Official Title:

Fecal Microbiota Transplantation in the Treatment of Inflammatory Bowel Disease: the Role of Selection of the Bai Nationality as Donor Source in Terms of Treatment Efficacy and Mechanisms

Date: S e p t e m b e r 8 , 2024

Study Protocol

1. Background

Inflammatory bowel disease (IBD) is a chronic, recurrent, non-specific intestinal disease, including ulcerative colitis (UC) and Crohn's disease (CD). Although biological therapy significantly improved the effect of the treatment of ulcerative colitis, but nearly two-thirds of the patient's response to drug treatment. Therefore, new treatments targeting the underlying pathophysiology of UC are critical. Given that alterations in the gut microbiome are strongly associated with disease activity in IBD, many studies have proposed microbiotoy-based therapies, in particular, Fecal microbiota transplantation (FMT) in the treatment of UC. Donor-to-donor variation in treatment with fecal microbiota transplantation may alter treatment efficacy. Therefore, screening high-quality donors can improve efficacy and minimize the risk of adverse effects.

Ulcerative colitis with the changes in the gut microbiota and biodiversity decrease and the change of the relative abundance of advantage bacterium group, a significant reduction in UC patients with intestinal flora diversity, at the species level, thick wall bacteria door bacteria (e.g., clostridium tender) ratio decreases, actinomyces door, the door deformation bacteria such as e. coli), enterobacteriaceae,

streptococcus, bacteroides ratio increased.A previous study by team of the investigators found that there were ethnic and regional differences in the incidence of IBD in Yunnan Province, and the incidence of Dai, Bai and Miao was lower than that of Han. Then the related factors were analyzed, among which, the ethnic characteristic diet of Yunnan ethnic minorities can improve the diversity of intestinal flora and viruses, increase the content of probiotics, and is a protective factor for the low incidence of UC.Based on this, donor-to-donor variation in the treatment of fecal microbiota transplantation may alter the therapeutic effect. Therefore, screening high-quality donors can improve efficacy and minimize the risk of adverse effects.Based on this, the investigators asked scientific questions: FMT in the treatment of IBD: the Bai nationality of Yunnan province may be a high quality donor.Our study is aims to FMT in the treatment of IBD, the role of selection of the Bai nationality as donor source in terms of treatment efficacy and mechanisms.

2. Objectives

2.1 Primary Outcome

The primary outcome is a composite of steroid-free clinical remission together with endoscopic remission or response at week12, defined as defined as a total Mayo score of ≤2 points with no individual sub-score >1 point, and at least a 1 point reduction from baseline in the

endoscopy sub-score.

2.2 Secondary Outcome

i.Steroid-free clinical remission (defined as combined Mayo sub-scores of 1 or less for rectal bleeding plus stool frequency)

ii.Steroid-free clinical response (defined as either a decrease of 3 points or more on the Mayo score, a 50% or greater reduction from baseline in combined rectal bleeding plus stool frequency Mayo sub-scores, or both)

iii.Steroid-free endoscopic response (defined as a Mayo endoscopy sub-score of 1 or less, with a reduction of at least 1 point from baseline)

iv.Changes in microbial composition (including bacteriome, virome and fungome), function and metabolite at weeks 0, 1, 8, 12

v.Change in microbiome of stool (including bacteriome, virome and fungome) at weeks 0, 1, 8, 12 and duration of change before reverting to baseline

vi.Difference in microbiome (including bacteriome, virome and fungome) compared between subjects in different treatment arm

vii.Proportion of microbiome (including bacteriome, virome and fungome) derived from recipient, donor or both in subjects who received FMT

viii.Difference in microbiome (including bacteriome, virome and

fungome) compared between subjects who have weight loss and those do not have weight loss

ix.Microbial factors (including bacteriome, virome and fungome) that are associated with percentage of body weight loss

x.Trans-kingdom correlation of microbial engraftment after FMT between bacteriome, virome and fungome

Groups (30 Patients/Group)	Samples	Before FMT Day Baseline(0)	Follow Up (Week) 1	Follow Up (Week) 8	Follow Up (Week) 12
Fresh (the Bai nationality Donor)	Stool and Urine	√	√	√	√
	Blood and Biopsies	√	√		√
Fresh (the Han nationality Donor)	Stool and Urine	√	√	√	√
	Blood and Biopsies	√	√		√

3. Design

3.1 FMT:

Fresh fecal bacterial solution was obtained through a fecal bacterial transplantation isolation system (FMT-6A-50/12-AS, Nanjing), which was directly infused into the ileocecal region through colonoscopy tube (100 mL each time) within 30 minutes.A total of 3 transplantations were performed on the 1st, 3rd and 5th day of admission, respectively. The fresh bacterial solution administered during the 3 FMT sessions was obtained from feces with a total weight of 100 g.

3.2 Mayo score

Item	Score	Evaluation
1. Stool frequency	0	Normal number of stools
	1	1-2 more than normal
	2	3-4 more than normal
	3	≥ 5 more than normal
2. Rectal bleeding	0	No blood seen
	1	Streaks of blood with stool in in less than half of the cases
	2	Obvious blood with stools in most of the time
	3	Blood alone passed
4. Endoscopic findings	0	Normal mucosa or inactive disease
	1	Mild activity (erythema, decreased vascular pattern, mild friability)
	2	Moderate activity (marked erythema, lack of vascular pattern, friability, erosions)
	3	Severe activity (spontaneous bleeding, large ulcerations)
5. Physician's global assessment	0	Normal
	1	Mild disease

	2	Moderate disease
	3	Severe disease

3.3 The clinical laboratory

Blood sampling for routine blood test, blood biochemistry, c-reactive protein (CRP) and blood sedimentation (ESR) detection.

3.4 Histologic assessment

Colonic biopsy will adopt a colonoscopy histologic evaluation.

6. Methods

Single-center randomized trial

6.1 Sample processing methods

The procedures of sample collections and subpackaging are as follows

① Study blood ： Red heparin tube 5mL+Purple heparin tube 4mL.Centrifuge at centrifugal force of 2500 rpm and temperature of 4℃ for 15min, and finally subpackage in 2ml cryogenic vials.

②Study stool ： The feces of patients are collected and packed into 2ml cryogenic vials.

③Study urine：Urine from patients was collected and then packed into a 15ml saliva tube.

④Study biopsies： Two of the most severe rectal (10cm local anus) lesions are selected and placed in 2ml cryogenic vials.

6.2 Fecal Calprotectin

Fecal calprotectin concentrations will be assessed in batches when

sufficient fecal samples have been collected for QUANTA Lite calprotectin ELISA assays. This is an enzyme-linked immunosorbent assay (ELISA) system that is based on colorimetric detection of polyclonal antibodies to calprotectin. The enzyme-linked immunosorbent assay ranged from 10 to 1800 mg/g. The required sample volume ranged from 50 to 150ml, and the test sensitivity was less than 10mg /g. The treatment arm of the subject will be analyzed in a blinded manner.

6.3 Microbiological Analysis

① Extraction of nucleic acids

DNA was isolated from the study samples using Maxwell® RSC PureFood GMO and Authentication Kit according to protocol. Fecal pellets were first added to CTAB buffer and vortex and heated for 5 min. Proteinase K and RNase A were added to the samples and incubated at 70℃. After centrifugal, collecting supernatant, transferred to Maxwell ® RSC instrument.

② Microbial and metabolomic analysis

The collected specimens will undergo laboratory microbiological analysis, including bacterial, viral, and fungal components of the human microbiome. Qiagen QIAamp®DNA Mini Kit, Qiagen QIAamp DNA Stool Mini Kit, Mobio UltraClean®Tissue & Cells DNA Isolation were used Kit, Maxwell®RSC PureFood GMO, and Authentication Kit perform different DNA enrichment and extraction methods for bacteria, viruses, and fungi.

Subsequent metagenomic sequencing was performed with the use of a Novoseq 6000 (2x300bp double-ended) system. Bacteria, fungi, and viruses were analyzed by methplan2, UNITE2-Bowtie2, and Kraken2, respectively. DESeq, Random Forest, and LEfSe linear discriminant analysis will be used to compare the differences in microbiome, metabolome, and metatranscriptome configurations between treatment groups, donors, and specimens at different time points. DESeq and Random Forest are executed in R via the DESeq and randomForest packages, respectively. Lefse analysis will be hutten hall lab galaxy server (http://huttenhower.sph.harvard.edu/y/). A DB-RDA analysis will be performed in R to describe the influence of other clinical factors on polysomy.

6.4 Studies in Animals

Animal studies may be performed using collected biological samples. For example, fecal samples collected from FMT donors and recipients can be gavaged into animal models to study the effects of the microbiota.

7. Clinical study approval document issued by the Ethics Committee of the First Affiliated Hospital of Kunming Medical University

Clinical study approval document issued by the Ethics Committee of the First Affiliated Hospital of Kunming Medical University

(2023) Lun L No.127

project name	Mechanism and clinical study of fecal bacteria transplantation for inflammatory bowel disease				
Application Authority (person)	The First Affiliated Hospital of Kunming Medical University				
Undertake the department	department of gastroenterology	Principal investigator	According to should rayZhang-qin li	professional ranks and titles	Graduate student of chief physician
Source of research funds	■ Government mouth Foundation Kou Company□ international co-operationD. Others (please indicate)				
Send review documents	1 Study protocol (Version No:1.0. Date: June 7,2023) 2 Informed Consent Form (Version 1.0, dated 07 June 2023) 3 Case Report Form (Version No.1.0, dated 07 June 2023) 4 CV of principal investigator and copy of GCP training certificate				
Ethical review methods	□ Conference Review				
	■ Quick Review				
juror	Du Qinglin and Zhang Ling				
Review comments from the Ethics Committee	Agreed to proceed at this center,				

The responsibilities, personnel composition, operating procedures and records of the EC include ICH-CCP and relevant laws and regulations of China.

matters need attention

1. The clinical test shall be implemented within 1 year from the date of approval of the ethics Committee, but not implemented during the fishing period. Several pieces of this review are be abolished.
2. The study should be performed with the protocol approved by the Ethics Committee and comply with the principles of CFD / CP and CP
3. From the date of consent to the study, conduct regular follow-up visits every 12 months (the review compliance may change according to the actual progress), please submit to the Ethics Committee one month before the expiration of the regular review (
4. During the study. Any modifications to the study protocol and the informed consent form. Please submit the relevant information in the amendment application form and the "submission document elimination form", and submit the amendment to the Ethics Committee before implementation.
5. In case of a serious adverse event or unexpected adverse event affecting the risk benefit ratio of the study, the Ethics Committee shall be reported to the CDA in writing, using the CHDA (Serious Adverse Event Report Form or the Ethics Committee (Serious AE / Unanticipated AE Report Form or other relevant report forms, but the foreign language report requires a Chinese abstract, and the Ethics Committee has the right to make new decisions on its evaluation.
6. Do not do or violate the program should be timely submitted (do not do or violate the program report form.7. Early termination of the study shall submit the Termination Report Form of the study protocol in time.
8. Submit after the completion of selenium research ("Study Conclusion Report Form".
9 Timely written report on important decisions of other ethics committees.

Chairman Signature of the
Chairman:good Ethics Committee of the
10th Affiliated Hospital of Kunming
Medical University (seal)

In 2023, August 24

Address: No.295, Xichang Road, Kunming, Yunnan province: 650032 Tel: 087i-65928584 Contact: Wang Ting

8. Statistical Analysis Plan

Continuous variables to mean (SD), said with a paired t test to compare the difference between the two groups. Classification variables will be compared using chi-square test or Fisher's exact test. The modified intention-to-treat analysis included all subjects receiving at least one FMT treatment. Patients who required treatment escalation, violated the study protocol, failed to discontinue corticosteroids at week 8, or discontinued the study for any reason were considered to have treatment failure. Each protocol analysis will include all patients who completed the 8-week masked study treatment course without protocol violation. Missing data will be used before the last observation to the method of calculation, so the missing values will be the last one is not the missing value replacement. A two-sided p value of less than 5% was considered to indicate statistical significance. Security will use descriptive statistical methods to collect data. All statistical tests were performed using SPSS Statistics version 20 (IBM Corporation, Armonk, NY).

9. Clinical study approval document issued by the Ethics Committee of the First Affiliated Hospital of Kunming Medical University

The First Affiliated Hospital of Kunming Medical University

Informed consent form for microflora transplantation (FMT / miniFMT)

surname and personal name: administrative or technical offices: Bed number: bed Hospitalization Number: Date:

Disease conditions and treatment recommendations:

1. Basic information of the patients:

diagnose:_____

age:_____Gender: _____ Allergic history:_____Pregnancy history: □ with □ with no

Number of previous FMT treatments: _____ (times), with or without adverse effects: □_ □ not have

FMT Pre-treatment examination:

ALT_____U /L ; AST_____U /L ; CRP____mg /L ; ESR____mm ;

EB -DNA_____Copy / ml; HCMV-DNA_____copy /ml;

Stool routine + occult blood: [];

HbsAg [ ]; Anti -HBs [ ]; HbeAg [ ]; Anti -Hbe [ ]; Anti -Hbc [ ];

Anti-HCV []; Anti-HIV 1 / 2 []; Syphilis: []; Others: []

2. Treatment recommendations

I have obtained the following explanations: microflora transplantation includes fecal bacteria transplantation (FMT) and formula microflora transplantation (miniFMT or SMT), the indications for microflora transplantation, foreign and domestic applications and research status, the benefits for the diagnosis and treatment of diseases, the chances of surgical (or regimen) treatment success,

Including: the benefits of functional intestinal flora reconstruction, the recent improvement or cure probability of the disease, the long-term evolution of the disease, different diseases and no

Differences in efficacy of the same subcategory of diseases, odds of success during treatment, and changes in efficacy during follow-up. The doctor has told me that I have one_ _____, Depending on the condition, faecal bacteria transplantation (FMT / miniFMT) treatment can be performed.

Our hospital has clearly informed the patient (close relatives / guardians) of the following contents:

(1) Although doctors have tried to eliminate possible risks, there may still be the risk of disease infection, "window period" problems that cannot be detected, wrong test results, and unpredictable risks; formula flora transplantation may also have unpredictable risks.

(2) Possible main risks and complications in the process of microflora transplantation and related diagnosis and treatment, including failure of operation, perforation, bleeding, abdominal infection, etc. In case of occurrence, agree to the doctor to give the corresponding diagnosis and treatment disposal.
(3) After the microflora transplant, my disease may be cured, partially effective, recurrent, or recurrent. Repeat implementation of microflora transplantation therapy may be required. I can choose to abandon this treatment strategy at any stage.
(4) For the diagnosis and treatment needs of the condition, the members of the diagnosis and treatment team can implement relevant alternative diagnosis and treatment measures.

Patients (close relatives / guardians) include the following contents:

(1) I agree to and authorize it_____(Physician signature)_ And its diagnosis and treatment team to implement the following possible options: via colonoscopy, on

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surname and personal name: administrative or technical offices: Bed number: bed Hospitalization Number: Date:

Gastrointestinal endoscopy, middle gastrointestinal endoscopy, gastric tube, nasojejunal tube, enema, oral capsule containing intestinal bacterial fluid, fistula tube, rectal graft tube (TET) (the involved endoscopy and anesthesia informed consent were signed separately).

(2) The type of transplanted bacteria I received was: □ fresh fecal bacteria □ frozen fecal bacteria □ formula bacteria.

(3) For faecal bacteria transplantation, I agree that the source of faecal bacteria is: □ relatives □ friends □_Other healthy people, as suggested by the doctor. For formula flora transplantation, I knew that the group selection was determined by the doctor.

(4) I agree that the hospital will preserve and use my fecal bacteria, body fluids and tissue specimens for teaching and medical research without involving personal identifiable information and privacy.

(5) Each doctor and patient shall hold one copy of this informed consent. I have been told that the property right of this document belongs to the institute and shall not be issued and distributed without permission.

(6) After full notification, I have read or passed my relatives have read and explained the above content, and I hereby sign the informed consent form.

Patients (close relatives / guardians) carefully promise that:

Patient (close relative / guardian) signature: Relationship: Time:_____year_____moon___ sun

Doctor Signature: Time:_____year_____moon_____sun

Department of Gastroenterology, the First Affiliated Hospital of Kunming Medical University2Informed consent form for microflora transplantation (FMT / miniFMT)

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Informed consent form for microflora transplantation (FMT / miniFMT)

surname and personal name: administrative or technical offices: Bed number: bed Hospitalization Number: Date:

Commitment content

(I) My name is XXX, and I have understood the process and related risks of fecal transplantation. I strongly require fecal transplantation treatment, and I am willing to take all the risks.

(Family member) I am XXX from XXX. I have understood the process and related risks of fecal transplantation, so I strongly demand the treatment of fecal transplantation, and I am willing to take all the risks.

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