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Original Article

Analysis of risk factors for early clinical recurrence of inflammatory bowel disease after fecal microbiota transplantation

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Abstract: Objective: To explore the risk factors for early clinical recurrence of inflammatory bowel disease (IBD) after fecal microbiota transplantation (FMT). Methods: A retrospective study was conducted on 192 patients with IBD who received FMT treatment in the Colorectal Disease Specialty/Intestinal Microecology Treatment Center of the Tenth People's Hospital Affiliated to Tongji University from February 2017 to June 2020. Univariate and multivariate logistic regression models were used to analyze the risk factors for early recurrence of inflammation. Feces from all participants were collected to extract the total bacterial genomic DNA. The V6-8 regions of the bacterial 16S rDNA gene were amplified by polymerase chain reaction (PCR), the PCR products were detected by the denaturing gradient gel electrophoresis (DGGE) method, and the intestinal flora was analyzed by DNA fingerprinting. Stool samples from all patients were tested for 9 bacteria, white blood cells (WBC) and platelet (PLT) counts, as well as the erythrocyte sedimentation rate (ESR) and serum C-reactive protein (CRP) level. Results: Of the 192 patients, 15 cases had inflammation recurrence during FMT and within one week after treatment, including 11 cases of ulcerative colitis (UC) and 4 cases of Crohn's disease (CD), with a total recurrence rate of 7.8%. High Mayo inflammatory activity score, Mayo endoscopic sub-item score (MES) =3 points, CRP>10 mg/L, anemia, albumin <30 g/L, absolute value of peripheral blood lymphocytes (PBL) <500/mm^3, and intolerance to enteral full nutrition were independent risk factors for recurrence during and after FMT in UC patients ($P<0.05$). Albumin <30 g/L and simultaneous use of immunosuppressive agents were associated with disease recurrence during and after FMT in CD patients. WBC, PLT, and CRP were all negatively correlated with Enterococcus (EC), and ESR was positively correlated with Saccharomyces boulardii (SB) ($P<0.01$). Conclusion: The low recurrence rate of IBD after FMT indicates the safety of FMT, but this procedure should be cautiously used in patients with severe intestinal barrier dysfunction and/or severe intestinal dysfunction.

Keywords: Fecal microbiota transplantation, inflammatory bowel disease, inflammation recurrence, risk factors

Introduction

Intestinal flora plays an important role in maintaining the stability of the intestinal environment and regulating the host mucosal immunity, the disturbance of which is associated with a variety of chronic inflammatory diseases. Fecal microbiota transplantation (FMT) is to transplant the flora of a healthy donor into the recipient's intestine, which can effectively rebuild the intestinal microecology, improve the intestinal environment, and restore the normal immune response of the host. FMT was initially used to treat recurrent Clostridium difficile infections, with a cure rate as high as 95.6%, which has been therefore applied in the treatment of a variety of chronic inflammatory diseases [1, 2]. Inflammatory bowel disease (IBD) refers to a group of intestinal disorders that cause chronic and recurrent inflammation of the gastrointestinal tract, including Crohn's disease (CD) and ulcerative colitis (UC), which is currently considered to be attributed to an abnormal immune response to intestinal flora in a genetically susceptible host [3]. In patients with IBD, the intestinal immune system is intolerant to the imbalance of intestinal flora, which leads to impaired intestinal mucosal barrier function, increased permeability, and the entry of pro-inflammatory substances such as anti-

gens and endotoxins in the intestinal cavity into the intestinal mucosa lamina propria, thereby inducing immune damage and inflammation. Intestinal ecological disturbance is an important initiation factor for the onset of IBD [3]. In recent years, FMT has been recommended for the treatment of IBD, with clinical evidence indicating an initial response rate and a final response rate of FMT for UC of 52.3% (33.5-70.8%) and 47.5% (29.4-65.8%) and those for CD of 34% (21.3-47.8%) and 39.6 (25.4-54.6%), respectively [4].

The entry of the antigens, endotoxins, and other components into the transplanted flora due to the impaired intestinal mucosal barrier function results in a stronger immune response to microbial antigens in IBD patients than healthy people, which may induce immune damage and trigger inflammation recurrence. Prior studies revealed that the total inflammatory recurrence rate of IBD after FMT was 14.9% (95% CI: 10-21%) [4, 5]. The use of probiotics in patients with IBD has indeed yielded some encouraging results. FMT is to re-establish intestinal flora balance after the flora disorders caused by antibiotics, with the strongest indication being refractory Clostridium difficile infection (rCDI). The understanding of the treatment progress of FMT in IBD can effectively guide the development of animal experiments and bring new hope to patients with clinically refractory IBD. In this retrospective study, the clinical data of 116 cases of UC and 76 cases of CD collected from February 2017 to June 2020 were retrospectively analyzed to explore possible risk factors for inflammation recurrence. This study innovatively investigated the risk factors for early clinical recurrence of IBD after FMT, which is conducive to disease prevention and early treatment, thus reducing the recurrence rate.

Materials and methods

Research participants

The clinical data of patients with IBD admitted to the Colorectal Disease Specialty/Intestinal Microecological Treatment Center of the Tenth People's Hospital Affiliated to Tongji University from February 2017 to June 2020 were retrospectively analyzed. Inclusion criteria: (1) Patients who were clinically confirmed with CD or UC; (2) Patients treated with FMT; (3) Patients with disease recurrence within one week after FMT treatment. Exclusion criteria: (1) Patients with unclear diagnosis of IBD; (2) Patients with previous total colectomy and ileoanal anastomosis; (3) Patients with incomplete clinical data. A total of 192 patients were included in this study.

This study was approved by the ethics committee of our hospital with the approval number of 2016-12-27.

Diagnostic criteria, definitions, and classification

Diagnosis and classification of IBD

IBD was diagnosed and classified referring to the *Chinese Consensus on Diagnosis and Treatment Standard of Inflammatory Bowel Disease* (Beijing, 2018).

Definition of clinical recurrence of IBD

I: The Crohn's disease activity index (CDAI) increased by more than 70 points for more one week, with a total score >220 points [6]; II: The Mayo inflammatory activity score for UC increased by >2 points for more one week and the Mayo endoscopic sub-item score (MES) was ≥2 points [7].

Feces samples (10±5 g) were collected from all patients on the day before microscopy, and were sent to the laboratory within 6 h after collection for sealing and storage at -20°C.

DNA extraction

The freeze-thaw + Bead Beater + kit DNA extraction method was employed for DNA extraction. First, 0.3 g of cryopreserved sample was weighed and transferred to a sterilized screw-capped tube containing 0.3 g zirconium beads for thorough mixing. Then, 1 mL of TN 150 and 150 μL of acid phenol were added and homogenized at 5000 r/min for 3 min using a Bead Beater. After cooling on ice, the fecal genomic DNA rapid extraction kit (Beijing Biotech Biotechnology Co., Ltd.) was used for extraction, and the operation was performed according to the kit instructions.

PCR amplification of genomic DNA

A pair of bacterial universal primers in the variable sequence regions of 16S rDNA were

designed to guide the PCR amplification reaction of the V6-8 regions of 16S rDNA [7]. The primers were synthesized by Shanghai Shenggong Biological Co., Ltd. The primer sequences were F968f-Gc: 5'-CGC CCG GGG CGC GCCCCG GGC GGG GCG GGG GCA CGG GGG GAA CGC GAA GAA CCT TAC-3'; R1401: 5'-CGG TGT GTA CAA GAC CC-3'. Reaction conditions: pre-denaturation at 94°C for 5 min; 94°C for 30 s, 56°C for 20 s, and 72°C for 1 min, for 30 cycles; extension at 56°C for 5 min. Reaction system (50 μL): dNtp 4 μL, Buffer 5 μL, Taq enzyme 0.3 μL, ddH_2O 37.7 μL, upstream and downstream primers and templates 1 μL each. The amplification results were detected by using 1.0% agarose gel electrophoresis (0.5 mL/L ethidium bromide staining), and the images were obtained with the UV gel imaging system. The theoretical length of the target fragment was about 470 bp. Repaired PCR amplification was performed when there was no specific amplification.

Repaired PCR amplification primer sequences were F968f-Gc: 5'-CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG GAA CGC GAA GAA CCT TAC-3'; R1401: 5'-CGG TGT GTA CAA GAC CC-3'. Reaction conditions: predenaturation at 94°C for 5 min, 94°C for 30 s, 56°C for 20 s, 72°C for 1 min, for 30 cycles; extension at 56°C for 5 min. Reaction system (50 μL): dNtp 4 μL, Buffer 5 μL, Taq enzyme 0.3 μL, ddH_2O 37.7 μL, upstream and downstream primers and templates 1 μL each.

Denaturing gradient gel electrophoresis (DGGE) and DGGE analysis for the amplified products of the V6-8 regions of bacterial 16S rDNA

8% polyacrylamide gel (containing propionamide, dipropionamide, urea, formamide, and glycerol) was used for DGGE with a denaturation gradient of 38-58%. Then electrophoresis was performed using a D code DGGE system (Bio-Rad) with a running buffer of 40 mmol/L Tris-ethanol (pH 8.0). Pre-electrophoresis was performed at 200 V for 5-10 min, and then the voltage was fixed at 85 V for electrophoresis for 16 h, followed by silver nitrate staining and image development with a UVI automatic gel imaging system (UV Itec, USA). For the appropriate bands on the DGGE gel, gel-cutting recovery for cloning and sequencing was performed.

Data analysis

Quantity One (Bio-Rad) software was used for similarity and diversity analyses of DGGE gel images (TIFF format). Cluster analysis was used for similarity analysis. Richness, and biodiversity analyses were adopted for diversity analysis.

The intestinal flora in patients with IBD was monitored, and its relationship with white blood cells (WBC), platelets (PLT), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) was observed. Various bacteria, WBC and PLT counts, as well as ESR and serum CRP levels in fecal specimens of all patients were detected.

FMT

Donor selection and management

Donor selection: The selection criteria of the donor were referred to the previous literature and the consensus of the donor selection experts established by the research team [8]. The criteria for FMT donors established in this study were different from those in European and American countries, with the age of the donor ranging from 18 to 30 years and BMI between 18.5-23.9 kg/m^2. The donor's single-gene dominant genetic disease gene was also tested. A negative result of the COVID-19 test of the donors was required after February 2020. Through long-term follow-up of the curative effect, a donor-recipient clinical curative effect screening model was established to dynamically observe the donor-recipient efficacy, and donors with an effective rate less than 20% were excluded.

Follow-up and management of donors: The research center was also equipped with full-time donor follow-up and management personnel. Follow-up and management covered the following domains: (1) Physiological condition: Heart rate, respiratory rate, pulse oxygen, sleep quality, daily defecation frequency, Bristol defecation score, and menstrual period (for female patients) were recorded; (2) Health status: The state of health (healthy or ill) of donors and the use of drugs were weekly reported; (3) Exercise: Moderate-intensity physical activity was required at least 5 days a week, with a total exercise time >150 min per week and 6000

walking steps daily; (4) Diet management: The donors' diet strictly followed *the Dietary Guidelines for Chinese Residents* (2016). An average of more than 12 kinds of foods a day and more than 25 kinds of foods a week were required, and the daily recipes were recorded and reported; (5) Intestinal flora regulation: Donors received 8 g of soluble dietary fiber (pectin) daily to regulate the intestinal flora; (6) Psychological evaluation: The psychological state of donors was scored by using the self-rating depression scale (SDS), self-rating anxiety scale (SAS), and Pittsburgh sleep quality index (PSQI) every 8 weeks; (7) Physical examination was carried out every 8 weeks. Through the follow-up data analysis, the poor living and eating habits of the donors were corrected in time, and the donors with diseases were excluded.

Preparation and quality control of bacteria liquid and capsules

Preparation of FMT bacteria liquid: The preparation method was referred to the expert consensus on standardized methodology of bacteria liquid preparation developed by the research team [8]. The intelligent microbial separation system-stool analysis pre-processing instrument was used to prepare standardized intestinal flora transplant bacteria solution. Specifically, the fresh feces were placed into a sterile mixer, stirred with normal saline (100 g feces: 300 mL sterile physiological saline), and filtered through 2.0 mm and 0.5 mm sieves successively. The fecal bacteria liquid was then collected and stored in a refrigerator at -20°C for short-term storage. The preparation process time was ≤60 min.

The preparation method of fecal bacteria capsules: It was referred to the expert consensus on standardization process for the clinical application of fecal bacteria capsules formulated by the research team [8]. The above-mentioned bacteria solution was centrifuged at 4°C to remove the supernatant, added with the freeze-dried protective agent and mixed with a shaker, to prepare a bacterial colony suspension for pre-freezing. The frozen sample was then freeze-dried, and the obtained bacterial powder was encapsulated.

Quality control: The intestinal flora activity of the recipient was >80%, and the number of live bacteria in a single transplantation was ≥1010 CFU. Samples with pathogenic bacteria, parasites, or multi-drug resistant bacteria were excluded from the study. Samples of each bacteria liquid preparation was kept for at least 6 months to facilitate traceability.

Transplantation process and method

Bowel preparation: (1) Standard decontamination protocol: Transintestinal pretreatment with vancomycin (0.5 g, 2 times/day) for 3 days was performed for transplantation. (2) Enhanced decontamination protocol: Before transplantation, vancomycin (0.5 g, 2 times/day), metronidazole (0.5 g, 2 times/day), fluconazole (0.2 g, 2 times/day), and gentamicin (80,000 units, 2 times/day) were used for pretreatment for a total of 6 days. (3) Elution: The intestinal tract was cleaned with polyethylene glycol 12-24 h before transplantation.

The selection and establishment of transplantation methods: They were detailed in the *Selection of bacterial colony transplantation pathways and the establishment of Chinese expert consensus on clinical application* [9]. The method of lower digestive tract transplantation included enema and colonoscopy, of middle digestive tract transplantation was transnasal jejunal tube transplantation, and of fecal capsule transplantation was oral administration of acid-resistant live bacteria capsules.

Research methods

IBD questionnaires were prepared to collect the data of the selected patients, including the demographic data, past medical history, clinical data, laboratory examinations, endoscopy, imaging examination data, and medication. After FMT, 192 patients who met the inclusion criteria were divided into clinical recurrence and non-recurrence groups according to their clinical outcomes during the follow-up period, to compare the clinical data and analyze the risk factors for the recurrence of IBD in patients undergoing FMT.

Statistical methods

SPSS 23.0 software was used for statistical analysis. The rank data were analyzed by the rank sum test. The count data were expressed by n (%) and processed by the X^2 test. Fisher's

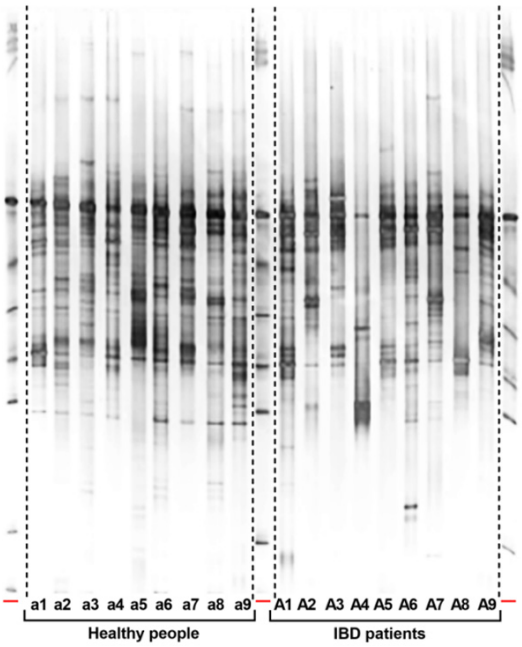

Figure 1. DGGE fingerprinting of fecal samples. Note: Lanes a1-a9 in the figure are PCR samples of total faeces from healthy patients and A1-A9 from IBD patients. Each lane represents a DGGE fingerprint of the total bacteria in the sample. Bands at different positions represent different dominant bacteria and the brightness of the band indicates the relative number of corresponding bacteria represented by the band. Each number represents: 1: Enterobacter, 2: Enterococcus, 3: Saccharomyces boulardii, 4: Small Clostridium, 5: Peptococcus, 6: Bacteroides, 7: Bifidobacterium lactis, 8: Lactobacillus, 9: Eubacterium. The red markers are Test. DGGE: denaturing gradient gel electrophoresis; IBD: inflammatory bowel disease.

exact probability method was used for univariate analysis, and the Logistic regression model was used for multivariate analysis. Pearson analysis was adopted for correlation analysis. $P<0.05$ was considered statistically significant.

Results

Figure 1 shows the PCR-DGGE profile analysis of total fecal bacteria DNA. In the figure, lanes a1-a9 are PCR samples of total feces of healthy people and A1-A9 of patients with IBD. Each lane represents the DGGE fingerprinting of the total bacteria in a sample. The bands in different positions represent different dominant bacteria, and the brightness of the band indicates the relative amount of the corresponding bacteria represented by the band.

In the experiment, a large number of bands were present in each lane in the experiment, but the number, position, and brightness of the bands varied, indicating a rich diversity of the bacteria and the difference in the dominant bacteria in each individual's feces. The cluster analysis of similarity is shown in **Figure 2**. In spite of the different degrees of flora similarity in the individual samples between the two groups, a relatively high flora similarity between the samples in the group can still be observed. There were large differences in the structure of the bacterial composition between the groups.

Correlation analysis of flora content and inflammatory indexes

Pearson correlation analysis was performed on WBC, PLT, ESR, CRP and 9 bacteria (Enterobacter [EMB], Enterococcus [EC], Saccharomyces boulardii [SB], Small Clostridium [SC], Peptococcus [PS], Bacteroides [BD], Bifidobacterium lactis [BL], Lactobacillus [LC], and Eubacterium [Es]). The results (**Figure 3**) showed that WBC, PLT, and CRP were negatively correlated with EC ($P<0.01$, $r=-0.513$, -0.478, -0.681, respectively), and ESR was positively correlated with SB ($P<0.01$, $r=0.791$).

Of the 192 patients, 15 cases had recurrence during FMT treatment and within one week after treatment, including 11 cases of UC and 4 cases of CD. Remission of recurrent UC was induced by hormone in 9 cases, total colectomy in 1 case, and infliximab in 1 case. All 4 cases of CD achieved remission induced by infliximab.

Univariate analysis showed that in UC patients, high Mayo inflammatory activity score, MES=3 points, intolerance to the target amount of enteral nutrition, recent use of hormones to induce remission, serum albumin <30 g/L, and peripheral blood lymphocyte deficiency were associated with a higher FMT postoperative recurrence rate ($P<0.05$). Thirteen patients who used biologic agents before FMT and 4 patients with extraintestinal manifestations had no recurrence (**Table 1**).

Univariate analysis demonstrated that in CD patients, the use of hormones to induce remission in the short-term and short-term bowel resection were associated with a higher recurrence rate following FMT ($P<0.05$). The recur-

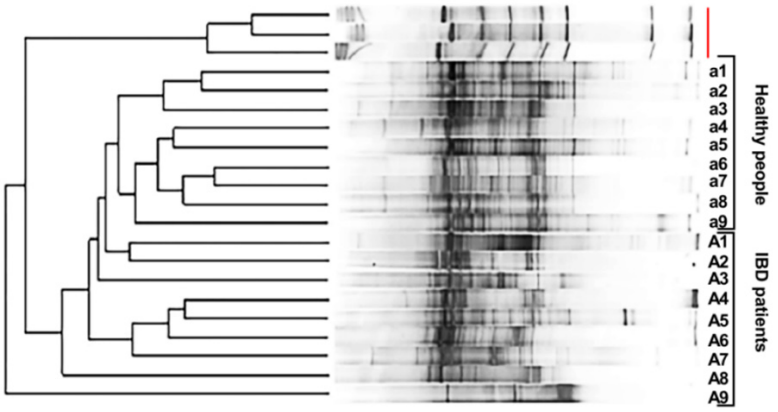

Figure 2. Cluster analysis of similarity of DGGE fingerprinting of fecal samples. Note: In the experiment, a large number of bands were present in each lane in the experiment, indicating a rich diversity of the bacteria and the difference in the dominant bacteria in each individual's feces. Each number represents: 1: Enterobacter, 2: Enterococcus, 3: Saccharomyces boulardii, 4: Small Clostridium, 5: Peptococcus, 6: Bacteroides, 7: Bifidobacteriumlactis, 8: Lactobacillus, 9: Eubacterium. The red mark is Test. DGGE: denaturing gradient gel electrophoresis; IBD: inflammatory bowel disease.

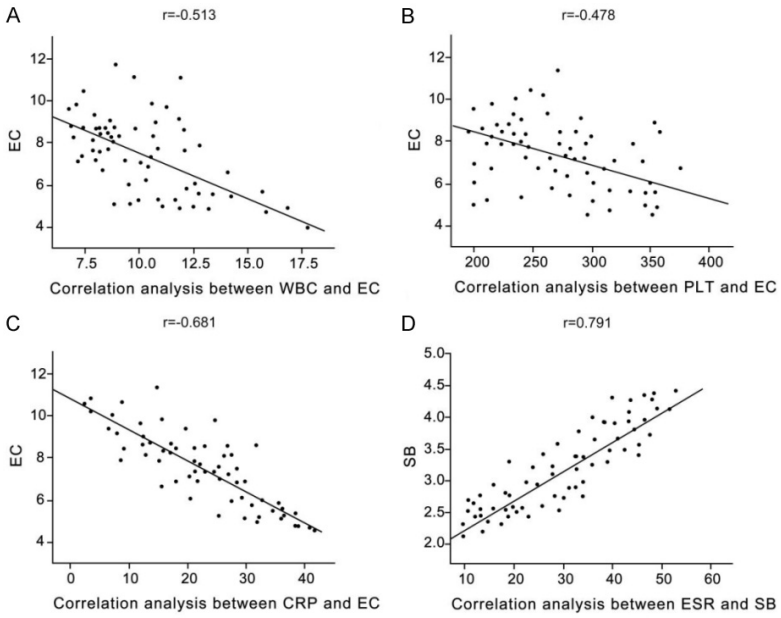

Figure 3. Correlation analysis between inflammatory indicators and bacterial counts. A. Pearson correlation analysis between WBC and EC. B. Pearson correlation analysis between PLT and EC. C. Pearson correlation analysis between CRP and EC. D. Pearson correlation analysis between ESR and SB. WBC: white blood cells; EC: Enterococcus; PLT: platelet; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; SB: Saccharomyces boulardii.

rence rate of severely active patients with high CDAI score reached 20.0% (2/10), which was significantly higher than that of the mild and moderate groups (3.0%, 2/66). The recurrence rate of patients who could not tolerate enteral full nutrition was significantly higher than that of the standard enteral nutrition group (75% vs. 5.6%); however, given the small number of relapsed patients, the above differences were not statistically significant. Patients with the disease site of L2 were more susceptible to relapse. The recurrence rate of patients with spontaneous use of immunosuppressants was 14.3% (2/14). Moreover, 1 of the 13 cases who used biological agents relapsed, 5 patients with extraintestinal manifestations showed no recurrence, and in 4 cases of recurrence, the fecal calprotectin was observed to be higher than 250 μg/g (**Table 2**).

Comparison of the approaches and methods of flora transplantation showed that single transplantation of a quarter of the bacteria solution was associated with a higher propensity for recurrence (16.1%) ($P<0.05$). The recurrence rate of inflammation in each group of different decontamination methods and transplantation routes was not statistically significant (**Table 3**).

Clinical data were collected and assigned values (**Table 4**), and logistic regression was used for analysis. The results demonstrated that high Mayo inflammatory activity score, MES=3 points, CRP>10 mg/L, anemia, albumin <30 g/L, absolute value of PBL<500/mm^3, and intolerance to enteral full nutrition were independent risk factors for disease recurrence during and after FMT in UC patients ($P<0.05$). The results showed that albumin <30 g/L and simultaneous use of immunosuppressive

Table 1. Univariate analysis of early inflammation recurrence after FMT in patients with UC

Clinical data	Non-recurrence group (n=105)	Recurrence group (n=11)	*P*-value
Disease site			
E1	34	3	1.000
E2	43	5	
E3	28	3	
Presence of extraintestinal manifestations	4	0	NA
Inflammatory activity score			
Mayo 0-2	19	1	0.023
Mayo 3-5	54	3	
Mayo 6-10	28	5	
Mayo 11-12	4	2	
Mayo endoscopic sub-item scores			
0-2	102	1	0.000
3	3	10	
Simultaneous use of biological agents	13	0	NA
Enteral nutrition tolerance	96	1	0.000
To the last hormone induction			
Within 4 weeks	3	8	0.001
Over 4 weeks	19	2	
Never	83	1	
Calprotectin >250 μg/g	99	10	1.000
With Clostridium difficile infection	9	2	0.235
Serological indicators before treatment			
CRP>10 mg/L	82	10	1.000
Hemoglobin <120 g/L (Female <110 g/L)	17	4	0.125
Albumin <30 g/L	21	6	0.031
Absolute value of peripheral blood lymphocytes <500/mm^3	4	8	0.008

Note: CRP: C-reactive protein.

agents were independent risk factors for disease recurrence during and after FMT in patients with CDI (P<0.05) (**Table 5**).

Discussion

FMT has been proven to be the most effective treatment for CDI by reconstructing the intestinal microbiome. Similar to Clostridium difficile enterocolitis, the onset of IBD has also been confirmed to be closely related to flora disorders, which results in FMT being a hotspot in the research of IBD in recent years. However, recent clinical studies have found that FMT may increase the risk of IBD. A study has found that the overall risk of recurrence of IBD reaches 14.3% (95% CI: 11-19%) [4]. Whereas, if only randomized controlled trials are included, the recurrence rate of IBD inflammation is 4.6%, with no significant heterogeneity among the studies [10], indicating that FMT is a relatively safe treatment in rigorously designed clinical studies.

In this study, MES, CRP, anemia, albumin, absolute value of PBL and intolerance to enteral full nutrition were found to be independent risk factors for disease recurrence during and after FMT in UC patients, while albumin and simultaneous use of immunosuppressive agents were associated with disease recurrence during and after FMT in CD patients.

A MES of 3 points indicates a serious intestinal mucosal barrier defect in UC patients. Patients with UC who cannot tolerate target enteral nutrition often experience severe gastrointestinal bleeding, high-flow diarrhea, severe abdominal pain and bloating, or toxic colitis. These conditions indicate that the patient has severe intestinal dysfunction with systemic inflammatory response that triggers inappropriate

Table 2. Univariate analysis of early inflammation recurrence after FMT in patients with CD

Clinical data	Non-recurrence group (n=72)	Recurrence group (n=4)	*P*-value
Disease site			
L1*	59	3	0.565
L2	3	0	
L3	10	1	
Presence of extraintestinal manifestations	5	0	NA
Disease behavior			
B1	54	1	0.041
B2	10	2	
B3	8	1	
Inflammatory activity score			
CDAI<150	26	1	0.198
CDAI 150-219	38	1	
CDAI 220-449	8	2	
CDAI≥450	0	0	
Simultaneous use of biological agents	12	1	1.000
Simultaneous use of immunosuppressive agents	12	2	0.500
Enteral nutrition tolerance	68	1	1.000
To the last hormone induction			
Within 4 weeks	0	1	0.000
Over 4 weeks	6	3	
Never	66	0	
Calprotectin >250 μg/g	60	4	0.125
To the last bowel resection			
Within 4 weeks	0	1	
Over 4 weeks	8	0	0.003
Never	64	0	
Serological indicators before treatment			
CRP>10 mg/L	33	3	1.000
Hemoglobin <120 g/L (Female <110 g/L)	33	2	0.500
Albumin <30 g/L	25	2	0.500
Absolute value of peripheral blood lymphocytes <500/mm^3	5	1	0.250

Note: *22 patients with L1 combined with L4B. CDAI: Crohn's disease activity index; CRP: C-reactive protein.

inflammatory responses to components such as xenoantigens and endotoxins in the transplanted flora, leading to activation of the primary disease. During the inflammatory activity of IBD, the activation of Bcl-2 and caspase-9 pathways may accelerate the apoptosis of peripheral blood mononuclear cells, with the degree of activation being proportional to the inflammatory activity [11]. Therefore, in many cases, a decrease in PBL (<500/mm^3) indicates the aggravation of mucosal inflammatory response and the impairment of intestinal barrier, leading to the deterioration of inflammation after transplantation. The mortality and colectomy rate increased significantly in IBD patients with CDI. Such patients have indications for FMT and require more cautious transplantation. The results of Qazi et al. showed that patients with IBD combined with CDI had a higher risk of disease deterioration after FMT [12]. This may be related to the higher baseline disease activity of patients with combined CDI infection, which further activates the mucosal immune response and alters the pathophysiological process of IBD [13]. However, no significant difference was found in the recurrence rate in patients with CDI, which may be due to the fact that this study provides more options in

Table 3. Univariate analysis of flora transplantation methods for early inflammation recurrence after transplantation

Bacteria transplantation method	Non-recurrence group (n=177)	Recurrence group (n=15)	*P*-value
Decontamination method			
No decontamination	8	2	0.108
Standard decontamination	53	6	
Enhanced decontamination	116	7	
Approach of flora transplantation			
Middle digestive tract	149	12	0.714
Lower digestive tract	14	2	
Fecal bacteria capsules	14	1	
Amount of bacterial solution per transplant			
1/4	47	9	0.005
1/2	74	5	
Full amount	56	1	

Table 4. Assignment table

Factor	Assignment
With recurrence (Y)	Recurrence =1, no recurrence =0
MES score	Including MES≥2 points =1, MES<2 points =0
Disease site	Disease site L1=1, L2=2, L3=3
Extraintestinal manifestations	Presence of extraintestinal manifestations =1, no extraintestinal manifestations =0
Calprotectin	Calprotectin >250 μg/g =1, calprotectin <250 μg/g =0
CDAI	CDAI<150=1, CDAI 150-219=2, CDAI 220-449=3, CDAI≥450=4
CRP	CRP>10 mg/L=1, CRP≤10 mg/L =0
Hemoglobin	Hemoglobin <120 g/L =1, hemoglobin ≥120 g/L=0
Albumin	Albumin <30 g/L =1, albumin ≥30 g/L =0
Absolute value of PBL	Absolute value of PBL<500/mm^3 =1, absolute value of PBL≥500/mm^3 =1
hormone-induced remission	Recent hormone-induced remission =1, no recent hormone-induced remission =0
Use of biological agents	Simultaneous use of biological agents =1, no simultaneous use of biological agents =0
Enteral nutrition tolerance	Enteral nutrition tolerance =1, no enteral nutrition tolerance =0

Note: CRP: C-reactive protein; CDAI: Crohn's disease activity index.

Table 5. Multivariate analysis of disease recurrence during and after FMT in CD patients

Indexes	B	SE	Wald	P	OR	95 CI%
MES score =3 points	4.452	1.788	5.721	0.001	2.354	1.365-5.467
CRP>10 mg/L	3.457	4.378	6.457	<0.01	1.876	0.648-6.644
Anemia	1.457	1.356	7.455	<0.01	2.678	1.354-5.684
Albumin <30 g/L	2.544	2.333	5.455	<0.01	3.47	1.356-6.687
Absolute value of (PBL) <500/mm^3	3.458	6.598	2.4758	<0.01	6.757	2.789-8.655
Intolerance to enteral full nutrition	4.571	7.658	1.752	<0.01	7.545	3.545-9.541
Simultaneous use of immunosuppressive agents	5.748	4.659	3.654	<0.01	1.457	0.787-3.758

Note: CRP: C-reactive protein; MES: Mayo endoscopic sub-item score; PBL: peripheral blood lymphocytes.

enhancing decontamination and intestinal aseptic pretreatment before transplantation. In patients with hormone-induced remission with formal dose reduction, patients are moderately to highly immunosuppressed when the transplantation is performed at the hormone usage exceeding 20 mg of prednisone equivalent, which may produce conditional pathogens in

the transplanted flora, triggering transient intestinal infection and activating inflammation.

CD patients who have undergone bowel resection in the past 4 weeks may still be in a postoperative stress state, manifested by dysfunction of intestinal digestion, absorption and transmission, and intestinal mucosal edema around the anastomosis and incision, which contributes to the susceptibility to relapse [14]. Among patients with CD, 70% of those who are intolerant to enteral nutrition often suffer from incomplete intestinal obstruction, with intestinal distend and accumulation of gas in the upper segment, resulting in retardation of bacterial growth during transplantation. Patients with non-fibrous stenosis may have severe inflammatory reactions, deep ulcers, and significant damage to the intestinal barrier at the site of obstruction, which may eventually lead to disease recurrence due to the prolonged staying of the flora. Those who have successfully induced remission by biological agents, on the other hand, may enjoy amelioration in intestinal flora structure and dysfunction, especially the recovery of the intestinal butyrate metabolism system. Persistent disturbance of the normal structure and function of the intestinal flora indicates the failure of induction therapy with anti-tumor necrosis factors, anti-integrins, and other biological agents [15, 16]. In a randomized controlled trial (RCT), Sokol et al. found that FMT can be used to maintain remission in CD patients with biologically-induced remission [17]. Nonetheless, little is known about the simultaneous implementation of FMT with biological agents in high-risk IBD patients. In this study, the number of patients who concurrently used biological agents was too small to validate the potential of the combined use of biological agents as a risk factor for aggravated inflammation.

The effects of different transplantation approaches have also been compared in the present study. Theoretically, a lower concentration of bacterial solution per unit area reaching the lesion and less exposure of xenoantigens indicate a lower possibility of exacerbated inflammation. The bacteria liquid may be diluted by the use of a nasal-jejunal tube for bacterial liquid transplantation, the oxygen-rich environment of the middle digestive tract, the dilution of high-concentration digestive juice, and the dilution of small intestinal juice. Previous clinical research has found that the less exposure of foreign bacteria per unit area of the intestinal mucosa may be the reason for the slightly lower recurrence rate of the middle digestive tract [18], which, nevertheless, cannot be verified in this study in light of the small sample size. In 4 RCTs using lower gastrointestinal tract transplantation, the overall recurrence rate of inflammation was only 4.6%, which excluded the local bacterial concentration from being a decisive factor for the recurrence of inflammation. In this research, patients with higher levels of inflammatory activity were empirically selected for transplantation with a smaller amount of bacterial solution. Patients who received 1/4 of the transplants accounted for 60% of the total relapsed patients, suggesting that reducing the amount of transplants fails to balance the risk of serious intestinal barrier defects.

A previous analysis revealed a potential relation between super donor and the success rate of transplantation and disease remission [19]. Our study only tracked the changes in clinical symptoms of patients within one week of treatment. The changing inclination of the recipient flora, changes in the content of key metabolites, and the corresponding relationship with the composition of the donor flora and metabolic pathways have not yet been elucidated. Therefore, further investigation is warranted to clarify the correlation of the success rate of FMT in the treatment of IBD with the risk of inflammation recurrence and the composition of the donor flora.

As far as we are aware, this is the first retrospective analysis to compare the risk factors of inflammation recurrence caused by FMT treatment of IBD. The low recurrence rate of IBD after FMT indicates the safety of FMT, but this procedure should be used with caution in patients with severe intestinal barrier damage and intestinal dysfunction. A clinical study has reported the effect of combined use of nonsteroidal anti-inflammatory drugs and combined use of biological agents on the success rate of FMT treatment and the rate of inflammation recurrence [20]. Whereas, as this study is a single-center retrospective study with limited evidence, a multi-center and larger sample size

study is warranted to further explore and develop standardized strategies to assess the risk of inflammation exacerbation before FMT.

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Disclosure of conflict of interest

None.

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