

FECAL MICROBIOTA TRANSPLANTATION AS TREATMENT FOR POST-INFECTIOUS OR ANTIBIOTIC- INDUCED IRRITABLE BOWEL SYNDROME: A PILOT STUDY

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Abstract – Objective: In 10-15% of patients with irritable bowel syndrome (IBS), the onset of symptoms follows a gastrointestinal infection or antibiotic use, which may be caused by alterations of the gut microbiota. We aimed to evaluate the effect of FMT in patients with post-infectious IBS or antibiotic-induced IBS.

Materials and Methods: We performed an open label pilot study (N=10) at the VU University medical center in Amsterdam from August 2016 through January 2017. Participants with therapy refractory post-infectious or antibiotic-induced IBS, with an IBS-Symptom severity score of at least 175 points were eligible. Donor feces was administered via a duodenal tube. Participants were followed for eight weeks via two validated questionnaires: IBS-SSS, and IBS-Quality of Life score (IBS-QOL). Fecal samples were obtained before and after FMT for microbiota analysis. FMT was considered clinically effective if participants reported an IBS-SSS improvement of at least 50 points compared to baseline, at eight weeks after FMT.

Results: FMT was effective in five participants. The median IBS-SSS of all patients improved from 340 (range 230-480) points at baseline to 205 (range 80-470) at eight weeks after FMT ($p=0.008$). The median IBS-QOL improved from 53% (range 21-77%) to 70% (21-93%) ($p=0.008$). In general, the microbiota composition of responders shifted to that of their corresponding donors. Non-responders initially also did, however, eight weeks after FMT, the microbiota composition had shifted back towards baseline.

Conclusions: FMT appears as a promising treatment for antibiotic-induced and post-infectious IBS. Based on these results, a randomized placebo controlled trial in this specific subgroup of IBS patients is warranted.

Keywords: Fecal microbiota transplantation, Irritable bowel syndrome, Microbiota.



INTRODUCTION

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder characterized by abdominal pain and discomfort associated with alterations in bowel habits, without underlying gastro-intestinal pathology¹. IBS strongly impairs quality of life, work productivity, and social function². The estimated worldwide prevalence of IBS in adults is approximately 11%³.

Although the underlying pathophysiology of IBS is not completely understood, it is generally regarded as a multifactorial disorder involving both host, and environmental factors. Host factors include an altered gastro-intestinal motility, visceral hypersensitivity, low-grade inflammation, a decreased barrier function, and an altered cognitive function^{4,5}. In recent years, alterations in the gut microbiota have also been linked to the pathophysiology of IBS; some studies^{6,7} have demonstrated changes in microbiota profiles in patients with IBS compared to healthy individuals. It is estimated that in about 10% of IBS patients, the onset of symptoms follows an episode of gastroenteritis, causing post-infectious IBS⁸. In addition, there is a strong association between IBS and prior use of antibiotics^{9,10}. This correlation supports the importance of the gut microbiota in IBS pathophysiology, providing a rationale for new treatment strategies targeting the gut microbiota, like Fecal Microbiota Transplantation (FMT)¹¹⁻¹³. FMT is defined as the transfer of fecal bacteria from a healthy individual into a recipient via a duodenal tube, colonoscopy, enema, or capsules. Effects of FMT rely on restoration of a disturbed intestinal microbiota by the healthy, balanced, donor feces. To date, results from RCTs have been conflicting¹⁴⁻¹⁹. We only included patients with post-infection or antibiotic induced IBS, because in these patients it is likely that they've developed symptoms as a direct consequence of an altered gut microbiota. The primary aim of this pilot study was to evaluate the effect of FMT in post-infectious and antibiotic-induced IBS.

PATIENTS AND METHODS

General Study Outline

From August 2016 through January 2017, we performed an open label pilot study at the VU University medical center (VUmc; Amsterdam, The Netherlands), to evaluate the effect of FMT in patients with post-infectious or antibiotic-induced IBS. The study was approved by the Medical Ethics Committee of the VUmc. All participants provided written informed consent.

Study Population

Participants (≥ 18 years) diagnosed with therapy refractory post-infectious or antibiotic-induced IBS as defined by the ROME III criteria, with clinical symptoms lasting over six months, an IBS-symptom severity score (IBS-SSS) of at least 175 points, and a negative screening for gastrointestinal pathology (e.g., celiac disease, Crohn's disease, ulcerative colitis) were eligible^{20,21}. Pregnancy and chronic use of antibiotics were exclusion criteria. If the participant had any acute medical condition on the day of FMT, the donor feces infusion was rescheduled. Participants were followed for a period of eight weeks.

Donor Feces

Donor feces suspensions were provided by the Netherlands Donor Feces Bank (NDFB; www.ndfb.nl)²². The donor-screening, feces collection, preparation, and storage of the donor feces suspension was described previously²². In summary, the NDFB recruits healthy volunteers (18-50 years old), with a body mass index between 18.5 and 25 kg/m². All donors are extensively screened by a questionnaire and personal interview concerning risk factors affecting general health or composition of the intestinal microbiota. Blood and feces are screened to identify potential pathogens transmissible by stool infusion. The collected feces (60 gram) is

homogenized with saline, sieved, and subsequently concentrated by centrifugation. Glycerol is added as cryoprotectant. All donor feces suspensions are stored at -80°C , and placed in quarantine for two months, until the donor has passed a second set of clinical, serological, and stool screening. A donor feces suspension is only released for clinical use after the donor has successfully passed the second screening. Donor feces of four different donors was used.

Fecal Microbiota Transplantation

On the day of FMT, a duodenal tube was placed through duodenoscopy. The donor feces solution was slowly administered through the duodenal tube. Participants did not receive pre-treatment or bowel lavage before FMT. One week after FMT, patients were questioned about the occurrence of short-term adverse events of FMT (e.g., diarrhea, abdominal cramps, nausea, vomiting), using a structured questionnaire provided by the NDFB.

Data Collection and Follow-up

Clinical outcome was assessed with two validated questionnaires: the IBS-SSS and IBS-Quality of Life score (IBS-QOL), conducted at baseline, and two, four, and eight weeks after FMT. The IBS-SSS evaluates the intensity of IBS symptoms during a 10 day period: abdominal pain (severity and duration), distension, satisfaction about bowel habit, and interference with life in general²¹. Each of the five questions generates a score from 10 to 100 points, leading to a total possible maximum score of 500 points. The IBS-QOL questionnaire includes 34 questions, each with a five-point scale. Total IBS-QOL scores were transformed to a 0-100 scale. A higher score indicated a better IBS specific quality of life.

Participants collected fecal samples at home one day before FMT, and two and eight weeks after FMT. Samples were immediately (at least within 30 minutes after defecation) stored in a container provided by the microbiology laboratory in the participants' own freezer. On the day of FMT, an aliquot of the donor feces suspension was also collected, and stored at -20°C . Eight weeks after FMT an outpatient follow-up visit was scheduled to discuss the effect of FMT. During this visit, participants also delivered their collected stool samples (samples remained frozen during transport and were immediately stored at -20°C).

Data Analysis

Total individual IBS-SSS scores before and after treatment were compared to determine treatment response. FMT was considered clinically effective if participants reported an IBS-SSS improvement of at least 50 points compared to baseline, at eight weeks after FMT (responder)^{21,23}. Wilcoxon signed-rank test was applied for paired nonparametric data, such as the IBS-SSS of all participants before and after FMT. Statistical analysis was performed using SPSS Statistics version 22 (IBM Corp., Armonk, NY, USA).

Analysis of Fecal Microbiota

Changes in microbiota composition, and microbiota diversity were analyzed with IS-pro, a microbiota profiling technique, based on the identification of species-specific length polymorphisms of the 16S-23S rDNA interspacer (IS) region, and phylum specific sequence polymorphisms of 16S rDNA²⁴. DNA was extracted from the fecal samples with the easyMag extraction kit according to the manufacturer's instructions (Biomerieux, Marcy L'Etoile, France)²⁴. Isolated DNA was analyzed with the IS-pro assay (IS-Diagnostics, Amsterdam, The Netherlands) according to the protocol provided by the manufacturer, as described previously^{24,25}. Dissimilarities between sample compositions of the donor and recipients were calculated as the cosine distance between each pair of samples²⁶. Diversity of fecal samples was calculated by the Shannon diversity index²⁷.

Several studies have examined the shift in microbiota profile towards that of the donor as a potential marker for clinical success. Shifts in microbiota profiles were calculated as the ratio between cosine distance of donor to recipient at baseline and the cosine distance of donor to recipient at 2 or 8 weeks after FMT:

$$\text{Shift of microbiota} = \frac{\text{cosine distance donor to recipient at baseline}}{\text{cosine distance donor to recipient at 2 or 8 weeks after FMT}}$$

A value of 1 indicated that the cosine distance between donor and recipient did not have changed two or eight weeks after FMT. A microbiota shift >1 corresponds with more similarity to donor communities.

RESULTS

Baseline Characteristics

From August 2016 through January 2017, five participants with post-infectious, and five participants with antibiotic-induced IBS were enrolled (Table 1). The participants had a median age of 38 years (range 22-58 years). The participants with antibiotic-induced IBS had a median IBS-SSS of 350 (range 280-480), and a median IBS-QOL score of 45% (range 21-60%). The participants with post-infectious IBS had a median IBS-SSS of 270 (range 230-370), and a median IBS-QOL score of 60% (range 49-77%). The duration of IBS symptoms ranged from 2 to 28 years.

Outcome

The primary outcome, being an IBS-SSS improvement of at least 50 points compared to baseline at eight weeks after FMT, was achieved in three patients with antibiotic-induced IBS and two patients with post-infectious IBS (50 to 290 points IBS-SSS improvement). Changes in IBS-SSS and IBS-QOL are shown in Figure 1. The median IBS-SSS of all patients improved from 340 (range 230-480) points at baseline to 205 (range 80-470) at eight weeks after FMT ($p = 0.008$). The median IBS-QOL improved from 53% (range 21-77%) to 70% (21-93%) ($p =$

TABLE 1. BASELINE CHARACTERISTICS.

#	Age	Gender	BMI	IBS medication	Etiology IBS	Duration of symptoms	Symptoms IBS	IBS-SSS	IBS-QOL
1	43	Male	21.0	None	AB	26	Constipation	370	60%
2	33	Male	26.0	Probiotics	AB	9	Mixed	350	25%
3	32	Male	20.9	Amitriptyline	PI	5	Diarrhea	330	66%
4	22	Male	22.8	Fibers Probiotics	AB	4	Diarrhea	280	45%
5	23	Female	17.1	None	PI	6	Diarrhea	370	49%
6	45	Female	21.5	Probiotics Iodine	PI	28	Constipation	230	60%
7	54	Male	26.5	Probiotics	PI	20	Diarrhea	250	57%
8	53	Male	32.1	None	AB	22	Diarrhea	480	21%
9	30	Male	24.3	None	AB	3	Mixed	350	46%
10	58	Female	22.0	None	PI	2	Diarrhea	270	77%

Scales: IBS-SSS: 50 (no symptoms) – 500 (severe symptoms); IBS-QOL: 0% (poor IBS specific quality of life) – 100% (good IBS specific quality of life). Abbreviations: IBS: irritable bowel syndrome; IBS-SSS: IBS symptom severity score; IBS-QOL: IBS quality of life score; BMI: body mass index in kg/m²; AB: antibiotic-induced IBS; PI: post-infectious IBS.

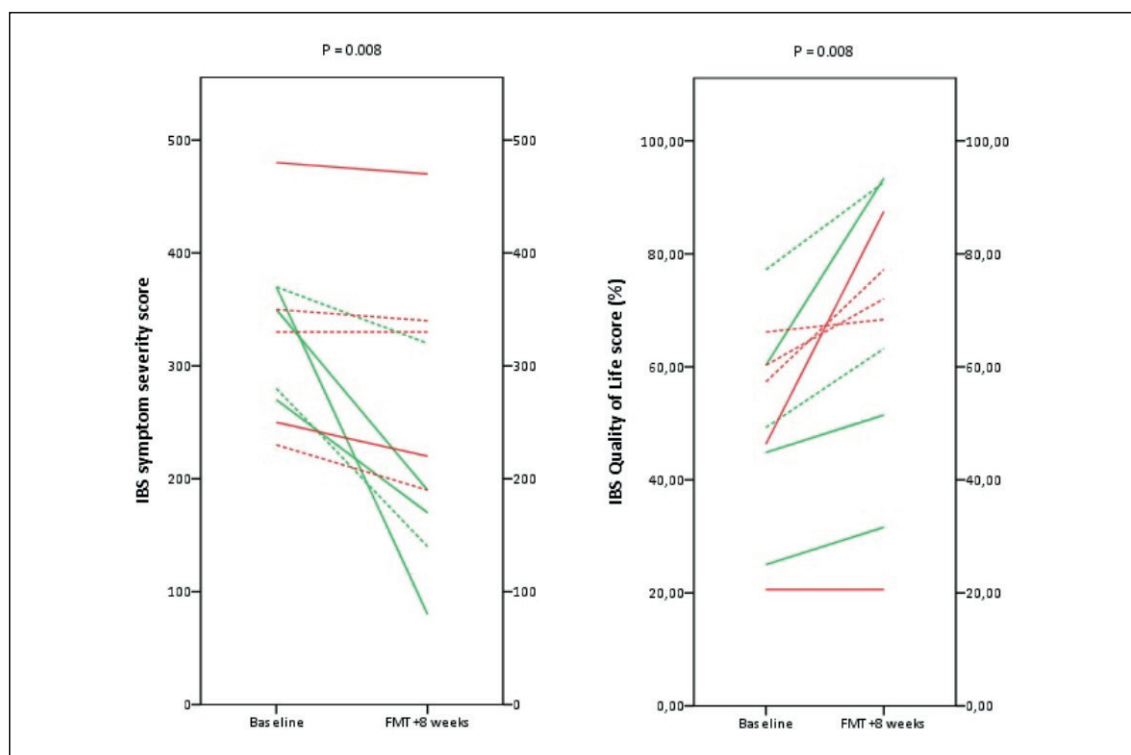


Figure 1. Changes in raw IBS symptom severity score and IBS Quality of Life score at 8 weeks after FMT. Statistical analysis performed with Wilcoxon's signed rank test. Green lines: responders (≥ 50 points improvement on the IBS symptom severity score); red lines: non-responders. Continuous lines: antibiotic induced IBS; dotted lines: post-infectious IBS.

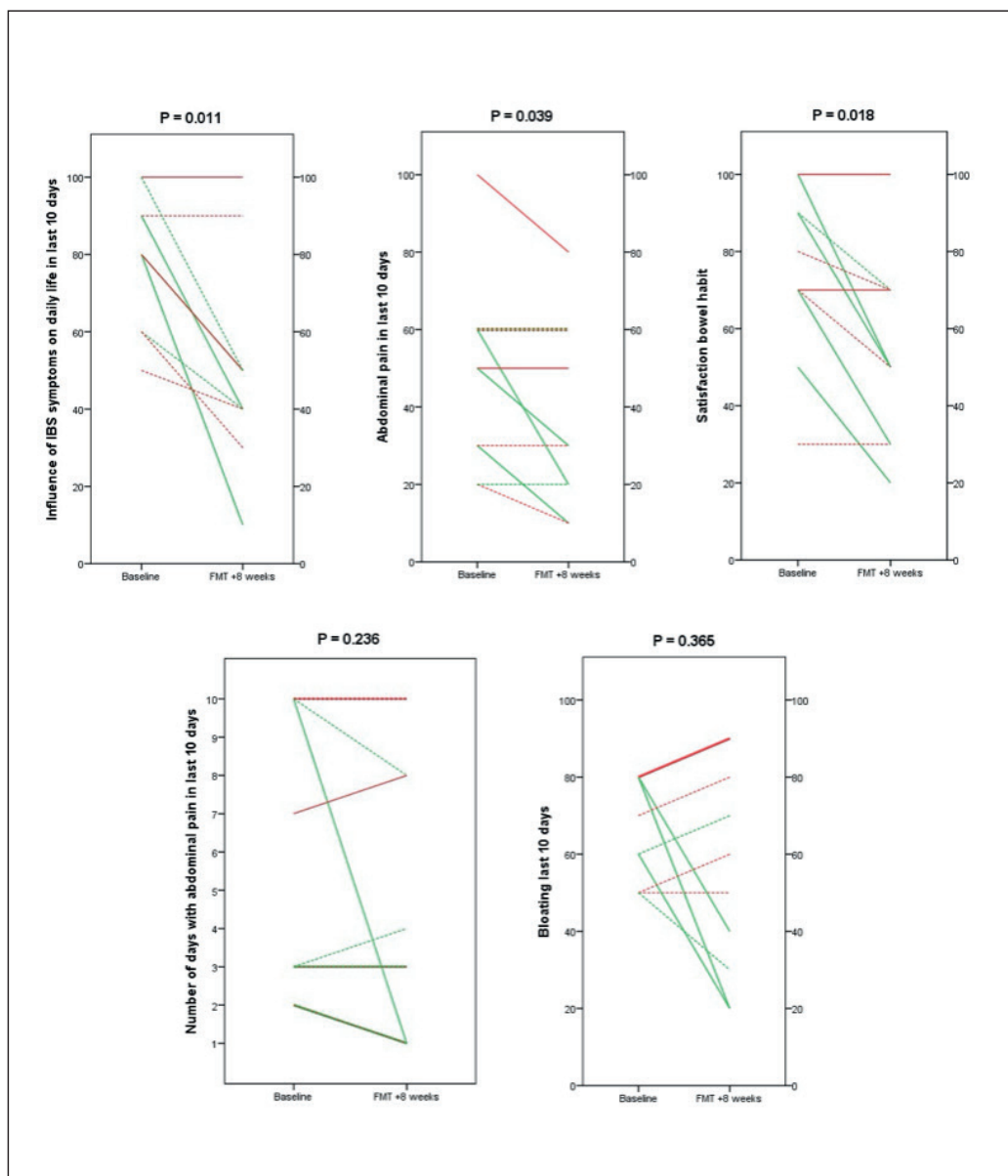
0.008). Also the severity of abdominal pain ($p = 0.039$), satisfaction about bowel habit ($p = 0.018$), and influence of IBS symptoms on daily life ($p = 0.011$) improved (Appendix A). There was no difference in bloating, or number of days with abdominal pain. FMT *via* a duodenal tube was well tolerated, and except for bloating, abdominal cramps, and nausea shortly and temporarily after FMT (self-limiting), no serious adverse events were reported.

Microbiota Analysis

Fecal microbiota analysis showed that the microbiota composition of most participants had shifted to that of their corresponding donor two weeks after FMT (Figure 2A). However, while in clinical responders this microbiota shift towards the donor profile continued (i.e., similarity to donor communities) between the two- and eight-week time point, in non-responders this shift was transient. Of note, at baseline, the cosine distance between donor and recipient of responders and non-responders did not differ (Figure 2B).

Four different donors were used in this pilot study. Three patients were treated with feces from donor 1; three from donor 2; two from donor 3; and two from donor 4. Interestingly, treatment with donor feces from donor 2 was successful in all three patients (Figure 2A). Compared with the other donors, donor 2 had a higher average total load of bacteria per 100 mg donor feces suspension (Figure 3). Especially Firmicutes and Proteobacteria were present in higher abundance. In addition, it should be noted that both patients who received donor feces from donor 3 did not respond to FMT; donor 3 had the lowest total bacteria load.

The Shannon diversity index of donors was higher than that of the participants at baseline (Figure 4). However, there were no major differences between participants at baseline and eight weeks after FMT. Importantly, in our cohort, changes in microbiota diversity did not seem to be associated with treatment response.



Appendix A. Changes in specific IBS related symptoms at 8 weeks after FMT. Statistical analysis performed with Wilcoxon’s signed rank test. Green lines: responders (≥ 50 points improvement on the IBS symptom severity score); red lines: non-responders. Continuous lines: antibiotic induced IBS; dotted lines: post-infectious IBS.

DISCUSSION

In this small open label pilot study of 10 patients with post-infectious or antibiotic-induced IBS, treatment with a single FMT (including 60 g of feces) administered through a duodenal tube resulted in clinical response in 5/10 participants. The median IBS-SSS of all participants improved with 40%; the IBS-specific quality of life with 17%. Microbiota analysis showed that clinical response to FMT was associated with a continued shift towards donor communities at eight weeks after FMT. Interestingly, all patients who received FMT from donor 2 were cured. This donor had a higher total load of bacteria, and caused a bigger change in the recipient’s microbiota than the other donors. From this result it may be hypothesized that effectiveness of donor microbiota may be related to the absolute amount of bacteria transferred. This

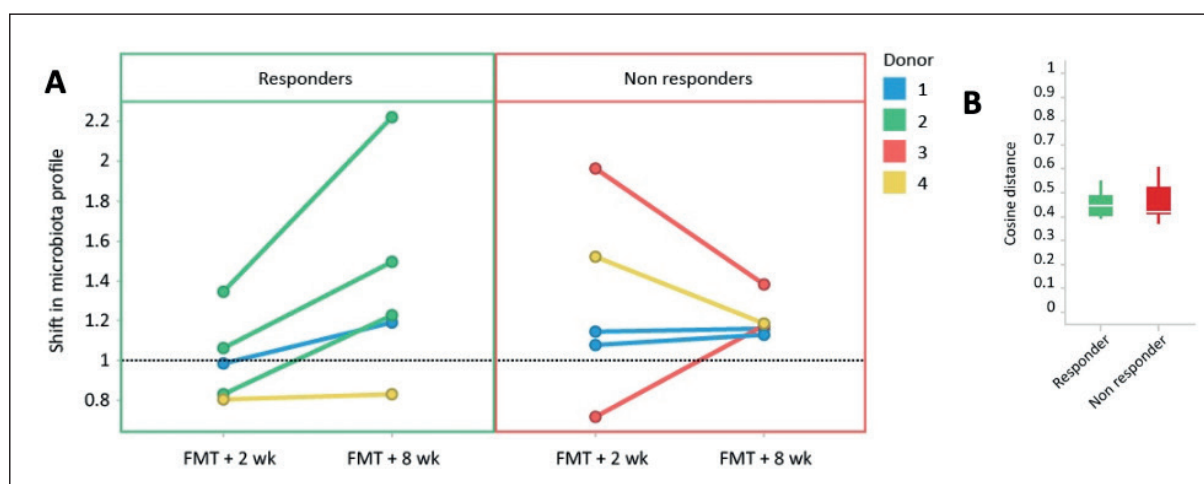


Figure 2. The extent of microbiota shifts towards the donor microbiota profile in responders and non-responders at 2 and 8 weeks after FMT. Microbiota shift is calculated as the ratio between cosine distance of donor to recipient at baseline, and the cosine distance of donor to recipient at 2 or 8 weeks after FMT. A higher (>1) value corresponds with more similarity to donor microbiota (A). Cosine distance between donor and recipient at baseline of responders and non-responders (B). Abbreviations: FMT: fecal microbiota transplantation.

hypothesis is supported by the findings of a recent double blind, placebo controlled RCT, in which they compared the treatment effect of 30 g FMT to 60 g FMT^{14,28}. El-Salhy et al¹⁴ concluded that the response rate was higher, and the effect stronger in patients who received 60 g transplants. In a second study, 10 patients not responding to a 30 g transplant were treated with a 60 g transplant. Seventy percent responded to the 60 g transplant, which suggests that the effect of FMT in IBS could be dose related²⁸.

It should be noted that in our study all three patients who received FMT from donor 2 belonged to the IBS-diarrhea group. This is in contrast with the results of the double-blind, placebo-controlled RCT conducted by Aroniadis et al¹⁹, who reported no symptom relief at 12 weeks post FMT in patients with diarrhea-predominant IBS. However, Aroniadis et al¹⁹ treated their patients with FMT capsules containing a total of 28.5 g of feces. The low amount of feces could have influenced their results. In the studies of El-Salhy et al¹⁴ no differences in effect rate between IBS-C and IBS-D patients was observed.

To date, four other RCTs have been published for FMT in IBS, reporting mixed results (including two abstracts not yet published as full manuscripts)¹⁵⁻¹⁸. In two of these RCTs donor FMT was associated with improvement of IBS symptoms 3 months post FMT^{15,17}. However, in the other two RCTs donor FMT was not associated with a significant improvement of symptoms^{16,18}. In a recent meta-analysis, published by Xu and colleagues, an overall clinical response rate of 49% (75/152) at 12 weeks post donor FMT was reported; in patients assigned to placebo the overall clinical response rate was 51% (52/102)²⁹. However, it is difficult to compare our results with previous reported results due to differences in numbers of patients included, the route of delivery, amount and type (fresh or frozen) of donor feces used, the use or not of bowel lavage prior to FMT, and the donors used.

Previous studies^{4,30-39} of the gut microbiota in IBS patients report alterations in the composition of the gut microbiota compared to healthy controls. Nonetheless, even though gut microbiota alterations seem to exist in IBS, results are inconsistent (sometimes even conflicting), and to date no uniform gut microbiota pattern in IBS patients has been shown^{4,6}. This also highlights the difficulty in finding robust microbiota markers associated with clinical symptoms of IBS^{4,40}. Several studies⁴¹⁻⁴³ have examined the shift in microbiota profile towards that of the donor as a potential marker for clinical success. In our study, FMT generally resulted in a shift toward the donor microbiota in the first two weeks after FMT. However, in the responders this shift was sustained between the two- and eight-week time point, while it was

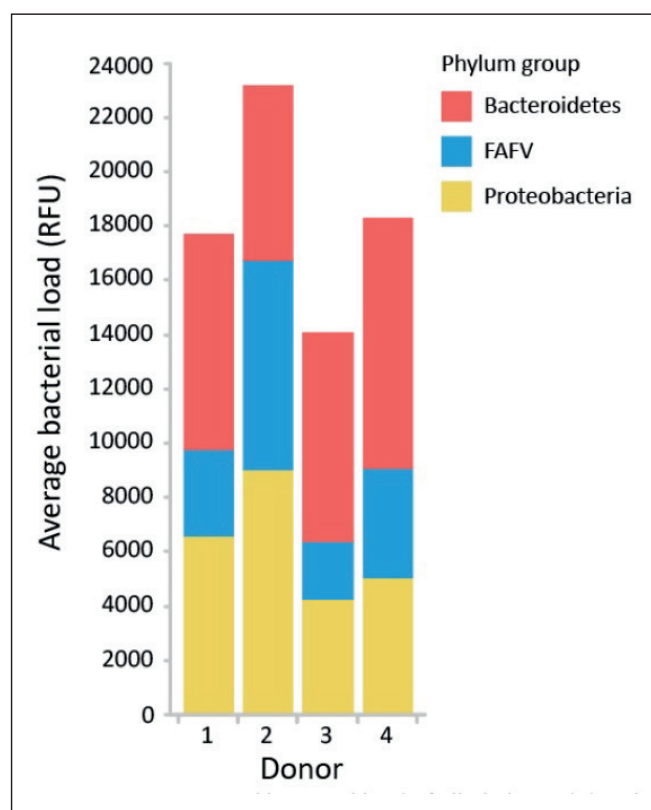


Figure 3. Average total bacterial load of all phyla, and distribution of phyla in donor fecal samples. Abbreviations: RFU: relative fluorescence units; FAFV: *Firmicutes, Actinobacteria, Fusobacteria, Verrucomicrobia*.

transient in the non-responders. This finding suggests that in patients with post-infection or antibiotic induced IBS, efficacy of FMT may be associated with continued shift towards donor communities. Halkjaer et al¹⁸ also reported a microbiota shift after FMT. However, this seemed not directly related to treatment response as in their study patients treated with placebo did report a better treatment response without a relevant microbiota shift. Overall, in our participants, the Shannon diversity index before FMT was lower than that of the donors. However, changes in Shannon diversity did not seem to be associated with treatment response in our small cohort which is consistent with the results of El-Salhy et al¹⁴.

The major limitation of our study is the open label design with small sample size. Previous published results suggest a significant placebo effect. This underlines that more and larger double-blind, placebo-controlled trials are necessary to provide clear answers about the efficacy of FMT in (post-infectious and antibiotic-induced) IBS, the factors predicting positive outcome, and the mechanisms behind any potential improvement of symptoms.

CONCLUSIONS

In conclusion, our pilot study provides further insight into the microbiota changes following FMT, and its efficacy for post-infection and antibiotic induced IBS. This pilot study showed that sustained change towards donor microbiota seems to be associated with better outcome of FMT in IBS patients. Furthermore, this study suggests that effectiveness of FMT could be donor-dependent and may be related to the absolute number of bacteria transferred. Large randomized, placebo controlled trials, including a specific subgroup of IBS patients to minimize heterogeneity, are needed to determine treatment effect, and to provide more reliable analyses of the association between clinical IBS symptoms, and microbiota composition. Additionally a randomized trial comparing FMT with and without bowel lavage is warranted.

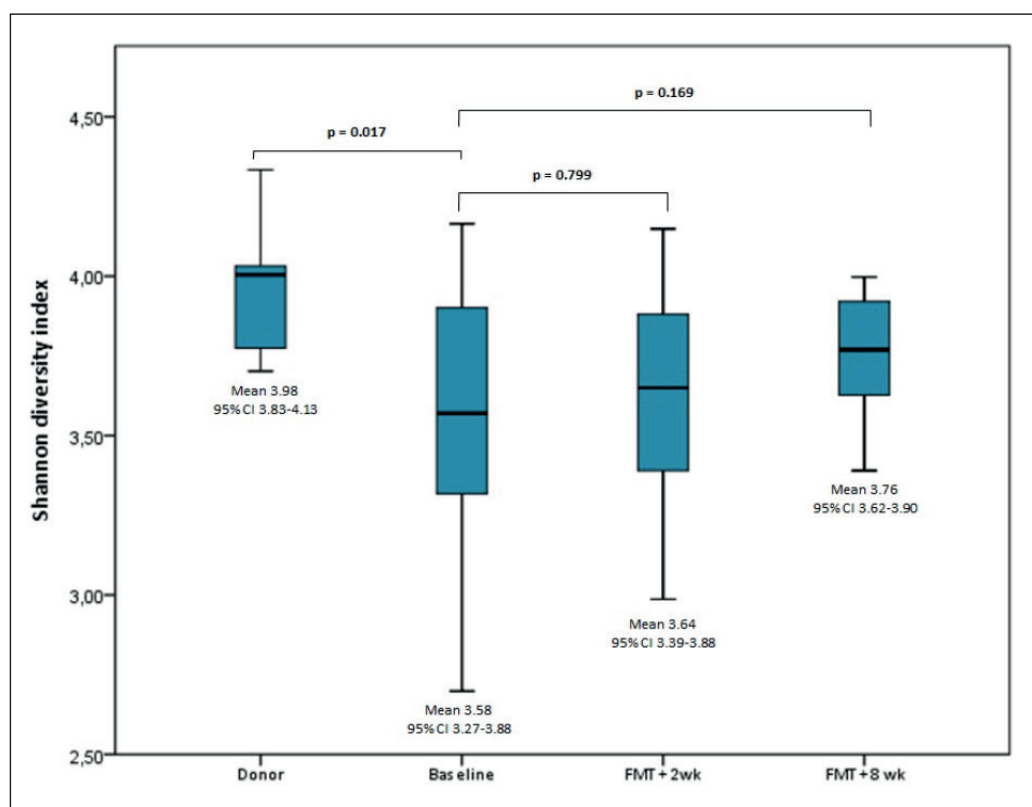


Figure 4. Microbiota diversity (Shannon diversity index) of donors and recipients. Statistical analysis performed with Wilcoxon's signed rank test (difference in Shannon diversity index between donor and recipient at baseline; differences between Shannon diversity index of recipients at baseline, two weeks, and eight weeks after FMT). Abbreviations: FMT: fecal microbiota transplantation.

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Author Contributions

YB, CVG, and CM conceptualized the pilot with input from ET, and JK. ET was involved in donor selection procedures. YB and CM were involved in patient recruitment, treatment and follow-up, AB performed the microbiota analysis. YB drafted the initial manuscript. ET, and JK revised the manuscript. AB, CM, and CVG extensively reviewed and revised the manuscript.

Conflict of interest

A.E. Budding has proprietary rights to the IS-pro technique, and is co-owner of the spin-off company IS-diagnostics. The other authors declare that they have no conflicts of interest.

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