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Relation of gut microbiota and postoperative gastrointestinal dysfunction in older patients with colon cancer undergoing elective colon resection: a protocol for a prospective, observational cohort study

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ABSTRACT

Introduction Gut microbiota (GM) appears critical for gastrointestinal symptoms, but whether alterations in GM are associated with increased risk of postoperative gastrointestinal dysfunction (POGID) in older patients with colon cancer (CC) undergoing elective colon resection remains unclear.

Methods and analysis This study aims to prospectively recruit 284 elderly patients with CC undergoing elective colon resection. GM of fresh faeces specimens is characterised using 16S rRNA gene sequencing. Data are collected preoperatively, daily postoperatively during the in-hospital stay, and follow-up visits are scheduled four times within 30 days after discharge. Associations with POGID will be investigated using logistic regression models to calculate ORs with 95% CIs. The models include the adjustment for age, sex, frequency of spicy diet, coffee drinking and tea drinking, tobacco and alcohol history, diabetes, obesity, gastroenteritis, preoperative gut microbial composition. Furthermore, we will use joint modelling for longitudinal data to study several outcome variables simultaneously.

Ethics and dissemination This study was approved by the Institutional Review Board of West China Hospital, Sichuan University (IRB Number: 20201334). The results will be disseminated through peer-reviewed publications or conference presentations.

Trial registration number It has been registered in PROSPERO, number CRD42019145032. It has been registered in the Chinese clinical trial registry, number ChiCTR2100043646.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This study is one of the first studies to investigate the preoperative gut microbiota (GM) composition of older patients with colon cancer undergoing elective colon resection to predict the risk of postoperative gastrointestinal dysfunction (POGID).
⇒ We identify the perioperative management to avoid a severe bias by following the Enhanced Recovery After Surgery (ERAS) protocol to control the baseline data.
⇒ The samples size, 284 samples, provides sufficient statistical power to detect relevant associations between GM and POGID.
⇒ We regulated the baseline data by selecting specified populations (the ethnic group of Han and Chengdu inhabitants) to reduce bias, taking into account the influence of ethnicity, area, environment and other factors on GM.
⇒ This single-centre study may be a limitation, exposing recruitment biases.

INTRODUCTION

Burden of postoperative gastrointestinal dysfunction (POGID) and unaddressed problems

In recent years, there has been a high incidence of gastrointestinal dysfunction (GID) after elective resection of a colon tumour, especially in old patients, which seriously impacts the recovery rate, reduces the quality of life and increases the length and cost of hospital stay.[1–3] Due to the lack of biomarkers that can precisely predict POGID and the complex aetiology of POGID, current therapeutic strategies are limited. Clinical treatment is limited to the management of symptoms.[4–6] However, researchers of POGID in patients are limited.[7] And few researchers have investigated the factor of GM.

Developmental origins of POGID

The condition of GID was first described in the 1960s by pathologists.[8 9] GID refers to gastrointestinal (GI) digestion and absorption disorders, including GI motility disorders and GI mucous membrane barrier damage.[9 10] GM and its metabolites can modulate GI motility via neuronal, hormonal and immunological signalling.[11] GI motility disorders and mucosal barrier damage affect one another. Once GI peristalsis weakens or

disappears, abnormal growth of GM aggravates the GI mucosa, which increases bacterial translocation from the lamina propria into the bloodstream and endotoxin uptake, eventually forming bacteremia. It, in turn, aggravates GI motility disorders, even causing toxic intestinal paralysis.[12–14] Postoperative ileus is the most common slowing or stopping of GI functions after surgery.[15] The clinical manifestations include abdominal pain, diarrhoea, abdominal distension, constipation and symptoms recur. At present, there are no specific treatments for POGID. Clinical treatment is limited to symptom management, and a variety of schemes are often used in combination.[16 17] The causative factors and pathogenesis of POGID are incompletely understood, but the operation factor, the side effect of opioids, undernutrition, psychosocial disturbance such as anxiety, depression, phobia, or somatisation, GM, and altered gut–brain axis function may contribute.[9 18–21] In addition, no consensus has been achieved with regard to the definition of POGID. Moreover, the Association of Coloproctology in Great Britain & Ireland identified POGID as a key research priority using a modified Delphi approach during a patient–clinician consensus process.[7 22]

Characterising of human gut microbiota (GM)

The human intestine is colonised by trillions of microorganisms, known collectively as GM, most of which are bacteria coevolved with the host in a symbiotic relationship.[23] It is estimated that more than 3.9×10^{13} bacteria colonise the human clone, and the ratio of bacteria to human cells is closer to 1:1.[24] Given the plasticity in microbial diversity and function, microbial-based therapeutic interventions, including diet-based modulation, targeted antibacterial approach to treatment, non-absorbable antibiotics, prebiotics, probiotics and synbiotics, as well as faecal microbial transplantation, potentially permit the development of novel strategies for POGID therapy.[20 25 26] In the context of precision medicine, the key clinical and scientific issues having not been solved for POGID in older patients with colon cancer (CC) undergoing elective colon resection including the alterations in GM composition and function should be characterised in next-generation gene sequencing.

Aim

GM appears critical for GI symptoms. Its role in modulating GI motility, gastric acid, epithelial secretion, maintaining epithelial barrier integrity and communication between the gut and the central nervous system may underlie its contribution to GI symptoms.[20 27 28] However, its pathogenesis remains unclear. In this protocol, we aim to investigate whether alterations in GM relate to POGID in older patients with CC undergoing elective colon resection.

METHODS AND ANALYSES

Study design and setting

This study is a prospective, observational, ongoing cohort study. A flow diagram of the study design is shown in figure 1. A total of 284 hospitalised patients with CC undergoing elective colon resection are being continually recruited from a hospital in Sichuan province, China. Data collecting is from 1 August 2021 to 1 January 2023. Data will be analysed from 1 January 2023 to 1 March 2023. All subjects from the same medical team follow the Enhanced Recovery After Surgery (ERAS) protocol (online supplemental file 1) to identify the perioperative management. The ERAS protocol reduces surgical stress, maintains postoperative physiological function, and enhances mobilisation after surgery. Data collected at baseline includes sociodemographic and lifestyle characteristics, medical information, underlying disease, surgery and tumor-associated information (table 1). Fresh faeces samples will be collected before surgery. Moreover, we will characterise GM of fresh faeces samples using 16S rRNA gene sequencing. Follow-up visits will be scheduled four times within 30 days after discharge. At each visit, outcome data will be collected, and diagnosis of POGID will be determined. Associations with POGID will be investigated using logistic regression models to calculate ORs with 95% CIs. The models included adjustment for age, sex, frequency of spicy diet, coffee drinking and tea drinking, tobacco and alcohol history, diabetes, obesity, gastroenteritis, preoperative gut microbial composition. Furthermore, we will use joint modelling for longitudinal data to study several outcome variables simultaneously.

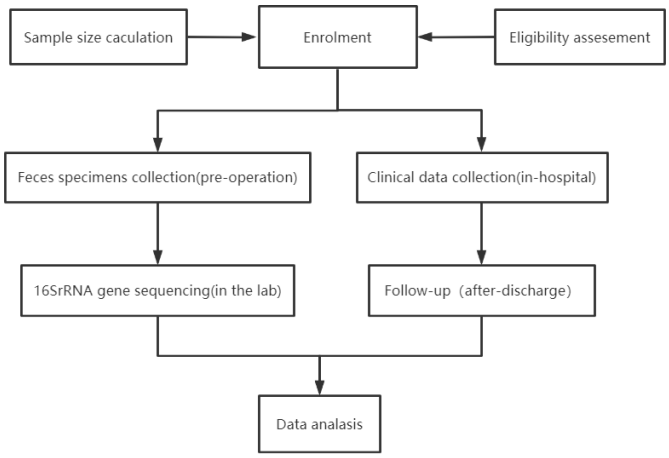

Figure 1 Flow diagram of this study.

Inclusion criteria

The inclusion criteria are as follows: (a) elderly patients over 60 years old; (b) patients willing to sign the informed consent and voluntary participation; (c) patients with incident primary CC and will undergo elective colon resection; (d) patients who are the ethnic group of the Han and Chengdu residents.

Exclusion criteria

Participants who meet the following criteria are excluded: (a) patients with mental illnesses who are unable to cooperate with the study; (b) patients with prior history of cancer and chemotherapy or radiotherapy, tumours accompanied by other malignant

Table 1 Baseline data collection at initial admission

Baseline information	Variate	Variable's description
Sociodemographic characteristics	Age	Years
	Sex	Biological (male/female)
	BMI	kg/m^2
	Obesity	Waist and hip circumference
Life style characteristics	Frequency of spicy diet	Times/weeks
	Frequency of coffee drinking	Times/weeks
	Frequency of tea drinking	Times/weeks
	Tobacco history	Years
	Alcohol history	Years
Medication history	Probiotics	Yes or no
	Antibiotics	Yes or no
	Steroids	Yes or no
Underlying disease	Hypertension	Yes or no
	Metabolic diseases: diabetes, obesity, hyperlipidemia	Yes or no
	Gastrointestinal diseases: polyps, gastroenteritis and recent gastrointestinal symptoms	Yes or no
Surgery-related information	Surgical method	Radical resection of right colon cancer, left colon cancer or sigmoid colon cancer
	Types of surgery	Laparotomy or laparoscopy
	Operation time	Hours
	Postoperative complications of non-gastrointestinal dysfunction: surgical wound bleeding or infection, etc	Yes or no
Tumor-associated information	Tumour staging	AJCC TNM staging
	Tumour size	cm^3

AJCC TNM staging, American Joint Committee on Cancer, AJCC (V.8) TNM staging system standard; BMI, body mass index.

tumours, or CC with distant metastasis; (c) patients treated by non-radical surgery such as palliative treatment; (d) antibiotics, corticosteroids or probiotics were used within 1 month before faeces specimen collection; (e) long-term use of immunosuppressives; (f) history of viral infection (ie, hepatitis B virus (HBV), hepatitis B virus (HCV) or HIV); and (g) ileus present at the time of admission.

Identifying the standard

The following participants are eliminated: (a) patients who cannot provide faeces samples before surgery; (b) loss of follow-up or incomplete data (missing data of leading observation indicators by more than 20%); and (c) faeces samples do not meet the gene sequencing standards.

Sample size calculation

According to the pre-experimental results, the incidence of POGID was 27% and 16% in the exposed and non-exposed groups, respectively, which was similar to published reports (eg, POGID occurred in 10%–20% of patients).[7] The type 1 error rate is 5%, and the power is 90%. The study needs to include 237 patients. In consideration of 20% attrition, the final sample size is 284 cases.

Outcome measures

The primary end outcome in this study is postoperative symptoms measured by the Porto Alegre Dyspeptic Symptoms Questionnaire (PADYQ). According to the PADYQ, with a total score of 44 points, scores greater than six will be diagnosed as POGID.[29 30] The secondary end outcomes include time to first bowel movement, time to first clear liquid diet, time of postoperative vomiting, time to fatus, severe nausea, constipation, diarrhoea, ileus, GI bleeding, sepsis, pain intensity, length and cost of hospital stay.[31] The first observation cut-off point is when POGID complications occur. The second observation cut-off point is 30 days after the operation, and recovery is assessed by telephone and WeChat follow-up on the 7th, 14th and 21st day after the operation.

Assessment of GM

Faeces samples collection is completed in the GI surgery centre, and 16S rRNA gene sequencing is undertaken in the microbiology laboratory.

Feces collection

The researchers are trained to collect fresh samples according to standard collection methods for faecal sampling.[32] After passing the training, the middle of faeces will be collected according to the aseptic requirements. Subjects urinate first before collection, and faeces will be collected in dry, anhydrous and sterile specimen tubes. Representative faeces will be collected in three parts (200 mg per part). And then store in 2 mL specimen tubes, respectively. Fresh faeces samples are placed in insulating polystyrene foam containers on an ice bath, transported from the hospital to the laboratory and stored at −80°C. The samples are collected and transported using the adopted method.[33]

DNA extraction, 16S rRNA gene amplification, illumina MiSeq sequencing and sequencing data analysis

Total bacterial genomic DNA will be extracted from faeces samples using a Genomic DNA Extraction Kit with magnetic beads (GenMagBio, Jiangsu, China). Bacterial 16S rRNA gene of distinct regions (V3–V4) will be amplified from the extracted DNA using specific primers 338F(5′-ACTCCTACGGGAGGCAGCA-3′) and 806R(5′-GGACTACHVGGGTWTCTAAT-3′) with the barcode. Sequencing libraries will be generated usingTruSeq DNA PCR-Free Sample Preparation Kit (Illumina, USA) following the manufacturer's recommendations, and index codes will be added. The library quality will be assessed on the Qubit V.2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. At last, the library will be sequenced on an Illumina NovaSeq platform, and 250 bp paired-end reads will be generated. Further details of sequencing methods and sequencing data analysis are available in online supplemental files 2 and 3.

Planned data analysis

Statistical analysis will be performed using SPSS V.25 software. Continuous variables with the normal composition of subjects' demographic information and basic disease information are expressed as mean±SD, and categorical variables are expressed as frequency and percentage. Categorical variables will be compared using X^2 analysis and Fisher's exact test. A t-test and rank-sum test will be used to compare the differences between continuous variables. All results will be considered significant at $p<0.05$. Logistic regression analysis will be used to predict the risk of POGID by age, sex, frequency of spicy diet, coffee drinking and tea drinking, tobacco and alcohol history, diabetes, obesity, gastroenteritis, preoperative gut microbial composition. Furthermore, we will use joint modelling for longitudinal data to study several outcome variables simultaneously.

Quality control

Aseptic operations will be carried out, faeces samples will be correctly retained and storage conditions will be established before the specimens are sent for examination. Patients' subject requirements and grouping will be blinded to the sample collectors, data inputters and statistical analysts. The details of preprocessing and quality control of sequencing data are available in online supplemental file 3. Personnel involved in data collection and entry received unified professional training. The final data will be verified by personnel engaged in the clinical specialty for more than 2 years.

Ethics and dissemination

Ethical considerations

This study was approved by the Institutional Review Board of West China Hospital, Sichuan University (IRB Number: 20201334). All patient information involved in this study will be coded and kept strictly confidential. The data obtained will only be used for scientific research purposes, and the patients' names and identities will not appear in any research report or public publication. Written informed consent will be obtained from each patient. The patients have the right to withdraw from this study at any time. If the patient refuses to participate in the study, their data collection will be stopped.

Dissemination

Findings will be disseminated through peer-reviewed publications or conference presentations.

Patient and public involvement

Patients and/or the public were not involved in the design, conduct, or reporting, or dissemination plans of this research.

DISCUSSION

This is one of the pioneering studies to use next-generation sequencing to investigate the preoperative GM composition of older patients with CC undergoing elective colon resection to predict the risk of POGID.[34] It may impact the clinical practice of advanced interference treatment in the foreseeable future. First of all, the identification of specific faecal bacteria related to POGID advances scientific knowledge on the cause of digestive symptoms. In addition, targeted prophylactic therapies may be developed to decrease the risk for POGID. Moreover, characterising GM in patients with POGID may allow a more targeted antibacterial, a probiotic-based or a nutrition-based approach to treatment.[27 35 36] Plus, these faecal bacteria may serve as biomarkers to identify older patients with CC undergoing elective colon resection at high risk for POGID and provide the basis for whether the patients are suitable for surgery under the condition of severe intestinal microbial dysbiosis.[37]

Accumulating evidence supports that GM is associated with obesity, diabetes, depression, kidney disease and other diseases.[38–41] Similar studies have shown that GI symptoms in patients with functional bowel disorders (FBDs) are associated with gut microbial diversity, including a significant decrease in the number of *Prevotella* species and enrichment of microbial ascorbate and aldarate metabolism, which play essential roles in the increase of luminal oxidative stress in symptomatic patients with FBDs. Nevertheless, it is not yet clear whether the reduction in diversity precedes the GI symptoms.[27] Vich Vila *et al*'s study showed that compared with control individuals in the general population, patients with irritable bowel syndrome (IBS) had more Firmicutes and less Bacteroidetes and patients with inflammatory bowel disease (IBD) had less Firmicutes and more Bacteroidetes than did controls. Controls had more Actinobacteria in their stool than did patients with IBD or IBS. Furthermore, GM composition can be used to distinguish IBD from IBS.[42] These similar studies suggest that GM may play a key role in gut health.

To date, 95 subjects are eligible for this project, and work is currently underway. After signing informed consent, each subject will be provided 50 RMB in financial compensation and the results of gut microbial composition will be provided for the patients free of charge.

Strengths and limitations of this study

Following the ERAS protocol to control the baseline data, we identify the perioperative management to avoid a severe bias. We further regulated the baseline data by selecting certain populations (the ethnic group of Han and Chengdu inhabitants) to reduce bias, taking into account the influence of ethnicity, area, environment and other factors on GM. Furthermore, the sample size of 284 samples gives enough statistical power to discover important relationships between GM and POGID. However, the fact that this is a single-centre study may be a limitation, as it exposes recruiting biases. In addition, we only go down to the species level, not the strain level, which is possible with metagenomic sequencing. We are also not looking at the presence of genes linked to bacterial virulence and antibiotic resistance.

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Competing interests None declared.

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