CHAPTER 11

Fecal Microbiota Transplantation as Treatment for Post-Infection and Antibiotic-Induced Irritable Bowel Syndrome: A Pilot Study


Submitted
ABSTRACT

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

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-infection 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.

Conclusion
FMT appears as a promising treatment for antibiotic-induced and post-infection IBS. Based on these results, a randomized placebo controlled trial is warranted.
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 pathology.\textsuperscript{1} IBS strongly impairs quality of life, work productivity, and social function.\textsuperscript{2} The estimated worldwide prevalence of IBS in adults is approximately 11%.\textsuperscript{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.\textsuperscript{4,5} In recent years, alterations in the gut microbiota have also been linked to the pathophysiology of IBS; numerous studies have demonstrated changes in microbiota profiles in patients with IBS compared to healthy individuals.\textsuperscript{6,7} With regard to environmental factors, it is estimated that in about 10% of IBS patients, the onset of symptoms follows an episode of gastroenteritis, causing post-infection IBS.\textsuperscript{8} In addition, there is a strong association between IBS and prior use of antibiotics.\textsuperscript{9,10} Both antibiotic-induced, and post-infection IBS may be caused by alterations in gut microbiota. 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).\textsuperscript{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, anecdotal data suggest the efficacy of FMT for IBS.\textsuperscript{14-17} The primary aim of this pilot study was to evaluate the effect of FMT in post-infection and antibiotic-induced IBS.
METHODS

General study outline
From August 2016 through January 2017, we performed an open label pilot study in the VU University medical center (VUmc; Amsterdam, The Netherlands), to evaluate the effect of FMT in patients with post-infection 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-infection 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. 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). The donor-screening, feces collection, preparation, and storage of the donor feces suspension was described previously. 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 general. 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 moved to -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).\textsuperscript{19,21} 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, Armonk, NY).

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 rDNA.\textsuperscript{22} DNA was extracted from the fecal samples with the easyMag extraction kit according to the manufacturer’s instructions (Biomerieux, marcy l’Étoile, France).\textsuperscript{22} 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.\textsuperscript{22,23} Dissimilarities between sample compositions of the donor and recipients were calculated as the cosine distance between each pair of samples.\textsuperscript{24} Diversity of fecal samples was calculated by the Shannon diversity index.\textsuperscript{25}

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 had not 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-infection, 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-infection 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.
### Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th>#</th>
<th>Age</th>
<th>Gender</th>
<th>BMI</th>
<th>IBS medication</th>
<th>Etiology</th>
<th>Duration symptoms (years)</th>
<th>Symptoms IBS</th>
<th>IBS-SSS</th>
<th>IBS-QOL</th>
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<td>Constipation</td>
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<td>2</td>
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<td>25%</td>
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<tr>
<td>3</td>
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<td>Amitriptyline</td>
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<td>Diarrhea</td>
<td>330</td>
<td>66%</td>
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<tr>
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<td>Fibers Probiotics</td>
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<td>4</td>
<td>Diarrhea</td>
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<tr>
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<td>PI</td>
<td>6</td>
<td>Diarrhea</td>
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<td>49%</td>
</tr>
<tr>
<td>6</td>
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<td>Female</td>
<td>21.5</td>
<td>Probiotics Iodine</td>
<td>PI</td>
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<td>Constipation</td>
<td>230</td>
<td>60%</td>
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<td>Diarrhea</td>
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<td>2</td>
<td>Diarrhea</td>
<td>270</td>
<td>77%</td>
</tr>
</tbody>
</table>

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

#### 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 patient with post-infection 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 = 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.
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.

**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).
Figure 2. The extent of engraftment of donor microbiota in responders and non-responders at 2 and 8 weeks after FMT. Engraftment is defined 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) engraftment corresponds with more similarity to donor microbiota (A). Cosine distance between donor and recipient at baseline of responders and non-responders (B).

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.
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.

Figure 4. Microbiota diversity (Shannon diversity index) of donors and recipients.
In this small open label pilot study of 10 patients with post-infection or antibiotic-induced IBS, treatment with a single FMT administered through a duodenal tube resulted in clinical response in 5 of 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. Additionally, our results suggest that donor feces with a higher total bacterial load resulted in better treatment outcome. This is the first study that used the validated IBS-SSS questionnaire to select symptomatic patients, and to determine treatment response.\textsuperscript{19,21,26}

Recent case-series of FMT in IBS reported a response rate of 70-75%.\textsuperscript{16,17} We report a lower response rate, which could be explained by several factors. First of all, numbers of patients in the case-series and in our pilot study are small, hence the seemingly different results may not be different. Secondly, we used different study endpoints. Due to the short time window (symptoms during the past 10 days), and the inclusion of IBS symptoms that are responsive to change, the IBS-SSS is recommended for treatment trials.\textsuperscript{21,26} The previous case-series used the primary endpoint ‘adequate relief of global IBS symptoms’ or ‘resolution or improvement of symptoms after FMT’ as outcome measure.\textsuperscript{16,17} The global assessment of symptoms, as intended in these endpoints, has the disadvantage that even when only minimal changes on IBS related symptoms are achieved, participants are classified as a responder, leading to a higher response rate.\textsuperscript{27} Additionally, it is suggested that a binary outcome like ‘adequate relief’ is confounded by initial IBS symptom severity, and poorly correlates with the amount of symptom improvement.\textsuperscript{28}
Thirdly, the route of delivery and amount of donor feces used may have influenced the outcomes. In the study of Holvoet et al., patients were treated with a fresh donor feces suspension (300cc), infused through colonoscopy.\textsuperscript{29} Pinn et al. treated their patients with 50-100cc donor feces suspension, infused into the distal duodenum or proximal jejunum by duodenoscopy.\textsuperscript{16} In our study, patients were treated with approximately 200cc donor feces suspension, containing 60 gram of feces. We infused the donor feces through a duodenal tube, because it is well tolerated by patients and less invasive compared to colonoscopy. In addition, because of duodenal delivery, bowel lavage prior to FMT was not necessary, increasing patient comfort. However, the lack of bowel lavage could also be an explanation for the difference in treatment effect, since one could hypothesize that bowel lavage prior to FMT could facilitate and increase engraftment of the donor microbiota. Holvoet et al. did offer bowel lavage before FMT, and loperamide both prior to and following FMT\textsuperscript{29}; for the study of Pinn et al. information on pre-treatment was missing.

Previous studies of the gut microbiota in IBS patients report an increase in the relative abundance of \textit{Firmicutes}, mainly \textit{Clostridium} cluster XIVa and \textit{Ruminococcaceae}, together with a reduction in the relative abundance of \textit{Bacteroidetes}.\textsuperscript{4,30-33} In addition, a decreased amount of \textit{Bifidobacteria}, and a lower microbiota diversity have been reported in patients with IBS.\textsuperscript{31,34-39} 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.\textsuperscript{4,6} This also highlights the difficulty in finding robust microbiota markers associated with clinical symptoms of IBS.\textsuperscript{4,40} 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 cohort.

Several studies have examined the shift in microbiota profile towards that of the donor as a potential marker for clinical success.\textsuperscript{41-43} 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 continued between the two- and eight-week time point, while it was transient in the non-responders. This finding suggests that in patients with IBS, efficacy of FMT may be associated with continued shift towards donor communities.

The major limitations of our study are the small sample size and the open label design. Especially with regard to microbiota analysis, numbers were too small to draw firm conclusions. It is estimated that the placebo effect in pharmaceutical randomized controlled trials in IBS is about 37.5%. Therefore larger double-blind, placebo-controlled trials are necessary to provide clear answers about the efficacy of FMT in (post-infection and antibiotic-induced) IBS. Additionally, the time between start of IBS symptoms and study participation ranged from two to 28 years. Information about the onset of IBS symptoms had to be recalled from memory by the participants. Although anamnestically all participants were diagnosed with post-infection or antibiotic-induced IBS, information about the causing organism or type of antibiotics was missing.

In conclusion, treatment with FMT might be promising for antibiotic-induced and post-infection IBS. IBS participants who clinically responded to FMT showed continued shift towards the donor microbiota profile, while non-responders did not. Large randomized, placebo controlled trials 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.
REFERENCES


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.