Supplemental Digital Content

Surgical Site Infection After Intracorporeal Anastomosis for Left-Sided Colon Cancer: A Multicenter Randomized Controlled Trial (STARS Trial)

Protocol and Statistical Analysis Plan Amendment History

CONTENTS

Protocol

A Multicenter Randomized Clinical Trial Comparing Surgical Site Infection after Total Laparoscopic and Laparoscopic-assisted Radical Surgery for Left Colon Cancer (STARS)

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Abstract

Title	A Multicenter Randomized Clinical Trial Comparing Surgical Site Infection after Total Laparoscopic and Laparoscopic-assisted Radical Surgery for Left Colon Cancer
Study Center	First Hospital of Jilin University Peking Union Medical College Hospital Cancer Hospital Affiliated to Fudan University Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine Beijing Friendship Hospital , Capital Medical University PLA General Hospital Daping Hospital Affiliated to Army Military Medical University Shengjing Hospital Affiliated to China Medical University Chinese Academy of Medical Sciences Cancer Hospital China - Japan Union Hospital of Jilin University The First Affiliated Hospital of China Medical University Nanfang Hospital of Southern Medical University
Study Purpose	The purpose of this study is to investigate the effects of intracorporeal anastomosis and extracorporeal anastomosis in laparoscopic-assisted radical left hemicolectomy on surgical site infection. Also consider perioperative recovery, safety, and oncology outcomes.
Study Type	A prospective, multicenter, non-inferiority, two-arms, single-blind, randomized controlled study
Patients Selection	Inclusion Criteria: (1) age between 18 and 80 years; (2) histologically or cytologically confirmed left-sided colon cancer (distal transverse colon, left colic flexure, descending colon, or proximal sigmoid colon); (3) clinical stage T1–4a, N0–2, and M0;

	(4) Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2; (5) suitable for laparoscopic colectomy; (6) no previous systemic chemotherapy or radiotherapy; (7) willing to provide written informed consent and comply with the research procedures.
	Exclusion criteria (1) cardiopulmonary dysfunction (NYHA cardiac function classification II-IV), liver dysfunction (MELD score greater than 12), or kidney dysfunction (serum creatinine above the upper limit of normal); (2) metastatic or multiple carcinoma; (3) patients with bowel obstruction, bowel perforation, or bowel bleeding requiring emergency surgery; (4) be accompanied with severe psychiatric illness; (5) be pregnant or during lactation; (6) fail to control diabetes mellitus (6.1–8.3 mmol/L); (7) patients with a history of taking hormone drugs; (8) planned synchronous abdominal organ resections; (9) end-to-end anastomosis.
	Exit criteria (1) After enrollment, patients who need emergency surgical resection due to intestinal obstruction, intestinal perforation, or intestinal bleeding. (2) Patients with distant metastases confirmed by intraoperative exploration or postoperative pathology. (3) Combined organ resection is required after intraoperative exploration. (4) Serious adverse events occurred preoperatively (according to the

	Common Terminology Criteria for Adverse Events, CTCAE version 5.0).
Intervention Methods	Intracorporeal anastomosis group: Total laparoscopic technology is used. The tumor is resected according to surgical specifications for radical colon cancer surgery. Mesentery resection is performed under laparoscopy, and anastomosis is completed under laparoscopy. A small incision is made to extract the specimen. Extracorporeal anastomosis group: Traditional laparoscopy-assisted technology is used. The tumor is resected according to surgical specifications for radical colon cancer surgery. A small incision is made in the middle of the abdomen to trim the mesentery, remove the specimen, and complete the anastomosis operation.
Primary Outcome Measures	The primary endpoint of this study was the surgical site infection within 30 days after surgery.
Follow-up Plan	The follow-up period for the primary endpoint was within 30 days after surgery. Follow-up is done through in-patient observation, outpatient consultations, and telephone inquiries. On the 30th day after surgery, patients are required to return to the hospital for blood routine, liver function, kidney function, urinary routine, lung CT, and abdominal CT examinations. It also documents the patient's surgery-related complications and grades over this period, as well as solutions. The total follow-up time for patients after surgery is 5 years. For patients with recurrent tumors, follow-up should continue for at least 3 years after recurrence or metastasis is discovered or until death.
Sample Size	354 cases

Statistical Methods	1 Full analysis set A comparative analysis of the primary outcomes will be conducted based on “modified intention-to-treat (mITT)”. In the mITT analysis, patients will be analyzed according to their randomization allocations. 2 Per-protocol analysis In per-protocol analysis, only cases treated strictly in accordance with random groups and assigned treatment plans will be included. Patients who haven't been treated with a randomly assigned protocol will be excluded from the analysis.

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1. Title

A Multicenter Randomized Clinical Trial Comparing Surgical Site Infection after Total Laparoscopic and Laparoscopic-assisted Radical Surgery for Left Colon Cancer (STARS)

2. Introduction

It is estimated that there were more than 1.09 million new cases of colon cancer and more than 500,000 deaths from colon cancer in 2018. In developed countries, the incidence of colon cancer is higher than in developing countries. Usually, cancers proximal to the splenic flexure are defined as right-sided and cancers at or distal to the splenic flexure are defined as left-sided (1). It is reported that left-sided colon cancer has different clinical and biological characteristics from right-sided colon cancer (2) and has a more favorable prognosis than the latter (3). Although neoadjuvant therapy and postoperative chemotherapy had been demonstrated to reduce the risk of tumor recurrence and death (4,5), surgery remains the main treatment for patients with potentially curable colon cancer(6). Currently, main surgical methods include traditional open surgery, minimally invasive surgery using laparoscopic techniques, and emerging robotic surgery. Compared with open surgery, laparoscopic surgery has achieved similar oncological outcomes in colon cancer or left-sided colon cancer (7,8). As for overall survival, laparoscopic surgery has been demonstrated oncologically safe, and was non-inferior to open surgery for patients with stage II or III colon cancer (9,10). However, surgical site infection (SSI) is one of the most common nosocomial infection in colonic surgery, with an incidence that varies between 5 and 30%(11,12). SSI is usually split into superficial incisional SSI, deep incisional SSI, and organ/space SSI(13,14). Data from the American College of Surgeons National Surgical Quality Improvement Program showed the overall SSI rate was 12.3%, and left resections had increased odds of superfici al SSI compared with right resections (15). There is an urgent need to determine the incidence of SSI, as well as strategies to reduce SSI rate(16). The aim of STARS trial is to evaluate the incidence of SSI and safety of the intracorporeal anastomosis after left-sided colon surgery.

3. Study Purpose and Endpoint

3.1 Study purpose

3.1.1 Main study purpose:

To investigate the effects of intracorporeal anastomosis and extracorporeal anastomosis in laparoscopic-assisted radical left hemicolectomy on surgical site infection.

3.1.2 Secondary study purpose

To investigate the difference in postoperative recovery, safety, and oncology outcomes between intracorporeal anastomosis and extracorporeal anastomosis in laparoscopic-assisted radical left hemicolectomy.

3.2 Study endpoint

3.2.1 Primary endpoint and its definitions

The primary endpoint is SSI within 30 postoperative days. SSI was defined according to the U.S. Centers for Disease Control and Prevention (Attachment 1). The overall SSI included any superficial and deep incisional SSIs or organ/space SSI. Any patient suspected of having organ/space SSI underwent abdominal computed tomography (CT) or digestive contrast radiography to further confirm anastomotic leakage. In our study, anastomotic leakage was categorized as organ/space infection.

3.2.2 Secondary endpoint and its definitions

The secondary outcomes included:

(1) Operating time: It is defined as the period from cutting the skin to suturing the skin or doing enterostomy. It is measured in minutes.

(2) Total blood loss during operation: It is defined as the blood loss during operation and is measured in milliliters.

(3) Conversion to open surgery: It is defined as an abdominal incision larger than that necessary for specimen extraction.

(4) Postoperative complications: Postoperative complications are investigated within 30 days after an operation. It includes fever of unknown origin, bowel obstruction, anastomotic leakage, SSI, other incisional complications, respiratory complications, urinary complications, cardiovascular and cerebrovascular complications, diarrhea, chylous fistula, intraperitoneal

hemorrhage, digestive hemorrhage, gastroparesis, and others (including bacteremia, cholecystitis, ion discharge, pancreatitis, and mental and behavioral abnormalities). Complications are graded according to the Clavien–Dindo classification (17). The diagnosis of anastomotic leakage is based on the patient's postoperative clinical symptoms, signs of abdominal infection, or drainage properties and is confirmed by imaging (angiography or computed tomography) or secondary surgery. Postoperative intraperitoneal/digestive hemorrhage is defined as bloody drainage/bloody stool > 100 mL in 1 h or evidence of hemorrhage on CT, endoscopy, or angiography. The respiratory complications include pulmonary complications or pleural effusion. Urinary complications include infections, retention, and leakage.

(5) Completeness of specimens: It is evaluated according to the West classification.(18) The resected specimens will be classified into three groups according to the plane of dissection: mesocolic plane, intramesocolic plane, and muscularis propria plane

(6) Number of lymph nodes dissected: The number of lymph nodes in the mesentery will be calculated. Additionally, the metastatic lymph nodes will be counted. The pathological TNM staging was performed according to the American Joint Committee on Cancer (AJCC) Cancer Staging Manual, 8th edition.

(7) Postoperative recovery: Postoperative recovery information includes time to first flatus, time to first defecation, time to start food intake, and postoperative hospital stay. These are measured in days.

(8) Length of incision: The incision length is measured with an aseptic ruler at the end of the surgery, after the incision is sutured. It is measured in millimeters.

(9) Visual analog score (VAS) of pain: Pain severity was assessed 48 hours after the operation using a ruler about 10 cm long. The ruler is numbered from 0 to 10. 0–3 points indicate no to mild pain. 4–6 points represent moderate pain. 7–10 points stand for severe pain.

(10) 3-year disease-free survival (DFS) and 5-year overall survival (OS): DFS was defined as the time from randomization until the discovery of local recurrence, distant metastasis, or death from the tumor. OS was defined as the time from randomization to death

due to any cause.

4. Study design

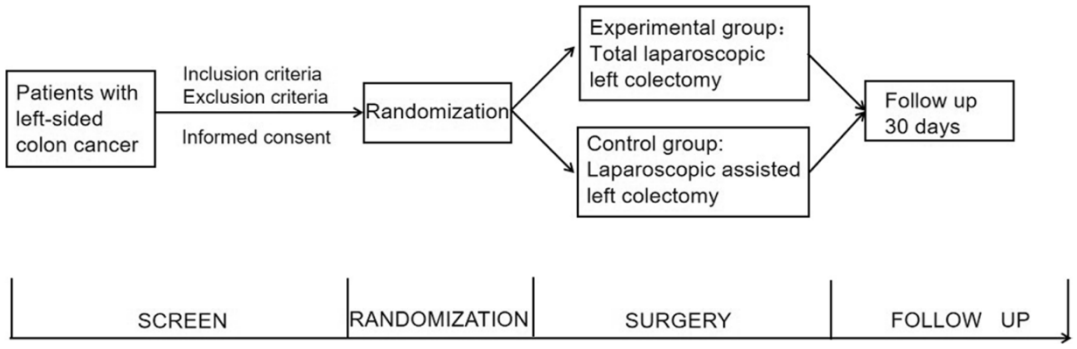
4.1 Overall study design

This is a prospective, multicenter, non-inferiority, two-arms, single-blind, randomized controlled study.

4.2 Study time frame

The subject recruitment period is 3 years, and the follow-up period lasts 5 years. The entire study, which includes study establishment, patient recruitment, patient follow-up, and data analysis, is expected to take 8 years. We'll conduct a primary endpoint analysis of the study after all patients finish their first month of post-operative follow-up. Additionally, a second analysis of results involving survival data will be done after all patients complete 5 years of follow-up.

Figure 1 the study design flowchart



5. Study population

5.1 Inclusion criteria

(1) age between 18 and 80 years;

(2) histologically or cytologically confirmed left-sided colon cancer (distal transverse colon, left colic flexure, descending colon, or proximal sigmoid colon);

(3) clinical stage T1–4a, N0–2, and M0;

(4) Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2;

(5) suitable for laparoscopic colectomy;

(6) no previous systemic chemotherapy or radiotherapy;

(7) willing to provide written informed consent and comply with the research procedures.

5.2 Exclusion criteria

(1) cardiopulmonary dysfunction (NYHA cardiac function classification II-IV), liver dysfunction (MELD score greater than 12), or kidney dysfunction (serum creatinine above the upper limit of normal);

(2) metastatic or multiple carcinoma;

(3) patients with bowel obstruction, bowel perforation, or bowel bleeding requiring emergency surgery;

(4) be accompanied with severe psychiatric illness;

(5) be pregnant or during lactation;

(6) fail to control diabetes mellitus (6.1–8.3 mmol/L);

(7) patients with a history of taking hormone drugs;

(8) planned synchronous abdominal organ resections;

(9) end-to-end anastomosis.

5.3 Exit criteria

(1) After enrollment, patients who need emergency surgical resection due to intestinal obstruction, intestinal perforation, or intestinal bleeding.

(2) Patients with distant metastases confirmed by intraoperative exploration or postoperative pathology.

(3) Combined organ resection is required after intraoperative exploration.

(4) Serious adverse events occurred preoperatively (according to the Common Terminology Criteria for Adverse Events, CTCAE version 5.0).

The data of patients who withdrew from the study were not used, and these patients were not allowed to re-enter the study.

6. Study treatment grouping

Experiment group: Total laparoscopic intracorporeal anastomosis group (IA group)

The tumor is resected according to surgical specifications for radical colon cancer surgery. Mesentery resection is performed under laparoscopy, and anastomosis is completed under laparoscopy. A small incision is made to extract the specimen after the anastomosis is completed.

Control group: Laparoscopy assisted extracorporeal anastomosis group (EA group)

The tumor is resected according to surgical specifications for radical colon cancer surgery. A small incision is made in the middle of the abdomen to trim the mesentery, remove the specimen, and complete the anastomosis. After completing the anastomosis, the incision will be sutured.

6.1 Randomization and allocation concealment

Randomization will be conducted immediately after recruitment. To guarantee randomization concealment adequately, and not be influenced either by the surgeon or the participants, randomization will be performed by a researcher who is not participating in this study from the Research Centre of Clinical Epidemiology (RCCE), Jilin University First Hospital. The random codes will be designed in a 1:1 ratio (experimental group or control group) using the R (version 3.3.3, University of Auckland, Oakland, New Zealand). The random codes will be sealed in an opaque envelope and handed to the researcher responsible for grouping in RCCE. The site investigators can submitted the participants' details to the RCCE and apply for randomization through text messages, e-mail, or telephone application. The researcher responsible for grouping in RCCE will review patient eligibility. When grouping, the envelope with the unique identification number for the participant will be opened, and the group assignment will immediately be delivered to the researcher of the study site by RCCE through text messages, e-mail, or telephone.

6.2 Blinding and unblinding

The clinical surgeons, outcome assessors, data recorders, and statisticians will all operate independently. Due to the nature of the surgical interventions, the surgeons will not be blinded to treatment allocation. However, the outcomes assessor who assess or analyze the end point will be blinded. The patients will also be blinded to group allocation to reduce the risk of bias. Unblinding will not be performed unless the finalization of the main data analysis or required for patient's safety. After the data analysis, we will have a blinded interpretation of the study results to minimize misleading data interpretation.

7. Intervention and study flow

7.1 Preparation procedures

Prior to the surgical intervention, patients are required to undergo a series of preparation steps to ensure optimal conditions for surgery and to minimize the risk of complications. On the evening before the operation, patients will consume a solution containing polyethylene glycol electrolytes to cleanse the bowel. This bowel preparation is crucial for visualization during surgery and to reduce the risk of infection. The day before surgery, patients will also be administered oral antibiotics, specifically metronidazole at a dosage of 800 mg and gentamycin sulfate at 160 mg, to target a broad spectrum of potential infectious agents. Furthermore, as a prophylactic measure against infection, mezlocillin sodium will be given 20 minutes prior to the induction of anesthesia.

7.2 Surgical Techniques:

General Surgical Procedures: On the day of surgery, patients are positioned supine on the operating table with their legs spread to allow ample space for the surgical team. The surgeon and assistant will be positioned on the right side of the operating bed, facilitating access to the surgical site. The initial incision, approximately 10mm in size, will be made above the umbilicus using an open technique, which allows for the introduction of the primary port for laparoscopic instruments. Subsequently, four additional ports will be strategically placed under laparoscopic guidance. Throughout the procedure, the principles of complete mesocolic excision (CME) and central vascular ligation will be adhered to. This involves a medial-to-lateral approach, where mesenteric vessels, including the inferior mesenteric vein, left colic artery, and one to two sigmoid arteries, are carefully exposed and divided at their origins using either absorbable or non-absorbable clips. The greater omentum is then separated from the transverse colon using a harmonic scalpel, and the splenic flexure and descending colon are mobilized.

7.2.1 Experimental Group Procedure

In the experimental group, the surgeon will use a 10-cm medical suture and methylene blue solution to mark the resection margin. The marginal vessels and mesentery will be divided inside the body. The proximal and distal colons are resected using a 60mm linear laparoscopic stapler. Side-to-side intracorporeal anastomotic techniques like anti-peristaltic, iso-peristaltic, or overlap methods will be applied (Figure 2-4). Once the anastomosis is

completed, the specimen is retrieved. The surgeon can place the specimen in a sterile plastic bag for retrieval. Alternatively, the surgeon can use a disposable incision retraction fixator to protect the wound. An abdominal drainage tube is inserted. Three different intracorporeal anastomotic methods are shown in Figures 2, 3, and 4.

7.2.1.1 Isoperistaltic Side-to-Side Anastomosis: This method involves making two small enterotomies on the antimesenteric borders, approximately 8 cm from the ends of the colon. The colonic ends are then positioned in the upper left abdomen. Each end of a 60mm linear laparoscopic stapler is inserted through the enterotomies, closed along the antimesenteric borders, and fired to create the anastomosis (Figure 2A). Subsequently, the common enterotomy is continuously sutured using a 3-0 knotless absorbable suture (Figure 2B).

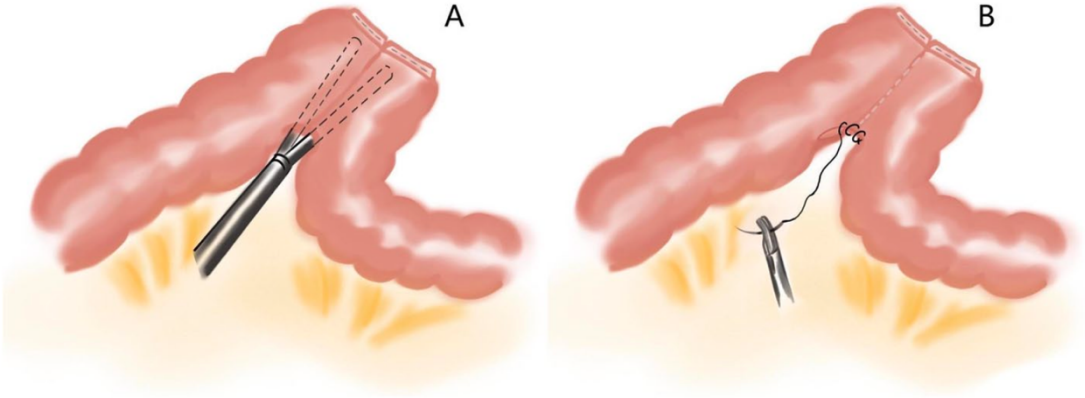


Fig. 2 A Two ends of a 60-mm linear laparoscopic stapler are inserted into the colons through enterotomies separately, then close along the antimesenteric borders and fire. B The common enterotomy is sutured continuously.

7.2.1.2 Antiperistaltic Side-to-Side Anastomosis: Similar to the isoperistaltic method, two small enterotomies are made on the antimesenteric borders of the colon ends. These ends are placed near the midline of the abdomen. The 60mm linear laparoscopic stapler is used in the same manner to close the antimesenteric borders and create the anastomosis (Figure 3A). The common enterotomy can then be closed using either a reloaded 60mm linear laparoscopic stapler positioned perpendicular to the colon ends (Figure 3B) or by continuous suturing with a 3-0 knotless absorbable suture.

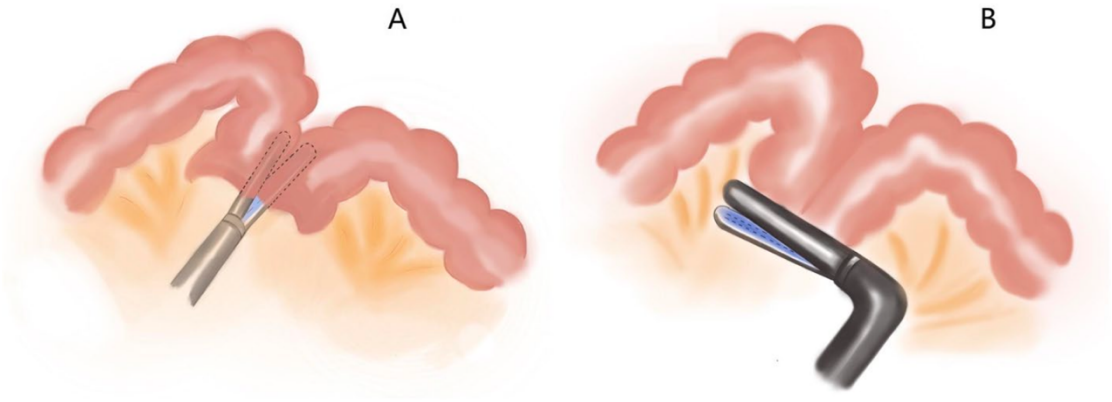


Fig. 3 A Two ends of 60-mm linear laparoscopic stapler are inserted into the colons through enterotomies separately, then close along the antimesenteric borders and fire. B The common enterotomy is closed by a reload 60-mm linear laparoscopic stapler perpendicular to colon ends

7.2.1.3 Overlap Side-to-Side Anastomosis: The choice between a cephalic or caudal approach for inserting the linear laparoscopic stapler depends on the tumor location and the ease of anastomosis. When the transverse colon end has limited mobility, the caudal approach offers greater convenience (Figure 4A). In this approach, one small enterotomy is made at the antimesenteric border of the transverse colon end, and another enterotomy is made approximately 8 cm from the descending or sigmoid colon end. The 60 mm linear laparoscopic stapler is inserted through the caudal trocars, with both ends being introduced into the colons via the enterotomies, closed along the antimesenteric borders, and fired to complete the anastomosis (Figure 4B). The common enterotomy is then continuously sutured using a 3-0 knotless absorbable suture. In the cephalic approach, the positions of the two enterotomies on the colons are made in opposition to those in the caudal cases.

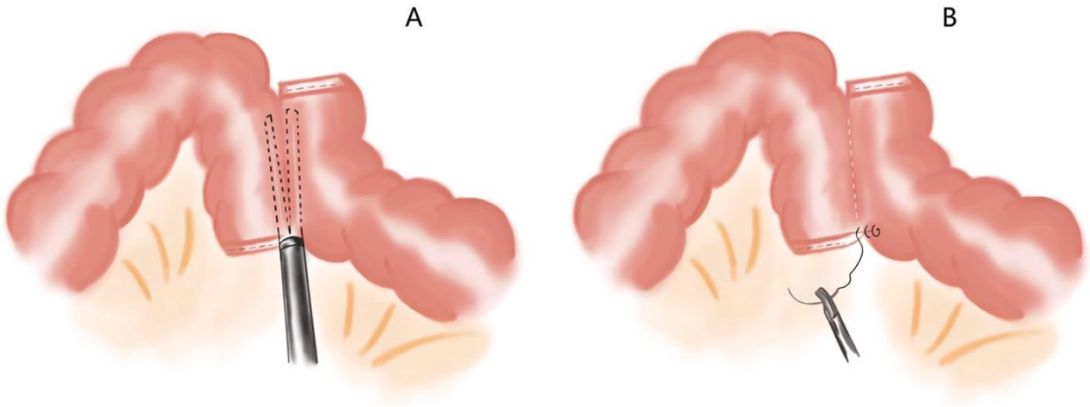


Fig. 4 A Two ends of 60-mm linear laparoscopic stapler are inserted into the colons through

the enterotomies separately, then close along the antimesenteric borders and fire. B The common enterotomy is sutured continuously

7.2.2 Control Group Procedures:

For patients in the control group, the surgeon uses wound edge protectors to exteriorize the colon through a small incision in the midline of the abdomen. A ruler and methylene blue solution are employed to mark the area for colon resection. This guarantees a 10-cm margin from the tumor. Guided by these markers, the marginal vessels and mesentery are divided outside the body. The method of anastomosis is at the surgeon's discretion. A side-to-side anastomosis (including antiperistaltic, isoperistaltic, or overlapping anastomosis) is performed. After completing the anastomosis, the incision is sutured. An abdominal drainage tube is inserted at the end of the operation.

Table 1 The time point and assessments of the STARS study

Time point	Study period							
	Screening	Treatment	Follow-up (month)					
			1	12	24	36	48	60
Informed consent	√							
Inclusion criteria	√							
Exclusion criteria	√	√	√	√	√	√	√	√
Baseline data	√							
Blood test	√							
Tumor marker test	√							
Chest CT	√							
Pelvic MRI	√							
Abdominal CT	√		√					
Electrocardiogram	√							
Colonoscopy	√							
Surgery		√						
Complications		√	√					
Recurrence/death			√	√	√	√	√	√

7.3 Concurrent treatment and follow-up.

Postoperative chemotherapy is recommended according to the NCCN guidelines for colon cancer treatment. The post-operative follow-up period must be in accordance with the time specified in this study (Table 1). Each participant will be followed until recurrence or death over a period of 5 years after the scheduled surgery.

The follow-up period for the primary endpoint is within 30 days after surgery. Follow-up will be conducted through in-patient observation, outpatient consultations, and telephone

inquiries. SSI presence will be assessed daily during hospitalization and weekly after discharge. At least two surgeons perform SSI surveillance. The outcome assessors are trained on the Centers for Disease Control and Prevention (CDC) definitions of SSI to produce good inter-rater reliability for the assessment of SSI. If SSI is suspected during follow-up, CT or abdominal ultrasonography will be done. All patients will have an abdominal or pelvic CT on the 30th day after surgery. If the CT shows evidence of SSI, the radiologist and surgeon will assess whether there is an SSI.

The patient's surgery-related complications and Clavien–Dindo grade within 30 postoperative days will be recorded. The overall follow-up period for patients after surgery is 5 years, which includes 5-year overall survival (OS) and 3-year disease-free survival (DFS). For patients with recurrent tumors, follow-up should continue for at least 3 years after recurrence or metastasis is discovered or until death.

Appropriate strategies will be used to improve patient adherence and ensure data integrity. When signing the informed consent form, all participants will be informed of the study procedures, potential benefits, and risks to make them fully understand the inconvenience and significance of data collection.

In addition, patients who have had abdominal surgery need to return to our hospital for other treatment of combined injuries, including suture removal. Our medical team will provide standard post-operative care for enrolled participants to promote their adherence.

Investigators will contact patients 1 week before the follow-up session to increase the attendance rate. Patients who miss a scheduled appointment will be contacted to reschedule another appointment within 1 week. Telephone follow-up calls, WeChat group discussions, and personal interviews will be used to maximize retention and complete follow-up.

7.4 Patients compliance and exit

To improve patient compliance, before randomization, physicians will communicate with patients in detail about surgical procedures, possible random groupings, relevant and evolving treatment, and patient safety and security conditions. They will obtain informed consent to ensure patients clearly understand the treatment and research procedures. The randomization time should be as close as possible to the time before surgery (the day before surgery) to

prevent accidental exclusion of patients after randomization. After randomization, patients can withdraw from the trial at any time. If a patient requests withdrawal from the study, the main researcher at their research center will communicate with them to avoid disrupting the balance of random groups due to exclusion. If the patient still insists on withdrawing, they will be removed from the trial. Clinical information relating to this patient will not be included in any data analysis related to this trial.

7.5 Deviation from the plan

Researchers involved in the trial will conduct corresponding surgical operations and data recording in line with the experimental requirements. Any event that strays from the original test plan must record the time and specific reason for occurrence to explain the deviation from the test plan.

8 Evaluation

8.1 Efficacy Evaluation

The methodologies for evaluating the efficacy of diverse anastomosis techniques encompass, but are not restricted to: intraoperative assessment and meticulous records of postoperative examinations, evaluation of postoperative symptoms and signs, and postoperative abdominal CT evaluation.

8.2 Safety Assessment

8.2.1 Baseline Signs and Symptoms

The baseline signs and symptoms include temperature, pulse, respiratory rate, and blood pressure. Surgeons also need to pay attention to specific symptoms such as dizziness, nausea, and vomiting. Additionally, hematological tests and imaging tests before surgery will be performed to ensure the patient group has no surgical contraindications and fully meets the inclusion criteria. These baseline data will serve as reference indicators for the evaluation.

8.2.2 Laboratory Safety Assessment

Continuous indicators show the postoperative measurements of each study subject and

the changes in values compared to the preoperative baseline. Laboratory indicators are classified as low, normal, and high based on the normal value range. They describe transitions from a normal or high baseline to a low value after the baseline, and from a normal or low baseline to a high value after the baseline. The lower or higher value after the baseline is calculated using the minimum or maximum observed value. The physician evaluates these abnormal indicators to determine their clinical significance. The proportion of subjects with "abnormal and clinically significant" changes is described.

8.2.3 Physical Examination and Vital Signs

Physical examination includes general examination, head examination, neck examination, chest and lung examination, heart and blood vessels examination, abdominal examination, spine and limbs examination, and nervous system examination. Vital signs consist of blood pressure, heart rate, and body temperature. Situations where vital signs deviate from the normal range or are abnormal are described and recorded.

9. Adverse event report

9.1 Definition of adverse events

Adverse events studied include intraoperative secondary injuries in patients and serious complications and deaths within 30 days after surgery. All adverse events reported spontaneously by test subjects or observed by staff will be recorded in a database and promptly reported to the test steering committee.

An adverse event is defined as any adverse experience that occurs in the subject during the study, regardless of whether it's considered associated with total laparoscopic anastomosis. All adverse events reported spontaneously by test subjects or observed by investigators are recorded. These include: 1) Accidental secondary damage during surgery. 2) Serious complications after surgery (grade III and above).

9.2 Severe adverse event

A serious adverse event includes any of the following adverse medical events: 1) events leading to death; 2) life-threatening events; 3) requiring hospitalization or prolonged hospitalization of patients; 4) Causing persistent or significant disability, or incapacity for

conduct.

9.3 Severity assessment

The severity of adverse events will be assessed using the Clavien—Dindo grading criteria for complications.

9.4 Relevance judgement

Determining the correlation between adverse events and surgical interventions will be made by researchers subjectively.

10. Data processing and storage

All relevant clinical data including demographic and medical information will be collected from the electronic medical record. The collected data will be encoded to protect the privacy of all participants and then be entered into an electronic case report forms (eCRFs) by trained research assistants in each sub-center. Double data entry will be performed to ensure that the data collected are accurate and verifiable from source documents. Input rules including field and range checks will also be set to minimize data entry errors. The research coordinator will periodically check the data distribution, verify outliers, and track missing data to improve data integrity and data validation. Data storage and backup will be managed by the edocdata platform (https://jlcrc.edocdata.com/), which will track all changes to the data and retains a history for each variable. Unauthorized persons or institutions will not be able to access any data stored on this server. When the trial is completed, the data will be locked and stored on edocdata platform, after which the researchers can no longer modify the data. Both paper and electronic documents will be preserved for at least 5 years after publication.

11. Quality management

Research center participation criteria: Surgeons involved in this study must complete at least 100 cases of laparoscopy-assisted left colectomy and have a minimum of 20 cases of intraoperative anastomosis in laparoscopy-assisted left colectomy. This study establishes a trial steering committee (TSC). The TSC is composed of surgeons with at least 10 years of experience in colorectal cancer surgery. Before enrolling in the research center, participating centers should provide surgical videos of two consecutive IA cases and two EA cases by the

same operator recently to determine if the surgery quality meets expectations and if they can qualify as enrolment units. The evaluation method is the Delphi method.

Evaluation of surgical quality during research: Surgery quality is assessed using videos and photographs. Surgical teams are required to take photographs of the laparoscopic investigation of the tumor site, surgical area after lymph node removal, and the anastomosis. The TSC evaluates the videos and photographs to assess surgical quality and ensure compliance with the allocated intervention. Any surgical procedures deviating from the randomized allocation plan are documented. If participating centers cannot obtain imaging data, they must document the reason for not being able to leave a video or photo.

Surgeons participating in the trial are required to record the surgical procedure and explain any surgical procedures that deviate from standardized procedures. The trial steering committee will evaluate the enrollment situation and quality of enrolled cases at each center every half a year and supervise and control the quality of enrolled cases at each center.

12. Statistical analysis

12.1 Method for determining sample size

This study is a multicenter, randomized, controlled, noninferiority trial specifically designed for patients within the age range of 18 to 80 years old who are diagnosed with left-sided colon cancer. In light of the previous data from our single center, it has been observed that the incidence of overall surgical site infection following extracorporeal anastomosis left colon cancer surgery stands at 23.3%, while the incidence of SSI after intracorporeal anastomosis is 16.7%. When there is a difference in the SSI rate of more than 5% within 30 days after surgery, intracorporeal anastomosis is regarded as inferior to extracorporeal anastomosis. Consequently, the sample size for this study is calculated based on non-inferiority, taking into account a difference of 5%, a one-sided level of significance of 0.025, a ratio of 1:1, a power of 0.80, and a 20% drop-out rate. In total, 354 patients are required for this study, with 177 patients in the experimental group and an equal number of 177 patients in the control group.

12.2 Analytical population

12.2.1 Full analysis set

The primary outcome will be comparatively analyzed based on “modified intention-to-treat (mITT)”. In the mITT analysis, patients will be analyzed according to their randomization allocations. If patients randomly assigned to the IA group actually receive EA during surgery, they will be analyzed according to the IA group in mITT analysis. If patients randomly assigned to group EA actually receive IA during surgery, they will be analyzed according to group EA. Patients who meet the exit criteria after randomization will not be included in the mITT population.

12.2.2 Per-protocol analysis

In per-protocol (PP) analysis, only cases treated strictly in accordance with random groups and assigned treatment plans will be included. Patients who haven't been treated with a randomly assigned protocol will be excluded from the per-protocol analysis.

12.3 Effect analysis and statistical method

12.3.1 primary endpoint analysis

In analyzing the primary outcomes, the rate of overall SSI will be reported as frequencies and percentages. We will calculate the absolute risk differences and the corresponding 95% confidence interval (CI) between two groups. If the absolute risk differences and the corresponding 95% CI are within the non-inferiority margin (<5%), non-inferiority is established. If not, IA is not non-inferior to EA. The analysis of the primary endpoint will be carried out in the mITT population, the PP population.

12.3.2 Secondary endpoint analysis

The analysis of the secondary endpoints will be carried out in the mITT population. In the analysis of secondary endpoints, categorical variables will be reported as frequencies and percentages. Groups will be compared using the $\chi 2$ test or Fisher’s exact test. Absolute risk differences and the corresponding 95% CI will be calculated. Continuous variables will be described as mean ± standard deviation or median (quartile). Groups will be compared using the t-test or Mann-Whitney U test, as appropriate. Hodges-Lehmann method will be used to calculate the difference in median and the corresponding 95% CI.

The comparison of 3-year DFS and 3-year OS between the two groups will utilize the

Kaplan–Meier curve and the Cox proportional risk model. Relevant risk factors will be adjusted in Cox proportional risk model.

The missing data in this trial is expected to be minimal. If it occurs, it will be treated as censored data and not imputed. All statistical tests were 2-sided, and statistical significance was set at P <0.05. All statistical analyses were performed using R or SPSS.

12.4 Interim analysis

There will be no interim analysis for this study

12.5 Terminal analysis

We will conduct an analysis of the main endpoints of the study and publish the relevant findings after all patients have completed the first month of follow-up after surgery. After all patients have completed 5 years of follow-up, we will analyze results involving survival data, and publish relevant research results.

12.6 Data Monitoring Committee

The data and safety monitoring board (DSMB) was composed of senior surgeons, ethicists, and statistical analysts. Each member of the DSMB is independent of this trial and is not likely to affect the competitive interests of the trial. The DSMB will receive and review the safety data for this trial. If the DSMB believes the number of adverse events in the trial is biased between groups, they will notify the chair of the trial steering committee.

There will be no interim analysis for this project. However, once the study begins, the DSMB will evaluate the results of the study every year. The assessment included primary and secondary endpoint records, adverse events, and loss of study samples. It also assesses differences with expectations, and potential conflicts with new findings and/or developments in the field of colon cancer. DSMB members are not allowed to share confidential information with anyone outside of the DSMB, with the exception of the trial steering committee.

The techniques used in this research institute are all techniques currently used in surgical treatment of colon cancer, and patients are not at higher risk. Therefore, this study did not set suspension parameters for the entire project. However, if in the DSMB's regular assessments, a center shows a huge difference in safety indicators compared to other centers and is significantly worse than the current international level, then the DSMB will submit its

recommendations in writing to the trial steering committee, requesting that the trial plan be revised, or the quality of cases in the corresponding center be re-evaluated, or even suspended or terminated the clinical trial enrollment of the corresponding center.

At the discretion of the DSMB during the trial process, the DSMB may decide to meet early to discuss testing procedures together about adverse events and promptly clarify any issues with key investigators.

13. Data collection and management

13.1 Electronic Case Report Form

An electronic case report form (eCRF) is developed by the lead investigator. The CRF table includes the primary endpoint and Secondary endpoints. Additionally, general baseline data on patients will be included.

All relevant clinical data including demographic and medical information will be collected from the electronic medical record. The collected data will be encoded to protect the privacy of all participants and then be entered into an eCRF by trained research assistants in each sub-center. Double data entry will be performed to ensure that the data collected are accurate and verifiable from source documents. Input rules including field and range checks will also be set to minimize data entry errors. The research coordinator will periodically check the data distribution, verify outliers, and track missing data to improve data integrity and data validation.

13.2 Data management

Data storage and backup will be managed by the edocdata platform (https://jlcrc.edocdata.com/), which will track all changes to the data and retains a history for each variable. Unauthorized persons or institutions will not be able to access any data stored on this server. When the trial is completed, the data will be locked and stored on edocdata platform, after which the researchers can no longer modify the data. Both paper and electronic documents will be preserved for at least 5 years after publication.

14. Ethics

14.1 Ethics Committee

This trial has been approved by the Ethics Review Committee of the First Hospital of

Jilin University (Approval number: 19K135-001). Each sub-center must conduct an ethical application before participating in this trial.

14.2 Patients information and informed consent

Eligible patients should receive in-person notification from the treating surgeon and written information about the trial. Each patient should provide informed consent in accordance with local ethics committee guidelines before entering the random allocation of studies.

The informed consent procedure should be carried out by the doctors of each centre. Information provided to patients with informed consent includes: 1) a statement about the study involved in the trial; 2) a full and fair explanation of the procedures to be followed; 3) a full explanation of the nature, anticipated length, and purpose of the study; 4) a description of any risk or discomfort that the patient can reasonably anticipate; 5) a description of any benefits that can be reasonably anticipated; 6) a statement that patient data will be treated with care and confidentiality and that the data will be kept for 15 years; 7) a statement about the patient's physical material will be kept for 15 years; 8) a statement about voluntary participation, where the statement will state the refusal Participation in the trial does not involve any punishment or loss of benefits the patient is entitled to, and patients can stop participating at any time without being penalized or losing benefits, in which case the patient will receive standard treatment with the same level of care.

15. Definition of Study Completion

The completion of the study was defined as all enrolled patients completed 5 years of follow-up. Follow-up outcomes include disease-free survival, recurrence survival, death, or loss of visits.

16. Study organizational structure

This study includes several medical centers. The research initiation center is First Hospital of Jilin University. Participating centers in clinical research include: Peking Union Medical College Hospital, Fudan University Cancer Hospital, Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Beijing Friendship Hospital Affiliated to

Capital Medical University, Chinese People's Liberation Army General Hospital, Daping Hospital Affiliated to Army Military Medical University, Shengjing Hospital Affiliated to Chinese Medical University, Chinese Academy of Medical Sciences Cancer Hospital, China - Japan Union Hospital of Jilin University, First Affiliated Hospital of China Medical University, Southern Medical University Southern College. The members of the TSC are heads of the corresponding departments of the above centers: Wang Quan, Lin Guole, Li Xinxiang, Feng Bo, Zang lu, Yao Hongwei, Du Xiaohui, Tong Weidong, Zhang Hong, Liu Qian, Xie Zhongshi, Wu Aiwen, and Deng Haijun. The members of the trial guidance team are attending physicians and above in the corresponding medical teams of each center. The DSMB is comprised of senior surgeons, ethicists, and statistical analysts. Each member of the DSMB is independent of this trial and is not likely to affect the competitive interests of the trial.

17. Confidentiality and Data Security

Participants are given a unique code to maintain their anonymity. Personal information documents are stored separately from assessment data in locked cabinets. Only the authorized research assistants and DSMB can access to the dataset. Unless there is an institutional or regulatory requirement, nobody else can access it. DSMB members can know the patient allocation and be aware of accumulated data. The DSMB will discuss the relevant data results with the TSC. DSMB members are not allowed to share confidential information with anyone outside the DSMB. The fully anonymized experimental data is stored on secure servers of Jilin University First Hospital.

18. Insurance and Compensation

Enrolled patients have all purchased medical commercial insurance jointly offered by our hospital and insurance companies before surgery. This insurance covers surgery-related complications and deaths. This study was funded by the Development Center for Medical Science & Technology, National Health Commission of the People’s Republic of China (W2017ZWS01 and WA2021RW19). The funder had no role in the study's design and will not have any role in data collection, analysis, interpretation, or manuscript writing.

19. Publication notes

No relevant data for this study will be published until the collection of primary outcomes is completed. We will conduct an analysis of the primary endpoints of the study and publish the relevant findings after all patients have completed the first month of follow-up after surgery. After all patients complete 5 years of follow-up, the results involving survival data will be analyzed again, and the relevant research results will be published.

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21. Attachments

Attachment 1: Definition and classification of SSI according to CDC

Surgical site infection (SSI) can be subdivided into infections of the subcutaneous tissue (superficial SSI), deep soft tissues such as fascial and muscle layers (deep SSI) and infections of organs or spaces (organ/space SSI) that occur within 30 days after surgery.

Superficial Incisional SSI	Infection occurs within 30 days after the operation and infection involves only skin or subcutaneous tissue of the incision and at least one of the following: 1. Purulent drainage, with or without laboratory confirmation, from the superficial incision. 2. Organisms isolated from an aseptically obtained culture of fluid or tissue from the superficial incision. 3. At least one of the following signs or symptoms of infection: pain or tenderness, localized swelling, redness, or heat and superficial incision is deliberately opened by surgeon, unless incision is culture-negative. 4. Diagnosis of superficial incisional SSI by the surgeon or attending physician. **Notes**: Do not report the following conditions as SSI: 1. Stitch abscess (minimal inflammation and discharge confined to the points of suture penetration).
Deep Incisional SSI	Infection occurs within 30 days after the operation and the infection appears to be related to the operation and infection involves deep soft tissues (e.g., fascial and muscle layers) of the incision and at least one of the following:

	1. Purulent drainage from the deep incision but not from the organ/space component of the surgical site. 2. A deep incision spontaneously dehisces or is deliberately opened by a surgeon when the patient has at least one of the following signs or symptoms: fever (>38°C), localized pain, or tenderness, unless site is culture-negative. 3. An abscess or other evidence of infection involving the deep incision is found on direct examination, during reoperation, or by histopathologic or radiologic examination. 4. Diagnosis of a deep incisional SSI by a surgeon or attending physician. **Notes**: 1. Report infection that involves both superficial and deep incision sites as deep incisional SSI. 2. Report an organ/space SSI that drains through the incision as a deep incisional SSI.
Organ/Space SSI	Infection occurs within 30 days after the operation and the infection appears to be related to the operation and infection involves any part of the anatomy (e.g., organs or spaces), other than the incision, which was opened or manipulated during an operation and at least one of the following: 1. Purulent drainage from a drain that is placed through a stab wound into the organ/space. 2. Organisms isolated from an aseptically obtained culture of fluid or tissue in the organ/space.

	3. An abscess or other evidence of infection involving the organ/space that is found on direct examination, during reoperation, or by histopathologic or radiologic examination. 4. Diagnosis of an organ/space SSI by a surgeon or attending physician.

Attachment 2: ASA Classification Criteria

It refers to the six levels into which patients are classified by the American Society of Anesthesiologists (ASA) prior to anesthesia based on their physical condition and risk to surgery.

classification	condition
Level I	Physical health, good development and nutrition, and normal functioning of organs.
Level II	In addition to surgical disease, there is mild coexisting disease with sound functional compensation.
Level III	Coexisting conditions are severe and physical activity is limited, but can still manage daily activities.
Level IV	Severe coexisting conditions, loss of ability to perform daily activities, and frequent life-threatening situations.
Level V	Dying patients who have difficulty sustaining life for 24 hours, with or without surgery.
Level VI	Confirmed brain death and his organs are intended for organ transplantation.

Attachment 3: ECOG Physical Fitness Assessment Criteria

Physical condition	Grading
Normal activity	0
Mild symptoms, comfortable living, able to do light physical activities	1
Can withstand the symptoms of the tumor and take care of one's own life, but not spend more than 50% of the time in bed during the day	2
The symptoms are severe. You spend more than 50% of your time in bed during the day, but you can still get up and stand, so you can take care of yourself for part of your life	3
Sick and unable to stay bedridden	4
Death	5

Attachment 4: Postoperative Complications Clavien-Dindo System Grading

Grading		Definition
I		There are complications of unnecessary medication, surgery, laparoscopic, and reflex intervention after surgery, but they include medications for antiemetics, fever-reducing drugs, pain relievers, diuretics, dielectrics, and physiotherapy, as well as incision infections opening at the bedside;
II		Patients requiring medication not including phase 1 medication, incisional infections require antibiotic treatment, blood transfusions, and parenteral nutrition included
III.		Surgical, laparoscopic, and interventional radiological treatment is required
	IIIa	Does not require general anesthesia
	IIIb	General anesthesia required
IV		Life-threatening complications (including central nervous system complications) that require IC (intermittent monitoring) or ICU treatment
	iVa	Insufficiency of one organ (including dialysis)
	ivB	multiple organ dysfunction
V		Death

Attachment 5: TNM Stages of Colon Cancer (AJCC, 8th Edition, 2018)

Primary tumors:

Tx: the primary tumor cannot be evaluated;

T0: no evidence of primary tumor;

Tis: Carcinoma in situ, intramucosal cancer (the tumor invades the lamina propria of the mucosa but does not break through the myofascial layer);

T1: The tumor invades the submucosal layer (the tumor invades the submucosal layer but does not involve the intrinsic muscle layer);

T2: the tumor invades the intrinsic muscle layer;

T3: the tumor penetrates the intrinsic muscle layer to the peri-colorectal tissue;

T4: the tumor invades the sublayer of the peritoneum or invades or adheres to nearby organs or structures;

T4a: Tumor penetrates the mesenteric peritoneum (including intestinal perforation at the tumor site visible to the naked eye, and continuous infiltration of the tumor through the inflamed area to the surface of the peritoneum layer);

T4b: Tumor directly invades or attaches to neighboring organs or structures.

regional lymph nodes (N)

Nx: regional lymph nodes cannot be evaluated;

N0: no regional lymph node metastasis;

N1: There are 1-3 regional lymph node metastases (tumor diameter in the lymph nodes is greater than 0.2 mm), or no regional lymph node metastasis, but there are any number of tumor nodules;

N1a: there is 1 regional lymph node metastasis;

N1b: There are 2-3 regional lymph node metastases;

N1c: no regional lymph node metastasis, but tumor nodules in subserous, mesenteric, or peri-peritoneal tissues;

N2: There are 4 or more regional lymph node metastases;

N2a: There are 4-6 regional lymph node metastases;

N2b: There are more than 7 regional lymph node metastases.

Distant Metastasis (M)

Mx: distant transfers cannot be evaluated;

M0: no distant metastasis on imaging, that is, there is no evidence of metastatic tumors in distant sites and organs (this classification should not be determined by pathologists);

M1: the presence of metastases in one or more distal parts, organs, or peritoneum;

M1a: distant metastases are limited to a single distant site or organ, but there is no peritoneal metastasis;

M1b: Distal metastases distributed to two or more distant sites or organs, no peritoneal metastases;

M1c: Peritoneal metastases, with or without other site or organ metastases.

		N0	N1/N1c	N2a	N2b
Tis	0				
T1		I	IIIA	IIIA	IIIB
T2		I	IIIA	IIIB	IIIB
T3		IIA	IIIB	IIIB	IIIC
T4a		IIB	IIIB	IIIC	IIIC
T4b		IIC	IIIC	IIIC	IIIC
M1a		IVA	IVA	IVA	IVA
M1b		IVB	IVB	IVB	IVB
M1c		IVC	IVC	IVC	IVC

Attachment 6: The VAS pain score

It is a straight line about 10 centimeters long. One end of the line is marked as "0", representing no pain, and the other end is marked as "10", representing the most severe pain imaginable. There are no other scale marks on the line, maintaining a simple visual presentation.

Self - assessment by the Patient: The patient marks a point on the line according to his or her current pain experience to represent the degree of pain. This process is completely completed by the patient independently to fully reflect his or her subjective pain experience. For example, if the patient feels mild pain, he or she may mark a point closer to the "0" end; if the pain is very severe, the mark will be closer to the "10" end.

Degree of Pain	VAS Score Range	Description
No Pain - Mild Pain	0 - 3 points	A score of 0 represents no pain at all; a score of 1 - 3 indicates mild pain, which has a relatively minor impact on daily life and generally does not require strong painkillers.
Moderate Pain	4 - 6 points	The pain begins to have a certain impact on daily life, intensifies during daily activities, and may require relief measures such as rest and the use of over-the-counter painkillers.
Severe Pain	7 - 10 points	Unbearable pain that has a severe impact on the quality of life, greatly interfering with daily activities, sleep, and emotions, and requires strong painkillers.

Attachment 7 Completeness of Specimens

Grading	Descriptions
Mesocolic plane	Smooth intact mesocolic excision.
Intramesocolic plane	The excision disrupts the mesocolon but do not reach down to the muscularis propria.
Muscularis propria plane	The excision disrupts the mesocolon and exposed the muscularis propria.

Protocol

A Multicenter Randomized Clinical Trial Comparing Surgical Site Infection after Intracorporeal Anastomosis and Extracorporeal Anastomosis for Left Colon Cancer (STARS)

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Abstract

Title	A Multicenter Randomized Clinical Trial Comparing Surgical Site Infection after Total Laparoscopic and Laparoscopic-assisted Radical Surgery for Left Colon Cancer
Study Center	First Hospital of Jilin University Peking Union Medical College Hospital Cancer Hospital Affiliated to Fudan University Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine Beijing Friendship Hospital , Capital Medical University PLA General Hospital Daping Hospital Affiliated to Army Military Medical University Shengjing Hospital Affiliated to China Medical University Chinese Academy of Medical Sciences Cancer Hospital China-Japan Union Hospital of Jilin University The First Affiliated Hospital of China Medical University Nanfang Hospital of Southern Medical University
Study Purpose	The purpose of this study is to investigate the effects of intracorporeal anastomosis and extracorporeal anastomosis in laparoscopic-assisted radical left hemicolectomy on surgical site infection. Also consider perioperative recovery, safety, and oncology outcomes.
Study Type	A prospective, multicenter, non-inferiority, two-arms, single-blind, randomized controlled study
Patients Selection	Inclusion Criteria: (1) age between 18 and 80 years; (2) histologically or cytologically confirmed left-sided colon cancer (distal transverse colon, left colic flexure, descending colon, or proximal sigmoid colon);

	(3) clinical stage T1–4a, N0–2, and M0; (4) Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2; (5) suitable for laparoscopic colectomy; (6) no previous systemic chemotherapy or radiotherapy; (7) willing to provide written informed consent and comply with the research procedures.
	Exclusion criteria (1) Have a history of malignant colorectal tumor or have been recently diagnosed with other malignant tumors. (2) Patients with intestinal obstruction, intestinal perforation, intestinal bleeding, etc. who need emergency surgery. (3) Patients who need to undergo combined organ resection or robot-assisted colectomy. (4) Patients who are receiving preoperative neoadjuvant therapy. (5) ASA grade≥IV and/or ECOG performance status score > 2. (6) Patients with severe impairment of liver and kidney function, cardiopulmonary function, coagulation disorders or accompanied by severe underlying diseases and unable to tolerate surgery. (7) Patients with a history of severe mental illness. (8) Pregnant or lactating women. (9) Patients who have a history of taking hormonal drugs. (10) Diabetic patients whose blood sugar cannot be controlled to be within 6.1 - 8.3 mmol/L. (11) Patients with other clinical and laboratory conditions that are considered by researchers as inappropriate for participating in this trial.
	Exit criteria (1) Patients with other non-tumor diseases that prevent them from

	continuing to receive this treatment regimen. (2) Patients who need emergency surgical resection due to intestinal obstruction, intestinal perforation, intestinal bleeding, etc. after being enrolled in the study. (3) Patients with distant metastasis confirmed by intraoperative exploration or postoperative pathology, including liver, pelvic cavity, ovary, peritoneum, distant lymph node metastasis, etc. (4) Patients who need combined organ resection as determined by intraoperative exploration. (5) Patients who request to withdraw from this study cohort for various reasons after being enrolled in the study, or who cannot complete the study plan and follow-up for various reasons.
Intervention Methods	Intracorporeal anastomosis group: Total laparoscopic technology is used. The tumor is resected according to surgical specifications for radical colon cancer surgery. Mesentery resection is performed under laparoscopy, and anastomosis is completed under laparoscopy. A small incision is made to extract the specimen. Extracorporeal anastomosis group: Traditional laparoscopy-assisted technology is used. The tumor is resected according to surgical specifications for radical colon cancer surgery. A small incision is made in the middle of the abdomen to trim the mesentery, remove the specimen, and complete the anastomosis operation.
Primary Outcome Measures	The primary endpoint of this study was the surgical site infection within 30 days after surgery.
Follow-up Plan	The follow-up period for the primary endpoint was within 30 days after surgery. Follow-up is done through in-patient observation, outpatient consultations, and telephone inquiries. On the 30th day after surgery,

	patients are required to return to the hospital for blood routine, liver function, kidney function, urinary routine, lung CT, and abdominal CT examinations. It also documents the patient's surgery-related complications and grades over this period, as well as solutions. The total follow-up time for patients after surgery is 5 years. For patients with recurrent tumors, follow-up should continue for at least 3 years after recurrence or metastasis is discovered or until death.
Sample Size	350 cases
Statistical Methods	1 Full analysis set A comparative analysis of the primary outcomes will be conducted based on “modified intention-to-treat (mITT)”. In the mITT analysis, patients will be analyzed according to their randomization allocations. 2 Per-protocol analysis In per-protocol analysis, only cases treated strictly in accordance with random groups and assigned treatment plans will be included. Patients who haven't been treated with a randomly assigned protocol will be excluded from the analysis. 3 As-treated analysis set In the as-treated analysis set, we'll analyze patients according to the actual treatment plan they received instead of the allocated plan for random groups.

TABLE OF CONTENTS

1. Title

A Multicenter Randomized Clinical Trial Comparing Surgical Site Infection after Intracorporeal Anastomosis and Extracorporeal Anastomosis for Left Colon Cancer

2. Introduction

It is estimated that there were more than 1.09 million new cases of colon cancer and more than 500,000 deaths from colon cancer in 2018. In developed countries, the incidence of colon cancer is higher than in developing countries. Usually, cancers proximal to the splenic flexure are defined as right-sided and cancers at or distal to the splenic flexure are defined as left-sided (1). It is reported that left-sided colon cancer has different clinical and biological characteristics from right-sided colon cancer (2) and has a more favorable prognosis than the latter (3). Although neoadjuvant therapy and postoperative chemotherapy had been demonstrated to reduce the risk of tumor recurrence and death (4,5), surgery remains the main treatment for patients with potentially curable colon cancer(6). Currently, main surgical methods include traditional open surgery, minimally invasive surgery using laparoscopic techniques, and emerging robotic surgery. Compared with open surgery, laparoscopic surgery has achieved similar oncological outcomes in colon cancer or left-sided colon cancer (7,8). As for overall survival, laparoscopic surgery has been demonstrated oncologically safe, and was non-inferior to open surgery for patients with stage II or III colon cancer (9,10). However, surgical site infection (SSI) is one of the most common nosocomial infection in colonic surgery, with an incidence that varies between 5 and 30%(11,12). SSI is usually split into superficial incisional SSI, deep incisional SSI, and organ/space SSI(13,14). Data from the American College of Surgeons National Surgical Quality Improvement Program showed the overall SSI rate was 12.3%, and left resections had increased odds of superfici al SSI compared with right resections (15). There is an urgent need to determine the incidence of SSI, as well as strategies to reduce SSI rate(16). The aim of STARS trial is to evaluate the incidence of SSI and safety of the intracorporeal anastomosis after left-sided colon surgery.

3. Study Purpose and Endpoint

3.1 **Study purpose**

3.1.1 Main study purpose:

To investigate the effects of intracorporeal anastomosis and extracorporeal anastomosis in laparoscopic-assisted radical left hemicolectomy on surgical site infection.

3.1.2 Secondary study purpose

To investigate the difference in postoperative recovery, safety, and oncology outcomes between intracorporeal anastomosis and extracorporeal anastomosis in laparoscopic-assisted radical left hemicolectomy.

3.2 **Study endpoint**

3.2.1 Primary endpoint and its definitions

The primary endpoint is SSI within 30 postoperative days. SSI was defined according to the U.S. Centers for Disease Control and Prevention (Attachment 1). The overall SSI included any superficial and deep incisional SSIs or organ/space SSI. Any patient suspected of having organ/space SSI underwent abdominal computed tomography (CT) or digestive contrast radiography to further confirm anastomotic leakage. In our study, anastomotic leakage was categorized as organ/space infection.

3.2.2 Secondary endpoint and its definitions

The secondary outcomes included:

(1) Operating time: It is defined as the period from cutting the skin to suturing the skin or doing enterostomy. It is measured in minutes.

(2) Total blood loss during operation: It is defined as the blood loss during operation and is measured in milliliters.

(3) Conversion to open surgery: It is defined as an abdominal incision larger than that necessary for specimen extraction.

(4) Postoperative complications: Postoperative complications are investigated within 30 days after an operation. It includes fever of unknown origin, bowel obstruction, anastomotic

leakage, SSI, other incisional complications, respiratory complications, urinary complications, cardiovascular and cerebrovascular complications, diarrhea, chylous fistula, intraperitoneal hemorrhage, digestive hemorrhage, gastroparesis, and others (including bacteremia, cholecystitis, ion discharge, pancreatitis, and mental and behavioral abnormalities). Complications are graded according to the Clavien–Dindo classification (17). The diagnosis of anastomotic leakage is based on the patient's postoperative clinical symptoms, signs of abdominal infection, or drainage properties and is confirmed by imaging (angiography or computed tomography) or secondary surgery. Postoperative intraperitoneal/digestive hemorrhage is defined as bloody drainage/bloody stool > 100 mL in 1 h or evidence of hemorrhage on CT, endoscopy, or angiography. The respiratory complications include pulmonary complications or pleural effusion. Urinary complications include infections, retention, and leakage.

(5) Completeness of specimens: It is evaluated according to the West classification.(18) The resected specimens will be classified into three groups according to the plane of dissection: mesocolic plane, intramesocolic plane, and muscularis propria plane

(6) Number of lymph nodes dissected: The number of lymph nodes in the mesentery will be calculated. Additionally, the metastatic lymph nodes will be counted. The pathological TNM staging was performed according to the American Joint Committee on Cancer (AJCC) Cancer Staging Manual, 8th edition.

(7) Postoperative recovery: Postoperative recovery information includes time to first flatus, time to first defecation, time to start food intake, and postoperative hospital stay. These are measured in days.

(8) Length of incision: The incision length is measured with an aseptic ruler at the end of the surgery, after the incision is sutured. It is measured in millimeters.

(9) Visual analog score (VAS) of pain: Pain severity was assessed 48 hours after the operation using a ruler about 10 cm long. The ruler is numbered from 0 to 10. 0–3 points indicate no to mild pain. 4–6 points represent moderate pain. 7–10 points stand for severe pain.

(10) 3-year disease-free survival (DFS) and 5-year overall survival (OS): DFS was defined as the time from randomization until the discovery of local recurrence, distant metastasis, or

death from the tumor. OS was defined as the time from randomization to death due to any cause.

4. Study design

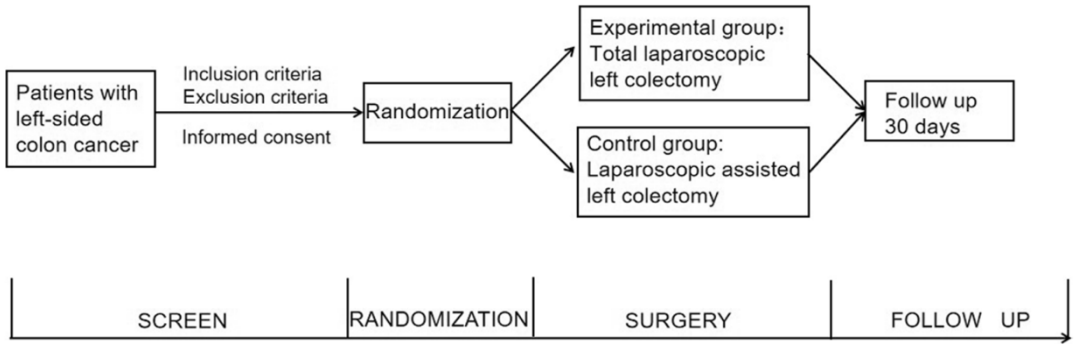
4.1 Overall study design

This is a prospective, multicenter, non-inferiority, two-arms, single-blind, randomized controlled study.

4.2 Study time frame

The subject recruitment period is 3 years, and the follow-up period lasts 5 years. The entire study, which includes study establishment, patient recruitment, patient follow-up, and data analysis, is expected to take 8 years. We'll conduct a primary endpoint analysis of the study after all patients finish their first month of post-operative follow-up. Additionally, a second analysis of results involving survival data will be done after all patients complete 5 years of follow-up.

Figure 1 the study design flowchart



5. Study population

5.1 Inclusion criteria

(1) age between 18 and 80 years;

(2) histologically or cytologically confirmed left-sided colon cancer (distal transverse colon, left colic flexure, descending colon, or proximal sigmoid colon);

(3) clinical stage T1–4a, N0–2, and M0;

(4) Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2;

(5) suitable for laparoscopic colectomy;

(6) no previous systemic chemotherapy or radiotherapy;

(7) willing to provide written informed consent and comply with the research procedures.

5.2 Exclusion criteria

(1) Have a history of malignant colorectal tumor or have metastatic or multiple carcinoma.

(2) Patients with intestinal obstruction, intestinal perforation, intestinal bleeding, etc. who need emergency surgery.

(3) Patients who need to undergo combined organ resection or robot-assisted colectomy.

(4) Patients who are receiving preoperative neoadjuvant therapy.

(5) ASA grade ≥ IV and/or ECOG performance status score > 2.

(6) Cardiopulmonary dysfunction (NYHA cardiac function classification II-IV), liver dysfunction (MELD score greater than 12), or kidney dysfunction (serum creatinine above the upper limit of normal);

(7) Patients with severe psychiatric illness.

(8) Pregnant or lactating women.

(9) Patients who have a history of taking hormonal drugs.

(10) Diabetic patients whose blood sugar cannot be controlled to be within 6.1 - 8.3 mmol/L.

(11) Patients with other clinical and laboratory conditions that are considered by researchers as inappropriate for participating in this trial.

5.3 Exit criteria

(1) Patients with other non-tumor diseases that prevent them from continuing to receive this treatment regimen.

(2) Patients who need emergency surgical resection due to intestinal obstruction, intestinal perforation, intestinal bleeding, etc. after being enrolled in the study.

(3) Patients with distant metastasis confirmed by intraoperative exploration or postoperative pathology, including liver, pelvic cavity, ovary, peritoneum, distant lymph node metastasis, etc.

(4) Patients who need combined organ resection as determined by intraoperative exploration.

(5) Patients who request to withdraw from this study cohort for various reasons after being enrolled in the study, or who cannot complete the study plan and follow-up for various reasons.

The data of patients who withdrew from the study were not used, and these patients were not allowed to re-enter the study.

6. Study treatment grouping

Experiment group: Total laparoscopic intracorporeal anastomosis group (IA group)

The tumor is resected according to surgical specifications for radical colon cancer surgery. Mesentery resection is performed under laparoscopy, and anastomosis is completed under laparoscopy. A small incision is made to extract the specimen after the anastomosis is completed.

Control group: Laparoscopy assisted extracorporeal anastomosis group (EA group)

The tumor is resected according to surgical specifications for radical colon cancer surgery. A small incision is made in the middle of the abdomen to trim the mesentery, remove the specimen, and complete the anastomosis. After completing the anastomosis, the incision will be sutured.

6.1 Randomization and allocation concealment

Randomization will be conducted immediately after recruitment. To guarantee randomization concealment adequately, and not be influenced either by the surgeon or the participants, randomization will be performed by a researcher who is not participating in this study from the Research Centre of Clinical Epidemiology (RCCE), Jilin University First Hospital. The random codes will be designed in a 1:1 ratio (experimental group or control group) using the R (version 3.3.3, University of Auckland, Oakland, New Zealand). The random codes will be sealed in an opaque envelope and handed to the researcher responsible for grouping in RCCE. The site investigators can submitted the participants' details to the RCCE and apply for randomization through text messages, e-mail, or telephone application. The researcher responsible for grouping in RCCE will review patient eligibility. When grouping, the envelope with the unique identification number for the participant will be opened, and the group assignment will immediately be delivered to the researcher of the study site by RCCE through text messages, e-mail, or telephone.

6.2 Blinding and unblinding

The clinical surgeons, outcome assessors, data recorders, and statisticians will all operate independently. Due to the nature of the surgical interventions, the surgeons will not be blinded to treatment allocation. However, the outcomes assessor who assess or analyze the end point will be blinded. The patients will also be blinded to group allocation to reduce the risk of bias. Unblinding will not be performed unless the finalization of the main data analysis or required for patient's safety. After the data analysis, we will have a blinded interpretation of the study results to minimize misleading data interpretation.

7. Intervention and study flow

7.1 Preparation procedures

Prior to the surgical intervention, patients are required to undergo a series of preparation steps to ensure optimal conditions for surgery and to minimize the risk of complications. On the evening before the operation, patients will consume a solution containing polyethylene glycol electrolytes to cleanse the bowel. This bowel preparation is crucial for visualization during surgery and to reduce the risk of infection. The day before surgery, patients will also be administered oral antibiotics, specifically metronidazole at a dosage of 800 mg and gentamycin sulfate at 160 mg, to target a broad spectrum of potential infectious agents. Furthermore, as a prophylactic measure against infection, mezlocillin sodium will be given 20 minutes prior to the induction of anesthesia.

7.2 Surgical Techniques:

General Surgical Procedures: On the day of surgery, patients are positioned supine on the operating table with their legs spread to allow ample space for the surgical team. The surgeon and assistant will be positioned on the right side of the operating bed, facilitating access to the surgical site. The initial incision, approximately 10mm in size, will be made above the umbilicus using an open technique, which allows for the introduction of the primary port for laparoscopic instruments. Subsequently, four additional ports will be strategically placed under laparoscopic guidance. Throughout the procedure, the principles of complete mesocolic excision (CME) and central vascular ligation will be adhered to. This involves a medial-to-

lateral approach, where mesenteric vessels, including the inferior mesenteric vein, left colic artery, and one to two sigmoid arteries, are carefully exposed and divided at their origins using either absorbable or non-absorbable clips. The greater omentum is then separated from the transverse colon using a harmonic scalpel, and the splenic flexure and descending colon are mobilized.

7.2.1 Experimental Group Procedure

In the experimental group, the surgeon will use a 10-cm medical suture and methylene blue solution to mark the resection margin. The marginal vessels and mesentery will be divided inside the body. The proximal and distal colons are resected using a 60mm linear laparoscopic stapler. Side-to-side intracorporeal anastomotic techniques like anti-peristaltic, iso-peristaltic, or overlap methods will be applied (Figure 2-4). Once the anastomosis is completed, the specimen is retrieved. The surgeon can place the specimen in a sterile plastic bag for retrieval. Alternatively, the surgeon can use a disposable incision retraction fixator to protect the wound. An abdominal drainage tube is inserted. Three different intracorporeal anastomotic methods are shown in Figures 2, 3, and 4.

Note that only these three anastomotic methods are allowed in intracorporeal anastomosis. If the anastomosis was completed by inserting the circular stapler through the anus after patients were assigned to the IA group, it is considered a conversion to EA. This is because the surgeons had to make an incision and insert the circular stapler anvil into the proximal colon with the abdomen open. In these cases, the anastomosis was not completed under laparoscopy.

7.2.1.1 Isoperistaltic Side-to-Side Anastomosis: This method involves making two small enterotomies on the antimesenteric borders, approximately 8 cm from the ends of the colon. The colonic ends are then positioned in the upper left abdomen. Each end of a 60mm linear laparoscopic stapler is inserted through the enterotomies, closed along the antimesenteric borders, and fired to create the anastomosis (Figure 2A). Subsequently, the common enterotomy is continuously sutured using a 3-0 knotless absorbable suture (Figure 2B).

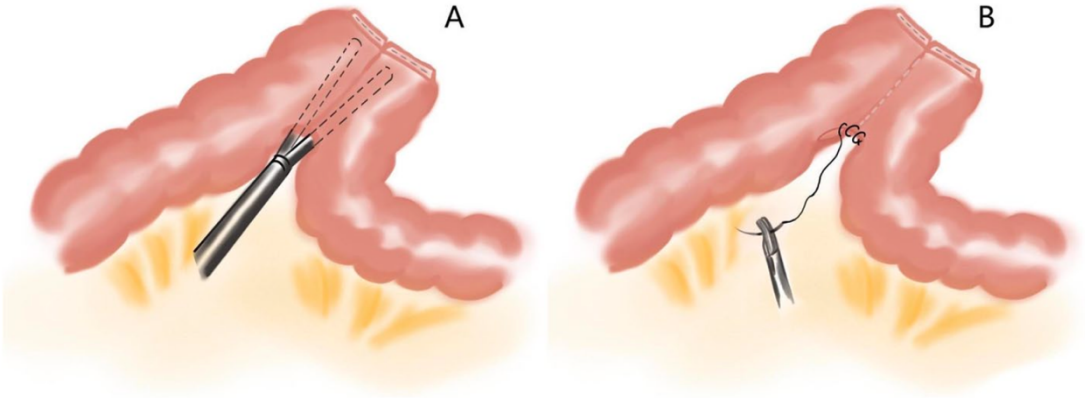


Fig. 2 A Two ends of a 60-mm linear laparoscopic stapler are inserted into the colons through enterotomies separately, then close along the antimesenteric borders and fire. B The common enterotomy is sutured continuously.

7.2.1.2 Antiperistaltic Side-to-Side Anastomosis: Similar to the isoperistaltic method, two small enterotomies are made on the antimesenteric borders of the colon ends. These ends are placed near the midline of the abdomen. The 60mm linear laparoscopic stapler is used in the same manner to close the antimesenteric borders and create the anastomosis (Figure 3A). The common enterotomy can then be closed using either a reloaded 60mm linear laparoscopic stapler positioned perpendicular to the colon ends (Figure 3B) or by continuous suturing with a 3-0 knotless absorbable suture.

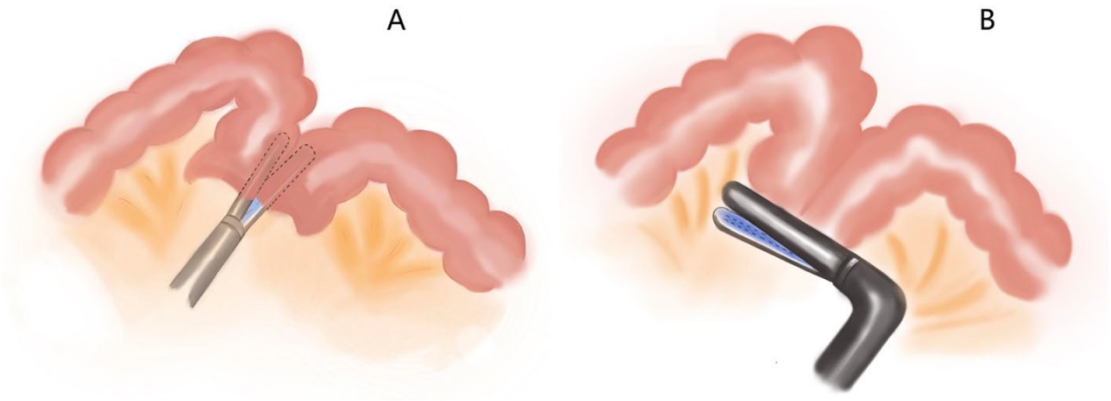


Fig. 3 A Two ends of 60-mm linear laparoscopic stapler are inserted into the colons through enterotomies separately, then close along the antimesenteric borders and fire. B The common enterotomy is closed by a reload 60-mm linear laparoscopic stapler perpendicular to colon ends

7.2.1.3 Overlap Side-to-Side Anastomosis: The choice between a cephalic or caudal

approach for inserting the linear laparoscopic stapler depends on the tumor location and the ease of anastomosis. When the transverse colon end has limited mobility, the caudal approach offers greater convenience (Figure 4A). In this approach, one small enterotomy is made at the antimesenteric border of the transverse colon end, and another enterotomy is made approximately 8 cm from the descending or sigmoid colon end. The 60 mm linear laparoscopic stapler is inserted through the caudal trocars, with both ends being introduced into the colons via the enterotomies, closed along the antimesenteric borders, and fired to complete the anastomosis (Figure 4B). The common enterotomy is then continuously sutured using a 3-0 knotless absorbable suture. In the cephalic approach, the positions of the two enterotomies on the colons are made in opposition to those in the caudal cases.

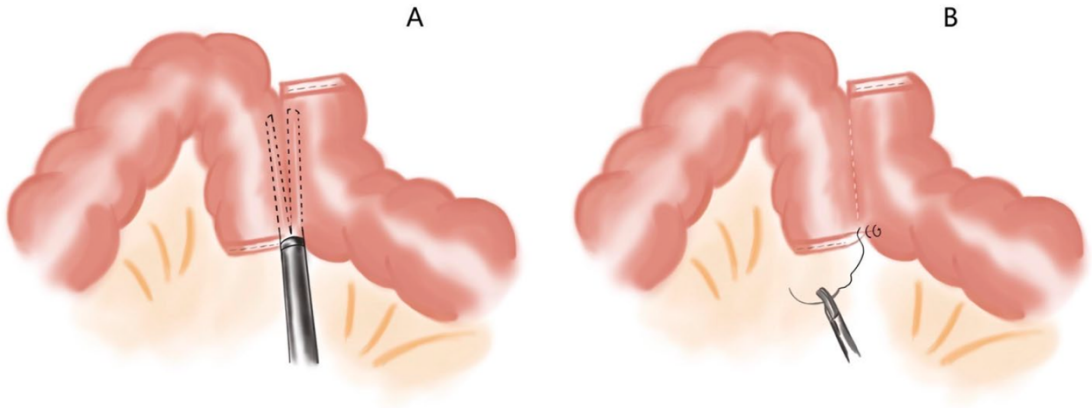
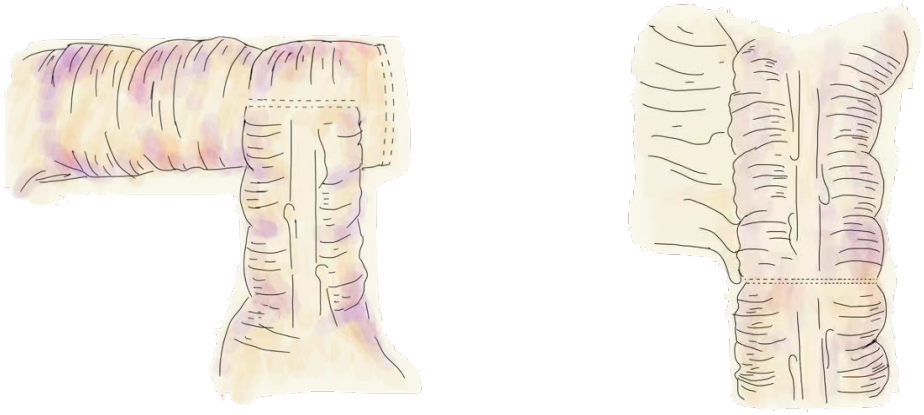


Fig. 4 A Two ends of 60-mm linear laparoscopic stapler are inserted into the colons through the enterotomies separately, then close along the antimesenteric borders and fire. B The common enterotomy is sutured continuously

7.2.2 Control Group Procedures:

For patients in the control group, the surgeon uses wound edge protectors to exteriorize the colon through a small incision in the midline of the abdomen. A ruler and methylene blue solution are employed to mark the area for colon resection. This guarantees a 10-cm margin from the tumor. Guided by these markers, the marginal vessels and mesentery are divided outside the body. The method of anastomosis is at the surgeon's discretion. A side-to-side anastomosis (including antiperistaltic, isoperistaltic, or overlapping anastomosis) is recommended. Side-to-end or end-to-end anastomosis (sewn by hand or by inserting a circular stapler through the anus or proximal colon) is also allowed (Figure 5). After completing the

anastomosis, the incision is sutured. An abdominal drainage tube is inserted at the end of the operation.



A B

Fig. 5 **A** Side-to-end anastomosis. It can be completed by inserting a circular stapler through the anus. Alternatively, it can be done by inserting the circular stapler through the end of the proximal colon and then using another linear stapler to close the end of the proximal colon after anastomosis. Hand sewing is also permitted. B end-to-end anastomosis. It can be completed by inserting a circular stapler through the anus. Alternatively, it can be done by sewing it by hand.

Table 1 The time point and assessments of the STARS study

Time point	Study period							
	Screening	Treatment	Follow-up (month)					
			1	12	24	36	48	60
Informed consent	√							
Inclusion criteria	√							
Exclusion criteria	√	√	√	√	√	√	√	√
Baseline data	√							
Blood test	√							
Tumor marker test	√							
Chest CT	√							
Pelvic MRI	√							
Abdominal CT	√		√					
Electrocardiogram	√							
Colonoscopy	√							
Surgery		√						
Complications		√	√					
Recurrence/death			√	√	√	√	√	√

7.3 Concurrent treatment and follow-up.

Postoperative chemotherapy is recommended according to the NCCN guidelines for colon

cancer treatment. The post-operative follow-up period must be in accordance with the time specified in this study (Table 1). Each participant will be followed until recurrence or death over a period of 5 years after the scheduled surgery.

The follow-up period for the primary endpoint is within 30 days after surgery. Follow-up will be conducted through in-patient observation, outpatient consultations, and telephone inquiries. SSI presence will be assessed daily during hospitalization and weekly after discharge. At least two surgeons perform SSI surveillance. The outcome assessors are trained on the Centers for Disease Control and Prevention (CDC) definitions of SSI to produce good inter-rater reliability for the assessment of SSI. If SSI is suspected during follow-up, CT or abdominal ultrasonography will be done. All patients will have an abdominal or pelvic CT on the 30th day after surgery. If the CT shows evidence of SSI, the radiologist and surgeon will assess whether there is an SSI.

The patient's surgery-related complications and Clavien–Dindo grade within 30 postoperative days will be recorded. The overall follow-up period for patients after surgery is 5 years, which includes 5-year overall survival (OS) and 3-year disease-free survival (DFS). For patients with recurrent tumors, follow-up should continue for at least 3 years after recurrence or metastasis is discovered or until death.

Appropriate strategies will be used to improve patient adherence and ensure data integrity. When signing the informed consent form, all participants will be informed of the study procedures, potential benefits, and risks to make them fully understand the inconvenience and significance of data collection.

In addition, patients who have had abdominal surgery need to return to our hospital for other treatment of combined injuries, including suture removal. Our medical team will provide standard post-operative care for enrolled participants to promote their adherence.

Investigators will contact patients 1 week before the follow-up session to increase the attendance rate. Patients who miss a scheduled appointment will be contacted to reschedule another appointment within 1 week. Telephone follow-up calls, WeChat group discussions, and personal interviews will be used to maximize retention and complete follow-up.

7.4 Patients compliance and exit

To improve patient compliance, before randomization, physicians will communicate with patients in detail about surgical procedures, possible random groupings, relevant and evolving treatment, and patient safety and security conditions. They will obtain informed consent to ensure patients clearly understand the treatment and research procedures. The randomization time should be as close as possible to the time before surgery (the day before surgery) to prevent accidental exclusion of patients after randomization. After randomization, patients can withdraw from the trial at any time. If a patient requests withdrawal from the study, the main researcher at their research center will communicate with them to avoid disrupting the balance of random groups due to exclusion. If the patient still insists on withdrawing, they will be removed from the trial. Clinical information relating to this patient will not be included in any data analysis related to this trial.

7.5 Deviation from the plan

Researchers involved in the trial will conduct corresponding surgical operations and data recording in line with the experimental requirements. Any event that strays from the original test plan must record the time and specific reason for occurrence to explain the deviation from the test plan.

8 Evaluation

8.1 Efficacy Evaluation

The methodologies for evaluating the efficacy of diverse anastomosis techniques encompass, but are not restricted to: intraoperative assessment and meticulous records of postoperative examinations, evaluation of postoperative symptoms and signs, and postoperative abdominal CT evaluation.

8.2 Safety Assessment

8.2.1 Baseline Signs and Symptoms

The baseline signs and symptoms include temperature, pulse, respiratory rate, and blood pressure. Surgeons also need to pay attention to specific symptoms such as dizziness, nausea,

and vomiting. Additionally, hematological tests and imaging tests before surgery will be performed to ensure the patient group has no surgical contraindications and fully meets the inclusion criteria. These baseline data will serve as reference indicators for the evaluation.

8.2.2 Laboratory Safety Assessment

Continuous indicators show the postoperative measurements of each study subject and the changes in values compared to the preoperative baseline. Laboratory indicators are classified as low, normal, and high based on the normal value range. They describe transitions from a normal or high baseline to a low value after the baseline, and from a normal or low baseline to a high value after the baseline. The lower or higher value after the baseline is calculated using the minimum or maximum observed value. The physician evaluates these abnormal indicators to determine their clinical significance. The proportion of subjects with “abnormal and clinically significant” changes is described.

8.2.3 Physical Examination and Vital Signs

Physical examination includes general examination, head examination, neck examination, chest and lung examination, heart and blood vessels examination, abdominal examination, spine and limbs examination, and nervous system examination. Vital signs consist of blood pressure, heart rate, and body temperature. Situations where vital signs deviate from the normal range or are abnormal are described and recorded.

9. Adverse event report

9.1 Definition of adverse events

Adverse events studied include intraoperative secondary injuries in patients and serious complications and deaths within 30 days after surgery. All adverse events reported spontaneously by test subjects or observed by staff will be recorded in a database and promptly reported to the test steering committee.

An adverse event is defined as any adverse experience that occurs in the subject during the study, regardless of whether it's considered associated with total laparoscopic anastomosis. All adverse events reported spontaneously by test subjects or observed by investigators are

recorded. These include: 1) Accidental secondary damage during surgery. 2) Serious complications after surgery (grade III and above).

9.2 Severe adverse event

A serious adverse event includes any of the following adverse medical events: 1) events leading to death; 2) life-threatening events; 3) requiring hospitalization or prolonged hospitalization of patients; 4) Causing persistent or significant disability, or incapacity for conduct.

9.3 Severity assessment

The severity of adverse events will be assessed using the Clavien—Dindo grading criteria for complications.

9.4 Relevance judgement

Determining the correlation between adverse events and surgical interventions will be made by researchers subjectively.

10. Data processing and storage

All relevant clinical data including demographic and medical information will be collected from the electronic medical record. The collected data will be encoded to protect the privacy of all participants and then be entered into an electronic case report forms (eCRFs) by trained research assistants in each sub-center. Double data entry will be performed to ensure that the data collected are accurate and verifiable from source documents. Input rules including field and range checks will also be set to minimize data entry errors. The research coordinator will periodically check the data distribution, verify outliers, and track missing data to improve data integrity and data validation. Data storage and backup will be managed by the edocdata platform (https://jlcrc.edocdata.com/), which will track all changes to the data and retains a history for each variable. Unauthorized persons or institutions will not be able to access any data stored on this server. When the trial is completed, the data will be locked and stored on edocdata platform, after which the researchers can no longer modify the data. Both paper and electronic documents will be preserved for at least 5 years after publication.

11. Quality management

Research center participation criteria: Surgeons involved in this study must complete at least 100 cases of laparoscopy-assisted left colectomy and have a minimum of 20 cases of intraoperative anastomosis in laparoscopy-assisted left colectomy. This study establishes a trial steering committee (TSC). The TSC is composed of surgeons with at least 10 years of experience in colorectal cancer surgery. Before enrolling in the research center, participating centers should provide surgical videos of two consecutive IA cases and two EA cases by the same operator recently to determine if the surgery quality meets expectations and if they can qualify as enrolment units. The evaluation method is the Delphi method.

Evaluation of surgical quality during research: Surgery quality is assessed using videos and photographs. Surgical teams are required to take photographs of the laparoscopic investigation of the tumor site, surgical area after lymph node removal, and the anastomosis. The TSC evaluates the videos and photographs to assess surgical quality and ensure compliance with the allocated intervention. Any surgical procedures deviating from the randomized allocation plan are documented. If participating centers cannot obtain imaging data, they must document the reason for not being able to leave a video or photo.

Surgeons participating in the trial are required to record the surgical procedure and explain any surgical procedures that deviate from standardized procedures. The trial steering committee will evaluate the enrollment situation and quality of enrolled cases at each center every half a year and supervise and control the quality of enrolled cases at each center.

12. Statistical analysis

12.1 Method for determining sample size

This study is a multicenter, randomized, controlled, noninferiority trial specifically designed for patients within the age range of 18 to 80 years old who are diagnosed with left-sided colon cancer. We adjusted the sample size according to our latest published multicenter retrospective data(19). We expected that the incidence of overall surgical site infection following extracorporeal anastomosis left colon cancer surgery stands at 13.3%, while the incidence of SSI after intracorporeal anastomosis is 8.0%. When there is an absolute risk difference in the SSI rate of more than 5% within 30 days after surgery, intracorporeal

anastomosis is regarded as inferior to extracorporeal anastomosis. Consequently, the sample size for this study is calculated based on non-inferiority, considering an absolute risk difference of 5%, a one-sided level of significance of 0.025, a ratio of 1:1, a power of 0.80, and a 20% drop-out rate. In total, 350 patients are required for this study, with 175 patients in the experimental group and an equal number of 175 patients in the control group.

12.2 Analytical population

12.2.1 Full analysis set

The primary outcome will be comparatively analyzed based on “modified intention-to-treat (mITT)”. In the mITT analysis, patients will be analyzed according to their randomization allocations. If patients randomly assigned to the IA group actually receive EA during surgery, they will be analyzed according to the IA group in mITT analysis. If patients randomly assigned to group EA actually receive IA during surgery, they will be analyzed according to group EA. Patients who meet the exit criteria after randomization will not be included in the mITT population.

12.2.2 Per-protocol analysis

In per-protocol (PP) analysis, only cases treated strictly in accordance with random groups and assigned treatment plans will be included. Patients who haven't been treated with a randomly assigned protocol will be excluded from the per-protocol analysis.

12.2.3 As-treated analysis set

In the as-treated (AT) analysis set, we'll analyze patients according to the actual treatment they received instead of the allocated plan for random groups.

12.3 Effect analysis and statistical method

12.3.1 primary endpoint analysis

In analyzing the primary outcomes, the rate of overall SSI will be reported as frequencies and percentages. We will calculate the absolute risk differences and the corresponding 95% confidence interval (CI) between two groups. If the absolute risk differences and the corresponding 95% CI are within the non-inferiority margin (<5%), non-inferiority is established. If not, IA is not non-inferior to EA. The analysis of the primary endpoint will be carried out in the mITT population, the PP population, and the AT population.

The independent risk factor of the primary endpoint will be analyzed in the AT population. High-risk variables identified through univariable analysis and clinical experience will be included in the multivariable analysis. Multivariable logistic regression will be employed to identify independent risk factors for SSI. The effect size will be estimated using odds ratios (ORs) with 95% confidence intervals.

12.3.2 Secondary endpoint analysis

The analysis of the secondary endpoints will be carried out in the mITT population. In the analysis of secondary endpoints, categorical variables will be reported as frequencies and percentages. Groups will be compared using the $\chi 2$ test or Fisher's exact test. Absolute risk differences and the corresponding 95% CI will be calculated. Continuous variables will be described as mean ± standard deviation or median (quartile). Groups will be compared using the t-test or Mann-Whitney U test, as appropriate. Hodges-Lehmann method will be used to calculate the difference in median and the corresponding 95% CI.

The comparison of 3-year DFS and 3-year OS between the two groups will utilize the Kaplan–Meier curve and the Cox proportional risk model. Relevant risk factors will be adjusted in Cox proportional risk model.

The missing data in this trial is expected to be minimal. If it occurs, it will be treated as censored data and not imputed. All statistical tests were 2-sided, and statistical significance was set at P <0.05. All statistical analyses were performed using R or SPSS.

12.4 Interim analysis

There will be no interim analysis for this study

12.5 Terminal analysis

We will conduct an analysis of the main endpoints of the study and publish the relevant findings after all patients have completed the first month of follow-up after surgery. After all patients have completed 5 years of follow-up, we will analyze results involving survival data, and publish relevant research results.

12.6 Data Monitoring Committee

The data and safety monitoring board (DSMB) was composed of senior surgeons, ethicists, and statistical analysts. Each member of the DSMB is independent of this trial and is not likely

to affect the competitive interests of the trial. The DSMB will receive and review the safety data for this trial. If the DSMB believes the number of adverse events in the trial is biased between groups, they will notify the chair of the trial steering committee.

There will be no interim analysis for this project. However, once the study begins, the DSMB will evaluate the results of the study every year. The assessment included primary and secondary endpoint records, adverse events, and loss of study samples. It also assesses differences with expectations, and potential conflicts with new findings and/or developments in the field of colon cancer. DSMB members are not allowed to share confidential information with anyone outside of the DSMB, with the exception of the trial steering committee.

The techniques used in this research institute are all techniques currently used in surgical treatment of colon cancer, and patients are not at higher risk. Therefore, this study did not set suspension parameters for the entire project. However, if in the DSMB's regular assessments, a center shows a huge difference in safety indicators compared to other centers and is significantly worse than the current international level, then the DSMB will submit its recommendations in writing to the trial steering committee, requesting that the trial plan be revised, or the quality of cases in the corresponding center be re-evaluated, or even suspended or terminated the clinical trial enrollment of the corresponding center.

At the discretion of the DSMB during the trial process, the DSMB may decide to meet early to discuss testing procedures together about adverse events and promptly clarify any issues with key investigators.

13. Data collection and management

13.1 Electronic Case Report Form

An electronic case report form (eCRF) is developed by the lead investigator. The CRF table includes the primary endpoint and Secondary endpoints. Additionally, general baseline data on patients will be included.

All relevant clinical data including demographic and medical information will be collected from the electronic medical record. The collected data will be encoded to protect the privacy of all participants and then be entered into an eCRF by trained research assistants in each sub-

center. Double data entry will be performed to ensure that the data collected are accurate and verifiable from source documents. Input rules including field and range checks will also be set to minimize data entry errors. The research coordinator will periodically check the data distribution, verify outliers, and track missing data to improve data integrity and data validation.

13.2 Data management

Data storage and backup will be managed by the edocdata platform (https://jlcrc.edocdata.com/), which will track all changes to the data and retains a history for each variable. Unauthorized persons or institutions will not be able to access any data stored on this server. When the trial is completed, the data will be locked and stored on edocdata platform, after which the researchers can no longer modify the data. Both paper and electronic documents will be preserved for at least 5 years after publication.

14. Ethics

14.1 Ethics Committee

This trial has been approved by the Ethics Review Committee of the First Hospital of Jilin University (Approval number: 19K135-001). Each sub-center must conduct an ethical application before participating in this trial.

14.2 Patients information and informed consent

Eligible patients should receive in-person notification from the treating surgeon and written information about the trial. Each patient should provide informed consent in accordance with local ethics committee guidelines before entering the random allocation of studies.

The informed consent procedure should be carried out by the doctors of each centre. Information provided to patients with informed consent includes: 1) a statement about the study involved in the trial; 2) a full and fair explanation of the procedures to be followed; 3) a full explanation of the nature, anticipated length, and purpose of the study; 4) a description of any risk or discomfort that the patient can reasonably anticipate; 5) a description of any benefits that can be reasonably anticipated; 6) a statement that patient data will be treated with care and confidentiality and that the data will be kept for 15 years; 7) a statement about the patient's physical material will be kept for 15 years; 8) a statement about voluntary participation, where

the statement will state the refusal Participation in the trial does not involve any punishment or loss of benefits the patient is entitled to, and patients can stop participating at any time without being penalized or losing benefits, in which case the patient will receive standard treatment with the same level of care.

15. Definition of Study Completion

The completion of the study was defined as all enrolled patients completed 5 years of follow-up. Follow-up outcomes include disease-free survival, recurrence survival, death, or loss of visits.

16. Study organizational structure

This study includes several medical centers. The research initiation center is First Hospital of Jilin University. Participating centers in clinical research include: Peking Union Medical College Hospital, Fudan University Cancer Hospital, Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Beijing Friendship Hospital Affiliated to Capital Medical University, Chinese People's Liberation Army General Hospital, Daping Hospital Affiliated to Army Military Medical University, Shengjing Hospital Affiliated to Chinese Medical University, Chinese Academy of Medical Sciences Cancer Hospital, China - Japan Union Hospital of Jilin University, First Affiliated Hospital of China Medical University, Southern Medical University Southern College. The members of the TSC are heads of the corresponding departments of the above centers: Wang Quan, Lin Guole, Li Xinxiang, Feng Bo, Zang lu, Yao Hongwei, Du Xiaohui, Tong Weidong, Zhang Hong, Liu Qian, Xie Zhongshi, Wu Aiwen, and Deng Haijun. The members of the trial guidance team are attending physicians and above in the corresponding medical teams of each center. The DSMB is comprised of senior surgeons, ethicists, and statistical analysts. Each member of the DSMB is independent of this trial and is not likely to affect the competitive interests of the trial.

17. Confidentiality and Data Security

Participants are given a unique code to maintain their anonymity. Personal information documents are stored separately from assessment data in locked cabinets. Only the authorized

research assistants and DSMB can access to the dataset. Unless there is an institutional or regulatory requirement, nobody else can access it. DSMB members can know the patient allocation and be aware of accumulated data. The DSMB will discuss the relevant data results with the TSC. DSMB members are not allowed to share confidential information with anyone outside the DSMB. The fully anonymized experimental data is stored on secure servers of Jilin University First Hospital.

18. Insurance and Compensation

Enrolled patients have all purchased medical commercial insurance jointly offered by our hospital and insurance companies before surgery. This insurance covers surgery-related complications and deaths. This study was funded by the Development Center for Medical Science & Technology, National Health Commission of the People's Republic of China (W2017ZWS01 and WA2021RW19). The funder had no role in the study's design and will not have any role in data collection, analysis, interpretation, or manuscript writing.

19. Publication notes

No relevant data for this study will be published until the collection of primary outcomes is completed. We will conduct an analysis of the primary endpoints of the study and publish the relevant findings after all patients have completed the first month of follow-up after surgery. After all patients complete 5 years of follow-up, the results involving survival data will be analyzed again, and the relevant research results will be published.

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21. Attachments

Attachment 1: Definition and classification of SSI according to CDC

Surgical site infection (SSI) can be subdivided into infections of the subcutaneous tissue (superficial SSI), deep soft tissues such as fascial and muscle layers (deep SSI) and infections of organs or spaces (organ/space SSI) that occur within 30 days after surgery.

Superficial Incisional SSI	Infection occurs within 30 days after the operation and infection involves only skin or subcutaneous tissue of the incision and at least one of the following: 1. Purulent drainage, with or without laboratory confirmation, from the superficial incision. 2. Organisms isolated from an aseptically obtained culture of fluid or tissue from the superficial incision. 3. At least one of the following signs or symptoms of infection: pain or tenderness, localized swelling, redness, or heat and superficial incision is deliberately opened by surgeon, unless incision is culture-negative. 4. Diagnosis of superficial incisional SSI by the surgeon or attending physician. **Notes**: Do not report the following conditions as SSI: 1. Stitch abscess (minimal inflammation and discharge confined to the points of suture penetration).
Deep Incisional SSI	Infection occurs within 30 days after the operation and the infection appears to be related to the operation and infection involves deep soft tissues (e.g., fascial and muscle layers) of the incision and at least one of the following:

	1. Purulent drainage from the deep incision but not from the organ/space component of the surgical site. 2. A deep incision spontaneously dehisces or is deliberately opened by a surgeon when the patient has at least one of the following signs or symptoms: fever (>38°C), localized pain, or tenderness, unless site is culture-negative. 3. An abscess or other evidence of infection involving the deep incision is found on direct examination, during reoperation, or by histopathologic or radiologic examination. 4. Diagnosis of a deep incisional SSI by a surgeon or attending physician. **Notes**: 1. Report infection that involves both superficial and deep incision sites as deep incisional SSI. 2. Report an organ/space SSI that drains through the incision as a deep incisional SSI.
Organ/Space SSI	Infection occurs within 30 days after the operation and the infection appears to be related to the operation and infection involves any part of the anatomy (e.g., organs or spaces), other than the incision, which was opened or manipulated during an operation and at least one of the following: 1. Purulent drainage from a drain that is placed through a stab wound into the organ/space. 2. Organisms isolated from an aseptically obtained culture of fluid or tissue in the organ/space.

	3. An abscess or other evidence of infection involving the organ/space that is found on direct examination, during reoperation, or by histopathologic or radiologic examination. 4. Diagnosis of an organ/space SSI by a surgeon or attending physician.

Attachment 2: ASA Classification Criteria

It refers to the six levels into which patients are classified by the American Society of Anesthesiologists (ASA) prior to anesthesia based on their physical condition and risk to surgery.

classification	condition
Level I	Physical health, good development and nutrition, and normal functioning of organs.
Level II	In addition to surgical disease, there is mild coexisting disease with sound functional compensation.
Level III	Coexisting conditions are severe and physical activity is limited, but can still manage daily activities.
Level IV	Severe coexisting conditions, loss of ability to perform daily activities, and frequent life-threatening situations.
Level V	Dying patients who have difficulty sustaining life for 24 hours, with or without surgery.
Level VI	Confirmed brain death and his organs are intended for organ transplantation.

Attachment 3: ECOG Physical Fitness Assessment Criteria

Physical condition	Grading
Normal activity	0
Mild symptoms, comfortable living, able to do light physical activities	1
Can withstand the symptoms of the tumor and take care of one's own life, but not spend more than 50% of the time in bed during the day	2
The symptoms are severe. You spend more than 50% of your time in bed during the day, but you can still get up and stand, so you can take care of yourself for part of your life	3
Sick and unable to stay bedridden	4
Death	5

Attachment 4: Postoperative Complications Clavien-Dindo System Grading

Grading		Definition
I		There are complications of unnecessary medication, surgery, laparoscopic, and reflex intervention after surgery, but they include medications for antiemetics, fever-reducing drugs, pain relievers, diuretics, dielectrics, and physiotherapy, as well as incision infections opening at the bedside;
II		Patients requiring medication not including phase 1 medication, incisional infections require antibiotic treatment, blood transfusions, and parenteral nutrition included
III.		Surgical, laparoscopic, and interventional radiological treatment is required
	IIIa	Does not require general anesthesia
	IIIb	General anesthesia required
IV		Life-threatening complications (including central nervous system complications) that require IC (intermittent monitoring) or ICU treatment
	iVa	Insufficiency of one organ (including dialysis)
	ivB	multiple organ dysfunction
V		Death

Attachment 5: TNM Stages of Colon Cancer (AJCC, 8th Edition, 2018)

Primary tumors:

Tx: the primary tumor cannot be evaluated;

T0: no evidence of primary tumor;

Tis: Carcinoma in situ, intramucosal cancer (the tumor invades the lamina propria of the mucosa but does not break through the myofascial layer);

T1: The tumor invades the submucosal layer (the tumor invades the submucosal layer but does not involve the intrinsic muscle layer);

T2: the tumor invades the intrinsic muscle layer;

T3: the tumor penetrates the intrinsic muscle layer to the peri-colorectal tissue;

T4: the tumor invades the sublayer of the peritoneum or invades or adheres to nearby organs or structures;

T4a: Tumor penetrates the mesenteric peritoneum (including intestinal perforation at the tumor site visible to the naked eye, and continuous infiltration of the tumor through the inflamed area to the surface of the peritoneum layer);

T4b: Tumor directly invades or attaches to neighboring organs or structures.

regional lymph nodes (N)

Nx: regional lymph nodes cannot be evaluated;

N0: no regional lymph node metastasis;

N1: There are 1-3 regional lymph node metastases (tumor diameter in the lymph nodes is greater than 0.2 mm), or no regional lymph node metastasis, but there are any number of tumor nodules;

N1a: there is 1 regional lymph node metastasis;

N1b: There are 2-3 regional lymph node metastases;

N1c: no regional lymph node metastasis, but tumor nodules in subserous, mesenteric, or peri-peritoneal tissues;

N2: There are 4 or more regional lymph node metastases;

N2a: There are 4-6 regional lymph node metastases;

N2b: There are more than 7 regional lymph node metastases.

Distant Metastasis (M)

Mx: distant transfers cannot be evaluated;

M0: no distant metastasis on imaging, that is, there is no evidence of metastatic tumors in distant sites and organs (this classification should not be determined by pathologists);

M1: the presence of metastases in one or more distal parts, organs, or peritoneum;

M1a: distant metastases are limited to a single distant site or organ, but there is no peritoneal metastasis;

M1b: Distal metastases distributed to two or more distant sites or organs, no peritoneal metastases;

M1c: Peritoneal metastases, with or without other site or organ metastases.

		N0	N1/N1c	N2a	N2b
Tis	0				
T1		I	IIIA	IIIA	IIIB
T2		I	IIIA	IIIB	IIIB
T3		IIA	IIIB	IIIB	IIIC
T4a		IIB	IIIB	IIIC	IIIC
T4b		IIC	IIIC	IIIC	IIIC
M1a		IVA	IVA	IVA	IVA
M1b		IVB	IVB	IVB	IVB
M1c		IVC	IVC	IVC	IVC

Attachment 6: The VAS pain score

It is a straight line about 10 centimeters long. One end of the line is marked as "0", representing no pain, and the other end is marked as "10", representing the most severe pain imaginable. There are no other scale marks on the line, maintaining a simple visual presentation.

Self - assessment by the Patient: The patient marks a point on the line according to his or her current pain experience to represent the degree of pain. This process is completely completed by the patient independently to fully reflect his or her subjective pain experience. For example, if the patient feels mild pain, he or she may mark a point closer to the "0" end; if the pain is very severe, the mark will be closer to the "10" end.

Degree of Pain	VAS Score Range	Description
No Pain - Mild Pain	0 - 3 points	A score of 0 represents no pain at all; a score of 1 - 3 indicates mild pain, which has a relatively minor impact on daily life and generally does not require strong painkillers.
Moderate Pain	4 - 6 points	The pain begins to have a certain impact on daily life, intensifies during daily activities, and may require relief measures such as rest and the use of over-the-counter painkillers.
Severe Pain	7 - 10 points	Unbearable pain that has a severe impact on the quality of life, greatly interfering with daily activities, sleep, and emotions, and requires strong painkillers.

Attachment 7 Completeness of Specimens

Grading	Descriptions
Mesocolic plane	Smooth intact mesocolic excision.
Intramesocolic plane	The excision disrupts the mesocolon but do not reach down to the muscularis propria.
Muscularis propria plane	The excision disrupts the mesocolon and exposed the muscularis propria.

CSP amendments summary

	Amendments in Version 2.0
Title	Change the title to "A Multicenter Randomized Clinical Trial Comparing Surgical Site Infection after Intracorporeal Anastomosis and Extracorporeal Anastomosis for Left Colon Cancer (STARS)"
Exclusion criteria	Change "planned synchronous abdominal organ resections;" to "Patients who need to undergo combined organ resection or robot-assisted colectomy".
	Change "metastatic or multiple carcinoma" to "Have a history of malignant colorectal tumor or have metastatic or multiple carcinoma".
	Add "Patients who are receiving preoperative neoadjuvant therapy."
	Add "ASA grade ⩾ IV and/or ECOG performance status score > 2".
	Add "Patients with other clinical and laboratory conditions that are considered by researchers as inappropriate for participating in this trial."
	Delete " end-to-end anastomosis."
Exit criteria	Delete "Serious adverse events occurred preoperatively (according to the Common Terminology Criteria for Adverse Events, CTCAE version 5.0). "
	Add "Patients with other non-tumor diseases that prevent them from continuing to receive this treatment regimen."
	Add "Patients who request to withdraw from this study cohort for various reasons after being enrolled in the study, or who cannot complete the study plan and follow-up for various reasons."
Experimental Group Procedure	Add a statement: "Note that only these three anastomotic methods are allowed in intracorporeal anastomosis. If the anastomosis was completed by inserting the circular stapler through the anus after patients were assigned to the IA group, it is considered a conversion to EA. This is because the surgeons had to make an incision and insert the circular stapler anvil into the proximal colon with the abdomen open. In these cases, the anastomosis was not completed under laparoscopy."
Control Group Procedures	Add a statement: "The method of anastomosis is at the surgeon's discretion. A side-to-side anastomosis (including antiperistaltic, isoperistaltic, or overlapping anastomosis) is recommended. Side-to-end or end-to-end anastomosis (sewn by hand or by inserting a circular stapler through the anus or proximal colon) is also allowed".
	Add figures and figure legends to explain the details of side-to-end anastomosis and end-to-end anastomsis
Analytical population	Add as-treated analysis set
Primary endpoint analysis	Add univariable analysis and multivariable analysis of SSI in as-treated population.
	Amendments in Version 2.1
Method for determining sample size	Re-estimate the sample size

Statistical analysis plan (SAP)

SAP version 1.0 (2023.12.05)

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

SSI	Surgical site infection
IA	Intracorporeal anastomosis
EA	Extracorporeal anastomosis
SAP	Statistical analysis plan
mITT	Modified intention-to-treat
PP	Per-protocol
AT	As-treated
CI	Confidence intervals
ORs	Odds ratios
HR	Hazard ratio
DFS	Disease-free survival
OS	Overall survival
VAS	Visual analog score

1 Introduction

The STARS trial is a multicenter, randomized, single-blind, non-inferiority study designed to compare the incidence of surgical site infection (SSI) following intracorporeal anastomosis (IA) versus extracorporeal anastomosis (EA) in patients undergoing surgery for left colon cancer. The primary objective of this trial is to determine whether IA is non-inferior to EA in terms of SSI rates within 30 postoperative days. While the aims, hypotheses, and design of the STARS trial have been detailed elsewhere, this statistical analysis plan (SAP) will focus on the forthcoming analysis and presentation of the trial data. This SAP is conducted based on the study protocol version 2.1. The comprehensive study design, including the primary and secondary endpoints, along with the methods for determining sample size and the approach to data management, are integral to the SAP and are critical for understanding the trial's methodology and interpretation of results.

2 Study design and objectives

This is a prospective, multicenter, non-inferiority, two-arm, single-blind, randomized controlled study. The study aims to investigate the effects of intracorporeal anastomosis and extracorporeal anastomosis in laparoscopic-assisted radical left hemicolectomy on surgical site infection. It also examines the difference in postoperative recovery, safety, oncology outcomes and survival. The trial was prospectively registered in clinicaltrial.gov (NCT04201717) and the study protocol (version 1.0) has been previously published,(1) and minor revisions has been made in study protocol version 2.0 and version 2.1. Details of exclusion criteria and study treatments can be found in study protocol version 2.1.

3 Sample size

The sample size is determined based on the rate of SSI. Based on our multicenter retrospective data,(2) we estimated the incidence of overall SSI following EA in laparoscopic left hemicolectomy stands at 13.3%, while the incidence of SSI after IA is 8.0%. When there is an absolute risk difference in the SSI rate of more than 5%

within 30 days after surgery, IA is regarded as inferior to EA. Consequently, the sample size for this study is calculated based on non-inferiority, taking into account an absolute risk difference of 5%, a one-sided level of significance of 0.025, a ratio of 1:1, a power of 0.80, and a 20% drop-out rate. In total, 350 patients are required for this study, with 175 patients in the IA group and an equal number of 175 patients in the EA group.

4 Statistical methods

4.1 General calculation rules

All statistical tests were 2-sided, and statistical significance was set at $P < 0.05$. All statistical analyses were performed using R or SPSS. P-values will be quoted to three decimal places. Confidence intervals (CI) will be two-sided and 95%. CI will also be quoted to one decimal place.

Categorical variables will be reported as frequencies and percentages. Percentages will be quoted to one decimal place. Chi-square tests in contingency tables will be replaced by Fisher's exact tests if any expected cell frequency is less than five. Absolute risk differences and the corresponding 95% CI will be calculated.

Continuous data with normal distribution were presented as mean (standard deviation) and analyzed by Student's t-test. Continuous variables with non-normal distribution were reported as medians (quartile) and compared using the Mann–Whitney U test. Hodges-Lehmann method will be used to calculate the difference in median and the corresponding 95% CI. Data will be quoted to one decimal place.

Survival data between two groups will be compared using a two-sided log-rank test stratified by pathological TNM (0–I, II, III) and age (≤65 years and >65 years). The hazard ratio (HR) for disease recurrence or death will be estimated using the stratified Cox proportional hazards model, including strata of pathological TNM (0–I, II, III) and age (≤65 years and >65 years). The 95% confidence interval for the HR will be constructed. Results from an unstratified analysis will also be provided. The Kaplan-

Meier methodology will be applied to estimate the median disease-free survival (DFS) and overall survival (OS) for each treatment arm. Kaplan-Meier curves will be developed.

4.2 Analysis populations

4.2.1 Modified intention-to-treat (mITT) population

In the mITT analysis, patients will be analyzed based on their randomization allocations. If patients randomly assigned to the IA group receive EA during surgery, they will be analyzed within the IA group in the mITT analysis. If patients randomly assigned to group EA receive IA during surgery, they will be analyzed according to group EA. Patients who meet the exit criteria after randomization won't be included in the mITT population.

4.2.2 Per-protocol (PP) population

In PP analysis, only cases treated strictly in accordance with random groups and assigned treatment plans will be included. Patients who haven't been treated with a randomly assigned protocol will be excluded from the per-protocol analysis.

4.2.3 As-treated (AT) population

In the AT analysis, we'll analyze patients according to the actual treatment they received instead of the allocated plan for random groups.

4.3 Analysis of endpoints

The analyses of primary endpoint and secondary endpoints will be conducted in mITT population, PP population and AT population.

Analyses for the primary outcome variable will be unadjusted. In analyzing the primary outcomes, the rate of overall SSI will be reported as frequencies and percentages. We will calculate the absolute risk differences and the corresponding 95% confidence interval (CI) between two groups. If the absolute risk differences and the corresponding 95% CI are within the non-inferiority margin (<5%), non-inferiority is established. If not, IA is not non-inferior to EA.

The independent risk factor of the primary endpoint will be analyzed in the AT population. High-risk variables identified through univariable analysis and clinical experience will be included in the multivariable analysis. Multivariable logistic regression will be employed to identify independent risk factors for SSI. The effect size will be estimated using odds ratios (ORs) with 95% confidence intervals.

4.4 **Baseline Characteristics**

The following baseline characteristics will be presented in mITT, PP and AT polulation:

- Sex, categorical variable;
- Age, continuous variable, measured in years;
- Body Mass Index, continuous variable, measured in kg/m^2;
- American Society of Anesthesiologists grading, categorical variable
- Eastern Cooperative Oncology Group performance status score, categorical variable;
- Tumor site, categorical variable, classified as distal transverse colon (distal 1/2 of transverse colon), splenic flexure of colon (junction of transverse colon and descending colon), descending colon (descending colon and the junction of sigmoid colon and descending colon), and proximal sigmoid colon (proximal 1/3 of sigmoid colon);
- NRS2002 score, continuous variable, measured in points
- Parenteral prophylactic antibiotics, categorical variable;
- Mechanical bowel preparation, categorical variable;
- Oral antibiotic bowel preparation, categorical variable;
- Postoperative antibiotic use time, categorical variable, divide by ≥3 days and <3 days;
- Clinical T stage, categorical variable;
- Clinical N stage, categorical variable;
- Clinical TNM stage, categorical variable;

4.5 Primary endpoint

The primary endpoint in this trial is surgical site infection (SSI) within 30 postoperative days. Cases that fail to complete the 30-day follow-up are treated as missing data. The SSI will be compared between groups in the mITT population, PP population, and AT population.

- Overall SSI, Categorical variable. The absolute risk differences and the corresponding 95% CI between two groups will be calculated. The 95% CI will be compared with the non-inferiority margin (5%).

The subtypes of SSI (Superficial or deep incisional SSI, and Organ/space SSI) will also be compared between the two groups. However, their 95% confidence interval will not be compared with the non-inferiority margin.

The independent risk factors for overall surgical site infection (SSI) will be analyzed in the AT population. In univariable analysis, continuous variables will be converted to categorical variables. Chi-square tests or Fisher's exact tests will be employed. The key variables are as follows.

- Sex (male, female);
- Age (≤65 years,>65 years);
- Body Mass Index (≤24kg/m^2, >24kg/m^2);
- American Society of Anesthesiologists grading (I, II, III);
- Eastern Cooperative Oncology Group performance status score (0, 1, 2);
- Tumor site (distal transverse colon, splenic flexure of colon, descending colon, and proximal sigmoid colon);
- NRS2002 score (≤2 points, > 2 points);
- Parenteral prophylactic antibiotics (Yes, No);
- Mechanical bowel preparation (No, only enema, only oral laxative, enema plus oral laxative);
- Oral antibiotic bowel preparation (Yes, No);
- Postoperative antibiotic use time(≥3 days, <3 days);

- Pathological TNM stage (0, I, II, III);
- Overflow of intestinal contents during anastomosis (Yes, No);
- Operating time (≤180mins, >180mins);
- Blood loss during operation(≤100ml, >100ml);
- Blood transfusion during operation (Yes, No);
- Prophylactic enterostomy (Yes, No);
- Anastomosis methods (Side-to-side anastomosis, End-to-side anastomosis, End-to-end anastomosis)
- Intervention (intracorporeal anastomosis, extracorporeal anastomosis)

Factors clinically relevant to SSI after univariable analysis will be included in multivariable analysis. Logistic regression will be used in multivariable analysis to identify independent risk factors for SSI. The effect size will be estimated using odds ratios (ORs) with 95% confidence intervals.

4.6 Secondary endpoints

The secondary endpoints will be compared between groups in the mITT population, PP population, and AT population.

- Operating time, continuous variable, measured in minutes;
- Total blood loss during operation, continuous variable, measured in milliliters;
- Conversion to open surgery, categorical variable;
- Postoperative complications, categorical variable. It will be analyzed according to the overall occurrence. The occurrence of its subtype (anastomotic leakage, bowel obstruction, etc.) will also be analyzed. Additionally, Clavien–Dindo classification will be analyzed (≤grade II, ≥grade III). (3)
- Completeness of specimens, categorical variable. It is evaluated according to the West classification (mesocolic plane, intramesocolic plane, and muscularis propria plane).(4)
- Number of lymph nodes dissected, continuous variable.
- The pathological TNM stage (as per the American Joint Committee on Cancer

Cancer Staging Manual, 8th edition), categorical variable. The T stage, N stage, and TNM stage will be analyzed separately.

- Postoperative recovery, continuous variable. It includes the time to first flatus, the time to first defecation, the time to start food intake, and the postoperative hospital stay. Additionally, the time to first ambulation and the time to remove the abdominal drainage tube, which are not included in the study protocol's design, will also be analyzed. These are measured in days.
- Length of incision, continuous variable. It is measured in millimeters.
- Visual analog score (VAS) of pain, it will be analyzed as categorical variable: no to mild pain (0–3 points); moderate pain (4–6 points) ,and severe pain (7–10 points).
- 3-year DFS: DFS is defined as the time from randomization until the occurrence of local recurrence, distant metastasis, or death from the tumor, whichever comes first. It is measured in months. For patients who are lost to follow-up, DFS will be censored at the time of the last available tumor assessment before the missing assessments.
- 5-year OS: OS was defined as the time from randomization to death due to any cause. It is measured in months. For patients lost to follow-up, OS will be censored at the time of the last available follow-up prior to the missing assessments.

4.7 Subgroup analysis

The subgroup analysis will be carried out only if the primary endpoint in the mITT population do not reach the non-inferiority margin. If the 95% CI of SSI is not within the non-inferiority margin, the primary endpoint will be summarized within the following subgroups.

- Sex (male, female);
- Age (≤65 years,>65 years);
- Body Mass Index (≤24kg/m^2, >24kg/m^2);
- Tumor site (distal transverse colon or splenic flexure of colon, descending colon,

proximal sigmoid colon);

- NRS2002 score (≤2 points, > 2 points);
- Mechanical bowel preparation (Yes, No);
- Oral antibiotic bowel preparation (Yes, No);
- Postoperative antibiotic use time(≥3 days, <3 days);
- Operating time (≤180mins, >180mins);

Subgroup analyses will be performed using mITT population. If intracorporeal anastomosis is proven to be noninferior to extracorporeal anastomosis regarding SSI, subgroup analysis will not be performed.

5 Missing data

Missing primary endpoint data will be treated as censored data and won't be imputed during analysis. Other missing data won't be imputed either. The missing data will be stated in the tables.

6 Interim analyses

No interim analyses are planned in this study.

7 Timing of analysis

We will conduct an analysis of the study's primary endpoint and publish the relevant findings after enrollment is completed and all patients have completed the first month of follow-up after surgery. When all patients complete 5 years of follow-up, the results involving survival data will be analyzed again, and the relevant research results will be published.

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