Fecal microbiota transplantation for maintenance of remission in ulcerative colitis patients: A double-blinded placebo-controlled trial.

Short title: FMT for maintenance of remission in UC patients

Lahtinen Perttu1,2, Jalanka Jonna3, Mattila Eero4, Tillonen Jyrki1, Bergman Paula5, Satokari Reetta3†, Arkkila Perttu2**,6**†**.**

†These authors contributed equally to this work and share last authorship

1. Department of Gastroenterology, Päijät-Häme Central Hospital, Lahti, Finland
2. Faculty of Medicine, University of Helsinki, Helsinki, Finland
3. Human Microbiome Research Program, Faculty of Medicine, University of Helsinki, Helsinki, Finland
4. Department of Infectious Diseases, Helsinki University Hospital, Helsinki, Finland
5. Department of Bioinformatics, Helsinki University Hospital, Helsinki, Finland
6. Department of Gastroenterology, Helsinki University Hospital, Helsinki, Finland

**Correspondence**: Perttu Lahtinen, Päijät-Häme Central Hospital, Keskussairaalankatu 7, 15850 Lahti, Finland. Tel. +358-3-819 11, e-mail: Perttu.lahtinen@phhyky.fi

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Contribution to the field statement:

Ulcerative colitis (UC) is associated with dysbiosis, i.e., undesired alterations of gut microbiota. Fecal microbiota transplantation given in repeated sessions has shown potential in maintaining remission of ulcerative colitis. However, a single treatment has not been studied previously for the maintenance of ulcerative colitis remission. Our study hypothesis was that restoring the gut microbiota via fecal microbiota transplantation may prevent relapses of colitis and help maintain remission. We have previously shown that a single fecal microbiota transplantation changes the gut microbiota of a recipient for at least a year.

We investigated fecal microbiota transplantation from a healthy donor versus placebo transplantation containing patients’ own fecal microbiota in48 patients with quiescent ulcerative colitis. Patients were followed for one year and symptoms were recorded bimonthly using the clinical Mayo score and colitis activity using the fecal calprotectin test.

In the treatment group, 54% of the patients remained in remission compared to 41% in the placebo group which was not a statistically significant difference. There was also no difference in the duration of remission after the intervention. The result of our study was negative; however, it guides future trials in search of an optimal fecal microbiota transplantation protocol.

**Author contributions:** Lahtinen wrote the paper; Lahtinen and Bergman analyzed the data and designed the figures; Arkkila, Satokari, and Mattila planned the study; Arkkila, Satokari, Lahtinen and Tillonen executed the study and collected most of the data; Arkkila, Lahtinen, and Tillonen assessed the colonoscopies; Satokari administered fecal banking and FMT treatments; Jalanka, Mattila, and Tillonen provided expertise in the study design and components of the article; All authors contributed to drafting the article and revised the manuscript for important intellectual content. All authors had access to the study data and reviewed and approved the final manuscript.

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**Summary**

**Background**

Fecal microbial transplantation (FMT) is a promising new method for treating active ulcerative colitis (UC), but knowledge of FMT for quiescent UC is scarce.

**Methods**

Forty-eight UC patients were randomized to receive a single FMT or autologous transplant in colonoscopy. The primary endpoint was set to the maintenance of remission, fecal calprotectin below 200 µg/g ,and a clinical Mayo score below three, throughout the 12-month follow-up. As secondary endpoints, we recorded the patient’s quality of life, fecal calprotectin, blood chemistry, and endoscopic findings at 12 months.

**Results**

The main endpoint was achieved by 13 out of 24 (54%) patients in the FMT group and by 10 out of 24 (41%) patients in the placebo group (log-rank test, P =.660). Four months after FMT, the quality of life scores decreased in the FMT group when compared to the placebo group (P =.017). In addition, the disease-specific quality of life measure was higher in the placebo group than in the FMT group at the same time point (P =.003). There were no differences in blood chemistry, fecal calprotectin, or endoscopic findings among the study groups at 12 months. The adverse events were infrequent, mild, and distributed equally between the groups.

**Conclusion**

There were no differences in the number of relapses between the study groups at the 12-month follow-up. Thus, our results do not support the use of a single FMT for maintenance of remission in UC.

ClinicalTrials. Gov,Trial registration number: NCT03561532.

Key words: Fecal microbiota transplantation, ulcerative colitis, quality of life, maintenance of remission, inflammatory bowel disease.

Introduction

Ulcerative colitis (UC) is a chronic inflammatory disease with an uncertain etiology. The pathophysiology is thought to involve an altered and exaggerated inflammatory response to commensal bacteria in genetically predisposed individuals.1 An increasing number of the population is affected by UC, and the prevalence is highest in North America and Northern Europe. For example, in Finland, the yearly incidence is over 25/100,000 and growing.2 The symptoms of UC include bloody diarrhea and abdominal gramps. The risk of colon cancer exceeds that of the general population, and the lifelong risk of colectomy remains elevated despite the new immune response targeting treatment options.3 Patients with UC show reduced quality of life compared to the general population even if the disease is quiescent.4

Ulcerative colitis is associated with decreased gut microbial diversity and stability, as well as altered microbiota composition and function.5 In conditions such as *Clostridioides difficile* infection and irritable bowel syndrome (IBS), fecal microbiota transplantation (FMT) has been shown to alter the patients’ gut microbiota in the long term to resemble that of healthy donors.6-8 During the last decade, FMT has become a recommended treatment option for recurrent *C. difficile* infection (rCDI).9 The efficacy of FMT for rCDI exceeds 90% using an optimal protocol.10 11 On this basis, it is worthwhile to investigate FMT in UC patients.

FMT has shown promising efficacy for active UC in placebo-controlled trials, although the methodology has varied between the studies.12-15 Repetitious FMTs have been the most frequently applied approach among these studies while the applied treatment protocols have been diverse otherwise. Some studies have applied a multi-donor approach and prepared each transplant from the feces of multiple donors.14 15 Anaerobic conditions for manufacturing the fecal transplant have been investigated with good results15, as well as administering a transplant to each patient as many as 40 times.14 One study showed a clear difference in the efficacy between donors, as transplants from one donor were more effective than the transplants from the other five donors.13

A recent randomized placebo-controlled trial from India investigated the efficacy of FMT in the maintenance of UC remission.16 In this study, FMT prevented relapses through the administration of transplants during bimonthly colonoscopies, making the implementation of the applied protocol very laborious in clinical practice. Additionally, the study population consisted of primary responders to FMT treatment; thus, the patients in the trial were a highly selective group.

Given that a single FMT alters the gut microbiota for the long term in rCDI 6 as well as in IBS patients, 8 we aimed to investigate the efficacy of a single FMT via colonoscopy to maintain remission in UC patients. Additionally, we aimed to investigate the potential differences in the quality of life, fecal calprotectin, and blood chemistry (blood count, liver enzymes, creatinine, and C-reactive protein), as well as endoscopic findings during the 12-month follow-up period.

Materials and methods

Study design

We randomized patients with UC in remission into two groups in a 1:1 ratio to receive either a fecal microbiota transplant from a healthy donor “FMT group” or an autologous transplant made from the patient´s own feces “placebo group”. To ensure blinding, all participants donated their stool for the preparation of the placebo transplant and the FMT group samples were discarded. A bowel lavage was performed using macrogol solution prior to colonoscopy. The transplant was administered into the cecum of the patient in colonoscopy at baseline.

After the baseline intervention, the patients were followed until a colonoscopy 12 months later. During the follow-up period, the participants were contacted at two, four, and eight months after the intervention where the clinical Mayo score 17 was recorded and blood samples were obtained. Questionnaires to assess the quality of life, the fifteen dimensions (15D) questionnaire, and the Inflammatory Bowel Disease Quality of Life (IBDQ) Questionnaire were completed at baseline as well as at four and 12 months.4Fecal calprotectin samples were obtained at seventimepoints (baseline and at 2, 4, 6, 8, 10, and 12 months).

The primary endpoint was considered sustained remission through the 12-month follow-up time. Remission was defined as a clinical Mayo score below three and fecal calprotectin below 200 µg/g. Additionally, an overt relapse between the measurement points leading to a course of steroids or escalation of maintenance therapy was considered a failure.

This randomized placebo-controlled study was conducted in Finland in the gastroenterology departments of Helsinki University Hospital, Helsinki and Päijät-Häme Central Hospital, Lahti. The ethical review board of Helsinki University Hospital approved the study (29/13/03/01/2014). The principles of the Declaration of Helsinki were followed. The trial was registered at ClinicalTrials.gov (NCT03561532).

Participants

Forty-eight patients (21-70 years old) diagnosed with UC were recruited for the study. The inclusion criteria stated that the patients had to be in remission, and the eligibility criteria included fecal calprotectin below 100 µg/g and a clinical Mayo score < 3 at the time of screening. The exclusion criteria included the use of antibiotics within three months prior to study entry, history of TNF-α blockers or other biologics, use of a high dose of corticosteroids (prednisolone ≥ 20 mg/d), and pregnancy. The patients were recruited from the primary and secondary health care of the Helsinki and Lahti regions.

At baseline, the majority of the patients were on mesalazine 22.

After the screening visit and before the start of the trial, some patients experienced minor activation of the disease. Eight patients, four in both groups, had a clinical Mayo score ≥ 3. Ten patients had fecal calprotectin ≥ 200 µg/g, three in the FMT group and seven in the placebo group. Participants with fecal calprotectin ≥ 200 µg/g or a clinical Mayo score ≥ 3 were analyzed separately as “subgroup B” (n=15), and the participants without signs of disease activity at baseline were included in “subgroup A” (n=33). Among all the recruited patients, sixteen patients had minor endoscopic colitis activity with an endoscopic Mayo score of 1 at baseline, while the rest of the patients had an endoscopic Mayo score of 0 at baseline.

Participant recruitment started in October 2014. At the beginning of the study, the inclusion criteria required a diagnosis of UC within six months. However, due to very slowly proceeding recruitment, an amendment to the study protocol was made and approved by the ethical board in October 2016 (HUS/1652/2016). Thereafter, patients with any disease duration were eligible. The recruitment remained slow even after the amendment. The study proceeded using fewer than the desired 80 participants due to time constraints. The follow-up of the last included patient was completed in May 2020. (CONSORT flow diagram in Supplementary Figure 1).

Donors

Transplants from three healthy donors were used in this study. The donors had normal body weight and were healthy without any diagnosed long-term illnesses or medications. All donors had a healthy lifestyle and a diet including animal products but rich in vegetables. They were screened according to the best practice at the time11; however, the donor screening guidelines have evolved since the start of the trial.9 We applied transplants from a female in her forties “Donor 1” and a young adult male “Donor 2” who had previously served as donors in our studies 6 8 and in routine clinical practice of FMT to treat rCDI, as well as a male in his fifties “Donor 3” who was a new donor.

Intervention

Half of the participants, 24 out of 48, received FMT via colonoscopy into the cecum as described previously.11 The fecal transplants were produced from 30 grams of feces from a healthy donor. We applied three universal donors, and fecal suspensions were prepared as previously described and stored at -80 °C.10 The remaining 24 participants in the placebo group were treated in an otherwise similar manner, but the fecal suspension was made using the participants’ own freshly donated stool.

Power calculation and estimated sample size

The relapse rates during the 12-month follow-up period were estimated to be 50% in the placebo group and 15% in the FMT group. Previous studies of FMT for maintenance of remission of UC were not published at the time of study design. Due to the lack of available studies, the estimation of outcomes was based on the knowledge concerning the maintenance of remission using mesalazine18 and extrapolating from FMT studies forrCDI11 in which over 90% efficacy had been achieved.

The calculated sample size using the z-test (95% confidence interval, α=0.05 and β=0.1, 90% power) to find a significant treatment effect was 33 patients in each group, and to cover possible dropouts, we aimed for a sample size of 40 participants per group, 80 participants in total.19

Randomization and blinding

The participants were randomized 1:1 to receive FMT or placebo. The randomization was executed in blocks of six participants by a study nurse not involved in the treatment of the patients. The participants and the treating personnel were blinded to the type of feces transplanted. The randomization was decoded only after all the patients had completed the 12-month follow-up.

Outcomes

The primary outcome was the maintenance of remission through the 12-month follow-up period. A relapse of colitis was considered a failure to achieve the primary outcome. A patient was considered to have a relapse if the clinical Mayo score was ≥ 3 or fecal calprotectin was ≥ 200 µg/g at any of the follow-up time points or if a participant experienced overt activation of colitis between the controls requiring a course of steroids, escalation of maintenance therapy, or a colonoscopy. The patients were followed until the time of the recorded relapse, after which they were dropped from the follow-up. The patients who remained in remission were followed until the study endpoint 12 months after the baseline.

The secondary endpoints were quality of life, endoscopic findings at 12 months, fecal calprotectin, and blood chemistry. The general quality of life was assessed with the 15 dimensions (15D) questionnaire, and disease-specific quality of life was assessed with the Inflammatory Bowel Disease Quality of Life Questionnaire (IBDQ) (McMaster University, Hamilton, Canada, license No. IBDQ22-081).4

The participants donated stool samples every second month (months 0, 2, 4, 6, 8, 10, and 12) for the detection of fecal calprotectin. Blood samples were obtained at baseline as well as at months 4, 8, and 12. The blood tests included blood count, liver enzymes, creatinine, and C-reactive protein. Fecal calprotectin values below 50 µg/g and CRP values below ten were not reported by the laboratory and were coded as null accordingly.

Statistical methods

Descriptive statistics are presented as the means with standard deviations (SD) for continuous variables and as frequencies and percentages for qualitative variables. Differences between the study groups in the maintenance of remission during the follow-up were assessed using the Kaplan‒Meier method. Associations of baseline characteristics with the maintenance of remission were analyzed with univariate Cox regression models. In addition, 15D scores are presented using profile plots, and differences between groups were assessed by t tests. Differences in endoscopic and microscopic colitis activity between the study groups were analyzed with the chi-square test (ꭓ2). P values <.05 were considered statistically significant for all analyses. SPSS version 27 (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp) was used for the statistical analysis.

Results

Baseline characteristics

Forty-nine patients were recruited for this study. After the screening visit and before randomization, one patient had overt activation of colitis and was excluded. This left 48 patients to be randomized with 24 in each group. The patient flow of the study is presented in Supplementary Figure 1 and the baseline characteristics of the patients are presented in Table 1. The placebo group had a longer duration of disease than the FMT group (114 vs. 39 months, P =.006). At baseline, the mean fecal calprotectin level was 115.8 (SD 184.7) in the placebo group and 66.4 (SD 108.6) in the FMT group (P=.261). The majority of the patients were on mesalazine: 21 out of 24 patients in the FMT group and 22 out of 24 in the placebo group. Four patients in the placebo group were on thiopurine but none were in the FMT group. At the study baseline, two patients in both groups were still on lower doses of tapering corticosteroid therapy. There were no statistically significant differences between the randomization groups within subgroups A and B where the patients had fecal calprotectin < 200 µg/g and a clinical Mayo score < 3 or fecal calprotectin ≥ 200 µg/g and a clinical Mayo score ≥ 3 at baseline, respectively (Table 1).

The main endpoint - maintenance of remission

The main endpoint of the study was the maintenance of remission through the one-year follow-up, which was achieved by 13 out of 24 patients (54%) in the FMT group and by 10 out of 24 (41%) patients in the placebo group. The difference between the groups was not statistically significant (log-rank test P=.660). A Kaplan‒Meier survival curve of relapses in the FMT and placebo groups is presented in Figure 1A.

A similar result was obtained when the patients were divided into subgroups according to the clinical Mayo score and fecal calprotectin at the baseline. In subgroup A, i.e., patients with a baseline clinical Mayo score below three and fecal calprotectin below 200 µg/g, six out of 16 patients relapsed in the placebo group, and seven out of 17 patients relapsed in the FMT group (P=.703, Figure 1B). Similarly, subgroup B, with patients with a baseline clinical Mayo score above three and fecal calprotectin above 200 µg/g, showed no difference between the placebo and FMT groups (P=.556) in the number of relapses; all 8 patients in the placebo group and 5 out of 7 patients in the FMT group relapsed (Figure 1C).

To study the possible effect of a specific donor on the patient’s outcome, we divided the patients into three groups according to the donor (Table 2) and compared these to the placebo. There were no statistically significant differences in the number of relapses between the different donors (log-rank, P=.517). At the 12-month follow-up, 41.7% of the patients remained in remission in the placebo group compared to 33.3% from Donor 1, 50% from Donor 2, and 62.5% from Donor 3 (Table 2).

We also analyzed the effect of essential baseline characteristics on the maintenance of remission between these donor groups which included the duration of disease status, fecal calprotectin, clinical Mayo score, total 15D score, and total IBDQ score. The mean duration of disease was 114 months in the placebo group, 5 months in the Donor 1 group, 52 months in the Donor 2 group, and 49 months in the Donor 3 group. The disease duration did not have a statistically significant effect on the maintenance of remission in any of the donor groups.

In the placebo group, lower maintenance of remission was associated with higher baseline fecal calprotectin (Cox reg, HR 1.003; CI 1.001-1.005;P=.010) and higher baseline clinical Mayo score (Cox reg, HR 1.498; CI 1.067-2.102; P=.020). In the Donor 2 group, a lower mean 15D total score at baseline was associated with lower maintenance of remission (Cox reg, HR 0.000; CI 0.000-0.374;P=.033). All other analyzed associations were statistically insignificant.

Secondary endpoint – Changes in the patient’s quality of life

We investigated the impact of FMT on patient quality of life as measured with the 15D questionnaire and disease-specific quality of life as measured with the IBDQ questionnaire.

The 15D total score was similar between the placebo and FMT groups at baseline (t test, P=.335) and 12-month follow-up after FMT treatment (P =.905). However, there was a significant difference in the 15D total score between the FMT and placebo groups (P=.017) four months after treatment. The mean change in the 15D total score from baseline to four months was -0.032 (slightly worse) in the FMT group and -0.009 (no change) in the placebo group. The estimation of the importance of change was performed as presented previously.20 The mean change in the 15D total score from baseline to 12 months was -0.008 (no change) in the FMT group and -0.015 (slightly worse) in the placebo group. Additionally, of the 15 dimensions, there were statistically significant differences in breathing (P=.049), usual activities (P =.042), and vitality (P =.006), all favoring the placebo group.

The disease-specific quality of life as measured with IBDQ 21 was also similar between the placebo and FMT groups at baseline (P=.519) and at 12 months (P =.868), but at four months, there was a difference in the IBDQ total score favoring the placebo group (P=.003, Table 3). Of the four IBDQ subcategories, there were statistically significant differences in the emotions (P=.008) and systemic (P=.010) subcategories.

Secondary endpoint - Blood chemistry and fecal calprotectin

Blood chemistry, blood count, liver enzymes, creatinine, and C-reactive protein were measured at four different timepoints. Fecal calprotectin was measured every second month at six different timepoints. There were no clinically significant changes in any of the blood tests compared to the baseline. All laboratory tests at each timepoint showed no statistically significant differences between the FMT and placebo groups (P >.05). The blood chemistry and fecal calprotectin values are collectively presented in Supplementary Table 2.

3.5 Endoscopic and microscopic colitis activity at 12 months

A colonoscopy was performed at the end of the trial, and pinch biopsies were obtained from all 23 patients who reached the primary endpoint and remained in clinical remission throughout the follow-up period. Endoscopic colitis activity was detected in two out of 13 patients in the FMT group and in two out of ten patients in the placebo group. Likewise, histological colitis activity was detected in the colon pinch biopsies in two out of 13 patients in the FMT group and two out of 10 patients in the placebo group indicating chronic inflammation. Thus, the number of patients who were in endoscopic and microscopic remission in the follow-up colonoscopy was 11 out of 13 in the FMT group and 8 out of 10 in the placebo group (ꭓ2, P =.772).

Adverse events

A similar number of patients experienced UC activation in the FMT and placebo groups(Figure 1). In addition to colitis activation, other adverse events were recorded in four patients in the FMT group and six patients in the placebo group.

In the FMT group, the adverse events included fatigue through the follow-up period, gastroenteritis at eight months after FMT, constipation at three weeks after FMT, a mild increase in liver enzymes and a subsequent diagnosis of primary sclerosing cholangitis. In addition, one patient reported fatigue and episodes of atrial fibrillation at the four-month timepoint, for which he underwent ablation treatment. This patient subsequently developed pneumonia.

In the placebo group, one patient with fibromyalgia reported back pain and colitis symptoms simultaneously. Another patient visited the emergency room at six months after the procedure and was diagnosed with mitral valve insufficiency. One patient with spondylarthritis experienced arthralgia during the follow-up. One patient experienced an escalation of bloating after the procedure, and two patients experienced prolonged mild respiratory infection.

Discussion

In this placebo-controlled trial, we examined the effect of a single FMT via colonoscopy on the maintenance of remission in ulcerative colitis patients. The primary endpoint was sustained remission through the one-year follow-up. A relapse of UC was regarded as a failure to achieve the primary endpoint. In addition to clinical symptoms, a clinical Mayo score above three and fecal calprotectin levels above 200 µg/g were considered colitis activation. There was no statistically significant difference in the number of patients with a relapse of UC during the follow-up period in the FMT and placebo groups. According to the results, a single FMT via colonoscopy was ineffective for maintaining UC in remission.

Studies with FMT for active as well as quiescent UC have been encouraging13 16, but the present data are not sufficient to justify treating UC patients with FMT in clinical practice. Our goal was to investigate whether manipulation of the gut microbiota with FMT early after UC diagnosis would help in the maintenance of remission and the effect on the course of the disease. When planning this study, we aimed to recruit patients whose UC was diagnosed within six months prior to the study baseline. However, due to slow recruitment, we made a change in the study protocol and started including patients with any duration of the disease. Additionally, another center, Päijät-Häme Central Hospital, joined the study in addition to Helsinki University Hospital. Nevertheless, the recruitment remained slow, and thus, we were only able to recruit 48 of the originally planned 80 patients within a reasonable time.

As a result of the change in the protocol, 31% of the patients fulfilled the initial inclusion criteria and had been diagnosed within the previous six months, of whom six were in the FMT group and nine were in the placebo group. Coincidentally, the patients with the longest duration of the disease were also randomized into the placebo group, resulting in a statistically significant difference in the duration of disease status between the randomization groups. The groups were similar to each other in all other parameters (Table 1).

Previously-randomized, placebo-controlled FMT trials investigating patients with UC have included patients with active colitis or patients who have reached clinical remission after several FMT sessions15 16 22. The patients in our study had UC in clinical remission but had not previously received FMT therapy. Between the recruitment and the study baseline, a portion of the patients elevated their calprotectin and clinical Mayo score values without overt colitis symptoms and were thus included in a subgroup analysis (Table 1). Overall, the population of our study represents UC patients in real-world clinical practice.

As a secondary endpoint, we aimed to investigate the effect of FMT on the patient’s quality of life. We measured this with the disease-specific IBDQ questionnaire and with the 15D questionnaire, which measures general health-related quality of life. Both questionnaires measure the quality of life in IBD patients with equal reliability.4 Interestingly, the placebo group presented higher quality of life scores four months after the treatment. This may refer to the extraintestinal influence of the gut microbiota, although the difference between the groups may partly be explained by the longer duration of disease in the placebo group and consequently better adaptation to the fluctuating symptoms of the disease. Indeed, the statistically significant differences concerned vitality, usual activities, and breathing in the 15D questionnaire, while intestinal symptoms did not differ between the groups. Additionally, in the IBDQ questionnaire, the subcategories of emotions and systemic symptoms were statistically significantly better in the placebo group at the four-month timepoint. We find disease duration and adaptation to be the most plausible explanation for the observed differences since the subscores of the FMT group increased and the differences between treatment groups disappeared at 12 months. However, changes in the microbiota composition and activity extrapolating to extraintestinal effects should also be addressed in future investigations. Previously, we observed a possible link between microbiota, general mental health, and depression in our FMT studies on IBS and rCDI.23 24

In line with our previous placebo-controlled FMT trial,23 the reported adverse events in this trial were evenly distributed between groups. There were no serious adverse events attributable to FMT replicating previous reports stating that FMT was safe when performed with high standards.25 Even as FMT appears safe in randomized controlled trials13 15 23 and evidence of long-term safety appears encouraging24, we find it highly important to continue gathering safety data of FMT from randomized trials as well as by collecting registry data from clinical practice. The interindividual variability of donors is high concerning microbiota profiles as well as other characteristics, and therefore, the science community and clinicians performing FMT for *C. difficile* infection need to stay alert for infectious complications and for possible rare short- and long-term adverse effects of FMT.26

Our study had some limitations. First, the number of studied patients remained rather low with only 48 patients due to slow recruitment. However, the remissions remained equally distributed between the groups, and no tendency for better or worse outcomes was detected. Second, after the patients experienced a relapse of UC, further data were not recorded. This decreased the amount of obtained data and complicated comparison of the secondary endpoints between the groups as there were fewer cases left for the analysis with each successive time point. However, after a relapse, some of the patients were given corticosteroids or the medication was changed which would have misrepresented the true effects of FMT or placebo. However, another drawback was that the patients in the placebo group had UC for a longer duration than those in the FMT group and were likely in a more stable phase of the disease. This may have impacted the results of the main endpoint as well as secondary endpoints; however, there were no statistically significant correlations between the duration of disease and the time to relapse or quality of life in either study group.

Our study also had clear advantages. A blinded placebo-controlled study design is a definite strength. We applied an autologous placebo, which assures very reliable blinding, and the same method has resulted in reasonable results in FMT trials for rCDI27 and in other conditions like irritable bowel syndrome23 and pouchitis28. However, it must be noted that the composition of fecal microbiota may change when it is exposed to oxygen, and in the case of patient samples, the duration of oxygen exposure could not be carefully controlled, unlike for the donor samples that were prepared and freeze-stored within two hours of defecation. The advantage of applying an autologous placebo is that it assures very reliable blinding. Other forms of placebo may be more easily detected by the patient or treating personnel. Another advantage of our study is the sufficiently long follow-up time, which allows the monitoring of the treatment effect durability.

Repeated FMT treatments could possibly enhance efficacy, as shown by Sood and colleagues, where repeated FMT treatments were associated with maintenance of remission.16 However, the study population was selected from responders to FMT given as induction of remission. Resultantly, a direct comparison to our results cannot be made. Repeated FMT treatments have shown the most promising results in UC to date, as induction of remission has been successful with repeated FMTs.13-15 Engraftment of the transplanted microbes may be more difficult in an active colitis environment than in a state of remission, and from this perspective, repeated FMT can be justified in active disease. Moreover, FMT may also exert its efficacy via host-derived biomolecules which exert immunoregulatory action or induce transcriptional changes in the affected intestinal epithelium. Action by nonpersisting biomolecules could also explain why multiple FMTs are needed for the induction of remission. On the other hand, if microbiota modulation is considered critical, a single FMT by colonoscopy with our protocol applying 30 g of donor feces has been shown to induce prolonged microbial engraftment in rCDI patients as well as in IBS patients. 6 23 Based on this information, the optimization of donor selection could possibly improve outcomes even with a single FMT given in remission. By optimizing donor selection, conditions for engraftment and functioning of beneficial microbiota may turn out important, particularly when FMT is given to patients in remission. Interestingly, in a preliminary study, dietary intervention was more effective than FMT in inducing remission of mild to moderate UC,29 and the combination of FMT and dietary modulation should also be addressed in future studies.

There are many open questions to be answered before we can determine whether FMT may be applied for the maintenance of remission in UC. More research is needed to define the optimal donor characteristics, patient population, and timing for FMT. Additionally, the best route of FMT administration remains undefined. While the colonoscopic route has shown promise16, FMT with capsules may be considered when high numbers of patients need to be treated.22

In conclusion, there were no statistically significant differences in the number UC relapses after a single FMT or placebo treatment; therefore, the main outcome of our study was negative. Our results do not support applying a single FMT for the maintenance of UC remission. However, these results must be interpreted with caution due to the small sample size, and larger studies are warranted.

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TABLE:

**TABLE 1** The baseline demographics of patients included in the analysis. Subgroup An inincluded patients with fecal calprotectin < 200 µg/g and a clinical Mayo score < 3 at baseline, and subgroup B included patients with fecal calprotectin ≥ 200 µg/g or a clinical Mayo score ≥ 3 at baseline. Standard deviations are shown in brackets.

|  |  |  |  |
| --- | --- | --- | --- |
|  |  |  |  |
| **All patients (N=48)** | **FMT** | **Placebo** | **P value** |
| Gender (M/F) | 14/10 | 12/12 | .562 |
| Age | 43.0 (12.9) | 43.1 (12.1) | .982 |
| Duration of the disease in months | 39.2 (51.0) | 114.0 (117.6) | **.006** |
| Calprotectin (µg/g) | 66.0 (108.6) | 115.8 (184.7) | .261 |
| 15D | 0.903 (0.095) | 0.928 (0.072) | .335 |
| IBDQ | 169.4 (28.8) | 162.7 (39.8) | .519 |
| **Subgroup A (N=33)** |  |  |  |
| Gender (M/F) | 8/9 | 8/8 | .866 |
| Age | 43.6 (13.0) | 44.8 (12.0) | .781 |
| Duration of the disease in months | 41.0 (56.2) | 125.4 (121.7) | **.015** |
| Calprotectin (µg/g) | 34.7 (46.3) | 18.9 (44.9) | .330 |
| 15D | 0.899 (0.106) | 0.939 (0.070) | .221 |
| IBDQ | 166.9 (28.6) | 171.4 (32.0) | .688 |
| **Subgroup B (N=15)** |  |  |  |
| Gender (M/F) | 4/4 | 5/2 | .608 |
| Age | 41.7 (13.6) | 39.8 (12.5) | .775 |
| Duration of the disease in months | 34.9 (38.7) | 91.3 (113.2) | .233 |
| Calprotectin (µg/g) | 142.3 (172.9) | 309.6 (208.3) | .117 |
| 15D | 0.915 (0.070) | 0.907 (0.078) | .830 |
| IBDQ | 175.0 (30.5) | 147.4 (49.3) | .223 |

Abbreviations: M, male; F, female; 15D, the total score of the 15 dimensions quality of life questionnaire; IBDQ, the total score of the inflammatory bowel disease quality of life questionnaire.

**TABLE 2** The number and percentage (in brackets) of patients who relapsed during the follow-up and of those who stayed in remission through the follow-up, divided according to the transplant donor and the placebo group.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Placebo** | **Donor 1** | **Donor 2** | **Donor 3** |
| **Number of patients** | 24 | 6 | 10 | 8 |
| **Relapsed, N (%)** | 14 (58.3%) | 4 (66.7%) | 5 (50.0%) | 3 (37.5%) |
| **Remission, N (%)** | 10 (41.7%) | 2 (33.3%) | 5 (50.0%) | 5 (62.5%) |

**TABLE 3** The inflammatory bowel disease questionnaire (IBDQ) mean total score and standard deviations in brackets. The P value was statistically significant at the four-month timepoint, favoring the placebo group.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **FMT** | **Placebo** | **P value** |
| **Baseline** | 169.4 (28.8) | 162.7 (39.8) | .519 |
| **4 months** | 172.2 (23.5) | 191.4 (19.8) | **.017** |
| **12 months** | 182.3 (25.1) | 186.2 (27.3) | .685 |

FIGURE LEGENDS

**FIGURE 1.** Kaplan‒Meier survival curve demonstrating the maintenance of remission defined as fecal calprotectin < 200 µg/g and a clinical Mayo score < 3 or an overt relapse between the measurement points. (A) All patients included in the analysis (log-rank test P=.660). (B) Subgroup A, i.e., the patients with fecal calprotectin < 200 µg/g and a clinical Mayo score < 3 at baseline (P=.703). (C) Subgroup B, i.e., the patients with fecal calprotectin ≥ 200 µg/g or a clinical Mayo score ≥ 3 at baseline (P=.556). Censored means the end of follow-up without relapse.

**FIGURE 2** The general quality of life (15D) of the complete study group shown according to each of the 15 dimensions and the mean total score and P value as expressed numerically within the picture. (A) 15D profiles at baseline (n = 48), (B) 15D profiles at 4 months (n = 30) and (C) 15D profiles at 12 months (n = 21). *\**P ≤ 0.05 and \*\*P ≤ 0.01.

**SUPPLEMENTARY**

**Supplementary Figure 1: Consort flow diagram**

**Supplementary Table 1.** The effect of baseline characteristics on the maintenance of remission analyzed with the Cox regression method. The baseline variables were analyzed one at a time and the presented numbers are crude values.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Baseline variable | Group | Mean | P value |  |
| Hazard ratio (95% CI) |
| 15D total score | Placebo | 0.928 | 0.350 | 0.02 (0.00; 82.50) |
| 15D total score | Donor 1 | 0.917 | 0.729 | 126.54 (0,00; 98E13) |
| 15D total score | Donor 2 | 0.894 | 0.033 | 0.00 (0.00; 0.37) |
| 15D total score | Donor 3 | 0.905 | 0.206 | 0,00 (0.00; 24E10) |
| IBDQ total score | Placebo | 162.7 | 0.09 | 0.99 (0.98; 1.00) |
| IBDQ total score | Donor 1 | 178.0 | 0.08 | 1.06 (0.99; 1.14) |
| IBDQ total score | Donor 2 | 172.6 | 0.17 | 0.97 (0.94; 1.01) |
| IBDQ total score | Donor 3 | 159.3 | 0.13 | 0.91 (0.80; 1.03) |
| Duration of disease | Placebo | 114.0 | 1.00 | 1.00 (1.00; 1.00) |
| Duration of disease | Donor 1 | 5.2 | 0.89 | 1.06 (0.47; 2.42) |
| Duration of disease | Donor 2 | 51.7 | 0.77 | 1.00 (0.99; 1.02) |
| Duration of disease | Donor 3 | 49.1 | 0.70 | 1.00 (0.98; 1.03) |
| Fecal calprotectin | Placebo | 115.8 | 0.01 | 1.00 (1.00; 1.00) |
| Fecal calprotectin | Donor 1 | 99.7 | 0.24 | 1.00 (1.00; 1.01) |
| Fecal calprotectin | Donor 2 | 73.1 | 0.42 | 1.00 (0.99; 1.01) |
| Fecal calprotectin | Donor 3 | 32.0 | 0.57 | 0.99 (0.96; 1.02) |
| Clinical Mayo score | Placebo | 0.9 | 0.02 | 1.50 (1.07; 2.10) |
| Clinical Mayo score | Donor 1 | 1.3 | 0.12 | 3.20 (0.74; 13.86) |
| Clinical Mayo score | Donor 2 | 1.1 | 0.14 | 1.67 (0.85; 3.28) |
| Clinical Mayo score | Donor 3 | 0.8 | 0.89 | 0.92 (0.32; 2.71) |

**Supplementary Table 2**: Laboratory values as the means and standard deviations (SD) in the FMT and placebo groups at timepoints of measurement, blood chemistry at 0, 4, 8, and 12 months, and fecal calprotectin at 0, 2, 4, 6, 8, 10, and 12 months. The number of measured patients (N) decreases at each timepoint as the patients drop out from the follow-up after a relapse of colitis; thus, measurements at the timepoint of relapse are included. Fecal calprotectin values below 50 µg/g and C-reactive protein values below 10 mg/l were coded as null.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | N  FMT | FMT,  Mean (SD) | N  placebo | placebo,  Mean (SD) | P value |
| Hemoglobin at baseline (g/l) | 24 | 142.0 (12.3) | 23 | 141.3 (8.8) | 0.836 |
| Hemoglobin at 4 months (g/l) | 18 | 144.0 (11.9) | 16 | 144.3 (11.7) | 0.951 |
| Hemoglobin at 8 months (g/l) | 15 | 143.2 (10.1) | 13 | 146.1 (9.5) | 0.447 |
| Hemoglobin at 12 months (g/l) | 13 | 148.0 (9.7) | 9 | 146.3 (13.8) | 0.743 |
| Leukocytes at baseline (10E9/l) | 24 | 5.5 (1.6) | 23 | 6.0 (1.5) | 0.238 |
| Leukocytes at 4 months (10E9/l) | 18 | 6.7 (2.0) | 16 | 6.0 (1.7) | 0.280 |
| Leukocytes at 8 months (10E9/l) | 15 | 6.2 (1.9) | 13 | 6.0 (1.5) | 0.732 |
| Leukocytes at 12 months (10E9/l) | 13 | 6.0 (2.4) | 10 | 7.0 (2.3) | 0.323 |
| Trombocytes at baseline (10E9/l) | 24 | 258.3 (53.4) | 23 | 270.2 (58.2) | 0.469 |
| Trombocytes at 4 months (10E9/l) | 18 | 266.2 (56.2) | 16 | 240.6 (79.3) | 0.280 |
| Trombocytes at 8 months (10E9/l) | 15 | 248.8 (44.1) | 13 | 261.4 (66.4) | 0.555 |
| Trombocytes at 12 months (10E9/l) | 13 | 242.2 (52.8) | 10 | 264.6 (76.9) | 0.415 |
| Creatinine at baseline (µmol/l) | 24 | 73.6 (14.4) | 24 | 70.9 (14.2) | 0.516 |
| Creatinine at 4 months (µmol/l) | 18 | 77.1 (13.2) | 16 | 75.0 (19.0) | 0.706 |
| Creatinine at 8 months (µmol/l) | 15 | 79.3 (16.1) | 13 | 75.2 (14.3) | 0.477 |
| Creatinine at 12 months (µmol/l) | 13 | 81.4 (10.5) | 10 | 80.7 (18.9) | 0.913 |
| Alanine transaminase at baseline (U/l) | 24 | 40.4 (52.8) | 24 | 28.5 (28.0) | 0.334 |
| Alanine transaminase at 4 months (U/l) | 18 | 42.6 (71.5) | 16 | 27.0 (11.1) | 0.395 |
| Alanine transaminase at 8 months (U/l) | 15 | 49.4 (85.7) | 13 | 27.5 (14.5) | 0.371 |
| Alanine transaminase at 12 months (U/l) | 13 | 32.6 (21.6) | 10 | 24.2 (7.6) | 0.255 |
| Alkaline phosphatase at baseline (U/l) | 24 | 63.7 (15.1) | 24 | 60.1 (17.6) | 0.453 |
| Alkaline phosphatase at 4 months (U/l) | 18 | 66.8 (14.8) | 16 | 66.2 (17.3) | 0.907 |
| Alkaline phosphatase at 8 months (U/l) | 15 | 66.8 (13.0) | 13 | 63.9 (14.5) | 0.584 |
| Alkaline phosphatase at 12 months (U/l) | 13 | 68.2 (12.3) | 10 | 64.6 (16.7) | 0.554 |
| C-reactive protein at baseline (mg/l) | 24 | 1.5 (4.1) | 24 | 2.8 (4.7) | 0.317 |
| C-reactive protein at 4 months (mg/l) | 18 | 2.2 (7.3) | 16 | 1.6 (3.8) | 0.772 |
| C-reactive protein at 8 months (mg/l) | 15 | 2.1 (6.7) | 13 | 3.5 (8.3) | 0.627 |
| C-reactive protein at 12 months (mg/l) | 13 | 2.5 (6.8) | 10 | 0.4 (1.3) | 0.415 |
| Fecal calprotectin at baseline (µg/g) | 24 | 66.0 (108.6) | 24 | 115.8 (184.7) | 0.261 |
| Fecal calprotectin at 2 months (µg/g) | 23 | 149.0 (325.7) | 22 | 74.0 (125.8) | 0.318 |
| Fecal calprotectin at 4 months (µg/g) | 18 | 29.1 (51.0) | 16 | 46.1 (86.4) | 0.483 |
| Fecal calprotectin at 6 months (µg/g) | 16 | 70.4 (111.3) | 14 | 49.8 (85.0) | 0.578 |
| Fecal calprotectin at 8 months (µg/g) | 15 | 375.4 (1295.5) | 13 | 32.0 (60.8) | 0.350 |
| Fecal calprotectin at 10 months (µg/g) | 13 | 23.7 (39.6) | 12 | 81.8 (136.3) | 0.154 |
| Fecal calprotectin at 12 months (µg/g) | 13 | 52.1 (123.9) | 10 | 98.2 (210.3) | 0.517 |

**The Study Flow Diagram**

## Follow-Up

Analyzed (n=24)  
♦ Excluded from analysis (n=0)

## Analysis

Analyzed (n=24)  
♦ Excluded from analysis (n=0)

Lost to follow-up (n=0)

Lost to follow-up (n=0)

## Enrollment

Allocated to FMT (n=24)

♦ Received FMT (n=24)

♦ Did not receive FMT (n=0)

## Allocation

Allocated to placebo (n=24)

♦ Received allocated intervention (n=24)

♦ Did not receive placebo (n=0)

Randomized (n=48)

Excluded (n=1)

♦  Relapsed before study

Assessed for eligibility (n=49)