Efficacy of single-layer continuous suture of the posterior wall in anastomosis involving a difficult location of the digestive tract

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Abstract. Surgery for digestive tract disease predominantly consists of reconstruction and anastomosis. Due to the difficult location, anastomosis is extremely challenging and the risk of complication increases accordingly. Traditional manual anastomosis and the application of a stapling device are insufficient. Therefore, the aim of this study was to investigate the feasibility and safety of a novel manual method in a difficult anastomotic location, consisting of a single-layer continuous suture in the posterior wall. In total, 15 beagle dogs were included in the study; eight underwent surgery with the novel manual method for reconstruction and anastomosis of the digestive tract, while seven underwent surgery with the stapler device as a control. The subsequent postoperative complications were observed and, three months later, the anastomotic ports were excised, and the pathological formation and morphological changes were evaluated. No statistically significant differences were identified between the total (50.0% vs. 57.1%; P=0.782) and anastomotic (0.0% vs. 28.6%; P=0.200) complication rates in the manual suture and staple suture groups, respectively. Compared with the control group, the operative expenditure was lower in the manual group (1726.7±33.5 vs. 2135.7±43.1 renminbi; P=0.001), the diameter of the anastomotic port was larger in the manual group (3.04±0.07 vs. 2.24±0.25 cm; P=0.004) and the thickness of the anastomotic port (in cm) was thinner in the manual group (2.94±0.06 vs. 5.07±0.85; P=0.002). Furthermore, the pathological formation of the anastomotic port in the manual group was improved. The results of the current study suggest single-layer continuous suture of the posterior wall in anastomosis of the digestive tract to be a novel method with feasibility and safety, particularly in difficult anastomotic locations.

Introduction

Surgery for digestive tract disease predominantly consists of reconstruction and anastomosis (1). The methods of anastomosis influence the outcome of surgery, postoperative quality of life and complications.

Traditional manual anastomosis is a complex surgery, consisting of double-layer interrupted suture, which requires an experienced surgeon, and has a higher incidence of postoperative complication. The incidence of stricture and leakage in traditional colorectal anastomosis is 4.5 and 1.8%, respectively (2). Furthermore, in certain types of surgery, such as esophagogastrectomy and colorectostomy, the surgery is more difficult due to special anatomic location and poor exposure of the back wall of the anastomosis, increasing the risk of complication accordingly.

In 1909, the first surgical stapler was developed and primarily used for dividing and stapling bowel segments (3). With the general application of the stapling device, an increasing number of proximal gastric resections via the abdomen have been performed for cardiac cancer. The stapling device has contributed to reduced surgery times in difficult locations (4) and decreased the rates of edema (5), infection (6), leakage and stricture of anastomosis (7), as well as morbidity of pulmonary complication (8). However, limitations of this method exist, and improper manipulation may cause partial incisions and tearing, which result in leakage and bleeding of the anastomosis (9,10). In addition, irregular suture of the mucosa may cause hyperplasia of granulation tissue and scar formation, which further increase the risk of stricture (11,12). In certain cases, with preoperative obstruction of the digestive tract, mucosal edema, thickened muscle layer and dysfunctional healing, the application of stapling device is constricted. The stapling techniques have been criticized in view of their apparent expense. Therefore, the identification of a novel manual method with simple and convenient characteristics for reconstruction in difficult locations is beneficial.

Single-layer continuous suture is a common method of blood vessel surgery (13). The advantage of this approach is its simplicity, and is particularly suited for vessel anastomosis in deep tissues. Single-layer suture has been confirmed to be tight and safe, similar to double-layer suturing in gastrointestinal anastomosis (14,15), and superior to conventional suturing in the colon (4). However, the efficacy of the method combined
with continuous suture in difficult anastomotic locations remains unknown.

In the present study, prospective and controlled experiments were performed, including single-layer continuous suture of the posterior wall, and double-layer interrupted suture of the anterior wall. The aim was to investigate the efficacy of the novel manual method in difficult anastomotic locations, and the method was found to be feasible and safe. This novel method may simplify the approach in complex surgery as a result of the special anatomic sites, and reduce expenditure.

Materials and methods

Animals. In total, 15 beagle dogs, including 10 females and five males, with a median weight of 9 kg (range, 7-12 kg), were included in the study. The dogs were fed with specialized dog food and supplied with an appropriate amount of water. The dogs were divided into two groups: A manual group and a staple group. The manual group underwent single-layer continuous suture, while the staple group underwent suture with a stapler device. The study was approved by the Xi'an Medical Experimental Animal Care Commission (Xi'an, China).

Procedures. The dogs were anesthetized by an intravenous injection of pentobarbital sodium (30 mg/kg; Sigma-Aldrich China, Inc., Shanghai, China), and an intratracheal tube was inserted and connected to a respirator during the surgery. A total of 800,000 IU of penicillin and 250 mg of metronidazole (both North China Pharmaceutical Group Corporation, Shanghai, China) were administrated intravenously at the initiation and end of the anesthesia.

Esophagogastric anastomosis. All animals were anesthetized by an intravenous injection of pentobarbital sodium (30 mg/kg); Sigma-Aldrich China, Inc., Shanghai, China), and an intratracheal tube was inserted and connected to a respirator during the surgery. A total of 800,000 IU of penicillin and 250 mg of metronidazole (both North China Pharmaceutical Group Corporation, Shanghai, China) were administrated intravenously at the initiation and end of the anesthesia.

Colon-to-rectum anastomosis. Bowel preparation consisted of fluids by mouth for three days prior to surgery. Laparotomy was performed through a midline incision, and the distal colon and rectum were disassociated. A circumferential dissection of the rectum was undertaken to the level of 6 cm from the dentate line, and ~5 cm of the rectum was resected. For the manual suture, the posterior wall of the anastomosis was sutured by single-layer continuous suture with 4-0 Prolene thread, and the priority wall was sutured by double-layer interrupted suture with silk thread as usual. For the staple suture, a purse-string suture was inserted and the anvil was placed on the proximal end of the rectum, while the distal colon end was used to introduce the staple. The central shaft of the stapler was then inserted through the antimesenteric border of the colon and the anvil was refitted. An end-to-side esophagogastric anastomosis was created with the button, the stapler was withdrawn and the distal colon end was closed with suture, while the abdominal wound was sutured in layers.

Following surgery, the dogs received intravenous injections of electrolyte solution and antibiotics for two days. On the third postoperative day, the dogs were allowed to drink water and a fluid meal.

Observations. Prior to and for 0.5 months following surgery, the anal temperature of each dog was measured every two days, and prior to and for three months following surgery, the feeding amount and weights were measured once a week. The complications of surgery, including wound infection, stricture and leakage of the anastomosis, were observed according to symptoms and signs.

Blood samples. Prior to and one month following surgery, blood samples were obtained from a rear-leg vein and drawn into ice-chilled glass tubes containing EDTA-2Na (1.25 mg/ml blood). Regular blood and liver function tests were then performed under the appropriate circumstances.

Specimen collection. At three months following surgery, each dog was anesthetized with pentobarbital sodium and laparotomized on the day following a 24-h fast. Next, full-thickness tissue specimens were obtained from the anastomotic port. The diameter of the anastomotic port and thickness of the wall were measured. In total, four tissue specimens were dissected from each anastomotic port symmetrically, and then dipped into 10% formalin solution. The tissues were embedded in paraffin, sectioned (3 µm thick) along the longitudinal axis of the intestine and stained with hematoxylin and eosin (H&E).

Morphological examinations of the anastomotic port. All slices with H&E staining were evaluated for the severity of inflammation, stricture and fibrosis by two independent pathologists under a light microscope (x100 magnification; Q550cw, Leica, Mannheim, Germany) of 10 fields. The standard scores were determined as follows: i) inflammation: 1, small amount of lymphocytes or granulocytes observed in <4 fields; 2, medium amount of lymphocytes or granulocytes observed in 4-7 fields; and 3, large amount of lymphocytes or granulocytes observed in >7 fields; ii) fibrosis: 1, small amount of fibrocytes; 2, medium amount of fibrocytes; and 3, large amount of fibrocytes; iv) irregular structure: 1, slight; 2, moderate; and 3, severe.

Statistical methods. Data were analyzed using SPSS 11.5 software (SPSS Inc., Chicago, IL, USA). Student's t-test was
used to analyze the differences between weight, temperature, feeding amount, surgery time and amount of bleeding. Fisher’s exact test was used for the comparison between the incidence rate and complications. All statistical tests were two‑sided and P<0.05 was considered to indicate a statistically significant difference.

Results

Total status of animal. A total of 15 dogs underwent surgery successfully. In the manual group, eight dogs underwent single-layer continuous suture of the posterior wall in esophagogastric anastomosis or colon rectum anastomosis, while in the staple group, seven dogs underwent staple anastomosis as controls. Following three months of observation, intra- and postoperative complications were identified, including bleeding, shock, leakage or stricture of anastomosis and infection (Table I).

Weight, temperature and diet. Prior to surgery, the mean weight of animals in the manual group was 9.0±0.6 kg, while this was 9.4±0.5 kg (P=0.658) in the staple group. At three months following surgery, the mean weight of the animals in the manual and suture groups was 10.1±1.7 and 12.2±0.7 kg, respectively (P=0.367; Table II). Similarly, no significant difference was identified between the mean weights of the two groups at other observation time points (Fig. 1A). Prior to surgery, the mean feeding amount of animals in the manual group was 176.7±10.9 g, and 180±14.1 g in the staple group (P=0.852). At one month following surgery, the mean feeding amount of the animals in the two groups was 172.0±20.3 versus 220±3.0 g (P=0.127). No significant difference was identified between the feeding amount of the two groups at other observation time points. (Fig. 1C).

Surgery time, amount of bleeding and expenditure. The mean surgical time in the manual group was 2.0±0.1 h, while this was 1.9±0.2 h in the staple group (P=0.915). The mean volume of blood lost during surgery was 132.2±23.9 ml in the manual group and 151.4±36.7 ml in the staple group (P=0.655) (Table II). The surgical expenditure included the apparatus, stapler, thread and drugs. The total surgical expenditure in

| Group | Anastomosis | Complication (yes/no) | Postoperative complication |
|-------|-------------|-----------------------|----------------------------|
| 1     | Staple      | EG                    | Yes                        | Infection and jaundice |
| 2     | Staple      | EG                    | Yes                        | Bleeding and shock     |
| 3     | Staple      | EG                    | Yes                        | Stricture of anastomosis |
| 4     | Staple      | EG                    | Yes                        | Leakage and infection  |
| 5     | Staple      | CR                    | No                         |                          |
| 6     | Staple      | CR                    | No                         |                          |
| 7     | Staple      | CR                    | No                         |                          |
| 8     | Manual      | CR                    | No                         |                          |
| 9     | Manual      | CR                    | No                         |                          |
| 10    | Manual      | EG                    | Yes                        | Infection               |
| 11    | Manual      | EG                    | Yes                        | Bleeding and shock      |
| 12    | Manual      | EG                    | Yes                        | Infection               |
| 13    | Manual      | EG                    | No                         |                          |
| 14    | Manual      | EG                    | No                         |                          |
| 15    | Manual      | EG                    | Yes                        | Infection               |

EG, esophagogastric anastomosis; CR, colon-to-rectum anastomosis.

Figure 1. Observations with regard to animal weight, temperature and feeding pre- and post-surgery. (A) The growth curves of the animals’ weight; no significant difference was identified between weight in the manual and staple groups. (B) The growth curves of the animals’ temperature; no significant difference was identified between temperature in the manual and staple groups. (C) The growth curves of animals’ feeding; no significant difference was identified between feeding in the manual and staple groups.
the manual group was significantly lower than that of the control group; 1726.7±33.5 versus 2135.7±43.1 renminbi (P=0.001) (Table II).

Blood test. Blood tests were performed prior to and one month following surgery. In the manual and staple groups, no significant difference was identified between the routine blood and liver function tests (Table III).

Complications of anastomosis. The incidence rate of the total complications in the manual group was 50%, and 57.1% in the staple group (P=0.782). The complications involving the
anastomosis port were 0 and 28.6%, with no significant difference (P=0.200) (Fig. 2A and B and Table IV).

**Morphological changes in the anastomotic port.** The diameter of the anastomotic port in the manual group was 3.04±0.07 cm, while this was 2.24±0.25 cm in the staple group (P=0.004). The thickness of the anastomotic port in the posterior wall of the manual group was 2.94±0.06 cm, which was thinner than that of the staple group (5.07±0.85 cm) (P=0.002), and also thinner than in its anterior wall.

Figure 2. Evaluation between complications and anastomotic ports. (A) Compared with the staple group (controls), the complication rate in the manual group was reduced, but the difference was not significant. (B) The complication rate of anastomosis in the manual group was significantly lower than that in the controls. *P<0.05, vs. the staple group. (C) The diameter of the anastomotic port in the manual group was larger than that in the controls. *P<0.05, vs. the staple group. (D) The thickness of the posterior wall in the manual group was smaller than that in the anterior wall of the manual group and the walls of the controls. *P<0.05 vs. the anterior wall of the manual group and the staple group. Data are presented as the mean ± standard error of the mean.

Figure 3. Tissues of the anastomotic port. (A) The anastomotic port of animal 3 following esophagogastric anastomosis with staple suture. The image shows stricture of the anastomotic port, with increased thickness and a diameter of 0.8 cm. (B) The anastomotic port of animal 15, following esophagogastric anastomosis with manual suture. The image shows a larger diameter and increased thickness of the anastomotic port. (C) The transversal inner surface of the anastomotic port following single-layer continuous suture.
Inflammation of the anastomotic port.

Pathological formation of the anastomotic port.

Discussion

In this study, single-layer suture was combined with continuous suture in back wall anastomosis at challenging surgical sites. The study showed that this combined suture is technically possible to perform, and that, under experimental conditions, the novel anastomosis appears to be as safe as stapled sutures. The postoperative complications were reduced or the same as those in stapled sutures; however, the expenditure was evidently reduced.

The predominant complications of anastomosis are leakage, stricture and infection. To date, the superiority of staple suture has remained controversial. It has been reported that in previous hand-sewn and staple groups, the incidence of leakage following esophagogastric anastomosis is 0-21.9 and 0-25.8%, respectively, and 0-19.5% and 0-32.8%, respectively, for stricture (16-24). In rectal cancer, leakage following low anterior resection with the double stapling technique is 2.6-17% (25-28), and no significant difference has been identified between the morbidity or mortality rates in hand-sewn and stapled techniques (14,29). The results of the current study demonstrated that the incidence rate of total complications was 50%, and anastomosis complication was 13.3%. The results were higher than those identified in the previously described studies, but lower than a study which reported postoperative minor complications in 70.9% of patients and serious complications in 22.6% of patients following esophagogastric anastomosis (30). The reasons of complication in the present study may have also involved the following: i) Preoperative preparation, due to the challenges of bowel preparation and implantation of the gastric tube; and ii) insufficient nutrition supply for animals following surgery. However, in the same conditions, the manual group demonstrated a lower rate of complications and higher safety than the staple group, providing evidence for its advantage.

The predominant challenges of anastomosis are due to exposure, particularly for anastomosis of the back wall in difficult locations. The anastomosis becomes invisible due to blockage by surrounding tissues, and even in the course of staple suture, the proceeding is not visible. However, continuous suture does not require a butt joint, which increases exposure to the back wall and contributes to convenience and safety.

Mechanical integrity and tissue viability are emphasized in gastrointestinal anastomosis. It has been shown in an experimental and comparative study, that single-layer anastomosis is as strong as two-layer suturing in the small intestine and colon, and may guarantee mechanical integrity (19). Tissue viability was also identified in the staple group, with a diameter of <1 cm, while the thickness of the wall was 1 cm (Fig. 3A).

Table IV. Complication of the two groups.

| Variables                  | Manual, n (%) | Staple, n (%) | P-value |
|----------------------------|---------------|---------------|---------|
| n                          | 8 (100.0)     | 7 (100.0)     |         |
| Bleeding shock             | 1 (12.5)      | 1 (14.3)      | 0.919   |
| Leakage                    | 0 (0.0)       | 1 (14.3)      | 0.467   |
| Stricture                  | 0 (0.0)       | 1 (14.3)      | 0.467   |
| Infection of abdominal     | 2 (25.0)      | 2 (28.6)      | 0.662   |
| Infection of wound         | 1 (12.5)      | 1 (14.3)      | 0.919   |

Fisher's exact test was used to assess the statistical difference between the manual and staple suture groups.

Table V. Pathological formation of the anastomotic port between the two groups.

| Group             | Score (mean ± SD) | P-value |
|-------------------|-------------------|---------|
| Fibrosis          |                   |         |
| Manual            | 1.92±0.78         | 0.354   |
| Staple            | 2.15±0.88         |         |
| Inflammation      |                   |         |
| Manual            | 1.58±0.83         | 0.063   |
| Staple            | 2.10±0.97         |         |
| Stricture         |                   |         |
| Manual            | 1.79±0.88         | 0.020   |
| Staple            | 2.40±0.75         |         |
| Total of slice    |                   |         |
| Manual            | 5.29±0.70         | 0.018   |
| Staple            | 6.65±1.95         |         |
| Total of samples  |                   |         |
| Manual            | 21.17±4.75        | 0.171   |
| Staple            | 26.60±7.33        |         |
| Anterior-posterior|                   |         |
| Anterior wall of manual | 11.00±2.00    | 0.030   |
| Posterior         | 9.50±2.51         |         |

Student's t-test was used to assess the statistical significance between tumor volume in the manual and staple suture groups. SD, standard deviation.

(4.22±0.16 cm) (P=0.036). However, no significant difference was identified between the staple group and the anterior wall of the manual group (P=0.179; Figs. 2C and D and 3A-C). Anastomotic stricture was also identified in the staple group, with a diameter of <1 cm, while the thickness of the wall was 1 cm (Fig. 3A).
suture), the blood flow of suture line increases in turn (9,10) and, therefore, the mechanism requires further investigation.

In the present study, no significant difference was identified between surgical time in the manual and staple suture groups. Continuous suture contributes to reduced surgical time, and does not increase complication in episiotomy (31). Staple anastomosis reduces surgical time by ≤30 min due to cutting and suturing tissues only once (32); however, to acquire a safe anastomosis, a sharp blade and a sufficient amount of tissue cutting is required, which may lead to surgical issues and prolong surgery time.

The complication of stricture has been found to closely correlate with the diameter of the anastomosis and the thickness of the wall. Few studies regarding anastomotic ports have included morphological observations. In the current study, a smaller diameter and increased thickness of the anastomotic port were identified in the staple group, with the thickness of the anastomotic wall in one sample reaching 1 cm, and a narrow diameter of <0.8 cm. Staple suture is a double-layer suture and, in certain cases, an additional strengthening suture is required to reinforce the suture with a third layer; however, this may cause too much tissue to turn inwards and induce stricture formation (10). Staplers with several external diameters allow the resection of various diameters and the dissection of various surface areas (33). However, selecting a stapler is almost impossible due to fixed types. By contrast, single-layer suture causes little inward tissue movement, which may reduce the anastomotic stricture, and continuous suture has been confirmed to contribute to the adjustment of the anastomotic diameter (34).

Fibrosis is an important factor for anastomosis stricture, and, in this study, a decreasing number of inflammation and fibroblast cells were identified by microscope, as well as a slightly irregular structure in the novel manual suture group compared with the staple suture and conventional manual suture groups. These results provided evidence that manual sutures may efficiently reduce anastomotic complication, and support the view that staple sutures increase the rate of postoperative anastomotic stricture (21). The results are consistent with a meta-analysis of randomized and controlled trials, comparing hand-sewn with stapled esophagogastric anastomosis in other studies (7).

In conclusion, the results of the current study suggest that single-layer continuous suture in anastomosis of the posterior wall of the digestive tract is a novel method with feasibility and safety. This novel method simplifies the surgical approach and can be easily applied clinically, in particular, it can be used in challenging anastomosis of special anatomic sites. In addition, this method reduces expenditure and deserves generalization in the future.
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