateral side fixations of the esophagus contribute to keep the anastomotic hole horizontally long as a valvuloplasty in esophagogastrostomy, suggesting a crucial role in reflux prevention (Fig. 2b; red arrows).

Until now, the safety and potential surgical merits of LPG have not fully been established in a well-designed, nationwide or randomized controlled phase II or III study [41]. Therefore, our study also had a limitation. First, this was a technical report with short-term outcomes, and the number of recruited patients was small. Second, the short-term outcomes of our simple hand-sewn esophagogastrostomy were fair; however, the long-term outcomes are unknown. Nevertheless, our non-flap hand-sewn technique for esophagogastrostomy is easy and safe and may be reliable as an anti-reflux procedure. We will report in the near future on the long-term clinical and nutritional outcomes and compare other LPG or LTG outcomes. In conclusion, we devised a simple non-flap hand-sewn technique for esophagogastrostomy with a 5-cm pseudo-fornix as a fundoplication, the posterior pressure mechanism by the remnant stomach and bilateral crus, and a flat-shaped anastomotic hole as a valvuloplasty in LPG. This technique may have a significant advantage that it can be performed laparoscopically with little stress.

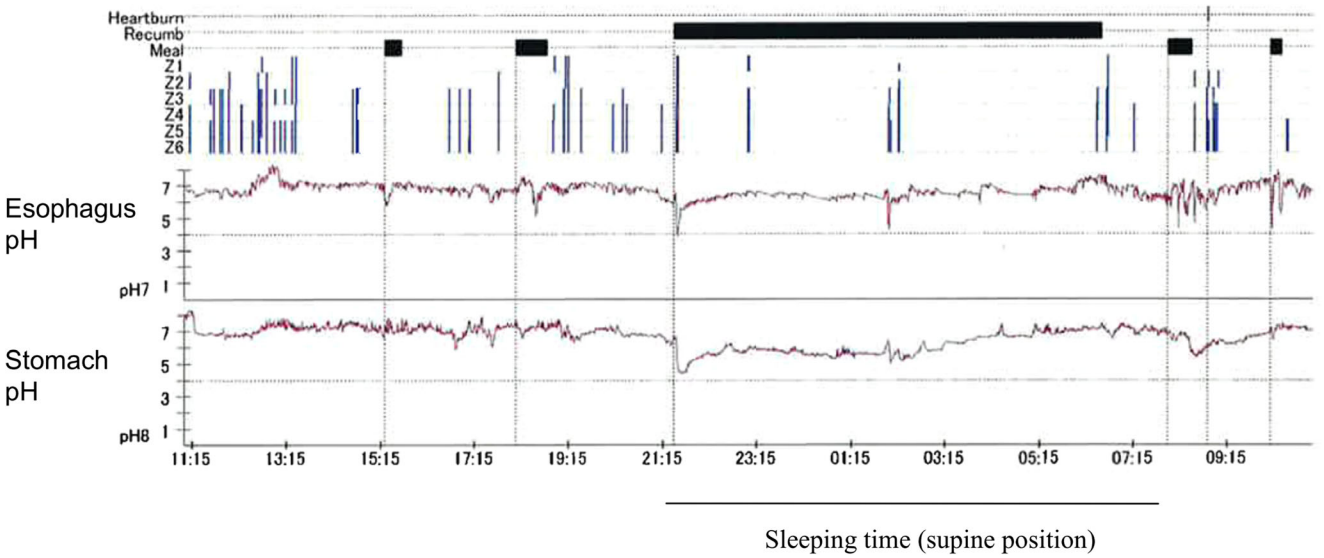

Fig. 4 The 24-h impedance-pH monitoring after LPG. The data of 24-h impedance- pH monitoring was collected since April 2019. This monitoring was performed at postoperative day 7 after gastrectomy

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Authors' contributions Shuhei Komatsu, Toshiyuki Kosuga, Takeshi Kubota, Tatsuya Kumano, Kazuma Okamoto, Daisuke Ichikawa, Yasuhiro Shioaki, and Eigo Otsuji performed the research and analyzed data, and Shuhei Komatsu wrote the paper.

Compliance with ethical standards

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

Research involving human participants and/or animals This study did not include any experiments on animals performed by any of the authors.

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

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