—Original Article—

Endoscopic ultrasound-guided puncture suture device *versus* metal clip for gastric defect closure after endoscopic full-thickness resection: A randomized, comparative, porcine study

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ABSTRACT

Objective: The secure closure of the wall defect is a critical stage of endoscopic full-thickness resection (EFTR). The aim of this study was to compare the closure of post-EFTR defects using an endoscopic ultrasound-guided puncture suture device (PSD) with the metal clip (MC) technique in a randomized, comparative, porcine study. **Methods:** We performed a randomized comparative survival study that included 18 pigs. The circular EFTR defects with a diameter of approximately 20 mm were closed with either a PSD or MC. Serum levels of interleukin-6 (IL-6) were determined preoperatively and on a postoperative day (POD) 1, 3, and 7. Three animals from each group were sacrificed at the end of the 7th, 14th, and 30th POD. Tissue samples retrieved from the closure sites were examined macroscopically and microscopically. **Results:** Resection and closure were performed in 18 pigs (100%) without major perioperative complications. The mean closure time was significantly longer in the MC group than in the PSD group (25.00 ± 3.16 min *vs.* 1.56 ± 0.39 min; $P < 0.05$). Preoperative and POD 7 serum levels of IL-6 did not differ between the two groups. However, on POD 1, the IL-6 levels were observed to be significantly greater in the MC group than in the PSD group ($P < 0.005$). No significant differences between the PSD and MC groups were observed at necropsy. **Conclusion:** In this *in vivo* porcine model, PSD is a feasible device that achieves post-EFTR defect closure with a much shorter closure time and with less immunological responses than the MC technique.

Key words: Endoscopic closure device, endoscopic full-thickness resection, endoscopic ultrasound, interleukin-6, metal clip

INTRODUCTION

Numerous animal experiments and several clinical trials demonstrate the advantages of the endoscopic full-thickness resection (EFTR) for the nonlifting lesions or neoplasms, re-resection of T1-carcinomas, and subepithelial tumors (SET).[1] In 2001, Suzuki and Ikeda first described full-thickness wall defects in the gastrointestinal (GI) tract after EFTR.[2] EFTR naturally results in such defects with the potential risk of intraperitoneal infection.

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A prerequisite for the entry of EFTR into routine endoscopic therapy is the ability to achieve an easy and reliable GI wall defect closure. Various endoscopic devices have been described for post-EFTR transluminal defect closure in animal studies and a few human clinical studies.[1,3-6] An optimal closure device, which could overcome the loss of insufflation and poor visualization during EFTR, remains a major challenge. We designed a new closure device, named puncture suture device (PSD) [Figure 1], which could handle these obstacles.

Surgical injury stimulates an acute inflammatory response and thereby the production of cytokines. Inflammatory cytokines, indirect measures of the host's acute inflammatory response, are commonly used to assess the magnitude of the acute phase response. The cytokine interleukin-6 (IL-6) is known to be a major mediator of the acute-phase response to inflammation.[7] Significantly lower levels of IL-6 are released after laparoscopic surgery than after conventional open surgery.[8-10]

Due to the recent increased interest in EFTR, the impact of different closures methods on host response is being studied. The aim of this randomized, comparative, porcine trial was to compare the outcome of post-EFTR defect closure of using the PSD with that of the metal clip (MC) technique. The study focused on operative times, and the postoperative acute systemic inflammatory response was evaluated as supporting evidence.

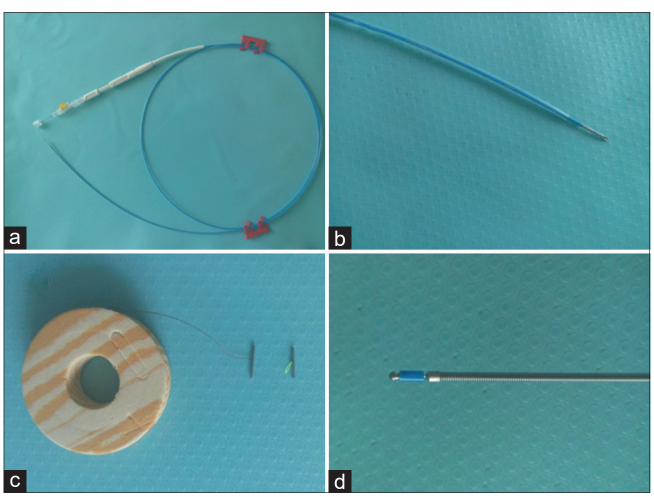

Figure 1. Puncture suture device. (a) A tissue anchor applier. (b) The hollow bore needle of the tissue anchor applier. (c) Two kinds of tissue anchors. (d) A knotting element applicator

METHODS

Animals and randomization

EFTR procedures were carried out in 18 female Bama miniature pigs weighing between 20 and 25 kg, divided into two equal groups that were randomly assigned to closure with either the PSD or MC. Prior to the procedure, all experimental animals were fed a liquid diet for 3 days and fasted for 24 h. Three animals from each group were sacrificed at the end of the 7th, 14th, and 30th postoperative day (POD). All procedures were performed under general anesthesia and endotracheal intubation. General anesthesia was achieved using propofol (2–4 mg/kg). The study was approved by the Institutional Review Board and Ethics Committee of China Medical University.

Procedure and closure technique

All animals underwent surgery while in the left lateral decubitus position. A single-lumen endoscope (EPK-i, Pentax, Tokyo, Japan) with a transparent cap was inserted through the pig's mouth, and a lavage was performed with normal saline until the stomach was free of solid particles. A circular gastric wall perforation of approximately 20 mm in diameter was resected endoscopically with a hook knife and an IT knife (Olympus Corporation, Tokyo, Japan) from the greater curvature of the stomach. Several superficial mucosal markings were made using electrocautery before the EFTR. The resected specimens (n = 18) were removed by forceps and their diameters were measured. EFTR was performed as Zhou *et al.* described.[11] An experienced endoscopist performed all EFTR procedures to reduce variation between the two groups. The post-EFTR gastric defect was closed immediately using MC (Olympus, Tokyo, Japan) or the PSD. During the procedure, a CO_2 insufflator was used. Pneumoperitoneum was relieved using a 20-gauge needle during or after the operation.

Metal clip closure

A single-lumen endoscope was advanced and the standard MC technique was performed as previously described [Figure 2].[12] To achieve a secure closure that contained a maximal thickness, we grasped the thickest borders of the defect as was possible. By rotating the jaws of the clip, the clips were placed correctly right across the defect. A more satisfactory attachment of the edges of the defect was achieved using the jaw

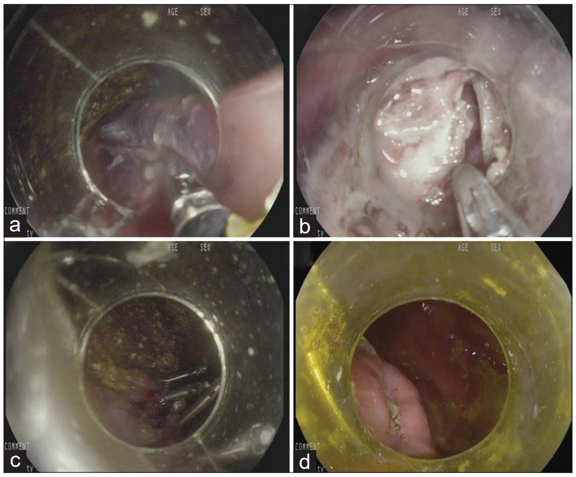

Figure 2. The defect of endoscopic full-thickness resection closure with metal clips. (a) A target circumferential incision was made as deep as the muscularis propria. (b) An artificial perforation observed during the endoscopic full-thickness resection. (c) The gastric wall defect was closed with clips. (d) A post-endoscopic full-thickness resection defect on the postoperative day 7

reopening function. MCs were sequentially placed to close the defect.

Puncture suture device closure

A linear array echoendoscope (EG-3830-UT, Pentax, Tokyo, Japan) guided the tissue anchors that were placed transmurally on the serosal side [Figure 3]. Color Doppler imaging was used to avoid interposed vessels at the puncture sites.[13] These anchors were spaced regularly around the periphery of the target lesion prior to EFTR. The locations of the anchors were confirmed after all were implanted. Then, the chosen tissue lesion, located within the gastric wall tissue anchors, was removed using EFTR. Finally, the sutures were locked together using a knotting element, thereby resulting in transmural suturing [Figure 4].

Blood and tissue samples

Venous blood samples were obtained the day before the operation and on POD 1, POD 3, and before euthanasia on POD 7. All animals were anesthetized prior to blood sampling. Blood samples were centrifuged at 3000 rpm. The serum was stored in pipettes and frozen at −80°C for further analyses. The porcine IL-6 ELISA KIT (Shanghong, Shanghai, China) was used to quantify levels of IL-6 using the sandwich ELISA method (enzyme immunoassay).

Follow-up and necropsy

Postoperatively, animals were recovered and kept in individual cages. The animals were allowed free access to water as soon they recovered from anesthesia, followed by a liquid meal on POD 1. Full, regular feeding was resumed for the remainder of the survival period. They were observed postoperatively for any clinical evidence of complications (as leakage, bleeding, etc.).

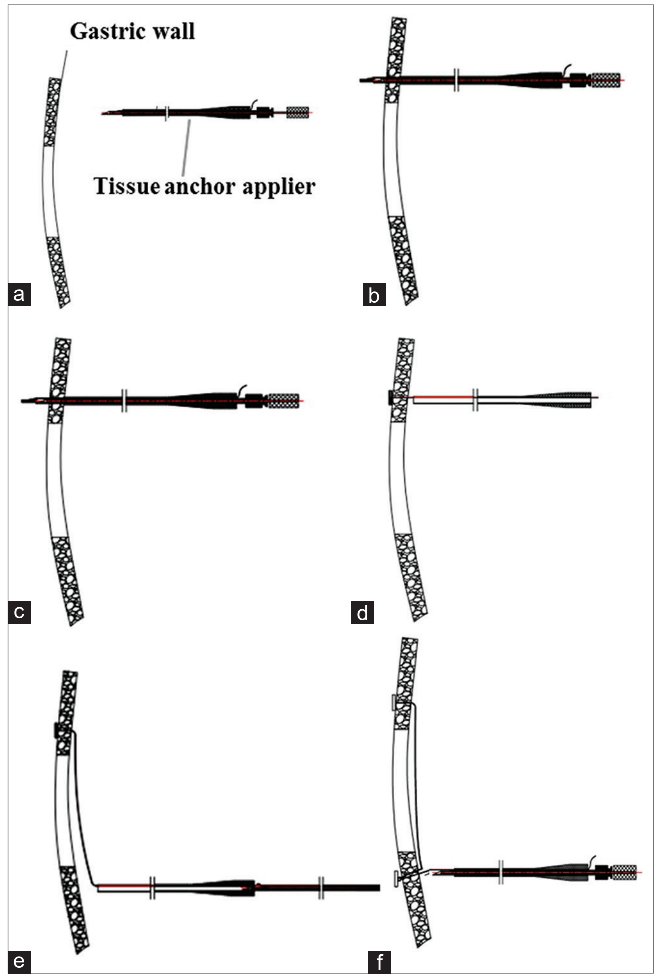

Figure 3. Schema of procedure steps of suturing with the puncture suture device. A hollow bore needle is loaded with the first tissue anchor and suture (a). The needle is advanced through the gastric wall, and the gastric is punctured a few millimeters away from the edge of the target tissue (b and c). By advancing the stylet, the tissue anchor and suture are released from the needle and are left in place on the serosa (d). The rest tissue anchors without long suture are placed and spaced regularly around the target lesion on the serosal side (e and f)

At necropsy, the abdominal cavity and the closure site were closely evaluated for peritonitis, abscesses, and adhesions. The specimens from gastric wall closure site were collected for macroscopic and histopathologic examination.

Statistical analysis

Data are expressed as a mean ± standard error of the mean. Continuous variables of the two groups were compared by *t*-test using SPSS version 22.0

(SPSS Inc., Chicago, IL, USA). A $P < 0.05$ was considered statistically significant.

RESULTS

Eighteen animals were randomly assigned to two study groups (9 PSD and 9 MC), and 18 procedures were completed as intended. No massive hemorrhage or peritonitis occurred. There were four minor bleeding episodes due to the resection incision of the EFTR. All of the bleeding episodes were limited to the intraoperative period and did not require any intervention. After placement of the knotting device or clips, the stomachs were fully distended using gas insufflation to confirm tight closure.

Characteristics of the operations are described in detail in Table 1. No significant differences were observed in sampled EFTR specimen size ($P > 0.05$) and the duration of EFTR ($P > 0.05$) between the PSD and MC groups. The mean closure time was significantly longer in the MC group than in the PSD group (25.00 ± 3.16 min *vs.* 1.56 ± 0.39 min; $t = 22.07$; $P < 0.05$). However, the PSD group also required a median time for anchor placement prior to EFTR of 13.22 ± 2.17 min. In addition, the PSD and MC groups differed significantly in the number of anchors or clips that were placed ($P < 0.05$).

Preoperative and POD 7 serum levels of IL-6 did not differ between the two groups. In the PSD group, levels of IL-6 increased significantly on POD 1, followed by a downward trend toward the preoperative level on POD 7. In the MC group, these levels also increased significantly on POD 1, then declined significantly on POD 7. On POD 1 and POD 3, the IL-6 levels were observed to be significantly greater in the MC group than in the PSD group ($P < 0.05$) [Table 2].

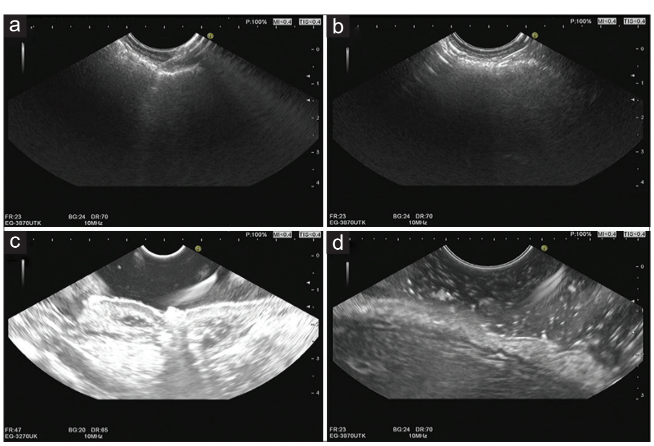

Figure 4. The defect of endoscopic full-thickness resection closure with puncture suture device. (a) The needle punctured the gastric wall. (b) The metal tissue anchor was placed on the serosal side. (c) The defect closed by puncture suture device. (d) Endoscopic ultrasound visualized a continuous muscular layer

All animals recovered well without severe complications, such as bleeding, pain, or signs of infection during the survival periods. At necropsy, no surrounding organ injury due to the anchors was observed in the PSD group. No abscesses and adhesions were observed in any of the pigs. Histologic examination of the closure sites on POD 7, 14, and 30 demonstrated the signs of healing. There were no signs of ischemic necrosis, local infection, or purulence. There was no significant difference between the PSD and MC groups in the pattern of inflammation and tissue repair.

DISCUSSION

In this study, we used a randomized animal experimental setting to compare two endoscopic techniques, the PSD and MC, for the closure of post-EFTR gastric wall defects. The results of the current study indicate that the MC group required longer closure time and consumed more clips. The successful closure with endoscopic clips is technically challenging; hence, it will probably require longer surgical experience with this technique.[12,14] It may be especially time-consuming

Table 1. Interleukin 6 values (pg/mL) compared between the studied groups

	PSD	MC	*t*	*P*
Preoperative	92.25±8.29	94.92±11.10	−0.578	0.571
POD 1	155.22±22.09*	176.11±17.36*	−2.230	0.040
POD 3	123.12±13.61*,#	143.33±21.07*,#	−2.418	0.028
POD 7	107.56±17.99	118.30±10.50*,#,**	−1.547	0.142
F	24.492	44.521		
P	<0.001	<0.001		

Compared with preoperative **P*<0.05, compared with POD 1, #*P*<0.05, compared with POD 3, ***P*<0.05. POD: Postoperative day, PSD: Puncture suture device, MC: Metal clip

Table 2. Results of endoscopic full-thickness resection closure with the puncture suture device and metal clip

	PSD	MC	*t*	*P*
The size of resection specimens (cm)	2.14±0.12	2.09±0.18	0.774	0.450
Tissue anchors or clips (*n*)	6.33±0.50	7.44±1.24	−2.500	0.030
EFTR time (min)	19.89±2.93	20.78±4.76	−0.477	0.640
Closure time (min)	1.56±0.39	25.00±3.16	−22.073	<0.001

EFTR: Endoscopic full-thickness resection, PSD: Puncture suture device, MC: Metal clip when the border of the defect is larger than the size of the jaws. The longer closure time is likely to increase the potential for gastric contents to flow into the abdominal cavity, which may result in an increased risk of pneumoperitoneum and intraperitoneal infection.[15] During EFTR, loss of insufflation and poor visualization are challenging obstacles that may lengthen closure time; however, placing PSD anchors prior to the making the defect results in a shorter closure time. After the resection, the knotting element is slid down through the endoscope channel, ignoring the poor view, and closing the defect effectively. The mean closure time was 1.56 ± 0.39 min (range: 1–2 min). Ye *et al*.[16] reported a retrospective study of closing post-EFTR defects using clips and an endoloop. In their 51 patients, the mean gastric SET diameter was 2.4 cm (range: 1.3–3.5 cm); 50 of them were resected successfully. The average procedure time was 52 min (range: 30–125 min). Guo *et al*.[17] reported a study of EFTR of gastric SETs with a mean tumor diameter of 12.1 mm (range: 6–20 mm). The mean time required to close these small defects using the over-the-scope clip (OTSC) was 4.9 min (range: L 2–12 min). OTSC require the withdrawal of the endoscope to load the suturing device, so valuable time is loss when the defect is vulnerable leaking GI contents and causing peritonitis.[18] However, the PSD facilitates closure of the defect immediately after EFTR, markedly reducing the duration of an open defect and the leakage risk interval.

In contrast to interrupted sutures, the PSD functioned similar to a "single strand continuous, running suture." Once the anchors were placed, the closure was simply and quickly performed by releasing the knotting element. Raju *et al*.[19] reported full-thickness resection of the colon using T-tags for defect closure. Successful closure of 2 cm defects was achieved in 19/20 pigs in a mean time of 41 min (range: 21–125 min) for four sutures. One animal failed to thrive, and necropsy revealed mild peritonitis and 2 mm defect at the closure site. Two of the 132 T-tags were inserted in adjacent viscera. In the PSD group, endoscopic ultrasound could control the penetration depth and avoid the risk of adjacent visceral injury.[20-23] Ultrasound guidance ensured that the anchors were placed transmurally, punctures of intramural vessels were avoided, and that the anchors were placed on the serosal side of the gastric wall.

Quicker closure may influence acute-phase inflammation by minimizing the gastric content spills; this hypothesis is supported by the higher plasma IL-6 levels in the MC group, compared with the PSD group. IL-6 is a cytokine with both pro- and anti-inflammatory functions, and its release into peripheral blood appears to be an early marker of injury severity following major trauma.[8,24] Levels of IL-6 in postmortem serum were shown to be useful objective indices of traumatic severity.[9] Within a few hours after surgery, the plasma concentrations IL-6 will increase.[7] Adachi *et al*.[25] found significantly higher postoperative levels of IL-6 after open surgery than after laparoscopic surgery in a retrospective study of 102 patients. Georgescu *et al*.[26] published a randomized controlled animal study of natural orifice translumenal endoscopic surgery (NOTES) and laparoscopic oophorectomy that demonstrated postoperative increases in IL-6 and IL-1 β levels in both groups; following NOTES, the inflammatory response was smaller. In this study, higher levels of serum IL-6 in the MC group on POD 1 were observed, and these data indicate that MC group received greater injury than the PSD group.

Furthermore, insufflation pressure and the choice of insufflation gas also affect the immunologic reaction.[27,28] To eliminate this source of variability between study groups, on-demand CO_2 insufflation through an endoscope was used in the present study.

In this study, the greater curvature of the stomach was chosen as the full-thickness resection site. Yang *et al*.[14] reported that tumors location in the greater curvature were significantly associated with a more challenging closure. They pointed out that the lack of neighboring support structures, air and fluid leakage into the peritoneal cavity, limited endoscopic view, and the mobility of the greater curvature all contribute to the greater difficulty of closure.

Limitations

this study was based on a small sample. Therefore, a larger survival study and additional analysis are needed to describe the true value of this closure technique before performing it in humans.

CONCLUSION

This randomized controlled animal trial demonstrates that larger post-EFTR gastric defects can be reliably closed using the PSD. Compared with the traditional MC technique, the PSD has shorter closure times and lower immunological responses.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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