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Fecal Microbiota Transplantation Reduces Symptoms in Some Patients With Irritable Bowel Syndrome with Predominant Abdominal Bloating: Short- and Long-Term Results from a Placebo-Controlled Randomized Trial

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CPE	Carbapenemase-producing <i>Enterobacterales</i>
ESBL	Extended Spectrum Beta Lactamase
FMT	Fecal Microbiota Transplantation
FODMAP	Fermentable Oligo-, Di- and Mono- saccharides and Polyols
IBD	Inflammatory Bowel Disease
IBS	Irritable Bowel Syndrome

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ABSTRACT

Background & Aims: Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder associated with intestinal dysbiosis. Given reported promising results of open-label fecal microbiota transplantation (FMT) therapy in patients with predominant abdominal bloating, we studied efficacy of this treatment in a randomized, placebo-controlled trial.

Methods: Patients with refractory IBS, defined as failure of ≥ 3 conventional therapies, were randomly assigned to single-dose nasojejunal administration of donor stools (n=43) or autologous stools (n=19) in a double-blind study, performed from December 2015 through October 2017, and followed for 1 year. IBS-related symptoms were assessed using a daily symptom diary to determine general abdominal discomfort, abdominal bloating, abdominal pain and flatulence on a scale of 1–6. Number of daily bowel movements, consistency of the stools, and the abdominal circumference were also recorded. Patients completed the IBS-specific quality of life questionnaire. Primary endpoints were improvement of IBS symptoms and bloating at 12 weeks (response). Secondary endpoints were changes in IBS-symptom-scores and quality of life. Stool samples were collected for microbiota amplicon sequencing. Open-label re-transplantation was offered after the trial.

Results: At week 12, 56% of patients given donor stool reported improvement in both primary endpoints compared with 26% of patients given placebo ($P=.03$). Patients given donor stool had significant improvements in level of discomfort (mean reduction of 19%; median score preFMT, 3.98; range, 2.13–6.00; median score post-FMT, 3.1; range, 1.29–5.90), stool frequency (mean reduction of 13%, median score preFMT, 2.10; range, 0.57–4.29; median score postFMT 1.7; range, 0.71–4.29), urgency (mean reduction of 38%; median score preFMT, 0.61; range, 0.00–1.00; median score postFMT, 0.37; range, 0.00–1.00), abdominal pain (mean reduction of 26%; median score preFMT, 3.88; range, 1.57–5.17; median score postFMT, 2.80; range, 1.14–4.94), flatulence (mean reduction of 10%; median score preFMT, 3.42; range, 0.71–6.00; median score postFMT, 3.07; range, 0.79–4.23), and quality of life (mean increase of 16%; median score preFMT 32.6; range, 11–119; median score postFMT, 43.1; range, 32.25–99). A significantly higher proportion of women given donor stool (69%) had a response than men (29%) ($P=.01$). Fecal samples from responders had higher diversity of microbiomes before administration of donor material than fecal samples from non-responders ($P=.04$) and distinct baseline composition ($P=.04$), but no specific marker taxa were associated with response. After single FMT, 21% of patients given donor stool reported effects that lasted for more than 1 year, compared with 5% of patients given placebo stool. A second FMT reduced symptoms in 67% of patients with an initial response to donor stool, but not in patients with a prior non-response.

Conclusions: In a randomized trial of patients with treatment-refractory IBS with predominant bloating, FMT relieved symptoms compared with placebo (autologous transplant), although

effects decreased over 1 year. A second FMT restored the response patients with a prior response. Response associated with composition of the fecal microbiomes before FMT; this might be used to as a biomarker to select patients for this treatment. [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02299973) no: NCT02299973

KEY WORDS: microbe, bacteria, prognostic factor, biomarker,

INTRODUCTION

Irritable bowel syndrome (IBS) is a highly prevalent functional gastrointestinal disorder that affects 7-21% of the general population (1). It is the most commonly diagnosed

gastrointestinal condition and has a chronic course that can impact social interactions, quality of life and productivity at work (2). Unpublished data show that IBS prevalence in Belgium is approximately 19% (Tack et al, 2009). IBS is a symptom-based condition that results from a complex interaction between genetic, psychological and environmental factors and leads to motility problems, abnormal secretions and altered visceral sensations (1). Several strands of evidence support an important role for the intestinal microbiota in IBS etiology. The transplantation of fecal microbiota from IBS patients to germ free rats can provoke IBS-like symptoms in these animals (3). In addition, IBS patients exhibit an altered microbiota composition (4–10) but, perhaps due to the high variability among studies, no consistent microbial IBS signature has been established. Within IBS patients, distinct phenotypic subgroups display differences in their intestinal microbiota ranging from a normal microbial composition to dysbiosis (11). One observation that appears consistent over several studies is the high *Firmicutes/Bacteroidetes* ratio of IBS patients compared to controls (12) yet, as transit time and stool consistency are directly associated to this parameter (13–15), stratification between constipation- and diarrhea-dominant IBS patients seems necessary. Nevertheless, these observations, along with the moderate success of antibiotics and probiotics in the treatment of IBS, has led to the recognition of the intestinal microbiota as a potential therapeutic target in the most recent ROME guidelines (16). Microbiota modulation may be most efficient in the subgroup of patients with severe bloating and flatulence, in which the disturbance of the microbial composition seems to be the most clear-cut (5,11).

The link between diet and symptom-induction is well-recognized in IBS. Flatulence and bloating are important symptoms of IBS. They can be linked to the build-up of intestinal gas content that is mediated by lifestyle, food intake and bacterial fermentation. The low FODMAP (Fermentable Oligo-, Di- and Mono- saccharides and Polyols) diet was developed to restrict rapidly fermentable, short-chain carbohydrates and to reduce functional gastrointestinal symptoms (17,18). Altering the FODMAP content of a diet also affects the composition of the fecal microbiota (19). However, low FODMAP intake was associated with changes that are generally considered unfavorable (20), such as

reduced total bacterial abundance and lower relative abundance of butyrate-producing *Clostridium* cluster XIVa (19).

As opposed to specific diets, transplantation of fecal microbiota (FMT) may represent a more efficient way of changing the intestinal microbiota. Because of its success in the treatment of *Clostridioides difficile* infections (21) this technique has gained significant interest, both in the scientific and popular media, and has been proposed as a potential treatment for a variety of diseases. To date, several reports of IBS patients treated using FMT exist, most of them anecdotal (22–24). Two recently published placebo-controlled trials provided conflicting results (25, 26) in mixed IBS populations.

As not all patients with IBS have microbial changes and the severity of the disturbance differs between subtypes, the therapeutic potential of FMT might differ between IBS patients.

Focusing on a specific IBS subtype, our group published an uncontrolled pilot trial successfully applying FMT in IBS patients with abdominal bloating (27). Successful transplantation was related to the degree of microbial enrichment and appeared to improve with the amount of successfully transplanted phylotypes, suggesting that the use of FMT in this subgroup of IBS patients might be more effective. The aim of this placebo-controlled randomized clinical trial was to investigate the potential for microbiota transplantation to treat IBS patients with severe bloating and to further map the associated microbiota changes.

MATERIALS AND METHODS

Patient inclusion

In this single center, placebo-controlled, double blind randomized controlled trial, patients with refractory IBS, aged between 18 and 75 years, were recruited in a tertiary hospital center. IBS was defined according to the ROME III criteria (28), refractory patients had to have failed at least 3 conventional therapies for IBS. Only patients with diarrhea-predominant or mixed-type IBS (IBS-D or IBS-M) that had symptoms of severe bloating were eligible for this trial with the severity of abdominal bloating assessed by a daily symptom diary (mean abdominal bloating sub-score of ≥ 3). Exclusion criteria were a previous diagnosis of coeliac disease, predominant constipation (IBS-C), lactose intolerance (unless on a stable diet for more than 3 months), prior gastro-intestinal surgery, antibiotic use within 3 months of inclusion or pregnancy. Patients with serious comorbidity and/or a severe psychiatric history were also excluded from the trial as per the including physician's discretion. Written informed consent was obtained from all patients and donors prior to study inclusion. This study was approved by the Ethics Committee of the Ghent University Hospital (EC2014/0631) and was registered on ClinicalTrials.gov (NCT02299973).

Randomization and study outline

Eligible patients were screened at the outpatient clinic. The severity of IBS and IBS related quality of life disturbance were assessed by means of a daily symptom diary collected over a period of minimum 14 days and by standardized questionnaires, respectively. Patients included were then randomly assigned (2:1) to active or placebo FMT by personnel not involved in the clinical assessment using a randomization website. Following FMT, patients were monitored for three months with scheduled visits to the outpatient clinic at 4-, 8- and 12-weeks post-transplantation. A follow-up consultation 1 year after the FMT was performed.

FMT procedure

Donor selection and active donor treatment preparation

Fresh donor stools were collected from two healthy male volunteers, specifically

selected from the pilot trial (27). Per protocol, donors were required to be in good overall condition, between 18 and 65 years of age, to have normal, regular bowel movements and to have no gastrointestinal symptoms. Exclusion criteria for donors included body mass index (BMI) > 30, antibiotic use in the past 6 months, chronic disease or abnormal screening results. Both donors were additionally selected based on clinical efficacy in the pilot trial (27) and having high microbial diversity, as assessed with 16S amplicon community profiling. Donors were subjected to a clinical examination at the start of the trial and were screened for various transmittable diseases at six-months intervals (Supplementary Table 1). Serological screening included testing for hepatitis A, B, C and E, HIV-1 and 2 and *Treponema pallidum*. Donor stools were screened by culturing for the presence of *Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, *Campylobacter* spp., *Clostridioides difficile* and *Aeromonas* spp. Additionally, specific screening for antibiotic-resistant strains was performed using the active detection of carbapenemase-producing *Enterobacterales* (CPE) and extended spectrum beta-lactamase (ESBL) producing organisms. Microscopic examination was performed to confirm the absence of eggs, cysts and/or larvae of parasites, and the presence of *Clostridioides difficile* toxins A and B was screened using an enzyme immuno-assay.

Fresh donor stools were collected on the day of transplantation, stored at 4°C and processed within 6 hours, as previously described (21). Briefly, 300 mL of 0.9% NaCl was added to the stool sample and thoroughly mixed using a handheld blender. The resulting suspension was filtered and transferred into 60 mL syringes. To be consistent with the placebo samples, a small amount of the suspension was mixed with glycerol and stored at -80°C for 16S amplicon sequencing of the administered active product. All procedures were performed under laminar airflow conditions.

During the course of the trial, donors were asked to stay on a stable diet. Donors were asked questions about changes in diet, changes in health, medication intake, changes in transit time (evaluated using Bristol stool score), and traveling abroad. With every donation of a stool sample, they were asked to report changes in any of these to a

health care professional.

Placebo treatment preparation

Stools from included patients were collected prior to the FMT procedure, stored at 4°C and processed within 6 hours as previously described. During the blending process glycerol 10% was added and the resulting suspension was stored at -80°C until the day of the FMT procedure.

Treatment allocation and randomization

For the placebo samples, samples were slowly defrosted four hours before usage. Donor samples were prepared on the day of the FMT. Non-study personnel performed the final allocation, standardizing the active and placebo transplants to make them identical in appearance and temperature. Study participants and investigators were blinded to treatment allocation.

FMT procedure

Patients underwent a bowel preparation prior to transplantation using macrogol. A nasojejunal probe was placed using the Cortrak® electromagnetic guidance system allowing for accurate placement of the tube in the distal duodenum or proximal jejunum. FMT was performed through nasojejunal administration of 300 mL of the donor or placebo treatment solution. Following transplantation, patients were kept fasting (e.g. no food or drinks allowed for 4 hours after FMT) and under close observation in an upright position for four hours.

Primary and secondary endpoints

The primary endpoint of this pilot study was the relief of general IBS symptoms and abdominal bloating at 12 weeks following the FMT (29). Adequate relief was defined as a positive answer to two key questions: '1) Do you feel improvement in your overall IBS symptoms since transplantation?' and '2) Do you feel improvement in your sensation of bloating since transplantation?'.

The secondary endpoints were: 1) changes in daily assessed IBS symptoms of abdominal discomfort, abdominal pain, abdominal bloating, flatulence, stool consistency, number of defecations and abdominal circumference (based on a daily symptom diary) 2) changes of quality of life; and 3) changes of fecal microbiota composition, 4) IBS related symptoms one year following FMT.

Evaluation of clinical endpoints

IBS-related symptoms were assessed using a daily symptom diary (Supplementary Table 2) evaluating general abdominal discomfort, abdominal bloating, abdominal pain and flatulence on a scale of one to six. In addition, the number of daily bowel movements, the consistency of the stools and the abdominal circumference (measured at the umbilicus by patients themselves at exactly the same time every day) were assessed as well. IBS-related quality of life was evaluated using a standardized, IBS-specific quality of life questionnaire (IBS-QoL) in the patient's native language. During the course of the trial, patients were kept on a stable diet.

Follow-up over time

Transplanted patients were prospectively followed during the year following FMT. Patients were sent a questionnaire at one year assessing evolution of their IBS-related symptoms. The assessment included the primary endpoint key questions '1) Do you feel improvement in your overall IBS symptoms since transplantation?' and '2) Do you feel improvement in your sensation of bloating since transplantation?' and a general questionnaire assessing overall IBS symptoms.

Cross-over and re-transplantation

In the cross-over part of the trial patients who received placebo treatment were offered a second FMT with healthy donor stools. Patients from the original active treatment arm who failed to show any improvement after transplantation (non-responders) were offered FMT with donor stools from another donor, while responders who lost response after a certain amount of time received a second transplantation using the same donor. Re-transplanted patients were followed for 12 weeks along a similar protocol as in the

original study.

Microbiome analysis

Consecutive fecal samples were collected for microbiome analyses. Stool samples of patients and donors were collected before the FMT (at baseline) and at selected time-points after treatment. Samples were immediately stored at -20°C and transferred to -80°C within one week. DNA was extracted from the frozen fecal samples using the PowerMicrobiome RNA Isolation Kit (MOBIO Laboratories Inc.) as previously described.(13) Bacterial and archaeal 16S rRNA genes were amplified using the 515F/806R primer set targeting the V4 hypervariable region according to Caporaso and colleagues(30). Sequencing was performed using the Illumina MiSeq platform with sequencing kit MiSeq v2, producing 250 bp paired-end reads.

16S RNA: Diversity analysis and enterotyping.

Amplicon data from the 16S rRNA gene were analyzed following the DADA2 pipeline specifications (31). Briefly, the first 30bp were removed, and the sequence length was set to 130bp and 200bp for the forward and reverse strands, respectively. The sequence error rate, dereplications, the inferred composition of the sample, and the chimera removal were done using the DADA2 default parameters. The taxonomic assignment was done utilizing the DADA2 RDP implementation (R packages “dada2” function “assignTaxonomy”) using as reference the rdp_train_set_16 (<https://zenodo.org/record/801828#.Xe-PctF7mQc>). The relative abundance table was summarized to genus level. Then, in order to correct the compositional effect inherent of high-throughput sequencing (HTS) technologies (32) the genus abundance matrix was corrected using the Center Log Ratio (CLR) transformation (R package “CoDaSeq” function “codaSeq.clr”).

The Principal Coordinates Analysis (PCoA) (function “dudi.pco” function “ad4”) was done based on the Euclidean distance matrix estimated from the CLR-transformed taxonomic table. The significance of the clinical variables over the microbiome was validated by applying the permutational multivariate analysis of variance using distance matrices

(ADONIS) test using 3000 permutations (R library “vegan” function “adonis”). The within-diversity of the samples was assessed using the inverse Simpson (R library “vegan” function “diversity”) index to avoid the previously described compositional biases.

The sample enterotyping was done following the previously published specification (13). Briefly, the 1106 samples from the Belgian Flemish Gut Flora Project (13) were used for mapping the taxonomic composition of the clinical trial. Then, the resulting genus matrix was rarefied to 10,000 reads and samples were clustered using the Dirichlet Multinomial Mixtures clustering method (R library “DirichletMultinomial” function “dmm”).

Success and Stability of the donor samples used over the two-year period.

The CLR-transformed matrix was used to estimate the Euclidean distance between continuous time-points. Then, the smoothed conditional means method (R library “ggplot2” function “geom_smooth”) was used to estimate the regression curve and its 0.95 confidence interval. The significance of the dispersion was given by the Welch's t-test. The survival plot was used to estimated measuring the Euclidean distance from donor to its recipient sample before the FMT trial.

Statistical analysis

Statistical analysis was performed using SPSS Statistics version 22 (Chicago, USA) and R version 3.6.1. Wilcoxon signed ranks test and the Mann-Whitney U test were used for analysis of paired and non-paired data, respectively. Two-tailed probabilities were calculated and *p*-values of less than 0.05 were considered statistically significant and were adjusted using the Benjamini & Hochberg correction. Bacterial community comparisons of the 16S data were done using the Vegan R package. Data were analyzed per the intention-to-treat protocol. All authors had access to the study data and reviewed and approved the final manuscript.

RESULTS

Characteristics of included patients

Between December 2015 and October 2017, 136 patients with refractory IBS were screened in this single-center, placebo-controlled, double-blinded randomized controlled trial. Seventy-three of them were excluded because of having insufficient symptoms of abdominal bloating, having predominant constipation or significant (psychiatric) comorbidity that deemed them unfit for participation (Figure 1). One patient was excluded from participation due to technical reasons (inability to obtain a safe position of the nasojejunal tube), leaving 62 patients to be randomized. Of the 43 patients randomized to the active treatment arm all completed the study protocol, while in the placebo arm (N=19) one patient committed suicide ten days following transplantation. Characteristics of the included study population are presented in Table 1. Median age and disease duration were comparable between treatment groups, however there was a male predominance in the placebo group compared to the active treatment group (respectively 59 vs 31%). All patients had undergone at least three conventional treatment attempts for IBS prior to inclusion in this trial. Ninety-four percent of patients had previously tried a low FODMAP diet, which was unsuccessful in 43% of them. The use of psychotropic drugs was limited to 11 patients (18%).

Fecal microbiota transplantation ameliorates symptoms of abdominal boating in patients with irritable bowel syndrome

The primary outcome, defined as self-reported adequate relief of general IBS symptoms and abdominal bloating at 12 weeks post FMT was achieved in 56% of patients (24/43) for both endpoints in the active treatment group versus 26% (5/19) of patients in the placebo treated group ($P = .03$, Figure 2A and B). Interestingly, there was a clear gender difference in the response to FMT, with female patients responding significantly better to active treatment compared to males (respectively 20/29 (69%) vs 4/14 (29%) ($P = .01$)). This gender difference was not present in the placebo group (respectively 2/8 (25%) vs 3/11 (27%), $P = .91$) (Supplementary table 3).

For the secondary outcomes, a significant reduction was seen in abdominal discomfort (mean reduction of 19% $P= .001$), the number of stools (-13%, $P= .02$), urgency (-38%, $P= .01$), abdominal pain -26%, $P= .001$) and flatulence (-10%, $P= .04$) in the active treatment group. No significant difference was seen in the placebo group. IBS-related quality of life improved significantly in the active treatment arm as opposed to patients that received placebo (+16%, $P= .03$) (Figure 2 C-D, Supplementary table 4).

Donors did not report major changes in diet, medication intake, changes in transit time or travel outside Europe. At the end of the cross-over period, however, in Donor 2 a health complication was recorded, leading to the immediate cessation of fecal donations for this trial. There was no significant difference in clinical efficacy between donors (Donor 2: 58% (15/26) vs Donor 1: 53 % (9/17) effective donations, $P= .76$) (Supplementary table 5).

Microbiota analyses

Samples used for microbial analyses

Fecal samples were collected of all participating patients and for 73% we could analyze the baseline sample before FMT and the sample that was used for transplantation (45 patient-donor pairs). For 3 patients, the sample that was used for transplantation was lacking while 9 patients failed to provide a baseline sample. For the 4 patients lost to follow-up, no samples were available for microbial analyses. An overview of the samples used in the analyses is available in supplement (Supplementary Table 6).

FMT-success is recipient dependent

Based on our preliminary study (27) we selected high diversity donors for this RCT. The bacterial diversity of the suspensions of the donors used for FMT was higher than that of the IBS patients ($P= .001$), with the suspensions of Donor 1 having an even higher diversity than the suspensions of Donor 2 ($P= .014$) (Figure 3A). When comparing the overall composition however, no strong overall differences between the suspensions used for transfer were found (Figure 3B), possibly due to higher variance in Donor 1 (see further).

Next, we analyzed the microbial diversity of the samples from IBS patients that received active FMT treatment, at baseline. IBS patients that responded to the FMT treatment had a higher microbial diversity at baseline (Figure 3C) as compared to those who failed active FMT treatment ($P = .04$). Moreover, when looking at the composition of the microbiota, a significant clustering of baseline samples was observed, with a significant difference in overall composition between responders and non-responders before treatment ($P = .044$) (Figure 3D). When performing in-depth analyses to identify specific biomarkers associated to success in the baseline samples of IBS patients, we could however not identify taxa that were predictive of success in our cohort.

Interestingly, also for IBS patients receiving placebo, a trend for distinct clustering for successful and non-successful autologous FMT was observed in samples at baseline ($P = .08$) (Supplementary Figure S1). When assessing the impact of gender, no significant baseline differences in diversity or richness were observed either (Supplementary Figure S2).

Based on available metadata collected from the patients, we assessed potential associations with FMT success. No baseline characteristics could be associated with treatment success, only a non-significant negative trend for appendectomy in active treatment was observed. Data were available for 31 patients who received active treatment and 7 out of them had a history of appendectomy, with 6 FMT failures and only 1 successful FMT, suggesting that appendectomy might preclude success of FMT in IBS. Further analyses failed to identify microbial taxa that were linked to appendectomy in this cohort. Despite the observed difference in response to active treatment based on gender, sex was not identified as an important confounder for microbiome composition.

Stability of microbial community composition of the donors

Based on the PCoA plot (Figure 3B), it became apparent that overall the fecal suspensions of Donor 2 were more similar to each other compared to the suspensions

of Donor 1. The lengthy study period gave us the unique opportunity to specifically analyze the stability of the community composition of the donor samples over time. The distances over the 2-years trial period between suspensions were much smaller for Donor 2 as compared to Donor 1 ($P=.03$). Remarkably, the distances between the fecal suspensions of different IBS patients that were used for placebo treatment, displayed less variation than the suspensions of Donor 1 over this period. In other words, the interpatient variation between 18 individual IBS patients recruited over 2 years, was less than the intrasubject variation observed in Donor 1 over this same time period (Figure 4A).

Similarity of microbial community composition between patient and donor

DMM enterotyping showed that suspensions from Donor 2 always clustered with the *Bacteroides1* enterotype. For Donor 1, 75% of suspensions used for transplant clustered with *Ruminococcaceae*, 20% with *Bacteroides1* and 5% with the *Bacteroides2* enterotype (Figure 4B). At baseline, most of the IBS patients clustered with the *Bacteroides1* enterotype (21 out of 50 patients). The second most common enterotype was *Bacteroides2* (11 out of 50 patients), closely followed by *Ruminococcaceae* ($n=10$) and *Prevotella* ($n=8$). No specific donor enterotype, nor donor-recipient enterotype matching could be linked to FMT success.

Although enterotype matching was not associated to success, the importance of overall closeness of donor and acceptor was assessed in the actively treated IBS patients in this cohort. When looking at the distance between donor and patient samples at baseline, a trend was observed with successful patient-donor pairs being slightly closer to each other at baseline (Figure 4C). Also, the survival analysis on similarity between patients and donors at baseline showed a similar non-significant trend suggesting that higher similarity between patient and donor at baseline might increase chances of successful FMT in IBS (Figure 4D).

No effect of FMT preparation on microbiome profile

We were able to assess the impact of the FMT preparation on the microbial composition in patients receiving placebo at compositional level. After all, when comparing the baseline sample with the sample used for transplantation, in patients who received an autologous FMT, the only difference between both samples was the FMT preparation. In total this comparison was possible for 18 subjects who received placebo treatment. When analyzing the difference between patients and their respective suspension using Bray Curtis dissimilarity, and the CLR Euclidean distance of the data, no differences were found (Supplementary Figure S3). When assessing in more detail, the processing of the fecal samples to suspensions for FMT had no impact on specific species either in this IBS placebo group. In 1 out of 17 IBS patients the DMM enterotype classification did differ between her sample and the product used for FMT as also displayed in Figure 4B. More specifically, the suspensions of this patient clustered with the *Bacteroides*1 enterotype although her baseline fecal sample clustered with the *Ruminococcaceae* enterotype.

Beneficial effects of FMT are limited over time but re-transplantation can salvage the effect; a subset of patients is recalcitrant also to repeated treatment

Patients who had a beneficial effect were followed over time. At 1 year following transplantation 79% (19/24, 5% missing data) of initial responders lost the effect, while 21% (5/24) had a continued response. The median time to loss of response was four months (3.5 months-12 months). Effects of FMT in the placebo group were lost more rapidly (Figure 5).

In the cross-over part of this trial, patients who received placebo treatment, who did not respond to active treatment or lost response over time were offered an open label, second microbiota transplantation one year later.

In the original placebo group, re-transplantation with donor stools (n=12) resulted in an overall response rate of 58% with overall improvement of abdominal bloating in 33% of patients (Table 2). There was a significant reduction in the IBS symptoms score and the number of daily defecations (Supplementary Table 7).

In the primary non-responder group, re-transplantation with a different donor resulted in an improvement of abdominal symptoms in only 25% of patients (Table 2) with no significant improvement in any of the secondary outcomes (Supplementary Table 8), suggesting that these patients resemble a group resistant to the effects of fecal microbiota transplantation.

Re-transplantation in patients who responded initially to FMT was successful in 67% of patients with significant improvement in abdominal discomfort, urge, abdominal pain and flatulence (Table 2, Supplementary table 9).

DISCUSSION

This is the first study to provide data on long-term follow-up for FMT in IBS, with effects of re-transplantation and insights in stratified response, combined with results on the changes to the gut microbial composition following FMT. In this single-center placebo-controlled randomized trial, single fecal microbiota transplantation was shown to provide adequate relief of symptoms and improve the quality of life of female patients with a refractory irritable bowel syndrome and predominant symptoms of abdominal bloating.

In this trial, FMT by nasojejunal administration appeared safe and was well-tolerated by the patients. Patients who received donor stools had a significantly larger improvement in IBS-related symptoms and abdominal bloating in particular compared to those who were transplanted with their own stools and this was accompanied by an improvement in the quality of life. Other studies have previously investigated the effects of FMT in IBS patients, however results have been inconsistent (33,34). In a mixed IBS population (N=90), Johnsen et al showed a positive effect of FMT performed by colonoscopy with adequate relief of IBS symptoms in 65% (26). Another large, placebo-controlled trial did

not find an effect of FMT in IBS patients with predominant diarrhea (IBS-D) (N=48), however, frozen stool capsules were used instead of fresh donor stools (35). Using a similar methodology with frozen FMT capsules, Halkjaer et al did not find a significant benefit of FMT in a mixed IBS population (25). Recently, El-Salhy showed a response rate of 89% in a mixed IBS population (N=165) using frozen stools from one well-characterized donor, administered via gastroscopy (36). Apart from providing more evidence for applying FMT in IBS patients, we also experienced that donor stool preparation might be an important factor for the efficacy of FMT. Indeed, unpublished data from our own lab suggest that freezing stools is associated with a reduction in diversity and might explain the lack of effect in studies using frozen FMT capsules.

This study is the fifth large RCT to be published investigating the effects of FMT in IBS patients and the first study adding information on patient and donor selection based on the microbiota. So far, two out of five RCT on FMT in IBS reported on the microbiota. In the study of Halkjaer et al, FMT was performed using a donor mix in a cohort with all three different IBS subtypes. Their main microbial findings were, in line with ours, that microbial diversity at baseline was higher in donors as compared to IBS patients and that diversity significantly increased in patients upon FMT with donor mix samples. The most recent published RCT in FMT for IBS also performed microbial analysis, however, they chose to apply a probe-based approach, designed to find dysbiosis signatures based on a preset number of DNA probes, limiting the comparability of their results with state-of-the-art amplicon sequencing results. In contrast to the present study, El-Salhy et al did not restrict their patient cohort to an IBS subtype and used a single donor (36). The latter precluded to further identify relevant features for donor selection.

Based on our microbial analyses, we were able to identify several factors that might increase treatment success in FMT for IBS patients. Based on our preliminary results (27) we selected two donors with high microbial diversity. Using these high-richness donors, we were able to find significant differences between FMT treatment and autologous FMT placebo. However, we observed here that high richness alone is not sufficient to select a donor. After all, we found a non-significant difference in effectiveness between both donors, with the best performing Donor 2 having a lower baseline diversity. Based on the PCoA we observed that samples from Donor 2 were more similar to each other than those of – higher richness – Donor 1. When assessing the stability of the donor microbiota over time, we found that Donor 2 was very stable over the two-year trial period, whereas the samples of Donor 1 were much more dissimilar, even compared to individual IBS patients over the same time period. This suggests that next to high diversity, stability of the microbial composition in the donors might be an important predictor of success. In this respect it would be worthwhile to follow-up stool bank donors over time, to assess their microbial stability as a potential marker for their FMT efficacy. This lack of temporal stability could represent an unbalanced ecosystem or represent the fact that the donor has non-stable physiological parameters (e.g. inflammation, transit time) relevant for a healthy gut microbiota. Of note, none of the latter factors were reported by either donor in this trial. Another asset of our study design using two successful donors, was the ability to switch donor for re-transplantation for the patients who initially failed to respond. We observed that non-responders remained non-responders, even after FMT with another successful donor. The latter suggests that some IBS patients might be refractory to FMT.

We here also made important progress in identifying which IBS patients might benefit from FMT. After all, for the IBS patients, we observed that higher diversity and overall bacterial composition at baseline was significantly associated to success but not linked to gender.

Furthermore, we observed a trend of resemblance to the donor to be predictive of success. However, we could not link a specific donor enterotype, nor donor-recipient enterotype matching to FMT success suggesting that stratification of the microbiota data in enterotypes did not match the compositional overlap that predefines successful FMT in our cohort.

Nevertheless, our data suggests that IBS patients clustering with healthy subjects, without a pronounced disease-related microbiome profile thus, might benefit most from FMT treatment. The less disturbed the microbiota, the more effective the FMT treatment appeared to be. In this respect it is interesting that also in the placebo group, a microbial distinction was apparent at baseline between responders and non-responders. As 'resetting' the microbiota using autologous FMT in these patients was enough to remove complaints, the use of autologous FMT in subgroups of IBS patients should be further assessed to enable the identification of patients who could benefit from such a 'reset'.

Despite the trend for less success with FMT in patients who had a history of appendectomy, we did not find any clinical parameter that was predictive of FMT success. However, it is remarkable that, in this trial, female patients responded better to FMT compared to males.

This is the first study to follow patients who underwent FMT over time. The long-term effect of a single FMT treatment disappears rather quickly in the majority of patients. Only 21% of successfully treated patients had a beneficial effect lasting longer than one year. Interestingly, re-transplantation can salvage the effect in a significant number of patients (67%), suggesting that repeated FMT might be a better way of inducing a lasting effect in IBS patients. Because of study design, we were not able to investigate what the effect of re-resetting (placebo treatment) the microbiota in the placebo-treated responders would be, but this should also be investigated in future studies. A recent small, open-label study suggested the same for 10 IBS patients, refractory to one-time FMT (36). This study supports this proof of concept that repeated FMT may be able to induce long-lasting efficacy in a subgroup of IBS patients. It supports the idea to initiate long-term bacterial therapy by means of capsules containing bacterial strains resembling the normal healthy gut microbiota.

Several limitations need to be considered when translating this study into the real-world setting. First, patients in this RCT were selected based on the Rome III criteria instead of the currently used fourth edition that has a more pronounced focus on the presence of abdominal pain. However, retrospectively, this would have not altered patient inclusion. Second, although the use of autologous FMT is the most accurate placebo-agent available in this type of studies, possible (negative) effects on the outcome cannot be completely ruled out. We have therefore assessed the impact of placebo-preparation on the molecular analyses of the samples and showed that preparation does not significantly alter the microbial composition. Yet, as DNA-based techniques do not differentiate between dead and living cells and only dominant groups are captured in 16S analyses with limited resolution, we cannot conclude at this stage that using aerobic procedures is to be recommended, nor that negative effects of autologous transplantation can be ruled out completely.

In conclusion, in this study we showed that single transplantation of fresh donor stools by nasojejunal administration can provide adequate relief of abdominal symptoms in patients with refractory IBS and severe abdominal bloating and although these effects are limited in time can be restored by re-transplantation. We identified several microbial factors associated with transplantation success including baseline microbial diversity and composition of the patient as well as potentially the stability of the donor stools and closeness of the patient's microbial composition to his dedicated donor.

Despite the positive results of this trial, applying FMT in every IBS patient is not guaranteed to lead to success and is subtype dependent. Yet, within the subgroup of abdominal bloating, not all patients will benefit from the treatment. This study provides one step closer to identifying IBS patients most likely to improve after FMT therapy.

Figure 1: Flowchart of patient inclusion

Figure 2: Primary and secondary endpoints at week 12 post-FMT. A. Number of patients who experienced adequate relief of general IBS symptoms and B. abdominal bloating, expressed as percentage of the total population. C. IBS symptom scores as indicated. D. IBS-related quality of life. Data expressed as mean $\pm$ SD. * $p < .05$; ** $p < .01$; *** $p < .001$.

Figure 3: Diversity and composition of IBS patients at baseline and microbial suspensions used for FMT. A. Difference in diversity of the fecal suspensions of Donor 1 and 2 as compared to IBS patients at baseline. B. Overall composition of the

suspensions used for transplantation. C. Difference in diversity between actively treated IBS patients that had a successful transplant versus the patients that failed FMT therapy. D. Difference between treatment success and failure in overall baseline composition of the actively treated IBS patients.

Figure 4: Donor stability and baseline patient-donor matching to increase FMT success.

A. Stability of the donor samples used over the two-year period. The different samples donated by Donor 1 during this trial were highly dissimilar to each other. In contrast, the composition of the different samples donated by healthy Donor 2 over the same period were very similar. The 'placebo' plot indicates how (dis)similar samples from independent IBS patients are. B. Proportion of the different enterotypes per treatment group, with left the enterotype of the fecal suspensions used for FMT and right the enterotypes of the recipients at baseline. C. Distance between patient-donor pairs at baseline per treatment group and per outcome of the FMT. D. Survival plot displaying the impact of similarity between patient-donor pairs at baseline and FMT success.

Figure 5 – Kaplan-Meier plot showing response decline in responders from the autologous and donor transplant groups

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Table 1: Patients' characteristics

	Active treatment	Placebo	p-value
N	43	19	NA
Age (median (range))	40 (25 – 59)	36 (18 – 63)	0.835
Sex	Male: n=13 (31%)	Male: n=11 (59%)	0.01
BMI (median (range))	23 (17 – 34)	20 (16 – 25)	0.133
IBS duration (years)	10 (2 – 40)	7 (1.5 – 25)	0.451
Failed FODMAPs	46% (n=20)	37% (n=7)	0.471
Psychotropic drug use	19% (n=8)	16% (n=3)	0.789
Baseline sample-pairs available for microbiota analysis	28 (65%)	17 (89%)	

Table 2 – Effects of a second open label FMT.

Response rate	Original placebo group (N=12)	Primary non-responders (N=7)	Primary responders with loss of response over time (N=9)
Improvement of overall IBS symptoms	58%	25%	67%
Improvement of abdominal bloating	33%	25%	33%

Baseline conditions donors:
Healthy donors versus IBS patients

Baseline conditions actively treated patients:
Non-responders vs responders

A

C

B

D

A

B

C

D

What you need to know:

Background and Context: Irritable bowel syndrome (IBS) is associated with intestinal dysbiosis. Fecal microbiota transplantation (FMT) therapy requires analysis in a randomized, placebo-controlled trial of patients with predominant abdominal bloating.

New Findings: In a randomized trial of 62 patients with treatment-refractory IBS with predominant bloating, FMT relieved symptoms in about half of patients (a higher percentage of women) compared with a quarter of patients given placebo (autologous transplant)—the effects decreased over 1 year.

Limitations: Studies are needed to identify intestinal and fecal components that mediate the response to FMT and to determine mechanism by which this treatment reduces symptoms of IBS.

Impact: FMT might be used in treatment of specific populations of patients with IBS.

Lay Summary: FMT reduces symptoms in some patients with IBS, but effects decrease with time. Studies are needed to identify which patients are most likely to respond and which components of the fecal material are responsible for the therapeutic effects.

SUPPLEMENTARY TABLES

Supplementary Table 1: Donor screening protocol for transmittable diseases

	Serology	Fecal samples
Bacteria	<i>Treponema pallidum</i>	<i>Clostridioides difficile</i>
		<i>Salmonella</i> spp.
		<i>Shigella</i> spp.
		<i>Campylobacter</i> spp.
		Antibiotic resistant bacteria
Viruses	HIV	Not routinely
	Hepatitis A (IgM)	
	Hepatitis B (HbsAg)	
	Hepatitis C (Anti HCV)	
Parasites	<i>Strongyloides stercoralis</i> *	Any parasite

* based on donor's history

Supplementary table 2: Daily symptom diary

WEEK :		NAAM :						
POSTINTERVENTION		Maandag	Dinsdag	Woensdag	Donderdag	Vrijdag	Zaterdag	Zondag
K1	Als u de symptomen van uw prikkelbaar darmsyndroom in de laatste week vergelijkt met hoe ze waren voor de transplantatie, heeft u dan de indruk dat de symptomen verbeterd zijn?							
K2	Als u het opgeblazen gevoel dat u de laatste week ervaren hebt vergelijkt met hoe het was voor de transplantatie, heeft u dan de indruk dat het opgeblazen gevoel verbeterd is?							
S1	Op een schaal van 0 tot 6, hoe storend waren de symptomen van uw ongemakken in de buik zoals u ze vandaag ervaren hebt (0 = helemaal niet, 1 = nauwelijks, 2 = min of meer, 3 = gemiddeld, 4 = belangrijk, 5 erg belangrijk, 6= heel erg belangrijk)							
S2	Aantal stoelgangen per dag							
S3	Consistentie van de stoelgang (1 = erg hard, 2 = hard, 3 = vast, 4 = los, 5 = waterig)							

WEEK :		NAAM :						
		Maandag	Dinsdag	Woensdag	Donderdag	Vrijdag	Zaterdag	Zondag
S4	Heeft u vandaag een gevoel van hevige aandrang voor stoelgang gehad? (Ja/neen)							
S5	Op een schaal 0-6, hoe uitgesproken waren uw buikpijnklachten vandaag? (0 = helemaal niet, 1 = nauwelijks, 2 = min of meer, 3 = gemiddeld, 4 = belangrijk, 5 erg belangrijk, 6 = heel erg belangrijk)							
S6	Op een schaal van 0-6, hoe uitgesproken was het opgeblazen gevoel dat u vandaag ervaren heb? (0 = helemaal niet, 1 = nauwelijks, 2 = min of meer, 3 = gemiddeld, 4 = belangrijk, 5 erg belangrijk, 6 = heel erg belangrijk)							
S7	Op een schaal van 0-6 hoe uitgesproken was de winderigheid die u vandaag ervaren hebt? (0 = helemaal niet, 1 = nauwelijks, 2 = min of meer, 3 = gemiddeld, 4 = belangrijk, 5 erg belangrijk, 6 = heel erg belangrijk)							
S8	Meet uw buikomtrek dagelijks op het zelfde tijdstip (bv. dagelijks om 20 uur). Gebruik een lintmeter die u aanbrengt rond de buik ter hoogte van de navel en noteer de omtrek in cm							

Supplementary table 3: Secondary outcomes divided by gender

	Male				Female			
	Placebo (Median diff)	P- Value	Donor (Median diff)	P- value	Placebo (Median diff)	P-value	Donor (Median diff)	P-value
Abdominal discomfort	-2.96%	0.944	-2.83%	0.055	+2%	0.398	-11.6%	0.000
Defecations	-0.25	0.515	-0.02	0.019	-0.53	0.600	-.039	0.004
Consistency	-1.67%	0.715	+0.67%	0.066	-1.3%	0.128	-4.68%	0.005
Urgency	-19.8%	0.161	-7%	0.398	+16%	0.463	-16.5%	0.000
Abdominal pain	-0.5%	0.314	3%	0.046	-0.5%	0.352	-18.8%	0.000
Bloating	-7.67%	0.314	-4.83%	0.142	+3.83%	0.237	- 8.33%	0.001
Flatulence	-0.83%	0.594	-6.5%	0.196	-4.16%	0.310	-6.67%	0.018
Circumference	-0.69	0.866	+1.84	0.386	+1.5	0.180	-0.24	0.588
IBS QoL	+0%	0.893	+8%	0.021	- 3 %	0.207	+ 7 %	0.000

Supplementary table 4: Secondary outcomes of transplantation

	Donor transplant		P	Autologous transplant		P
	PreFMT	PostFMT		PreFMT	PostFMT	
N	42	42		19	19	
IBS discomfort	3.98 (2.13- 6.00)	3.1 (1.29- 5.90)	P=0.001	3.84 (1.76- 4.57)	3.31 (1.09- 5.14)	P=.478
N of stools	2.10 (0.57- 14.29)	1.7 (0.71- 4.29)	P=0.02	2.40 (1.01 – 6.43)	2.3 (1.00- 3.96)	P=.334
Stool consistency	3.40 (1.23- 4.44)	3.13 (0.78- 4.32)	P=.06	3.41 (2.70- 4.00)	3.29 (2.85- 4.07)	P=0.277
Urge	0.61 (0.00- 1.00)	0.37 (0.00- 1.00)	P=.01	0.70 (0.00- 1.00)	0.53 (0.00 – 1.00)	P=.638
Abdominal pain	3.88 (1.57- 5.17)	2.80 (1.14- 4.94)	P=.001	3.79 (2.06- 4.86)	3.57 (0.93- 4.86)	P=.205
Abdominal bloating	3.76 (3.00- 6.00)	3.29 (1.29- 6.00)	P=.06	3.45 (3.00- 4.71)	3.36 (1.00- 5.14)	P=.959
Flatulence	3.42 (0.71- 6.00)	3.07 (0.79- 4.23)	P=.04	3.00 (2.32- 5.05)	3.02 (1.00- 4.17)	P=.62
Abdominal circumference	85.7	85.3 (53.8-	P=.894	86.3	86.6 (50.2-	P=.530

	(53.9-127.9)	127.5)		(50.1-101.9)	101.8)	
IBS QoL	32.6 (11-119)	43.1 (32.25-99)	P=.03	29.1 (22-61)	32.6 (22-69)	P=.18

Supplementary table 5: secondary outcomes per donor

	Donor 1 (Median Diff)	Donor 2 (Median Diff)	P-value
Abdominal discomfort	-0.685 (-11.4%)	-0.670 (-11.2%)	0.789
Defecations	-0.475	-0.360	0.500
Consistency	+0.02 (+3.3%)	0.00 (+0.0%)	0.162
Urgency	-0.165 (-16.5%)	-0.300 (-30%)	0.483
Abdominal pain	-0.715 (-11.9%)	-0.810 (-13.5%)	0.663
Bloating	-0.475 (-7.9%)	-0.530 (-8.83%)	0.534
Flatulence	-0.620 (-10.3%)	-0.270 (-4.50%)	0.947
Circumference	-0.60	+0.86	0.524
IBS QoL	+9%	+7 %	1.000

Supplementary table 6: Fecal samples available for microbial analyses

Number	Donor 1	Donor 2	Placebo
Patients total	16	24	19
Fecal samples before FMT	15	19	17
Samples used for transplantation	12	22	18
Baseline-transplant pairs	11	17	17

Supplementary table 7: Secondary outcomes of re-transplantation in the original placebo group

	Pre transplantation (median)	12 weeks post FMT (median)	p
N	10	8	
IBS discomfort	4 (1,71 – 5,57)	2,68 (0,89 – 5,57)	p = 0,176
N of stools	2,86 (1,57 – 4,14)	1,64 (1 – 4,29)	p = 0,034
Stool consistency	3,28 (2,57 – 8,29)	3,22 (2,06 – 4)	p = 0,345
Urge	0,71 (0 – 1)	0,61 (0 – 1)	p = 0,655
Abdominal pain	3,8 (1,71 – 5,43)	2,93 (0,94 – 5,29)	p = 0,176
Abdominal bloating	3,43 (1,86 – 6)	2,47 (1,22 – 5,14)	p = 0,128
Flatulence	3,29 (2 – 5,14)	3,81 (2,29 – 5)	p = 0,735
Abdominal circumference	85,5 (77 – 92,50)	87 (75 – 95,64)	p = 0,599
IBS-QoL	44,85 (21,32 – 72,79)	66,55 (19,12 – 83,09)	p = 0,150

Supplementary table 8 – Secondary outcomes of re-transplantation in the primary non-responder group

	Pre transplantation (median)	12 weeks post FMT (median)	p
N	7	5	
Missing data	1	3	
IBS-SSS (score/500)	370 (310 – 440)	380 (270 – 390)	p = 0,414
IBS discomfort	4 (3,29 – 4,57)	4,15 (3,29 – 4,43)	p = 0,593
N of stools	1,87 (1 – 4,57)	2 (1,29 – 2,86)	p = 0,144
Stool consistency	3,15 (2,63 – 4,29)	3,86 (3,25 – 4,43)	p = 0,068
Urge	0,71 (0,29 – 0,86)	0,56 (0,29 – 1)	p = 0,465
Abdominal pain	3,93 (3,29 – 4,57)	3,79 (3,43 – 4,43)	p = 1
Abdominal bloating	3,72 (3,13 – 4,43)	3,29 (3,29 – 4,14)	p = 0,285
Flatulence	3,75 (2,57 – 4,29)	3,21 (2,43 – 5,29)	p = 0,715
Abdominal circumference	85,86 (81 – 91,13)	87 (80 – 91,14)	p = 0,273
QoL score	46,32 (29,41 – 60,29)	32,35 (26,68 – 69,85)	p = 0,893

Supplementary table 9 – Secondary outcomes of re-transplantation in the loss-of-response group

	Pre transplantation (median)	12 weeks post FMT (median)	p
N	9	9	
Missing data	3	3	
IBS-SSS (score/500)	370 (200 – 430)	250 (90 – 360)	p = 0,018
IBS discomfort	4,5 (3,43 – 6)	2,86 (1 – 4,42)	p = 0,043
N of stools	2,98 (1,43 – 15,4)	2,14 (1 – 4,7)	p = 0,893
Stool consistency	3,43 (2 – 4,90)	3 (2,42 – 3,64)	p = 0,225
Urge	0,71 (0,29 – 1)	0,14 (0 – 0,5)	p = 0,043
Abdominal pain	4 (3,25– 5,80)	2,14 (1 – 4,43)	p = 0,043
Abdominal bloating	3,14 (1,25 – 5,9)	2,43 (1,14 – 5)	p = 0,225
Flatulence	3,7 (2,29 – 5,80)	3,29 (1,5 – 4,6)	p = 0,043
Abdominal circumference	88,1 (78,71 – 97,29)	89,97 (77 – 102,4)	p = 0,498
QoL score	35,29 (13,24 – 54,41)	61,03 (16,18 – 90,44)	p = 0,345

SUPPLEMENTARY FIGURES

Recipients from Placebo and time-point 0
Aitchison distance

Supplementary Figure S1: Composition of samples from placebo patients at baseline: Difference between treatment success and failure in overall baseline composition of the placebo treated IBS patients.

Supplementary Figure S2: Composition of samples at baseline according to gender: Difference between treatment success and failure in overall baseline composition of the IBS patients according to gender.

Differences between fecal samples and suspensions in the placebo group
Aitchison distance

Supplementary figure 3: FMT preparation does not significantly alter the microbiota composition in the placebo samples