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Effect of Fecal Microbiota Transplantation on 8-Week Remission in Patients With Ulcerative Colitis
A Randomized Clinical Trial

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IMPORTANCE High-intensity, aerobically prepared fecal microbiota transplantation (FMT) has demonstrated efficacy in treating active ulcerative colitis (UC). FMT protocols involving anaerobic stool processing methods may enhance microbial viability and allow efficacy with a lower treatment intensity.

OBJECTIVE To assess the efficacy of a short duration of FMT therapy to induce remission in UC using anaerobically prepared stool.

DESIGN, SETTING, AND PARTICIPANTS A total of 73 adults with mild to moderately active UC were enrolled in a multicenter, randomized, double-blind clinical trial in 3 Australian tertiary referral centers between June 2013 and June 2016, with 12-month follow-up until June 2017.

INTERVENTIONS Patients were randomized to receive either anaerobically prepared pooled donor FMT (n = 38) or autologous FMT (n = 35) via colonoscopy followed by 2 enemas over 7 days. Open-label therapy was offered to autologous FMT participants at 8 weeks and they were followed up for 12 months.

MAIN OUTCOMES AND MEASURES The primary outcome was steroid-free remission of UC, defined as a total Mayo score of ≤2 with an endoscopic Mayo score of 1 or less at week 8. Total Mayo score ranges from 0 to 12 (0 = no disease and 12 = most severe disease). Steroid-free remission of UC was reassessed at 12 months. Secondary clinical outcomes included adverse events.

RESULTS Among 73 patients who were randomized (mean age, 39 years; women, 33 [45%]), 69 (95%) completed the trial. The primary outcome was achieved in 12 of the 38 participants (32%) receiving pooled donor FMT compared with 3 of the 35 (9%) receiving autologous FMT (difference, 23% [95% CI, 4%-42%]; odds ratio, 5.0 [95% CI, 1.2-20.1]; *P* = .03). Five of the 12 participants (42%) who achieved the primary end point at week 8 following donor FMT maintained remission at 12 months. There were 3 serious adverse events in the donor FMT group and 2 in the autologous FMT group.

CONCLUSIONS AND RELEVANCE In this preliminary study of adults with mild to moderate UC, 1-week treatment with anaerobically prepared donor FMT compared with autologous FMT resulted in a higher likelihood of remission at 8 weeks. Further research is needed to assess longer-term maintenance of remission and safety.

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Visual Abstract

Editorial page 151

Video and Supplemental content

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Ulcerative colitis (UC) is a chronic inflammatory bowel disease characterized by colonic mucosal inflammation occurring at the interface between the luminal contents and the mucosal immune system. UC is increasingly common worldwide and has a high rate of persistent or relapsing symptoms[1] characterized by bloody diarrhea, anemia, and abdominal pain. UC is associated with a risk of colectomy[2] and an increased risk of colorectal cancer relative to the general population.[3] Although there is growing evidence implicating the colonic microbiome in UC pathogenesis,[4,5] most therapies target the immune response rather than the luminal microbial environment.[6]

In studies conducted since 2013, fecal microbiota transplantation (FMT) was an extremely effective treatment for recurrent or refractory *Clostridium difficile* infection.[7-10] This has encouraged research examining FMT as a potential therapy for other diseases possibly influenced by the microbiome. FMT is proposed to treat UC by modifying the colonic ecosystem, but the potential biochemical and/or immune mechanisms by which this may occur are unknown. FMT has demonstrated variable efficacy in treating active UC in 3 randomized clinical trials using aerobically prepared stool suspensions with relatively high treatment intensities.[11-13]

Most colonic bacteria and archaea are obligate anaerobes and are extremely oxygen sensitive; thus, they may be diminished or eliminated when stool is processed under aerobic conditions.[14] If oxygen-sensitive organisms or their metabolites contribute to the clinical effect of FMT, preserving their viability may enhance the clinical effect. The objective of this study was to investigate whether using anaerobically prepared stool with a lower treatment burden would be effective at inducing remission in active UC.

Key Points

Question Can a short duration of fecal microbiota transplantation (FMT) using anaerobically prepared pooled stool suspension induce remission in active ulcerative colitis?

Findings In this randomized clinical trial that included 73 adults with mild to moderately active ulcerative colitis, the proportion achieving steroid-free remission at 8 weeks was 32% with donor FMT vs 9% with autologous FMT, a significant difference.

Meaning Anaerobically prepared fecal microbiota transplantation may be effective in treating ulcerative colitis, but further research is needed to assess longer-term efficacy and safety.

Methods

Study Design, Setting, and Patients

A randomized, double-blind clinical trial of FMT that enrolled 73 patients with active UC was conducted between June 2013 and June 2016 at 3 Australian centers. Participants were followed up for 12 months until June 2017. All participants were 18 years of age or older and gave written informed consent. The ethics committee at each site approved the protocols. The full protocol appears in Supplement 1.

Eligible patients had active UC with a total Mayo score[15] of 3 to 10 points and an endoscopic subscore of ≥2. The total Mayo score is a composite of clinical and endoscopic markers and ranges from 0 to 12 (0 = no disease and 12 = most severe disease). Patients were excluded if they had severe disease defined by either a total Mayo score of 11 to 12 or Truelove and Witts criteria[16] (passing >6 bloody stools/day plus ≥1 of the following: temperature >37.8°C, pulse >90 bpm, hemoglobin <10.5 g/dL, or erythrocyte sedimentation rate >30 mm/h). Other exclusion criteria were previous colonic surgery, gastrointestinal infection, pregnancy, anticoagulant therapy, or current use of antibiotics or probiotics.

Stable dosing of UC maintenance therapy was required prior to enrollment: 4 weeks for 5-aminosalicylic acid, 6 weeks for thiopurines and methotrexate, and 8 weeks for biological agents. Patients could enroll taking an oral dose of prednisolone ≤25 mg, with a mandatory taper of 5 mg per week. Participants unable to cease oral prednisolone by week 8 were considered FMT nonresponders.

Patient screening included total Mayo score comprised of symptom and sigmoidoscopy assessment. Stool was collected for autologous FMT, fecal calprotectin, microbiota, and metabolome analysis and infective screening (microscopy, culture, and *C difficile* toxin mRNA). Baseline Simple Clinical Colitis Activity Index score (range, 0-19; 0 = no symptoms and 19 = most severe symptoms),[17] medical history, demographic details, a survey of patient perception and acceptability of FMT, and a 3-day diet diary including a weighed record of all food and fluid consumed for 2 weekdays and 1 weekend day were recorded. Blood was taken for complete blood examination, electrolytes and liver function, C-reactive protein, and peripheral blood mononuclear cell populations.

Donor Selection and Stool Processing

Donors were sought by advertisement. Strict criteria applied to potential donors to minimize risks of disease transmission as previously described[18] (eTable 1 in Supplement 2). Potential stool donors sequentially underwent a screening questionnaire, medical interview, and examination followed by blood and stool testing; 76 potential donors were screened, with 19 (25%) fulfilling the screening strategy. Stool was pooled and blended from 3 to 4 donors at 16 collection time points, producing 16 distinct batches. Each stool batch provided treatment for 1 to 7 participants. Treatment batches consisted of pooled stool (25%) blended with normal saline (65%) and glycerol (10%) under anaerobic conditions, and aliquoted into 3 containers for each recipient and frozen immediately at −80°C. The container for colonoscopic delivery contained 50 g of stool in 200 mL and the 2 containers for enema delivery contained 25 g of stool in 100 mL. Autologous stool containers had identical ratios and volumes of stool, saline, and glycerol but they were processed under aerobic conditions.

Randomization

Accrued participants were randomized 1:1 using a computer-generated simple randomization algorithm (http://www.random.org) to receive either pooled donor stool FMT (dFMT) or autologous FMT (aFMT). The randomization and blinding

procedure was conducted by nursing staff who were not present at FMT administration. The randomization record was kept in a separate document to the patient record and other study data such that participants and clinicians performing the procedures and assessing the primary and secondary end points were blinded to the therapy received.

Interventions

Participants received 3 L of polyethylene glycol bowel preparation the evening before and loperamide, 2 mg orally, immediately prior to colonoscopy. At colonoscopy, 200 mL of fecal suspension of either donor stool or autologous stool was delivered into the right colon. Two further 100-mL aliquots of the same fecal suspension were administered by enema in the following 7 days. The total weight of stool administered over the 3 FMT procedures was 100 g. Recipient stool samples were collected at baseline (week 0) and weeks 4, 8, and 52 for microbiome, metabolome, and fecal calprotectin assessment. Biopsies were taken at colonoscopy at weeks 0 and 8 for lamina propria mononuclear cell (LPMC) analysis.

At the week 8 colonoscopy, following an assessment of the primary and secondary end points of remission, unblinding of randomization occurred, and aFMT participants received open-label donor FMT induction by colonoscopy followed by 2 dFMT enemas over the following 7 days. The same inflammatory bowel disease–specialized gastroenterologist performed and assessed both colonoscopies for each patient. Participants who did not undergo the week 8 assessment, required rescue therapy, or were unable to wean oral steroids were considered to have not achieved the primary outcome of steroid-free remission.

Outcomes

Primary Outcome

The primary outcome was steroid-free remission of UC as defined as a total Mayo score of ≤2 (range, 0-12) with an endoscopic Mayo score of ≤1 (range, 0-3) at week 8.

Secondary Outcomes

There were several secondary outcome measures. Clinical response (measured by a ≥3-point reduction in total Mayo score at week 8 and 12 months), clinical remission (measured by a Simple Clinical Colitis Activity Index score ≤2 at week 8 and 12 months), and endoscopic remission (measured by a Mayo score of <1 at week 8 and 12 months) were compared for participants receiving dFMT with those receiving aFMT. Patients' perception and acceptability of FMT were assessed using a written questionnaire completed by patients prior to enrollment and at 12 months (eAppendix 5 in Supplement 2). Adverse events were assessed at week 8 and 12 months by patient survey.

Changes from baseline in peripheral blood and colonic LPMC populations (assessed by flow cytometry) following FMT were evaluated at week 8, stratified by both change in total Mayo score following FMT and randomization. LPMCs were isolated enzymatically from left colonic biopsies and peripheral blood mononuclear cells isolated from blood by density gradient centrifugation as previously described[19,20] and processed immediately for analysis of immune cell populations by flow cytometry (eAppendix 3 in Supplement 2).

Changes in fecal-associated microbiota following FMT (at 8 weeks and 12 months) were assessed by 16S ribosomal RNA sequencing, stratified by both change in total Mayo score following FMT and randomization. The durability of engraftment of these species acquired following dFMT was assessed by quantifying these species at 12 months. The V4 hypervariable region of the 16S ribosomal RNA gene was amplified and raw sequencing data processed into operational taxonomic units at 97% similarity in stool samples from individual donors, pooled stool batches, and FMT recipients taken at weeks 0, 4, 8, and 52 (eAppendixes 1 and 2 in Supplement 2).

Fecal short-chain fatty acid (SCFA) analyses were not a prespecified secondary end point but they were assessed during microbiome analysis. These were performed via the tube filtration method using high-performance gas chromatography as previously described.[21]

Sample Size

Sample size was calculated using a Z test with pooled variance for the difference of 2 independent proportions. The estimated remission rate in the aFMT group was set at 26% and the remission rate in the dFMT group at 60% (based on case series[22]). With 64 patients, there would be 80% power to detect a 34% difference between groups. Type 1 error was set at 5% (2-sided).

Statistical Analysis

Baseline demographic, medication, and dietary factors are presented using means (SDs) or frequencies (percentages) as appropriate, unless otherwise stated. Baseline levels of butyrate and dietary fiber were compared between donors and participants with UC using nonparametric Mann-Whitney-Wilcox tests. Nutrient intake was analyzed using FoodWorks 9 software package (Xyris).

The primary analysis compared steroid-free remission of UC at week 8 between treatment groups using a Fisher exact test. Individuals were analyzed in the group to which they were allocated (intention to treat). A post hoc linear mixed-effects logistic regression was performed estimating the effect of treatment (fixed effect) on remission. Nonnested random intercepts were included to account for batch effects (individuals receiving the same donor mix) and site effects (treating institution). Secondary dichotomous clinical outcomes were also compared using Fisher exact tests and identical mixed-effects logistic regression models. Change in total Mayo score (week 8 minus week 0) was assessed using linear mixed-effects regression with randomization, baseline score, and steroid use as fixed effects and nonnested random intercepts per batch and site, as above.

Assessment of treatment effect on immunological markers was also assessed using linear mixed-effects regressions with week 8 values as outcome, treatment group, and baseline values as fixed effects. Random intercepts were included for each group of individuals receiving the same donor mix (batch effects) and post hoc nonnested random intercepts were included for each treating institution (site effects).

Treatment effect models on immunological markers were extended to include change in Mayo score (week 8 minus week 0) as a fixed effect. The estimate of treatment effect on calprotectin and SCFAs, which had an extra assessment at week 4, was similarly modeled but with both week 4 and week 8 assessments as outcome. Logistic mixed-effects regressions were used to assess associations with microbiome diversity and zero-inflated negative binomial mixed-effects regressions used to assess associations with microbiome abundance. Organisms defined as being associated with dFMT were those for which the change was statistically significant at both weeks 4 and 8 with a *P* value <.01. The details of SCFA and microbiome models are presented in eAppendix 4 in Supplement 2.

Interactions between baseline factors and week 8 Mayo score were assessed by including a pairwise interaction between the factor and treatment allocation as a fixed effect in the mixed-effects regression models with Mayo score as outcome. Similarly, associations between week 8 Mayo scores and change in SCFA were assessed by including, as fixed effects, the estimated change in SCFA (see eAppendix 4 in Supplement 2 for details). Associations between baseline total Mayo scores and both baseline SCFA and immunological measures were assessed using linear regressions with Mayo scores as outcome, adjusting for oral steroid use. In these models, individuals missing week 8 Mayo score were excluded from the analyses and the calprotectin, SCFA measures, and immunological markers were log transformed. Due to the small number of individuals missing baseline covariate data (at most n = 6), these missing values were imputed using cohort means.

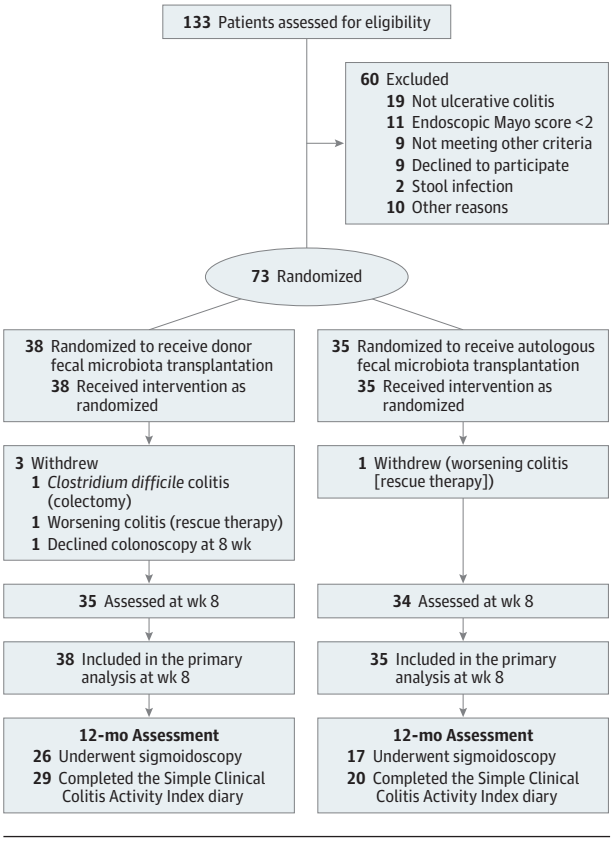
Individuals missing the week 8 Mayo assessment were assumed missing at random imputed using multiple multivariate fully conditional imputation by chained equations (100 imputations, 20 iterations each). In addition to the variables used in the mixed-effect regressions (baseline Mayo score, randomized allocation, use of steroids, donor mix, and treating institution), patient characteristics (sex, age at diagnosis, and age at study entry), disease characteristics (extent of disease and baseline endoscopic Mayo score), and medication use (oral 5-aminosalicylate, topical 5-aminosalicylate, immunomodulatory, and biologic drugs) were included in the imputation.

For all linear models, visual inspection of residual and (for mixed-effects) random-effect distributions were performed. A 2-tailed $P < .05$ was considered significant. No adjustment for multiple testing was performed as all secondary analyses were considered exploratory. Analyses were performed in R version 3.5.0 using *lme4*, *mice*, and *glmmTMB* packages (R Foundation for Statistical Computing).

Results

Between June 2013 and June 2016, 133 patients were assessed for eligibility; 73 were randomized, 38 to dFMT and 35 to aFMT. Three participants withdrew from the dFMT group and 1 from the aFMT group, leaving 69 participants who completed the week 8 assessment (**Figure 1**). Baseline patient demographics, clinical data, and measures of disease activity and inflammation appeared well balanced between the 2 treatment groups (**Table 1**).

Figure 1. Flow of Patients in Trial of Fecal Microbiota Transplantation for Ulcerative Colitis



Primary Outcome

The primary end point of steroid-free remission was achieved in more participants who received dFMT compared with aFMT (12/38 [32%] vs 3/35 [9%]; difference, 23% [95% CI, 4%-42%]; odds ratio [OR], 5.0 [95% CI, 1.2-20.1]; $P = .03$) (**Table 2**).

The mean total Mayo score decreased in both groups at week 8 (aFMT, −1.2 [95% CI, −1.9 to −0.5] and dFMT, −3.5 [95% CI, −4.3 to −2.7]). The change in total Mayo score for each participant is represented in **Figure 2**.

Secondary Outcomes

8 Weeks

Clinical response was also observed in more participants receiving dFMT than aFMT (21/38 [55%] vs 8/35 [23%]; difference, 32% [95% CI, 10%-54%]; OR, 4.3 [95% CI, 1.5-11.9]; $P = .007$), as was clinical remission (18/38 [47%] vs 6/35 [17%]; difference, 30% [95% CI, 7%-51%]; OR, 4.5 [95% CI, 1.5-13.5]; $P = .01$) (Table 2). Steroid-free endoscopic remission occurred in 4 of 38 participants (11%) receiving dFMT vs 0 of 35 (0%) receiving aFMT (difference, 11% [95% CI, −1% to 27%]; $P = .12$) (Table 2). At 8 weeks, 34 of 35 participants (97%) in the aFMT group received dFMT.

Table 1. Baseline Characteristics of the Study Groups

Characteristic	Donor Fecal Microbiota Transplantation (n = 38)	Autologous Fecal Microbiota Transplantation (n = 35)
Sex, No. (%)		
Women	18 (47)	15 (43)
Men	20 (53)	20 (57)
Age, median (IQR), y		
At diagnosis	30.5 (22-48)	29 (21-39)
At randomization	38.5 (28-52)	35 (25-46)
Duration of disease, median (IQR), y	4.9 (1.6-9.6)	5.8 (2.4-11)
Left-sided disease only, No. (%)[a]	23 (61)	22 (63)
Total Mayo score, mean (SD)[b]	7.2 (1.7)	7.4 (1.9)
Medication, No. (%)		
Oral steroids	8 (21)	11 (31)
Oral 5-ASA	33 (87)	24 (69)
Topical 5-ASA	11 (29)	7 (20)
Immunomodulator[c]	14 (37)	15 (43)
Biologics[d]	3 (8)	4 (11)
Inflammatory markers, median (IQR)		
CRP, mg/L	2.8 (1.3-7.2)	2.3 (0.8-10)
WBC count, /μL	6200 (5300-7300)	7900 (6100-8900)
Fecal calprotectin, mg/kg	566.5 (372.5-2687.5)	774 (221-1768)
Diet, mean (SD)[e]		
Protein, g	97 (38)	109 (42)
Carbohydrate, g	230 (70)	221 (102)
Total Fat, g	76 (33)	86 (34)
Saturated Fat, g	29 (16)	32 (15)
Sugars, g	90 (36)	103 (74)
Starch, g	139 (56)	115 (54)
Fiber, g	19 (8)	21 (8)
Calcium, mg	700 (467)	718 (447)
Iron, g	11.1 (6.5)	10.8 (4.4)
Energy, kJ	8742 (2574)	9049 (3111)
Sulfate, mg	1768 (2110)	2073 (3191)

Abbreviations: 5-ASA, 5-aminosalicylate; CRP, C-reactive protein; IQR, interquartile range; WBC, white blood cell.

SI conversion factor: To convert CRP to nmol/L, multiply by 9.524.

[a] Left-sided disease only defined as disease not extending proximal to the splenic flexure.

[b] Total Mayo score is a composite of clinical and endoscopic parameters. It ranges from 0 to 12 (clinical remission ≤2; mild disease, 3-6; moderate disease, 7-10; and severe disease, 11-12).

[c] Immunomodulators were either azathioprine or 6-mercaptopurine.

[d] Biologics were either infliximab or vedolizumab.

[e] Dietary information was acquired via 3-day diet diary conducted prior to patient receiving fecal microbiota transplantation.

12 Months

At 12 months, 72 of 73 participants had received dFMT, 69 of 73 (95%) were contactable, and 9 of 69 (13%) had undergone colectomy. Flexible sigmoidoscopy was performed on 26 of 38 patients (68%) randomized to the dFMT group and 11 of 26 (42%) were in clinical and endoscopic remission. Five of the 12 participants (42%) who achieved the primary end point of steroid-free remission at week 8 following dFMT maintained remission at 12 months (eTable 2 in Supplement 2).

Patient Acceptability

Prior to FMT, 65 of 69 participants (94%) and at 12 months following FMT, 57 of 60 (95%) thought that 1-week induction therapy with dFMT would be acceptable to patients with UC (eTables 3 and 4 in Supplement 2).

Immune Analysis

Lamina propria B cell (β = 0.46 [95% CI, 0.06-0.87]; P = .03) and dendritic cell (β = 0.43 [95% CI, 0.04-0.82]; P = .03) populations were positively associated with total Mayo score at baseline. Conversely, natural killer cells (β = −0.50 [95% CI, −0.91 to −0.09]; P = .02) were negatively associated with total Mayo score at baseline. However, dFMT or dFMT adjusted for total Mayo score were not significantly associated with change in any lamina propria cell populations at week 8 (eTable 5 in Supplement 2).

Microbial Diversity, Abundance, and Durability

At baseline, blended donor stool showed the most microbial diversity (measured by operational taxonomic units) followed by individual donor stool then stool of patients with UC. Diversity increased following dFMT compared with aFMT at weeks 4 and 8 (**Figure 3** and eTable 6 in Supplement 2). There was no significant association between change in total Mayo score following dFMT and baseline diversity (β = 0.6 [95% CI, −4.8 to 5.9]; P = .84) nor change in diversity at week 8 (β = −20.3 [95% CI, −50.7 to 11.2]; P = .23).

The 10 bacteria and the archaea *Methanobrevibacter smithii* whose increased abundance were most strongly associated with dFMT at weeks 4 and 8 were all anaerobic (eTable 7 in Supplement 2). The abundance of these organisms remained relatively stable from weeks 4 to 8; however, by 12 months, there was variability in abundance of many of these organisms (eTable 8 in Supplement 2). Increased abundance of *Anaerofilum pentosovorans* and *Bacteroides coprophilus* species was strongly associated with disease improvement following dFMT (eTable 9 in Supplement 2).

Other Outcomes

Metabolome

Change from baseline in stool concentrations of butyrate and other SCFAs was not significantly different between treatment groups at weeks 4 or 8 (eTable 10 in Supplement 2). Stool SCFA concentrations were not associated with any observed dFMT treatment effect (eTable 11 in Supplement 2).

Post Hoc Outcomes

We did not detect an interaction between age at diagnosis or randomization, disease duration, disease distribution, sex, medication use (other than oral steroid), nor macronutrient intake with a change in total Mayo score following dFMT (eTable 12 in Supplement 2). In Supplement 2, raw patient data are available in eTables 16-19; eTable 15 includes information on fecal calprotectin levels, and eTable 20 and the eFigure include information on butyrate-producing species and genera.

Table 2. Outcome Measures Comparing Donor Fecal Microbiota Transplantation (FMT) With Autologous FMT at Week 8

Outcome	No./Total No. (%)		Absolute Percentage Gain Over Autologous FMT, % (95% CI)[a]	Mixed-Effect Odds Ratio (95% CI)	*P* Value[b]
	Donor FMT (n = 38)	Autologous FMT (n = 35)			
Primary Outcome[c]					
Steroid-free remission of ulcerative colitis at wk 8[d]	12/38 (32)	3/35 (9)	23 (4 to 42)	5.0 (1.2 to 20.1)	.03
Secondary Outcomes[c]					
Clinical response[e]	21/38 (55)	8/35 (23)	32 (10 to 54)	4.3 (1.5 to 11.9)	.007
Clinical remission[f]	18/38 (47)	6/35 (17)	30 (7 to 51)	4.5 (1.5 to 13.5)	.01
Endoscopic remission[g]	4/38 (11)	0/35 (0)	11 (−1 to 27)	NA[h]	.12
Other Outcomes					
Mean change in total Mayo score from wk 0 to wk 8 (SD)	−1.2 (2.1)	−3.5 (2.5)	−33 (−48 to −17)	−2.4 (−3.5 to −1.2)	<.001

Abbreviation: NA, not available.

[a] Absolute percentage gain refers to donor FMT over autologous FMT.

[b] *P* value applies to odds ratio.

[c] The primary and secondary outcomes at week 8 between treatment groups were assessed on an intention-to-treat basis using a Fisher exact test. A post hoc logistic mixed-effects analysis was performed estimating the effect of treatment (fixed effect) on remission. Nonnested random intercepts were included to account for batch effects (individuals receiving the same donor mix) and site effects (treating institution).

[d] Steroid-free remission was defined as a total Mayo score of ≤2 (range, 0-12) with an endoscopic Mayo score of ≤1 (range, 0-3).

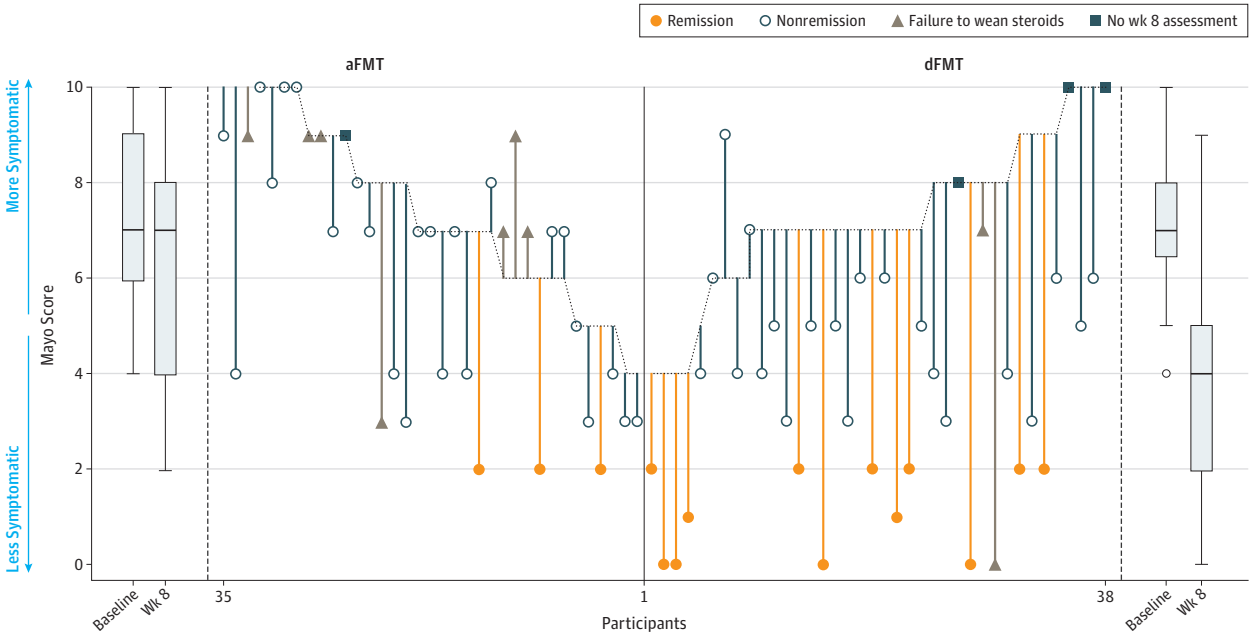
[e] Clinical response was measured by a ≥3-point reduction in total Mayo score at week 8.

[f] Clinical remission was measured by a Simple Clinical Colitis Activity Index score ≤2 at week 8.

[g] Endoscopic remission was measured by a Mayo score of <1 at week 8.

[h] Unable to calculate odds ratio for endoscopic remission.

Figure 2. Change in Total Mayo Score for Patients



The parallel line plot shows change in Mayo score for individual patients. For each participant, a line starts at their baseline total Mayo score and finishes at their week 8 Mayo score. Boxplots of baseline and week 8 Mayo scores per treatment group present the median and interquartile range (25th to 75th percentiles) with whisker length equal to 1.5 interquartile range. aFMT indicates autologous fecal microbiota transplantation; dFMT, donor fecal microbiota transplantation.

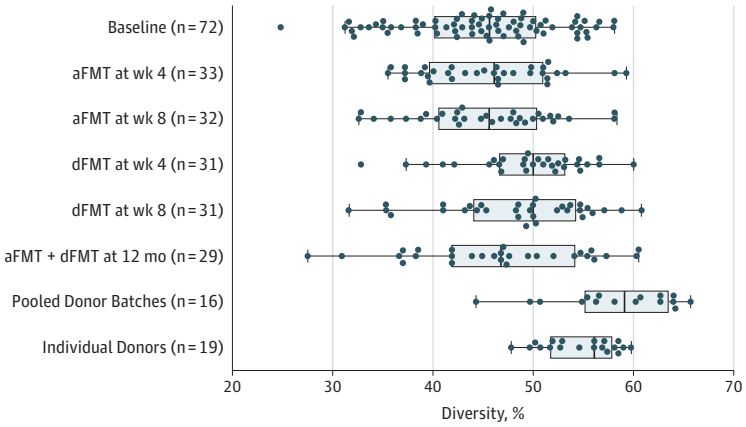
Adverse Events

Week 8

There were 3 serious adverse events in the dFMT group (1 worsening colitis, 1 *C difficile* colitis requiring colectomy, and 1 pneumonia) and 2 serious adverse events in the aFMT group (both worsening colitis).

Three participants developed new anemia (aFMT, 2; dFMT, 1), 2 had mild elevation in alkaline phosphatase (aFMT, 0; dFMT, 2), and 4 had mild elevations of alanine aminotransferase (aFMT, 3; dFMT, 1). Overall, there were no significant differences from baseline in serum creatinine, alanine aminotransferase, alkaline phosphatase, bilirubin, or

Figure 3. Colonic Bacterial Diversity in Patients With Ulcerative Colitis



Colonic bacterial diversity in patients with ulcerative colitis at baseline, 4 and 8 weeks after either donor fecal microbiota transplantation (dFMT) or autologous FMT (aFMT), combined groups at 12 months, and with individual donors and pooled donor stools. Diversity was assessed as the percentage of the total number of identified species.

hemoglobin at week 8 between donor and autologous FMT groups (eTable 13 in Supplement 2).

12 Months

At least 1 adverse event was reported by 31 of 61 participants (51%) who returned the questionnaire (13 reported worsening colitis and 9 of these underwent colectomy). There were 8 reported infections and 5 immune-related diseases (2 new cases of psoriatic arthritis and 1 each of enteropathic arthritis, Crohn disease, and allergy to infliximab) that developed in the 12-month follow-up period. During this time, 13 participants reported weight gain; 8, weight loss; and 40, weight unchanged (eTable 14 in Supplement 2).

Discussion

The main finding of this study was that a 3-dose, 1-week induction course of dFMT was more likely to induce clinical and endoscopic remission in participants with active UC at week 8 compared with aFMT. The study also showed a significant difference in favor of dFMT for the secondary end points of clinical remission and clinical response.

Important differences between this study and previous trials of FMT for UC are the short duration and low intensity of the induction regime. Paramsothy et al[13] demonstrated efficacy of dFMT over placebo with an intensive regime that involved a single colonoscopic delivery of FMT to the right colon followed by enemas 5 days per week for 8 weeks. This is a high treatment burden that would likely limit applicability to practice. The other studies did not use colonoscopic delivery; Moayyedi et al[12] demonstrated efficacy of dFMT over placebo using a weekly FMT enema for 7 weeks and Rossen et al[11] reported no significant difference between dFMT and aFMT using a nasoduodenal infusion of FMT at weeks 0 and 6. In addition to being efficacious, the low-intensity regime was also considered acceptable to most participants; of the surveyed participants who received the short induction course of FMT over 1 week in this study, 95% found it to be acceptable therapy for UC.

A unique feature of this study was the use of anaerobic stool processing, a method that has been previously demonstrated to preserve viable anaerobes.[23] Previous FMT studies[11-13] used aerobic stool processing methods; however, it has been demonstrated that many obligate anaerobes, such as *Faecalibacterium prausnitzi*, are lost with aerobic stool processing but are preserved with anaerobic stool processing.[14] All of the organisms positively associated with the observed treatment response in this study were anaerobes (mostly obligate anaerobes). Preservation of donor-derived anaerobes may explain the similar clinical effect seen with this low-intensity treatment study when compared with other protocols with more intensive regimes.[12,13] The use of pooled stool increased the diversity of microbes in each aliquot and this may also have increased the chance that dFMT contained organisms with the potential to correct a functional deficit in the microbiome of people with active UC. Sequencing analysis indicated that the abundance of organisms that changed significantly from baseline to week 4 remained stable to week 8, but abundances varied by 12 months. This pattern paralleled the observed treatment effect.

To our knowledge, this is the first study to assess bacterial metabolites as well as mucosal and blood immune cell populations following FMT in UC. These are exploratory (hypothesis-generating) analyses conducted to explore potential mechanistic effects of FMT. There was no correlation between stool butyrate concentrations and either dFMT effect or disease activity of UC. There was a significant association between mucosal immune populations and disease activity; however, there was no significant correlation between mucosal immune populations and dFMT. It is plausible that the treatment effect of dFMT resulted from the acquisition of metabolic functional capacity from donor microorganisms and was not driven by a primary immunological effect; however, further dedicated studies are required to validate these findings.

Limitations

This study has several limitations. First, the 12-month data are limited by the crossover design, being open label, and incomplete ascertainment and therefore are observational only.

Second, there was a significant loss of follow-up at 12 months compared with 8 weeks. Third, due to both power limitations and the risk for type 1 error, secondary outcome and subgroup analyses should be considered exploratory. Fourth, central video reading of colonoscopy was not undertaken; however, autologous stool is a more effective blind to the endoscopist and preferable to water-based placebo stool used in previous trials.[12,13] Fifth, there was not a prespecified antibiotic "washout period" prior to study entry. It is therefore possible that some participants took antibiotics prior to the trial and this may bias the initial microbiome assessment. Sixth, stool handling was not under completely anaerobic conditions outside of the anaerobic chamber. However, the processing methods used in this study have been demonstrated to preserve the viability of anaerobic organisms.[23] Seventh, the study was not powered to assess safety and thus further larger studies are required to assess this.

Conclusions

In this preliminary study of adults with mild to moderate UC, 1-week treatment with anaerobically prepared donor FMT compared with autologous FMT resulted in a higher likelihood of remission at 8 weeks. Further research is needed to assess longer-term maintenance of remission and safety.

ARTICLE INFORMATION

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