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

Beibei Sun, Jintao Guo, Nan Ge, Siyu Sun, Sheng Wang, Xiang Liu, Guoxin Wang, Linlin Feng
Endoscopy Center, Shengjing Hospital, China Medical University, Liaoning Province, China

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-section 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.

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Sun B, Guo J, Ge N, Sun S, Wang S, Liu X, et al. Endoscopic ultrasound-guided puncture suture device versus metal clip for gastric defect closure after endoscopic full-thickness resection: A randomized, comparative, porcine study. Endosc Ultrasound 2016;5:263-8.

Address for correspondence
Dr. Siyu Sun, Endoscopy Center, Shengjing Hospital, China Medical University, No. 36, Sanhao Street, Shenyang, Liaoning Province, 110004 China. E-mail: sun-siyu@163.com
Received: 2016-05-21; Accepted: 2016-05-25
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. 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. Significantly lower levels of IL-6 are released after laparoscopic surgery than after conventional open surgery. 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.

**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. 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 CO₂ 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]. 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...
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 \(-80^\circ\text{C}\) for further analyses. The porcine IL-6 ELISA KIT (Shanghai, 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.).

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 ($25.00 \pm 3.16$ min vs. $1.56 \pm 0.39$ min; $t = 22.07$; $P < 0.05$). However, the PSD group also required a median time for anchor placement prior to EFTR of $13.22 \pm 2.17$ 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, 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].

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. 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

---

**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.
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 intra-peritoneal infection.\textsuperscript{[13]} 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.\textsuperscript{[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.\textsuperscript{[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: 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.\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[8,24]} Levels of IL-6 in postmortem serum were shown to be useful objective indices of traumatic severity.\textsuperscript{[9]} Within a few hours after surgery, the plasma concentrations IL-6 will increase.\textsuperscript{[7]} Adachi et al.\textsuperscript{[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.\textsuperscript{[26]} published a randomized controlled animal study of natural orifice transluminal endoscopic surgery (NOTES) and laparoscopic oophorectomy that demonstrated postoperative increases in IL-6 and IL-1β 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.\textsuperscript{[27,28]} To eliminate this source of variability between study groups, on-demand CO\textsubscript{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.\textsuperscript{[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.
Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES
1. Schmidt A, Meier B, Caca K. Endoscopic full-thickness resection: Current status. World J Gastroenterol 2015;21:9273-85.
2. Suzuki H, Ikeda K. Endoscopic mucosal resection and full thickness resection with complete defect closure for early gastrointestinal malignancies. Endoscopy 2001;33:437-9.
3. Fritscher-Ravens A, Hampe J, Grange P, et al. Clip closure versus endoscopic suturing versus thoracoscopic repair of an iatrogenic esophageal perforation: A randomized, comparative, long-term survival study in a porcine model (with videos). Gastrointest Endosc 2010;72:1020-6.
4. Park PO, Bergström M, Rothstein R, et al. Endoscopic sutured closure of a gastric natural orifice transluminal endoscopic surgery access gastrotomy compared with open surgical closure in a porcine model. A randomized, multicenter controlled trial. Endoscopy 2010;42:311-7.
5. Schurr MO, Baur FE, Krautwald M, et al. Endoscopic full-thickness resection and clip defect closure in the colon with the new FTRD system: Experimental study. Surg Endosc 2015;29:2434-41.
6. Kobayashi M, Sumiyama K, Ban Y, et al. Closure of iatrogenic large mucosal and full-thickness defects of the stomach with endoscopic interrupted sutures in in vivo porcine models: Are they durable enough? BMC Gastroenterol 2015;15:5.
7. Ohzato H, Yoshizaki K, Nishimoto N, et al. Interleukin-6 as a new indicator of inflammatory status: Detection of serum levels of interleukin-6 and C-reactive protein after surgery. Surgery 1992;111:201-9.
8. Stensballe J, Christiansen M, Tønnesen E, et al. Early IL-6 and IL-10 response in trauma is correlated with injury severity and mortality. Acta Anaesthesiol Scand 2009;53:515-21.
9. Mimasaka S, Funayama M, Hashiyada M, et al. Significance of levels of IL-6 and IL-8 after trauma: A study of 11 cytokines post-mortem using multiplex immunoassay. Injury 2007;38:1047-51.
10. Watt DG, Horgan PG, McMillan DC. Routine clinical markers of the magnitude of the systemic inflammatory response after elective operation: A systematic review. Surgery 2015;157:362-80.
11. Zhou PH, Yao LQ, Qin XY, et al. Endoscopic full-thickness resection without laparoscopic assistance for gastric submucosal tumors originating from the muscularis propria. Surg Endosc 2011;25:2926-31.
12. Grupka MJ, Benson J. Endoscopic clipping. J Dig Dis 2008;9:72-8.
13. Guo J, Sun B, Wang S, et al. Diagnosis of lymphoma by endoscopic ultrasound-assisted transendoscopic direct retroperitoneal lymph node biopsy: A case report (with video). Endosc Ultrasound 2015;4:69-72.
14. Yang F, Wang S, Sun S, et al. Factors associated with endoscopic full-thickness resection of gastric submucosal tumors. Surg Endosc 2015;29:3888-93.
15. Schmidt A, Bauder M, Riecken B, et al. Endoscopic resection of subepithelial tumors. World J Gastrointest Endosc 2014;6:592-9.
16. Ye LP, Yu Z, Mao XL, et al. Endoscopic full-thickness resection with defect closure using clips and an endoloop for gastric subepithelial tumors arising from the muscularis propria. Surg Endosc 2014;28:1978-83.
17. Guo J, Liu Z, Sun S, et al. Endoscopic full-thickness resection with defect closure using an over-the-scope clip for gastric subepithelial tumors originating from the muscularis propria. Surg Endosc 2015;29:3356-62.
18. Raju GS, Shibukawa G, Ahmed I, et al. Endoluminal suturing may overcome the limitations of clip closure of a gaping wide colon perforation (with videos). Gastrointest Endosc 2007;65:806-11.
19. Saxena P, Lakhtakia S. Endoscopic ultrasound guided vascular access and therapy (with videos). Endosc Ultrasound 2015;4:168-75.
20. Bhutani MS, Arora A. New developments in endoscopic ultrasound-guided therapies. Endosc Ultrasound 2015;4:319-23.
21. Weng W, Wang S, Liu W, et al. The application of linear endoscopic ultrasound in the patients with esophageal anastomotic strictures. Endosc Ultrasound 2015;4:126-31.
22. Gebhard F, Pletsch H, Steinbach G, et al. Is interleukin 6 an early marker of injury severity following major trauma in humans? Arch Surg 2000;135:291-5.
23. Guo J, Sun B, Wang S, et al. Diagnosis of lymphoma by endoscopic ultrasound-assisted transendoscopic direct retroperitoneal lymph node biopsy: A case report (with video). Endosc Ultrasound 2015;4:69-72.
24. Yang F, Wang S, Sun S, et al. Factors associated with endoscopic full-thickness resection of gastric submucosal tumors. Surg Endosc 2015;29:3888-93.
25. Schmidt A, Bauder M, Riecken B, et al. Endoscopic resection of subepithelial tumors. World J Gastrointest Endosc 2014;6:592-9.
26. Ye LP, Yu Z, Mao XL, et al. Endoscopic full-thickness resection with defect closure using clips and an endoloop for gastric subepithelial tumors arising from the muscularis propria. Surg Endosc 2014;28:1978-83.
27. Guo J, Liu Z, Sun S, et al. Endoscopic full-thickness resection with defect closure using an over-the-scope clip for gastric subepithelial tumors originating from the muscularis propria. Surg Endosc 2015;29:3356-62.
28. Raju GS, Ahmed I, Bordelon P. Colonoscopic full-thickness resection of colon in a porcine model as a prelude to endoscopic surgery of difficult colon polyps – A novel technique. Gastrointest Endosc 2009;70:159-65.
29. Saxena P, Lakhtakia S. Endoscopic ultrasound guided vascular access and therapy (with videos). Endosc Ultrasound 2015;4:168-75.
30. Bhutani MS, Arora A. New developments in endoscopic ultrasound-guided therapies. Endosc Ultrasound 2015;4:319-23.
31. Weng W, Wang S, Liu W, et al. The application of linear endoscopic ultrasound in the patients with esophageal anastomotic strictures. Endosc Ultrasound 2015;4:126-31.
32. Gebhard F, Pletsch H, Steinbach G, et al. Is interleukin 6 an early marker of injury severity following major trauma in humans? Arch Surg 2000;135:291-5.
33. Guo J, Sun B, Wang S, et al. Diagnosis of lymphoma by endoscopic ultrasound-assisted transendoscopic direct retroperitoneal lymph node biopsy: A case report (with video). Endosc Ultrasound 2015;4:69-72.
34. Yang F, Wang S, Sun S, et al. Factors associated with endoscopic full-thickness resection of gastric submucosal tumors. Surg Endosc 2015;29:3888-93.
35. Schmidt A, Bauder M, Riecken B, et al. Endoscopic resection of subepithelial tumors. World J Gastrointest Endosc 2014;6:592-9.