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Immunomodulatory Effects of Fecal Microbiota Transplantation in Steroid-refractory Acute Gastrointestinal Graft-versus-host Disease Following Allogeneic Hematopoietic Stem Cell Transplantation

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ABSTRACT

Steroid-refractory acute gastrointestinal graft-versus-host disease (SR GI-GvHD) is a severe complication following allogeneic hematopoietic stem cell transplantation (allo-HSCT), characterized by intestinal immune dysregulation, microbiota disruption, and poor outcomes. Fecal microbiota transplantation (FMT) may restore intestinal immune homeostasis through microbiota modulation.

This single-center retrospective cohort study included 26 patients with hematologic malignancies who developed SR GI-GvHD after allo-HSCT. Eleven patients received conventional second-line immunosuppressive therapy, while 15 additionally received FMT. Treatment assignment was based on patient preference and shared decision-making. Clinical remission, symptom recovery, nutritional indicators, Eastern Cooperative Oncology Group (ECOG) performance status, quality of life assessed using the EORTC QLQ-C30, and survival outcomes were compared between groups.

The complete remission rate was higher in the FMT group than in the control group (73.3% vs 54.5%). FMT significantly shortened the times to diarrhea resolution, dietary recovery, and complete remission. Patients receiving FMT showed greater improvements in serum albumin levels, Nutritional Risk Screening 2002 scores, and ECOG performance status. Significantly greater improvements in emotional functioning, global health status, and appetite loss were also observed. Overall and progression-free survival showed favorable trends following FMT, although the differences were not statistically significant. FMT-related adverse events were limited mainly to mild gastrointestinal symptoms and transient fever, which resolved with symptomatic management. No grade 3 or 4 FMT-related adverse events occurred.

FMT combined with second-line immunosuppressive therapy may improve clinical remission, nutritional status, functional recovery, and quality of life in patients with SR GI-GvHD. Larger prospective randomized trials are warranted to confirm these exploratory findings.

Keywords: Allogeneic hematopoietic stem cell transplantation; Fecal microbiota transplantation; Graft-versus-host disease; Immune dysregulation; Intestinal microbiota; Quality of life

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INTRODUCTION

Acute graft-versus-host disease (aGVHD) occurs in 30% to 70% of patients after allogeneic hematopoietic stem cell transplantation (HSCT) and is one of the most common complications following HSCT.[1] The gastrointestinal (GI) tract is the most common visceral organ to be affected and is a major cause of death following transplantation. The most severe complications of GI-GvHD are classified as grade III–IV and significantly worsen patients' survival and decrease their quality of life.[2,3] GI-aGVHD mainly manifests as intractable diarrhea, abdominal pain, nausea and vomiting, and in severe cases, gastrointestinal bleeding and intestinal obstruction may occur, leading to severe malnutrition, immune dysfunction, and even life-threatening conditions.[4]

Glucocorticoids are the primary choice of treatment for aGVHD. However, up to 50% of patients develop steroid-refractory (SR) disease. SR GI-GvHD was defined as failure to achieve a complete or partial response after at least 3 days of treatment with methylprednisolone at a dose of ≥2 mg/kg/d (or equivalent corticosteroid), or disease progression during corticosteroid therapy, according to established criteria from the NIH Consensus Conference and previous landmark studies.[5,6] Patients with SR disease have a very dire prognosis, with a median overall survival of 30% at 2 years postdiagnosis.[5,6] Second-line immunosuppressive therapy (such as tacrolimus, mycophenolate mofetil, antithymocyte globulin, etc) has limited efficacy and increases the risk of infection.[7]

Interest has recently grown regarding the involvement of gut microbiota in the pathogenesis of aGVHD. Recent work has shown that aGVHD is associated with significant gut dysbiosis that is characterized by decreased microbial diversity, diminished SCFA-producing organisms, and increased potential pathogens.[8,9] The gut microbiota influence the development of GVHD by controlling intestinal immune homeostasis, influencing barrier integrity in the gut, and affecting various metabolic processes in the region.[10,11]

Fecal microbiota transplantation (FMT) restores gut microecological balance, has become the standard treatment for recurrent *Clostridioides difficile* infection, and has shown good efficacy in inflammatory bowel disease, metabolic syndrome, and other diseases.[12–14] Previous studies have shown that FMT can be used as a treatment for refractory gut dysbiosis and steroid-resistant GvHD, with favorable efficacy.[15,16]

This study aimed to evaluate the clinical efficacy and safety of FMT in the treatment of acute GVHD after HSCT and its impact on patients' nutritional status, performance status, and quality of life through a retrospective analysis, providing evidence-based medical evidence for clinical practice.

MATERIALS AND METHODS

Patient Data

This was a single-center, retrospective cohort study performed after a positive vote by the hospital's internal ethics committee. The requirement for written informed consent was waived due to the retrospective nature of the study. Patient enrollment and treatment assignment (FMT vs control/alternative treatment) were based on patient preference and shared decision–making after thorough discussion of potential benefits and risks, rather than randomization. This study was conducted in accordance with the STROBE statement for reporting observational studies. As this was an exploratory retrospective study rather than a confirmatory randomized controlled trial, no formal sample size calculation was performed a priori. Instead, the sample size was determined by the total number of consecutive patients who met the eligibility criteria during the study period from 2020 to 2024. All eligible patients who provided informed consent during the clinical care process were included. The study included 26 patients who developed grade IV GI-GvHD after allo-HSCT during the period of 2020 to 2024. According to the International Consortium grading for Acute GI GvHD, all patients were categorized as steroid-refractory GI-GvHD.

The inclusion criteria were as follows: (1) age ≥14 years; (2) received allogeneic hematopoietic stem cell transplantation (allo-HSCT) for hematologic malignancies; (3) diagnosed with grade IV GI-GvHD according to the International Consortium grading system; (4) confirmed as SR GI-GvHD, defined as no response or disease progression after at least 3 days of methylprednisolone (≥2 mg/kg/d) or equivalent corticosteroid therapy; (5) persistence of diarrhea (≥500 mL/d or ≥3 episodes/d) for more than 7 days despite corticosteroid treatment.

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The exclusion criteria were as follows: (1) active uncontrolled infection, including bacteremia, fungemia, or severe pneumonia; (2) active gastrointestinal bleeding, ileus, or toxic megacolon; (3) severe neutropenia (absolute neutrophil count $<0.5 \times 10^9$/L) without recovery trend; (4) severe thrombocytopenia (platelet count $<20 \times 10^9$/L) with active bleeding tendency; (5) history of anaphylaxis to FMT preparation components; (6) pregnancy or lactation.

Grouping and Treatment

Eleven patients in the control group received second-line immunosuppressive therapy, including tacrolimus, mycophenolate mofetil, CD25 monoclonal antibody (basiliximab), and ruxolitinib. The choice of second-line agent was determined by the treating physician based on individual patient characteristics and prior medication history, following institutional guidelines. Tacrolimus was administered at an initial dose of 0.03 mg/kg/d by continuous intravenous infusion, adjusted to maintain trough levels of 5 to 15 ng/mL. MMF was given at 15 mg/kg twice daily orally. Ruxolitinib was initiated at 5 mg twice daily, with dose adjustment based on platelet counts and clinical response. CD25 monoclonal antibody (basiliximab) was administered at 20 mg on days 1 and 4. The treatment duration varied according to clinical response and tolerability. Fifteen patients in the FMT group received FMT via nasojejunal tube (n=12) or oral capsules (n=3) in addition to second-line immunosuppressive therapy with the same agent selection and dosing protocols as the control group. All patients who received FMT opted for this therapy after detailed counseling regarding its potential benefits and risks. The number of FMT sessions per patient was determined by clinical response at the 28-day evaluation and the occurrence of relapse after initial response, following the retreatment criteria detailed in the FMT Regimen section below.

FMT Regimen

Donor screening was conducted according to standardized protocols, including: (1) medical history interview to exclude chronic diseases, gastrointestinal disorders, infection risk factors (eg, high-risk sexual behavior, drug use), and antibiotic/probiotic use within 3 months; (2) blood tests for HBV, HCV, HIV, syphilis, CMV, EBV, and SARS-CoV-2; (3) stool tests for routine analysis, occult blood, enteric pathogens (*Salmonella*, *Shigella*, *Campylobacter*, etc), *Clostridioides difficile* toxin, ova/parasites, and enteric viruses (rotavirus, norovirus, adenovirus). Donors with any positive finding or acute infection within 4 weeks were excluded. All fecal preparations were processed and stored under GMP-like conditions. The preparation consisted of 50 g of donor stool homogenized in 250 mL of sterile normal saline, filtered through sequential sieves, and centrifuged. The final suspension was aliquoted into 50 mL syringes for nasojejunal infusion or encapsulated for oral administration. All preparations were stored at −80 °C until use, and thawed at 37 °C immediately before administration. Microbiological safety testing was performed on each batch, including aerobic and anaerobic bacterial cultures, fungal cultures, and toxin assays. For the FMT group, the route of administration was nasojejunal tube in 12 patients and oral capsules in 3 patients. The volume of liquid FMT was 150 to 200 mL per session, and capsule FMT consisted of 30 to 40 capsules per session (each containing 0.5 g of fecal material). Liquid fecal preparations were purchased from Nanjing Fecal Bank, and fecal capsules were purchased from Shanghai BioTeng Biotechnology Company. Antibiotics were stopped at least 12 hours before the procedure. A proton pump inhibitor (PPI) was administered intravenously at least 1 hour before, or oral PPI was given once the night before and once on the morning of the procedure. Metoclopramide 10 mg was injected intramuscularly at least 0.5 hours before. Fecal samples were taken out 1 hour before treatment, thawed and rewarmed (do not open the cap), rewarmed in a 37°C water bath to finally reach 35°C to 37°C, and then infused into the intestine through a nasoduodenal tube within 2 to 4 minutes. Fecal capsules were swallowed directly after preparation was completed.

Retreatment criteria for FMT were as follows: Patients who achieved partial remission (PR) or stable disease (SD) at the 28-day efficacy evaluation, or those who achieved complete remission (CR) but subsequently relapsed (defined as recurrence of diarrhea≥500 mL/d or ≥3 episodes/d after initial CR), were eligible for repeat FMT. A minimum interval of 14 days between FMT sessions was observed. All repeat FMT procedures followed the same preparation and administration protocol as the initial FMT. Among the 15 FMT-treated patients, 7 received a single FMT session, 6 received 2 sessions, and 2 received 3 sessions. Due to the limited sample size, subgroup analysis to evaluate the impact of treatment frequency on outcomes

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was not statistically feasible; however, clinical responses were observed across patients receiving different numbers of FMT sessions, and this heterogeneity is acknowledged as a limitation of the study.

Efficacy and Safety Evaluation

Daily diarrhea volume and frequency, abdominal pain score, and GvHD score were recorded after FMT. Efficacy was evaluated at 28 days after FMT as the time point to assess the effectiveness and safety of FMT. Complete remission (CR) was defined as disappearance of diarrhea, abdominal pain, and/or bleeding, or a reduction in stool volume ≥500 mL within 3 days; partial remission (PR) was defined as incomplete disappearance of bloody stools but a reduction of >50% compared to the most severe stage, and/or an average daily stool volume >500 mL but a reduction of more than 500 mL compared with the most severe stage; progressive disease (PD) was defined as new symptoms or worsening of original symptoms, and/or an increase in stool volume of more than 50%; the rest were defined as stable disease (SD).[9] Safety was evaluated based on adverse events during FMT and follow-up. Adverse events were graded according to the CTCAE v5.0 criteria. Attribution of adverse events to FMT versus underlying disease or concomitant medications was determined by 2 independent investigators. Infection surveillance included weekly blood cultures, serum galactomannan testing, and CMV/EBV DNA monitoring. Intensive care unit (ICU) admissions and sepsis episodes were recorded. Safety assessment was stopped 100 days after the first FMT, or at the time of patient death/withdrawal.

Nutritional and Performance Assessments

Nutritional Risk Screening 2002 (NRS2002): to assess nutritional risk; ECOG performance status score: to assess performance status; serum albumin: measured by bromocresol green method.

Quality of Life Assessment (EORTC QLQ-C30)

To evaluate the impact on the patient's improved quality of life, the European Organisation for Research and Treatment of Cancer Quality of Life Core Questionnaire (EORTC QLQ-C30) was employed. This scale has 30 items and includes 5 functional (physical, role, cognitive, emotional, and social) domains, and 3 symptom domains (fatigue, pain, nausea and/or vomiting) and 6 single symptoms (eg, dyspnea, insomnia, appetite loss, constipation, diarrhea, and financial difficulties) and quality of life/global health status. Domain scores are linearly transformed to a 0 to 100 scale using the formula: Transformed score=[(Raw score−possible minimum)/(possible maximum−possible minimum)]×100. A higher score on the functional and global health status domains indicates a better function or quality of life. A higher score on the symptom domains indicates development of symptoms. Missing data were handled according to the EORTC Scoring Manual: for multi-item domains, if at least half of the items were completed, the domain score was calculated using the mean of the completed items; otherwise, the score was treated as missing. Single-item missing responses were excluded from analysis. Questionnaires with more than 20% missing items were excluded. All scores were done and interpreted as per the advised EORTC score.

Follow-up and Survival Analysis

The follow-up deadline was October 1, 2025. Follow-up was conducted by telephone.

Overall survival (OS): interval from diagnosis to patient death or follow-up deadline; progression-free survival (PFS): interval from start of treatment to relapse due to any cause, death, or follow-up deadline.

Statistical Methods

Continuous variables were summarized as mean±standard deviation or median with interquartile range or minimum–maximum range, as indicated. Between-group comparisons used independent-samples t tests or Mann–Whitney U tests, as appropriate. Categorical variables were summarized as counts and percentages and compared using chi-square or Fisher's exact tests. Survival outcomes were evaluated using Kaplan–Meier curves and log-rank tests, with hazard ratios and 95% confidence intervals estimated using Cox regression. Between-group differences in quality-of-life changes were assessed using ANCOVA. All tests were two-sided, with $p<0.05$ considered statistically significant. Analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA) and R version 4.3.0 (R Foundation for Statistical Computing, Vienna, Austria).

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RESULTS

Baseline Patient Characteristics

A total of 26 patients with steroid-resistant GI-GvHD were enrolled in this study (Figure 1). In the control group of 11 patients, the median age was 38 years (14–60 years), including 8 males and 3 females. There were 5 cases of acute myeloid leukemia, 4 cases of myelodysplastic syndromes, and 2 cases of acute lymphoblastic leukemia. Haploidentical related donor transplantation was performed in 10 cases, and matched sibling donor transplantation in 1 case. All patients received peripheral blood stem cell transplantation. Seven patients received mBu/Cy + ATG, 1 received Cla + mBu/Cy + ATG, and 3 received mBu/Cy + HMA. In the FMT group of 15 patients, the median age was 28 years (15–59 years), including 8 males and 7 females. Eight patients had acute myeloid leukemia, 4 had myelodysplastic syndrome, and 3 had acute lymphoblastic leukemia. Twelve patients received haploidentical related donor transplantation, 2 patients received matched related donor transplantation, and 1 patient received unrelated donor transplantation. Peripheral blood stem cell transplantation was used in all patients. Eleven cases received mBu/Cy + ATG, 2 cases Flu + mBu/Cy + ATG, 1 case Cla + mBu/Cy + ATG, and 1 case received mBu/Cy + HMA. GvHD prophylaxis was given to all 26 patients with ATG + cyclosporine + MMF + short-term methotrexate.

All 26 patients received the same GVHD prophylaxis regimen consisting of antithymocyte globulin (ATG), cyclosporine (CsA), mycophenolate mofetil (MMF), and short-term methotrexate (MTX). The doses and schedules were identical across all patients: ATG (2.5 mg/kg/d on days −4 to −2), CsA (3 mg/kg/d continuous infusion from day −1, adjusted to maintain trough levels of 200 to 400 ng/mL), MMF (15 mg/kg twice daily from day 1 to day 30), and MTX (15 mg/m² on day +1, followed by 10 mg/m² on days +3, +6, and +11). This uniform intensified prophylaxis protocol was applied to all patients regardless of donor type due to the high-risk characteristics of the study population (all patients had hematologic malignancies and subsequently developed steroid-refractory GI-GvHD).

In the control group, the median time to GI-GvHD onset was 43 days (minimum–maximum, 28–196 days) after transplantation, with 3 cases occurring within 30 days, 6 cases between 30 to 100 days, and 2 cases after 100 days. In the FMT group, the median time to GI-GvHD onset was 36 days (minimum–maximum, 18–186 days) after transplantation, with 1 case occurring within 30 days, 10 cases between 30 to 100 days, and 4 cases after 100 days. There were no significant differences between the 2 groups in terms of age, sex, primary disease, transplant type, conditioning regimen, time to GvHD onset, etc (p>0.05), indicating comparability. At baseline, the median stool volume was 1200 mL/d (IQR 900–1500) in the control group and 1100 mL/d (IQR 850–1450) in the FMT group (p=0.612). The median diarrhea frequency was 8 times/d (IQR 6–10) in the control group and 7 times/d (IQR 5–9) in the FMT group (p=0.538). All patients had received methylprednisolone at ≥2 mg/kg/d for a median of 5 days (IQR 4–7) before enrollment, with no significant difference between groups (p=0.724). Prior second-line therapies before enrollment included ruxolitinib (control: 2 patients; FMT: 3 patients) and mycophenolate mofetil (control: 4 patients; FMT: 5 patients) (Table 1).

FMT Efficacy and Safety

Fifteen patients underwent FMT, of which 7 patients received FMT once, 6 patients received FMT twice, and 2 patients received FMT 3 times. The indications for repeat FMT were: (1) PR or SD at day 28 post-FMT (n=5); (2) relapse after initial CR (n=3). No patient received more than 3 FMT sessions. Due to the limited sample size (n=15 in the FMT group), formal subgroup analysis to compare outcomes by FMT session frequency (1 vs 2 vs 3 sessions) was not statistically feasible, as the small numbers in each subgroup precluded meaningful comparisons. In the FMT group of 15 patients, there were 11 CR (73.3%), 3 PR (20.0%), and 1 PD (6.7%). The patient with PD developed transplant-associated thrombotic microangiopathy and intestinal bleeding. In the control group, there were 6 CR (54.5%), 4 PR (36.4%), and 1 PD (9.1%). In the FMT group, 5 patients developed nausea, 3 developed vomiting, 1 developed fever, and 1 developed biliary tract infection. These patients improved after symptomatic treatment (Table 2). No grade 3 or 4 FMT-related adverse events occurred. No FMT-attributed bacteremia, sepsis, or ICU admission was observed during follow-up. One patient in the FMT group developed a biliary tract infection that was deemed unrelated to FMT based on temporal association and negative stool cultures.

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Time to Disease Resolution

The time to symptom resolution in the FMT group was significantly shorter than that in the control group (Table 3). The time to diarrhea resolution in the FMT group was shortened by 41% compared with the control group (8.5 days vs 14.3 days, p=0.008), the time to resume diet was shortened by 29% (15.1 days vs 21.2 days, p=0.042), and the time to CR was shortened by 29% (17.9 days vs 25.2 days, p=0.036).

Changes in Nutritional Indicators

Nutritional status assessment revealed that the albumin level in the FMT group increased significantly (+8.36±6.77 g/L, p<0.001), but it was not significant in the control group (+2.03±6.40 g/L, p=0.317) with a statistically significant difference between groups (p=0.026) (Table 4). Nutritional risk screening 2002 (NRS2002) scores decreased significantly in both groups, but the FMT group decreased to 3 (3, 3) and the control group to 3 (3, 4), with a significant difference between groups (p=0.037). The ECOG score decreased from 4 to 2 in the FMT group and to 3 in the control group, with a more significant improvement in performance status in the FMT group (p=0.007) (Table 4).

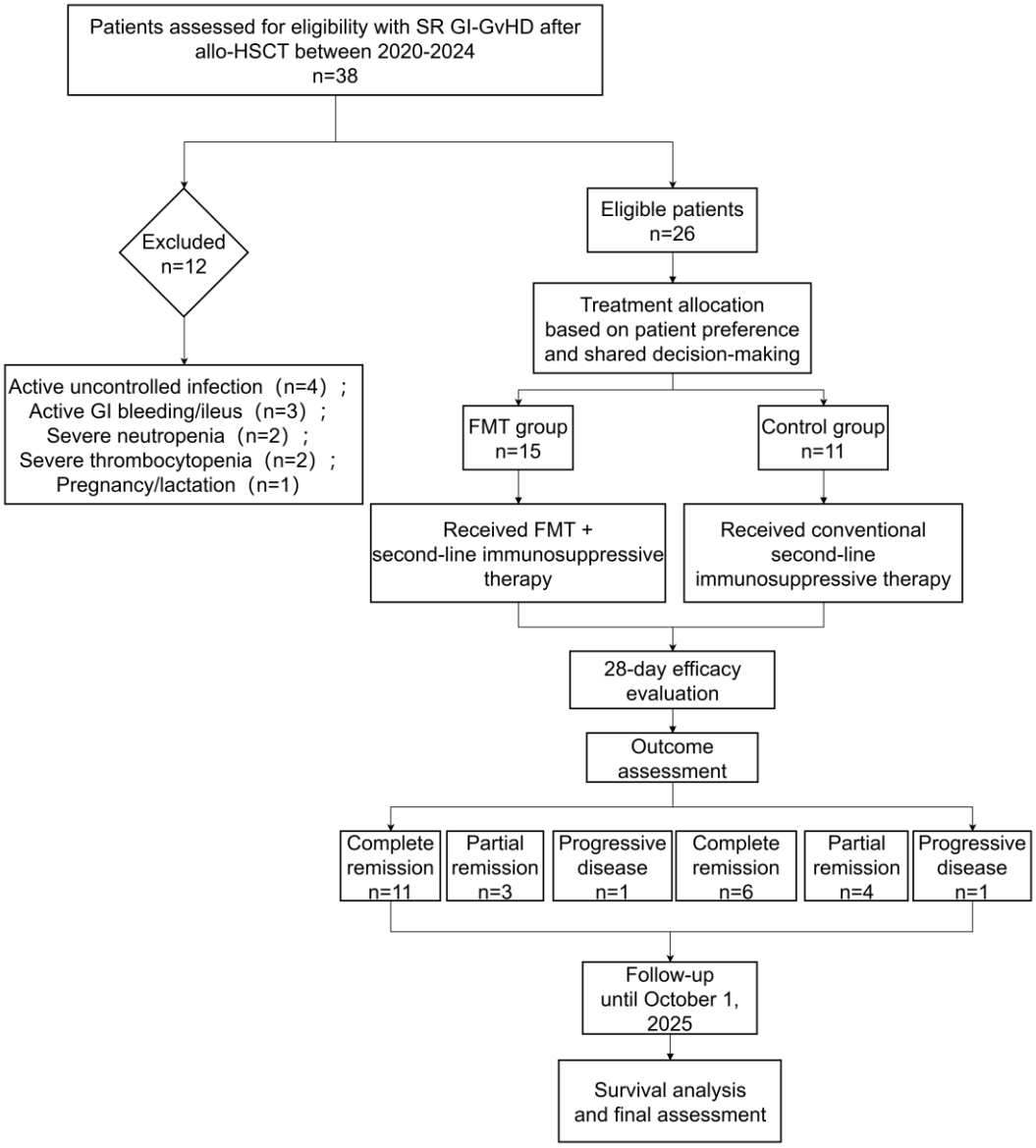

Figure 1. Patient screening flowchart.

Table 1. Clinical characteristics of 26 patients with steroid-resistant acute intestinal graft-versus-host disease.

Characteristics	Control group (n=11)	FMT group (n=15)	*p*
Age, median (range), y	38 (14–60)	28 (15–59)	0.312
Sex, n (%)			0.847
Male	8 (72.7)	8 (53.3)	
Female	3 (27.3)	7 (46.7)	
Disease, n (%)			0.654
AML	5 (45.5)	8 (53.3)	
MDS	4 (36.4)	4 (26.7)	
ALL	2 (18.2)	3 (20.0)	
Transplant type, n (%)			0.723
HRD	10 (90.9)	12 (80.0)	
MSD	1 (9.1)	2 (13.3)	
URD	0	1 (6.7)	
Conditioning regimen, n (%)			0.257
mBu/Cy + ATG	7 (63.6)	11 (73.3)	
Flu + mBu/Cy + ATG	0	2 (13.3)	
Cla + mBu/Cy + ATG	1 (9.1)	1 (6.7)	
mBu/Cy + HMA	3 (27.3)	1 (6.7)	
Time to GvHD onset, n (%)			0.891
<30 d	3 (27.3)	1 (6.7)	
30–100 d	6 (54.5)	10 (66.7)	
>100 d	2 (18.2)	4 (26.6)	
Baseline GvHD severity			
Stool volume, median (IQR), mL/d	1200 (900–1500)	1100 (850–1450)	0.612
Diarrhea frequency, median (IQR), times/d	8 (6–10)	7 (5–9)	0.538
Methylprednisolone duration before enrollment, median (IQR), d	5 (4–7)	5 (4–7)	0.724
Prior second-line therapies before enrollment, n (%)			
Ruxolitinib	2 (18.2)	3 (20.0)	1.000
Mycophenolate mofetil	4 (36.4)	5 (33.3)	1.000

ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; ATG: antithymocyte globulin; Flu: fludarabine; FMT: fecal microbiota transplantation; GvHD: graft-versus-host disease; HMA: hypomethylating agent; HRD: haploidentical related donor; IQR: interquartile range; mBu/Cy: modified busulfan and cyclophosphamide; MDS: myelodysplastic syndromes; MSD: matched sibling donor; URD: unrelated donor. Data are presented as n (%), median (minimum–maximum) for age, or median (interquartile range) for baseline GvHD severity indicators, as indicated.

Table 2. FMT times, efficacy and adverse events in Control and FMT groups.

Characteristics	Control group (n=11)	FMT group (n=15)
FMT times, n	NA	NA
1	NA	7
2	NA	6
3	NA	2
Efficacy, n (%)	NA	NA
CR	6 (54.5)	11 (73.3)
PR	4 (36.4)	3 (20.0)
PD	1 (9.1)	1 (6.7)
FMT-related adverse events, n	NA	NA
Nausea	NA	5
Vomiting	NA	3
Constipation	NA	0
Fever	NA	1
Hematochezia	NA	1
FMT-related infection	NA	1

[a]CR: complete remission; FMT: fecal microbiota transplantation; PD: progressive disease; PR: partial remission. Data are presented as counts (n) or counts with percentages [n (%)], as indicated.

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Table 3. Time to disease resolution.

Category	Control group (n=11)	FMT group (n=15)	Intergroup difference	*p*
Time to CR, d	25.2±7.4	17.9±7.5	−7.3 d (−29%)	0.036
Time to resume diet, d	21.2±5.8	15.1±6.1	−6.1 d (−29%)	0.042
Time to diarrhea resolution, d	14.3±4.1	8.5±3.2	−5.8 d (−41%)	0.008

[a]CR: complete remission; FMT: fecal microbiota transplantation. Times are presented as mean±standard deviation. Intergroup differences are expressed in days and as percentage differences relative to the control group.

Table 4. Changes in nutritional and performance indicators.

Category	Control group (n=11)		FMT group (n=15)		Intergroup *p*
	GvHD	GvHD CR	GvHD	GvHD CR	
Weight, kg	56.82±10.10	54.82±9.93	55.73±13.26	55.40±11.64	0.671
BMI, kg/m²	20.09±2.30	19.49±3.43	20.26±5.04	20.04±4.77	0.722
NRS2002	6 (6, 6)	3 (3, 4)[a]	6 (6, 6)	3 (3, 3)[a]	0.037
ECOG	4 (4, 4)	3 (2, 3)[a]	4 (4, 4)	2 (1, 2)[a]	0.007
Albumin, g/L	26.06±4.26	28.09±6.09[a]	25.08±3.79	33.44±7.66[a]	0.026

[a]$p<0.05$. [b]BMI: body mass index; CR: complete remission; ECOG: Eastern Cooperative Oncology Group; FMT: fecal microbiota transplantation; GvHD: graft-versus-host disease; NRS2002: Nutritional Risk Screening 2002. Weight, BMI, and albumin are presented as mean±standard deviation; NRS2002 and ECOG scores are presented as median (25th percentile, 75th percentile).

Quality of Life Scores

Quality of life was assessed using the EORTC QLQ-C30 (Table 5). In the unadjusted comparisons, the FMT group showed greater improvements in emotional functioning (p=0.039), global health status (p=0.025), and appetite loss (p=0.018). The reported ANCOVA results also favored the FMT group for physical functioning (p=0.027), emotional functioning (p=0.023), global health status (p=0.044), and appetite loss (p=0.015). Other between-group differences were not statistically significant.

Functional Dimensions

Symptom scores generally decreased after treatment in both groups. In the unadjusted between-group comparisons, the FMT group showed a greater reduction in appetite loss (difference in change, −29.50 points; 95% CI, −52.27 to −6.72; p=0.018). Between-group differences in the remaining symptom domains and financial difficulties were not statistically significant. Constipation scores remained zero in both groups (Table 5).

Symptom Dimensions

Fatigue: Control group: from 100.00 (median 100.00) before treatment to 53.53 (44.44) after treatment (p=NA); FMT group: from 100.00 (100.00) before treatment to 42.22 (44.44) after treatment (p=NA). Intergroup difference in improvement was −11.31 points (95% CI, −32.58 to 9.95; Cohen d=−0.41; p=0.309).

Nausea and Vomiting: Control group: from 28.79 (median 33.33) before treatment to 7.58 (0.00) after treatment (p=0.014); FMT group: from 26.67 (33.33) before treatment to 7.78 (0.00) after treatment (p<0.001). Intergroup difference in improvement was 2.32 points (95% CI, −13.97 to 18.62; Cohen d=0.12; p=0.770).

Pain: Control group: from 100.00 (median 100.00) before treatment to 30.30 (16.67) after treatment (p=NA); FMT group: from 98.89 (100.00) before treatment to 16.67 (0.00) after treatment (p<0.001). Intergroup difference in improvement was −12.53 points (95% CI, −34.56 to 9.50; Cohen d=−0.44; p=0.274).

Dyspnea: Control group: from 18.18 (median 0.00) before treatment to 12.12 (0.00) after treatment (p=0.341); FMT group: from 31.11 (33.33) before treatment to 11.11 (0.00) after treatment (p=0.023). Intergroup difference in improvement was −13.94 points (95% CI, −33.35 to 5.47; Cohen d=−0.52; p=0.199).

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Insomnia: Control group: from 66.67 (median 66.67) before treatment to 24.24 (0.00) after treatment (p<0.001); FMT group: from 68.89 (66.67) before treatment to 24.44 (33.33) after treatment (p<0.001). Intergroup difference in improvement was −2.02 points (95% CI, −21.84 to 17.80; Cohen d=−0.08; p=0.851).

Appetite Loss: Control group: from 100.00 (median 100.00) before treatment to 60.61 (66.67) after treatment (p=NA); FMT group: from 100.00 (100.00) before treatment to 31.11 (33.33) after treatment (p=NA), with greater improvement in the FMT group. Intergroup difference in improvement was −29.50 points (95% CI, −52.27 to −6.72; Cohen d=−1.01; p=0.018).

Constipation: Control group: from 0.00 (median 0.00) before treatment to 0.00 (0.00) after treatment (p=NA); FMT group: from 0.00 (0.00) before treatment to 0.00 (0.00) after treatment (p=NA). Intergroup difference in improvement was 0.00 points (95% CI, 0.00 to 0.00; Cohen d=0.00; p=NA).

Diarrhea: Control group: from 100.00 (median 100.00) before treatment to 24.24 (33.33) after treatment (p=NA); FMT group: from 100.00 (100.00) before treatment to 15.55 (0.00) after treatment (p=NA). Intergroup difference in improvement was −8.69 points (95% CI, −31.39 to 14.01; Cohen d=−0.30; p=0.455).

Financial Difficulties: Control group: from 81.82 (median 100.00) before treatment to 51.51 (33.33) after treatment (p=0.005); FMT group: from 60.00 (66.67) before treatment to 26.67 (33.33) after treatment (p<0.001). Intergroup difference in improvement was −3.03 points (95% CI, −22.78 to 16.72; Cohen d=−0.12; p=0.758).

Survival Analysis

The median follow-up duration for the entire cohort was 18.5 months (IQR, 11.2–28.4 months): 20.3 months (IQR, 12.5–30.1) for the FMT group and 15.8 months (IQR, 9.8–24.6) for the non-FMT group. Censoring was applied to patients who were alive without the event of interest at the last follow-up (October 1, 2025); 8 patients (53.3%) in the FMT group and 5 patients (45.5%) in the control group were censored for OS analysis. As of the last follow-up, 7 patients (46.7%) in the FMT group died, and 6 patients (54.5%) in the non-FMT group died. The median OS was not reached in the FMT group, while the median OS in the non-FMT group was 11.0 months. The 2-year OS rate was 59.3% in the FMT group and 45.5% in the non-FMT group. Kaplan-Meier survival analysis showed a trend toward improved OS in the FMT group compared with the non-FMT group, but the difference between the 2 groups was not statistically significant (HR=0.63; 95% CI, 0.21–1.88; log-rank p=0.395). (Figure 2).

For progression-free survival (PFS), 7 patients (46.7%) in the FMT group had disease progression or death, and 6 patients (54.5%) in the non-FMT group had disease progression or death. The median PFS was not reached in the FMT group, but the median PFS in the non-FMT group was 5.0 months. The 2-year PFS rate was 55.8% in the FMT group and 40.9% in the non-FMT group. In the PFS survival curve, the curve in the FMT group appeared to be better than that in the non-FMT group, but the difference was not statistically significant (HR=0.57; 95% CI, 0.19–1.70; log-rank p=0.296).

Given the potential confounding factors and the small sample size, we further performed multivariable Cox regression analyses to adjust for clinically relevant covariates, including age (continuous), disease type (acute leukemia vs other), and prior lines of therapy. After multivariable adjustment, the HR for OS was 0.58 (95% CI, 0.18–1.85; p=0.358), and the HR for PFS was 0.52 (95% CI, 0.16–1.68; p=0.271). The adjusted HRs remained below 1, consistent with the unadjusted analyses, suggesting a persistent trend toward survival benefit with FMT, although statistical significance was not reached.

The FMT group showed a trend toward improvement: although the differences were not statistically significant (p>0.05), the HRs in the FMT group were both <1 (OS, 0.63; PFS, 0.57), suggesting that FMT may have potential survival benefit. The 2-year survival rate increased by about 15 percentage points: the 2-year OS rate and PFS rate in the FMT group were both about 15 percentage points higher than those in the non-FMT group. Median survival was significantly prolonged: the median OS and PFS in the FMT group were not reached, while the median OS in the non-FMT group was only 11 months, and the median PFS was only 5 months. Due to the small sample size of 26 cases in the present research, its statistical power is relatively restricted. Large-scale trials with more participants are suggested to confirm our findings.

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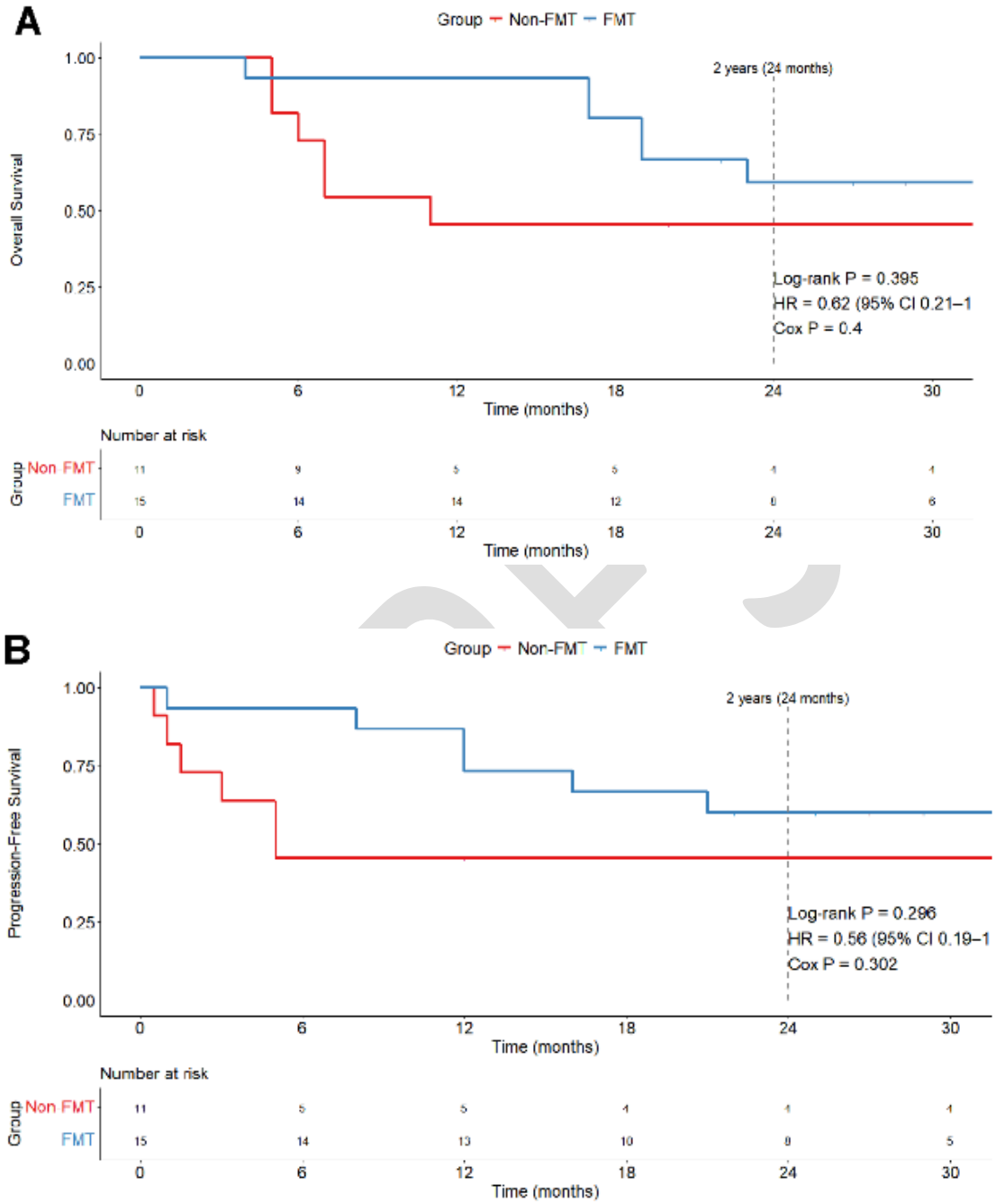

Figure 2. Kaplan-Meier survival curves for patients in the FMT and non-FMT groups. A. Overall survival (OS). B. Progression-free survival (PFS). The number of patients at risk at each time point is shown below the survival curves. Censoring is indicated by tick marks (+). CI: confidence interval; FMT: fecal microbiota transplantation; HR: hazard ratio.

Table 5. QLQ-C30 scores in patients with steroid-resistant acute intestinal GvHD.

Category	Control group (n=11)		FMT group (n=15)		Unadjusted analysis			ANCOVA		
	GvHD	GvHD CR	GvHD	GvHD CR	t	*p*	*d* (95% CI)	t	*p*	*d* (95% CI)
Functional	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Physical Functioning	10.3±9.6	62.4±25.9[a]	8.0±10.5	76.4±27.7[a]	1.69	0.104	0.67 (−2.40 to 35.05)	25.78	0.027[a]	1.07 (4.66 to 46.90)
Role Functioning	21.2±15.1	68.2±28.3[a]	17.8±14.7	67.8±28.5[a]	0.33	0.748	0.13 (−14.96 to 21.02)	10.04	0.361	0.42 (−11.01 to 31.09)
Emotional Functioning	17.4±13.2	46.2±25.1[a]	15.0±11.9	66.1±28.8[a]	2.19	0.039[a]	0.87 (1.26 to 43.38)	28.89	0.023[a]	1.10 (5.87 to 51.90)
Cognitive Functioning	22.7±22.7	48.5±24.1[a]	22.2±17.4	61.1±28.6[a]	1.72	0.099	0.68 (−1.76 to 28.02)	13.66	0.133	0.70 (−3.44 to 30.76)
Social Functioning	0.0±0.0	27.3±23.9[a]	1.1±4.3	46.7±28.3[a]	1.76	0.092	0.70 (−1.65 to 38.21)	16.20	0.229	0.57 (−9.38 to 41.78)
Global Health Status	0.8±2.5	40.2±18.2[a]	1.1±2.9	59.4±21.3[a]	2.39	0.025[a]	0.95 (3.58 to 34.30)	20.82	0.044[a]	0.97 (1.80 to 39.85)
Symptom	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Fatigue	100.0±0.0	53.5±27.1[a]	100.0±0.0	42.2±27.6[a]	−1.04	0.309	−0.41 (−32.58 to 9.95)	−16.22	0.215	−0.57 (−41.12 to 8.67)
Nausea and Vomiting	28.8±30.8	7.6±13.7[a]	26.7±18.7	7.8±13.9[a]	0.30	0.770	0.12 (−13.97 to 18.62)	−4.31	0.369	−0.41 (−13.49 to 4.87)
Pain	100.0±0.0	30.3±28.7[a]	98.9±4.3	16.7±28.2[a]	−1.12	0.274	−0.44 (−34.56 to 9.50)	−20.81	0.132	−0.72 (−46.74 to 5.13)
Dyspnea	18.2±22.9	12.1±22.5	31.1±23.5	11.1±20.6[a]	−1.32	0.199	−0.52 (−33.35 to 5.47)	−11.51	0.244	−0.55 (−30.32 to 7.30)
Insomnia	66.7±21.1	24.2±33.6[a]	68.9±23.5	24.4±29.5[a]	−0.19	0.851	−0.08 (−21.84 to 17.80)	−0.37	0.976	−0.01 (−24.87 to 24.13)
Appetite Loss	100.0±0.0	60.6±29.1[a]	100.0±0.0	31.1±29.5[a]	−2.53	0.018[a]	−1.01 (−52.27 to −6.72)	−34.72	0.015[a]	−1.18 (−60.40 to −9.05)

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Table 5. Continued...

Category	Control group (n=11)		FMT group (n=15)		Unadjusted analysis			ANCOVA		
	GvHD	GvHD CR	GvHD	GvHD CR	t	*p*	*d* (95% CI)	t	*p*	*d* (95% CI)
Constipation	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	NA	NA	NA (0.00 to 0.00)	0.00	NA	0.00 (0.00 to 0.00)
Diarrhea	100.0±0.0	24.2±30.2[a]	100.0±0.0	15.6±27.8[a]	−0.76	0.455	−0.30 (−31.39 to 14.01)	−20.43	0.109	−0.75 (−44.34 to 3.49)
Financial Difficulties	81.8±31.1	51.5±31.1[a]	60.0±36.1	26.7±31.4[a]	−0.31	0.758	−0.12 (−22.78 to 16.72)	−11.36	0.318	−0.49 (−33.09 to 10.37)

[a]$p<0.05$. [b]ANCOVA: analysis of covariance; CI: confidence interval; CR: complete remission; FMT: fecal microbiota transplantation; GvHD: graft-versus-host disease; QLQ-C30: Quality of Life Questionnaire Core 30. Scores are presented as mean ± standard deviation.

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DISCUSSION

This retrospective cohort study analyzed the clinical efficacy, safety, and quality of life impact of FMT for SR-GI-GvHD post-allogeneic hematopoietic stem cell transplantation (allo-HSCT). Results showed, statistically, that the FMT group was better than the control group with respect to the rate of complete remission, reduction of symptoms, the improvement of nutritional status, and quality of life. While no statistically significant differences in OS and PFS were found between the 2 groups, a favorable trend toward survival benefit was observed in the FMT group. This was an exploratory study and provides preliminary evidence for clinical practice in patients with SR-GI-GvHD.

In the FMT group, the majority, 73.3%, achieved complete remission, which was much higher than the 54.5% in the control group. These findings align with the advancements in the application of FMT in the management of GvHD. The core of gut changes in GvHD, with the loss of the normal gut flora, the loss of microbial diversity, and the bloom of gut flora that has the potential to be pathogenic, is the gut dysbiosis. The loss of normal gut flora is corrected by the infusion of gut flora from a healthy individual, and gut dysbiosis is corrected. FMT changes the gut dysbiosis in GvHD, accelerates the differentiation of regulatory T cells (Tregs), and causes a decrease in intestinal damage. Mechanistically, FMT may restore intestinal microbial diversity, particularly butyrate-producing bacteria such as *Faecalibacterium prausnitzii* and *Roseburia*, which are known to enhance intestinal barrier integrity through tight junction protein upregulation and mucin production.[7] Furthermore, FMT may reduce pro-inflammatory cytokines (eg, TNF-α, IL-6) and promote IL-10-producing Tregs, thereby modulating local and systemic immune responses.[8] The gut–brain axis may also be involved, as microbiota-derived metabolites such as short-chain fatty acids and tryptophan derivatives can influence neuroinflammation and mood regulation, potentially explaining the observed improvements in emotional functioning and global health status. FMT was first mentioned in the context of SR-GvHD and Kakihana's study.[17] The study showed that FMT has a positive impact on the diversity of the gut flora and remission of the symptoms. These positive outcomes reported support the findings of Bilinski et al[18] who showed that 60% to 80% of individuals with refractory GvHD respond to FMT.

In this study, the FMT group had significantly shorter times to diarrhea resolution, dietary resumption, and complete remission compared with the control group, suggesting that FMT accelerates clinical symptom control. This advantage may be related to the rapid reconstruction of gut microecological balance and restoration of intestinal barrier function by FMT. A study by Goloshchapov et al[19] showed that FMT could significantly increase gut microbial α-diversity within 7 days after transplantation and restore the abundance of beneficial bacteria such as butyrate-producing bacteria, thereby rapidly improving intestinal inflammation. In addition, FMT may reduce pro-inflammatory cytokine levels and promote mucosal repair by regulating the local intestinal immune microenvironment.[20]

Nutritional assessment was an important part of this study. The results showed that the increase in albumin level in the FMT group was significantly higher than that in the control group, and the improvements in NRS2002 score and ECOG score were more significant. This finding is closely related to the recovery of intestinal function. Intractable diarrhea and intestinal absorption dysfunction caused by GI-GvHD are the main causes of post-transplant malnutrition. FMT improves intestinal mucosal barrier function and promotes nutrient absorption, effectively reversing the malnourished state. Högenauer et al[21] pointed out that the gut microbiota participates in multiple aspects of host nutritional metabolism, including vitamin synthesis, short-chain fatty acid production, and mineral absorption. FMT improves patients' overall nutritional status by restoring these functions. In addition, improvement in performance status may affect patient prognosis through various pathways, including enhancing tolerance to subsequent treatments, reducing infection risk, and improving quality of life.[22]

An assessment of quality of life was a novel inclusion of this study. Using the EORTC QLQ-C30 scale, it was evident that the FMT group significantly surpassed the control group concerning physical, emotional, and general health status, and appetite loss. Most significantly, the rapid response of FMT in regard to the appetite loss parameter significantly correlated with the amelioration of intestinal symptoms. Improvements in emotional function and general health status indicated that FMT affected the patients' psychological well-being through the gut-brain axis. In the last few years, the microbiota-gut-brain axis has become one of the most important areas of research

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focused on the gut microbiome. The gut microbiota communicates with the central nervous system in a bidirectional manner and directs the function of systems and networks along the neural, endocrine, and immune pathways. FMT could potentially improve the patients' perception of anxiety and depression as well as their health through the regulation of patient perception along this gut-brain axis. A study by Valentin-Domelier et al[23] showed that FMT improved depression and anxiety scores in patients with inflammatory bowel disease, and this improvement was independent of relief of intestinal symptoms. A systematic review by Sherwin et al[24] further confirmed that gut microbiome interventions have a positive impact on mental and psychological health, with mechanisms possibly involving vagus nerve signaling, neurotransmitter metabolism, and neuroinflammation regulation.

Although survival analysis in this study did not show statistically significant differences in OS and PFS between the FMT group and the control group, in-depth analysis still revealed exploratory trends suggesting potential benefits of FMT. First, the HRs in the FMT group were both less than 1 (OS, 0.63; PFS, 0.57), suggesting that FMT may reduce the risk of death and disease progression, although the confidence intervals were wide. Second, the 2-year OS rate and PFS rate in the FMT group were about 15 percentage points higher than those in the control group, a difference that is clinically meaningful. Third, the median OS and PFS in the FMT group were not reached, while the median OS in the non-FMT group was only 11.0 months, and the median PFS was only 5.0 months, suggesting a potential advantage that warrants further investigation. The lack of statistical significance is likely due to limited statistical power from the small sample size (n=26), and the observed HRs < 1 should be interpreted as hypothesis-generating rather than confirmatory. These findings underscore the need for larger, adequately powered randomized controlled trials to definitively assess survival benefits. The relatively short follow-up time was also an important factor; some patients in the FMT group were still in follow-up, and the long-term survival advantage may not have fully emerged. In addition, some patients in the control group may have received other salvage therapies after treatment failure, affecting the observation of between-group differences.

In this study, FMT-related adverse events were mainly mild gastrointestinal reactions and low-grade fever, all of which resolved after symptomatic treatment, and there were no serious adverse events directly related to FMT. This favorable safety profile reflects our rigorous donor selection, meticulous preparation procedures, and prompt recognition and management of complications. The standardized operation specifications proposed by Bakken et al[25] emphasize that standardization of donor screening, fecal preparation, and transplantation route is key to ensuring safety. This study used third-party unrelated donor fecal preparations, and through strict quality control, no donor-derived infections occurred. Future studies should further explore the feasibility of autologous FMT or transplantation of specific bacterial consortia to reduce infection risk and improve predictability of efficacy.[26]

The main limitations of this study include: first, the single-center retrospective design with non-randomized treatment allocation, which introduces inherent selection bias despite comparable baseline characteristics; second, the small sample size (n=26) which limits statistical power for survival analyses and precludes robust multivariable adjustment for all potential confounders—specifically, we were unable to perform multivariable analyses adjusting for donor type, conditioning regimen, and GvHD onset timing simultaneously due to the limited number of events, and we therefore acknowledge that residual confounding from these factors cannot be excluded; third, the lack of longitudinal microbiome sequencing data (eg, 16S rRNA or metagenomic analysis) is a major limitation, as we are unable to confirm successful donor engraftment, track microbial diversity recovery, or correlate specific microbial changes with clinical responses—consequently, the biological effects of FMT were inferred indirectly from clinical outcomes, symptom resolution, and nutritional recovery rather than from direct microbial profiling; fourth, the absence of donor–recipient microbial matching analysis; fifth, the relatively short follow-up time for some patients, which may not capture late survival differences; sixth, the use of third-party unrelated donor feces rather than donor-recipient matched or autologous preparations, which may affect engraftment efficiency; seventh, the potential for unmeasured confounding, including differences in supportive care, nutritional support, and infection prophylaxis between groups—specifically, details regarding enteral nutrition, parenteral nutrition, albumin supplementation, and dietary management were not systematically recorded, and although all patients were managed under uniform institutional protocols, we

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cannot exclude the possibility that unmeasured differences in nutritional support may have contributed to the observed nutritional recovery outcomes; and eighth, the lack of adjustment for multiple comparisons in the QLQ-C30 analysis, which may increase the risk of false-positive findings. These limitations should be carefully considered when interpreting the results. Future research should adopt multicenter randomized controlled designs, expand sample size, and extend follow-up time. Integrating multi-omics technologies such as 16S rRNA sequencing, metagenomics, and metabolomics to deeply analyze the mechanism of action of FMT and identify microbial markers for predicting efficacy is an important direction for achieving precision FMT therapy.[27] In addition, the optimal timing of FMT still needs to be optimized. In this study, some patients received FMT relatively late after GvHD diagnosis, which may have affected efficacy. Early intervention may restore the microecological balance before intestinal damage becomes irreversible and improve remission rates. A study by Koyama et al[28] suggested that patients receiving FMT early in GvHD (within 2 weeks of diagnosis) had higher remission rates. In addition, the combination strategy of FMT with immunosuppressants also deserves exploration, including the timing and frequency of FMT and its synergistic effects with different second-line drugs.[29]

In summary, this exploratory study suggests that FMT combined with second-line immunosuppressants for the treatment of SR-GI-GvHD can significantly improve the clinical remission rate, accelerate symptom recovery, improve nutritional status, performance status, and quality of life, and has a good safety profile. As an innovative treatment strategy targeting the gut microbiome, FMT provides a promising new option for the treatment of SR-GvHD. Although large-scale and extended research is required to validate the survival benefit, and the survival findings remain exploratory pending confirmation in larger cohorts, existing evidence substantiates the significance of FMT in the holistic management of GvHD. To optimally harness the clinical potential of FMT, the future realm of research in this regard should consider addressing the gaps in the understanding of the mechanisms involved, even as evaluations, optimization, and tailoring the specific strategies that FMT could incorporate.

FMT combined with second-line immunosuppressants for the treatment of steroid-refractory acute gastrointestinal graft-versus-host disease after allo-HSCT can significantly improve the complete remission rate (73.3% vs 54.5%), accelerate symptom resolution (diarrhea resolution time shortened by 41%), improve nutritional status (albumin increase 4.1 times that of the control group), performance status (more significant improvement in ECOG score), and quality of life (significant improvements in emotional function, global health status, and appetite loss), and shows a trend toward survival benefit (2-year OS and PFS rates both increased by about 15 percentage points), although this was not statistically significant and should be considered exploratory, with a good safety profile. FMT provides a new effective strategy for the treatment of SR-GI-GvHD. However, given the single-center design and small sample size of this study, the generalizability of these findings to other transplant centers with different donor screening protocols, fecal preparation methods, patient populations, and healthcare systems requires confirmation through larger, multicenter studies. The use of commercial third-party donor feces rather than institutionally prepared or donor-recipient matched materials may also influence the reproducibility of these results across different settings.

STATEMENT OF ETHICS

This study was conducted in accordance with the Declaration of Helsinki and was reviewed and approved by the Ethics Committee of the Affiliated Huai'an Hospital of Xuzhou Medical University. Given the retrospective nature of the study and the use of existing clinical data, the requirement for written informed consent for participation in the retrospective analysis was waived by the Ethics Committee. Before fecal microbiota transplantation, written informed consent for the clinical procedure was obtained from all patients or, for participants younger than 18 years, from their parents or legal guardians. All patient information was anonymized before analysis to protect confidentiality.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

Not applicable.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

AI ASSISTANCE DISCLOSURE

Not applicable.

REFERENCES

1. Hematopoietic Stem Cell Application Group CSOH. Chinese expert consensus on the diagnosis and treatment of acute graft-versus-host disease after hematopoietic stem cell transplantation (2024). Zhonghua Xue Ye Xue Za Zhi. 2024;45(6):525–33.
2. Zeiser R, Blazar BR. Acute graft-versus-host disease—biologic process, prevention, and therapy. N Engl J Med. 2017;377(22):2167–79.
3. Malard F, Holler E, Sandmaier BM, Huang H, Mohty M. Acute graft-versus-host disease. Nat Rev Dis Primers. 2023;9(1):27.
4. Taur Y, Xavier JB, Lipuma L, Ubeda C, Goldberg J, Gobourne A, et al. Intestinal domination and the risk of bacteremia in patients undergoing allogeneic hematopoietic stem cell transplantation. Clin Infect Dis. 2012;55(7):905–14.
5. Penack O, Marchetti M, Aljurf M, Arat M, Bonifazi F, Duarte RF, et al. Prophylaxis and management of graft-versus-host disease after stem-cell transplantation for haematological malignancies: updated consensus recommendations of the European Society for Blood and Marrow Transplantation. Lancet Haematol. 2024;11(2):e147–59.
6. Kim NH, Hamadani M, Abedin S. New investigational drugs for steroid-refractory acute graft-versus-host disease: a review of the literature. Expert Opin Inv Drug. 2024;33(8):791–9.
7. Van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, et al. Duodenal infusion of donor feces for recurrent Clostridioides difficile. New England J Med. 2013;368(5):407–15.
8. Paramsothy S, Nielsen S, Kamm MA, Deshpande NP, Faith JJ, Clemente JC, et al. Specific bacteria and metabolites associated with response to fecal microbiota transplantation in patients with ulcerative colitis. Gastroenterology. 2019;156(5):1440–54.
9. Kootte RS, Levin E, Salojärvi J, Smits LP, Hartstra AV, Udayappan SD, et al. Improvement of insulin sensitivity after lean donor feces in metabolic syndrome is driven by baseline intestinal microbiota composition. Cell Metab. 2017;26(4):611–9.
10. Spindelboeck W, Schulz E, Uhl B, Kashofer K, Aigelsreiter A, Zinke-Cerwenka W, et al. Repeated fecal microbiota transplantations attenuate diarrhea and lead to sustained changes in the fecal microbiota in acute, refractory gastrointestinal graft-versus-host-disease. Haematologica. 2017;102(5):e210–3.
11. De Vos WM, Tilg H, Van Hul M, Cani PD. Gut microbiome and health: mechanistic insights. Gut. 2022;71(5):1020–32.
12. Cammarota G, Ianiro G, Tilg H, Rajilić-Stojanović M, Kump P, Satokari R, et al. European consensus conference on faecal microbiota transplantation in clinical practice. Gut. 2017;66(4):569–80.
13. Malik S, Naqvi SAA, Shadali AH, Khan H, Christof M, Niu C, et al. Fecal microbiota transplantation (FMT) and clinical outcomes among inflammatory bowel disease (IBD) patients: an umbrella review. Digest Dis Sci. 2025;70(5):1873–96.
14. Lopetuso LR, Deleu S, Puca P, Abreu MT, Armuzzi A, Barbara G, et al. Guidance for fecal microbiota transplantation trials in ulcerative colitis: the Second ROME Consensus Conference. Inflamm Bowel Dis. 2025;31(9):2408–19.
15. Weber D, Tariq M, Hazenberg M, Poeck H, Malard F. Microbiome, GvHD, and immune reconstitution in allogeneic hematopoietic cell transplantation. Bone Marrow Transpl. 2026;61(4):385–94.
16. Peled JU, van den Brink MRM. Fecal transplantation in hematopoietic transplantation. Journal of Clinical Oncology : Official Journal of the American Society of Clinical Oncology. 2023;41(34):5320–23.
17. Kakihana K, Fujioka Y, Suda W, Najima Y, Kuwata G, Sasajima S, et al. Fecal microbiota transplantation for

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patients with steroid-resistant acute graft-versus-host disease of the gut. Blood. 2016;128(16):2083–8.
18. Biliński J, Jasiński M, Basak GW. The role of fecal microbiota transplantation in the treatment of acute graft-versus-host disease. Biomedicines. 2022;10(4):837.
19. Goloshchapov OV, Olekhnovich EI, Sidorenko SV, Moiseev IS, Kucher MA, Fedorov DE, et al. Long-term impact of fecal transplantation in healthy volunteers. BMC Microbiol. 2019;19(1):312.
20. Imdad A, Pandit NG, Zaman M, Minkoff NZ, Tanner-Smith EE, Gomez-Duarte OG, et al. Fecal transplantation for treatment of inflammatory bowel disease. Cochrane Db Syst Rev. 2023;4(4):CD012774.
21. Högenauer C, Hammer HF, Krejs GJ, Reisinger EC. Mechanisms and management of antibiotic-associated diarrhea. Clin Infect Dis. 1998;27(4):702–10.
22. Gratwohl A, Brand R, Frassoni F, Rocha V, Niederwieser D, Reusser P, et al. Cause of death after allogeneic haematopoietic stem cell transplantation (HSCT) in early leukaemias: an EBMT analysis of lethal infectious complications and changes over calendar time. Bone Marrow Transpl. 2005;36(9):757–69.
23. Gîlcă-Blanariu GE, Șchiopu CG, Ștefănescu G, Mihai CTLA, Diaconescu S, Afrăsânie VA, et al. The intertwining roads between psychological distress and gut microbiota in inflammatory bowel disease. Microorganisms. 2023;11(9):2268.
24. Sherwin E, Sandhu KV, Dinan TG, Cryan JF. May the force be with you: the light and dark sides of the microbiota-gut-brain axis in neuropsychiatry. CNS Drugs. 2016;30(11):1019–41.
25. Bakken JS, Borody T, Brandt LJ, Brill JV, Demarco DC, Franzos MA, et al. Treating Clostridioides difficile infection with fecal microbiota transplantation. Clinical Gastroenterology and Hepatology: The Official Clinical Practice J Am Gastroenterol Association. 2011;9(12):1044–49.
26. Petrof EO, Gloor GB, Vanner SJ, Weese SJ, Carter D, Daigneault MC, et al. Stool substitute transplant therapy for the eradication of Clostridium difficile infection: 'RePOOPulating' the gut. Microbiome. 2013;1(1):3.
27. Zmora N, Zilberman-Schapira G, Suez J, Mor U, Dori-Bachash M, Bashiardes S, et al. Personalized gut mucosal colonization resistance to empiric probiotics is associated with unique host and microbiome features. Cell. 2018;174(6):1388–405.
28. Koyama M, Mukhopadhyay P, Schuster IS, Henden AS, Hülsdünker J, Varelias A, et al. MHC class II antigen presentation by the intestinal epithelium initiates graft-versus-host disease and is influenced by the microbiota. Immunity. 2019;51(5):885–98.
29. Gatti-Mays ME, Redman JM, Collins JM, Bilusic M. Cancer vaccines: enhanced immunogenic modulation through therapeutic combinations. Hum Vacc Immunother. 2017;13(11):2561–74.

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