Randomised clinical trial: Faecal microbiota transplantation versus autologous placebo administered via colonoscopy in irritable bowel syndrome

Short title: Faecal microbiota transplantation in IBS

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Summary:

Background

Irritable bowel syndrome (IBS) has been associated with microbial dysbiosis.

Aim

To investigate the efficacy of faecal microbiota transplantation (FMT) in the treatment of IBS.

Methods

Forty-nine IBS-patients were randomized to receive autologous or allogenic FMT via colonoscopy. The primary endpoint was a sustained, minimum of 50-point, reduction of the IBS Symptom Severity Score. The secondary outcomes were levels of anxiety and depression, changes in quality of life, gut microbiota and faecal water content as assessed with validated questionnaires, intestinal microbiota composition and stool dry weight.

Results

The primary endpoint, a sustained reduction of IBS symptoms as compared to the baseline, was not achieved in either group. However, there was a transient reduction in the mean IBS Symptom Severity Score in the FMT group at 12 weeks after the treatment as compared to baseline ($P = 0.01$). The groups did not differ in the number of patients achieving clinical response at 12 weeks. In the FMT-treated patients, microbial composition had changed to resemble that of the donor and the stool water content decreased significantly as compared to the baseline. The depression score decreased in patients with a reduction in IBS symptoms after FMT, but not in those placebo-treated patients nonetheless experiencing a reduction in IBS symptoms.

Conclusions

FMT provided only a transient relief of symptoms, although it induced a sustained alteration in the microbiota of IBS patients. Therefore, FMT delivered by a single infusion via colonoscopy cannot be recommended as a treatment for IBS in clinical practice.

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Key words: Microbiome, depression, anxiety, quality of life, transit time, follow-up time.

1 INTRODUCTION

Irritable bowel syndrome (IBS) is a common gastrointestinal disorder. The global prevalence of IBS is approximately 10%, although estimates vary greatly depending on the studied population and which diagnostic criteria are used [1,2]. The widely applied diagnostic criteria Rome III-IV divide the patient phenotypes according to their most dominant bowel characteristics into diarrhoea predominant, constipation predominant, mixed and unsubtyped IBS.

The aetiology of IBS is unknown although many theories have been proposed. Altered gut motility, epithelial hyperpermeability, low grade inflammation, visceral hypersensitivity, epigenetics and genetics, altered gut-brain interaction and psychological stressors have all been reported in patients with IBS[1]. In some IBS-patients, psychological factors play a role in the severity and persistence of symptoms; these may also affect the decision to seek health care and alter the response to the provided treatment [3]. IBS has been particularly associated with anxiety and depression, for example, IBS-patients have a three-fold risk of suffering from either depression or anxiety in comparison to the general population [4].

The expanding understanding of the microbiota's multiple functions for human health and its ways of interacting with the host highlight the possible role played by gut microorganisms in the pathogenesis of IBS [5]. It is known that the incidence of IBS is markedly increased after infectious gastroenteritis [6,7]. Furthermore, several studies have detected alterations in the gut microbiota composition between IBS patients and healthy controls, however a microbiota typical for IBS patients has not been conclusively defined [5,8,9]. Further evidence of the microbial component in IBS is provided by the fact that consumption of microbiota modifying agents such as antibiotics [10]

and probiotics [11] has been shown to be effective in reducing IBS symptoms. Regardless of the suspected causal relation between IBS and the intestinal microbiota, it is difficult to obtain convincing supportive evidence due to the multifactorial aetiology of the disorder, the significant heterogeneity in patients as well as the complexity of the gut microbiota. We postulated that the role of the microbiota in IBS could be clarified by altering the composition of the intestinal microbiota in IBS patients by transplanting them with a complex microbial population i.e. faecal microbiota transplantation (FMT).

FMT has been shown to be highly effective in treating recurrent Clostridioides difficile infections (rCDI). Although comparative trials between different administration routes are lacking, in this patient group, colonoscopy is often considered as the optimal route[12]. The excellent treatment efficacy in rCDI-patients and its favourable safety profile have encouraged investigators to seek other avenues for FMT research [13]. Data from rCDI-patients indicates that FMT may also relieve functional GI-symptoms as our recent results revealed that rCDI-patients treated with FMT experienced less severe GI-tract symptoms over the long-term than rCDI-patients treated with antibiotics only [14]. Furthermore, FMT for rCDI alters the microbiota of the recipient to resemble that of the donor, a change lasting for at least 12 months [15,16]. Currently, the long-term engraftment of the transplanted microbiota has not been as well documented in patient groups other than rCDI.

There are five recent placebo-controlled trials investigating FMT in IBS with conflicting results [17-21]. In two of these trials, a single faecal transplantation was administered via coloscopy [17,19], in two of the studies, faecal capsules were given for 3 to 12 days [18,20] and yet in another trial, FMT was

administered via gastroscopy[21]. In summary, FMT via colonoscopy resulted in a modest, but transient, decrease in IBS symptoms [17,19], the FMT capsules did not exert any benefits [18,20] but FMT via gastroscopy achieved a clear benefit with an up to 89.1% response rate[21]. Although all of these three administration routes, i.e. capsules taken orally for 12 days or a single FMT via colonoscopy or gastroscopy, altered the microbiota of IBS patients towards that of the donor [18], a concurrent decrease in the symptoms was observed only when FMT was administered via colonoscopy [19] or gastroscopy[21]. Given the mixed results, the efficacy of FMT in IBS seems to depend not only on the microbiota of the donor, but perhaps also on some other element of the stool, or on the survival of key elements of the microbiota while the stool is processed.

It is unclear whether FMT can sustainably alter the microbiota of IBS patients and how the microbial changes relate to IBS symptoms. Thus, the aim of this study was to evaluate the long-term efficacy of FMT administered via colonoscopy in reducing IBS symptoms in a randomised, double-blinded and placebo-controlled clinical trial, where the patients were monitored for one year. We also studied the impact of FMT on the quality of life, depression and anxiety of IBS patients. Furthermore, we measured the long-term changes in microbiota and stool consistency after a single FMT.

2 MATERIALS AND METHODS

2.1 Study design

We randomised 55 IBS patients to receive FMT from a universal donor (FMT group) or an autologous transplant (placebo group). After the randomization, one participant withdrew

without giving a reason and an additional five participants were excluded due to comorbidities such as lymphocytic colitis, proctitis and bile acid diarrhoea. Therefore, 49 IBS patients were included in the final analysis subdivided into 23 in the FMT group and 26 in the placebo arm (Supplementary Figure 1). All patients were treated via a single colonoscopy with 30 grams of faecal material administered into the caecum as described previously [22]. For the FMT treatment, faecal suspensions from a single universal donor were prepared and stored at -80 ºC until the day of the treatment [23] to ensure homogenous FMT material for the whole FMT group. The placebo group was treated with an autologous sample, where the patient´s own stool, donated within six hours prior to the colonoscopy and prepared as an infusion on the same day, was administered via colonoscopy. To ensure blinding, both groups provided their stool for the preparation of placebo, and if the patient was randomized to the FMT group, the stool sample was discarded. The donor screening procedures and characteristics are detailed below.

The IBS symptoms and mental wellbeing as well as the quality of life of the patients were evaluated with validated questionnaires; IBS symptom severity score (IBS-SSS) [24], Rome-III questionnaire [25], IBS Quality of Life -questionnaire (IBS-QoL) [26], Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI) [27] and a health-related quality of life questionnaire, 15-D [28]. The patients completed questionnaires at baseline on the day of the FMT treatment as well as at the follow-up time points on weeks 4, 8, 12, 26 and 52 after the treatment; 15D and IBS-QoL -questionnaires were only filled in on weeks 0, 8, 26 and 52. Stool samples for the microbiota analysis were collected on all six study visits. The patients were not given any restrictions regarding nutrition or medications during the follow-up. The primary endpoint of the study was

that there should be sustained relief of IBS symptoms throughout the 52-week follow-up period i.e. a 50-point decrease in the IBS-SSS total score as compared to the baseline.

This study was approved by the ethical committee of Helsinki University Hospital (registration nro. 40/13/03/01/2015) and all participants signed an informed consent. All authors had access to the study data and have reviewed and approved the final manuscript.

2.2 Sample size

The sample size was calculated based on the assumption of a 40 % placebo effect and a clinically significant 40 % treatment effect over placebo. A two group χ^2 test with a 0.05 two-sided significance level with 80 % power to detect this difference yielded a sample size of 26 in each group. The decrease of 50 points in IBS-SSS score was considered as a significant and clinically meaningful change in the severity of the symptoms. The confidence interval was selected to be 95 % (α=0.05 and β=0.1).

2.3 Randomisation

The patients were randomised into the study groups at the first contact point, before any evaluation of the IBS-subgroup or the severity of symptoms with Rome III and IBS-SSS questionnaires. The patients were randomised 1:1 to receive FMT either from a donor or as an autologous placebo. The randomization was done in blocks of six by a study nurse who was not involved in the treatment of the patients. The patient and the hospital personnel were blinded to

the type of faeces being transplanted. Decoding was only performed when all the patients had completed the 52-week follow-up.

2.4 Patients

Adult patients (18-73 years old), diagnosed by an experienced gastroenterologist to have IBS and remaining symptomatic despite receiving conventional treatment, were recruited into the study. After the enrolment, the patients completed the Rome III questionnaire. At this stage, there were three patients not fulfilling the Rome III criteria for IBS due to the fluctuating nature of their symptoms. The participating patients were recruited from occupational health care, Porvoo hospital, Helsinki municipal hospital, and University Hospital of Helsinki from August 2015 to July 2017. The patients had received conventional treatments according to their predominant symptoms but without obtaining satisfactory relief. Patients were excluded from the trial if they were unable to provide written informed consent, had an organic gastrointestinal diagnosis such as inflammatory bowel disease or if they were pregnant (Consort flow diagram in Supplementary Figure 1).

2.5 The donor

A single universal donor, a young adult male who was in good general health and normal weight was used as the faecal donor. He had been delivered through vaginal childbirth, had not had antibiotics during the previous year, and he was not a health care worker. The donor was pre-screened according to the previously defined criteria [22]. The donor did not have any long-term diagnoses and was not using any permanent medications. He did not have a history of high-risk

sexual behaviour, use of illicit drugs or had recently travelled to areas with high incidence of infectious diarrhoea.

The donor was screened with the following diagnostic tests. Blood: HIV, hepatitis A, B, and C. Faeces: Culture of faecal bacterial pathogens (Salmonella, Yersinia, and Campylobacter) and antibiotic resistant bacteria (MRSA, ESBL), detection of Clostridioides difficile toxin, Helicobacter pylori, and faecal parasites (ova and protozoa).

2.6 Outcomes

The primary endpoint of the study was a sustained relief of IBS symptoms throughout the 52-week follow-up period. This was defined by a 50-point decrease in the IBS-SSS total score. The secondary outcomes included changes in quality of life, depression, anxiety, gut microbiota composition and stool consistency. Adverse events were recorded.

2.7 Microbiota analysis

The microbial DNA was extracted with a previously described method [29] where the mechanical lysis was followed up by high-throughput DNA extraction using KingFisher Flex 96 (Thermo Fisher Scientific). The microbial composition was analysed by sequencing the V3-V4 region of the 16S ribosomal gene using MiSeq sequencing. The resultant reads were pre-processed using the R-package mare pipeline [30,31]. After pre-processing, there were on average 47 029 (standard deviation, SD=8 475) reads per sample. The reads were annotated to taxonomic assignments using USEARCH (version 8.1.1756) and SILVA 16S rRNA reference database version 115 [32].

2.8 Statistical methods

Statistical analyses were performed using either the R software program (R core team, version 3.5.2.) or the SPSS software program (IBM Statistics, version 26). Results are shown as mean (standard deviation, SD) unless otherwise stated. The statistical difference between the treatment groups was calculated using t-test or ANOVA with Benjamini-Hochbergs correction for multiple testing when the data was normally distributed; for nonparametric data, this was calculated using Wilcoxon signed rank test or Kruscal-Wallis tests. The difference between baseline and follow-up time points (Δ) was calculated for all questionnaire data. The correlation between recorded symptoms and microbial similarity between donor and recipient was estimated with Spearman correlation. Variations in the microbiota composition were assessed with Bray-Curtis dissimilarities and visualized using Principal co-ordinate analysis (PCoA).

3 RESULTS

3.1 Patient characteristics at baseline

A total of 49 IBS patients were included in the final analysis; 23 in the FMT group and 26 in the placebo (Supplementary Figure 1). Randomisation was conducted prior to the Rome III classification, therefore there were more diarrhoea predominant patients in the placebo group (Table 1). The majority of the 49 patients had diarrhoea predominant IBS (n=25), 21 patients had either mixed or unsubtyped IBS, three patients were in remission at baseline, and none of the patients had constipation predominant IBS. The severity of IBS symptoms as evaluated by IBS-SSS, was similar in both groups. On average, the symptom severity was moderate in both groups ranging from mild to severe. The depression scores (BDI) were similar between the groups and on average, the values ranged from mild depression to not depressed. The placebo group had

249 significantly higher levels of anxiety (BAI) as compared to the FMT group, which was due to three
250 outlier subjects with very high baseline scores (Supplementary Figure 2). There were no other
251 statistical differences between the groups at the baseline.

252

253 3.2 The primary end point – decrease in IBS-SSS

254 The primary endpoint of the study was a sustained reduction of IBS symptoms defined by a decline
255 in the IBS-SSS score of 50 points or more as compared to the baseline. This was achieved at week
256 12 in 11 out of 23 patients in the FMT group and in 11 out of 26 patients in the placebo group, and
257 there was no significant difference between the groups. However, in the FMT group at week 12,
258 the mean of IBS-SSS was reduced significantly as compared to the baseline (paired t-test, P = 0.01,
259 95% CI 16.39 to 108.62, Figure 1). The mean IBS-SSS value of the FMT group declined by 62 points
260 from the baseline value of 270 points down to 189 points at 12 weeks after FMT, which was the
261 lowest mean IBS-SSS score encountered in the study (Table 2). At the other time points, the IBS-
262 SSS scores did not differ significantly either as compared to the baseline or between the FMT and
263 placebo groups.

264

265 3.3 Secondary endpoints

266 We investigated how the patients' mental health, including depression and anxiety, quality of life
267 in general, as well as disease specific quality of life, were affected by the FMT treatment in
268 comparison to those receiving placebo. We found no significant differences between the total
269 scores of IBS-QoL or 15D at any time point or between the groups (Figure 2A). There were no

significant changes in the reported depression (BDI) or anxiety (BAI) scores after the FMT between the placebo and FMT groups or between the time points within the groups.

We noted a significant correlation between the measured change in IBS symptoms (Δ IBS-SSS) and the change in depression (Δ BDI) and the change in the quality of life (Δ 15D) when examining both treatment groups together. This association was stronger in both cases in the FMT group. In the FMT group, the change in IBS-SSS had a significant correlation with the change in BDI (ρ = 0.354, $P < 0.001$, Supplementary figure 3), whereas this was not statistically significant in the placebo group. The Δ IBS-SSS and Δ 15D displayed a strong and significant correlation in the treated group (ρ = 0.55, $P < 0.001$) and weaker, but still significant correlation in the placebo group (ρ = 0.28, P = 0.002). There was no correlation between the changes in IBS symptoms (IBS-SSS) and anxiety (BAI).

3.4 Responders to the therapy

We studied more closely the differences between those patients who responded to the FMT treatment and compared them to the non-responders. The responders were considered as those patients who reported a reduction of IBS-SSS of 50 points or more at any recorded time point as compared to the baseline. Conversely, the non-responders were the patients whose IBS-SSS score decreased by less than 50 points or even increased as compared to the baseline. In both study groups, the number of responders was lower than the number of non-responders at all the other time points except at 12 weeks after FMT, where the number of responders exceeded the number of non-responders in the FMT group (Table 3). The number of patients who responded to the

treatment did not vary significantly between the two groups or between the follow-up time points.

We also aimed to clarify if the responder status would affect the quality of life and mental health of the patients. We calculated the mean of events at all the time points excluding the baseline value. We noted that only in the FMT group were the reported 15D scores significantly higher in the responders as compared to the non-responders (P = 0.05, Figure 3B). Moreover, only the responders in the FMT group displayed a significantly lower BDI depression score as compared to the non-responders (P = 0.05, Figure 3A), whereas in the placebo group, there was no difference in the level of depression irrespective of how the patients responded to the treatment. The mean BDI score of the responders in the FMT group was 6.6, referring to not depressed, whereas the mean BDI scores of other subgroups were higher referring to mild depression, i.e. in the responders in the placebo group 10.2; in the non-responders in the FMT group 11.0 and in the non-responders in the placebo group 12.8 (Figure 3A).

3.5 Microbiota results

To characterize FMT-induced microbiota changes, we collected stool samples before the FMT and five times during the 52-week follow-up and performed a microbiota analysis by high-throughput sequencing of the V3-V4 region of the bacterial 16S rRNA gene. First, we analysed changes in the microbiota richness and the level of similarity with the donor's microbiota and finally assessed changes in microbiota profiles within the study groups (Figure 4). At baseline, the microbial richness in both groups was lower than that of the donor (Figure 4A). The microbial richness increased significantly only in the FMT group, whereas in the placebo group, the microbial richness

315 remained unchanged (Figure 4A). However, the increase in microbial richness was not reflected as
316 an increase in the microbial diversity (Supplementary Figure 4).

317

318 The comparison of microbiota similarity to the donor revealed that the microbial community of
319 the FMT treated patients had been altered to resemble that of the donor after FMT and this
320 similarity remained throughout the follow-up (Figure 4B). In contrast, the similarity of microbiota
321 to the donor did not change and remained low in the placebo group (Figure 4B). The within-group
322 PCoA revealed that the microbiota of patients in the placebo group remained unchanged
323 throughout the study (Figure 4C), whereas a clear shift in the microbiota profile in the FMT group
324 was observed and the baseline samples clustered separately from the post-FMT follow-up-samples
325 ($P < 0.05$, Figure 4D).

326

327 3.6 The stool consistency

328 The faecal water content is an objective way of assessing stool consistency and it has been
329 reported that the faecal water content correlates with transit time [33]. In order to define the effect
330 of FMT on stool water content and intestinal transit time, we performed a stool dry weight
331 analysis. In both groups, the stool samples at the baseline as well as at 12, 26 and 52-week time
332 points were dried, and the stool water content was calculated. The stool water content decreased
333 in both groups, but the only statistically significant changes occurred in the FMT group at the time
334 points of 26 weeks (t-test, FMT-treated $P = 0.005$, placebo $P = 0.33$) and 52 weeks (FMT-treated P
335 $= 0.001$, placebo $P = 0.07$) as compared to the baseline. There were no significant changes in the
336 placebo group nor were there any differences between the groups.

3.7 Adverse events

There was no significant difference in the total number of adverse events between the groups and no severe adverse events occurred in either group. In summary, 35% (17/49) of all patients experienced adverse events after the intervention, (FMT 7/23, placebo 10/26, χ^2, P = 0.49). The patient-reported adverse events were mild and transient GI symptoms, loose stools or bloating and transient fever. Altogether, four patients reported transient fever after the transplant, all in the placebo group (P = 0.045). One patient in the placebo group had a yeast vulvitis. The adverse events are listed in Table 4.

4 DISCUSSION

In this placebo-controlled trial, we evaluated the effect of a single FMT treatment on the symptoms of adult IBS patients. We defined the primary endpoint as a sustained 50-point reduction in the IBS-SSS, which is considered to represent a significant reduction in IBS symptom severity. In the FMT group, the mean of IBS-SSS was reduced significantly three months after the FMT as compared to the baseline, whereas those in the placebo group experienced no marked reduction of the mean IBS-SSS at any time point. Therefore, the results are in line with the first placebo-controlled FMT study on IBS[17], which showed that a single FMT treatment in colonoscopy decreased IBS symptoms at three months after FMT, but not beyond. However, in our study, the number of patients achieving a 50-point reduction of IBS-SSS was not significantly different between the groups and the symptom scores between the two groups did not differ significantly at any time point.

The primary end point of our study was ambitious i.e. a marked reduction in the IBS symptoms at every time point after the FMT throughout the 52-week trial period. In contrast, the follow-up times of drug trials in IBS have typically been shorter than year-long study[10]. The study of Johnsen et al. had an equally long follow-up time of 52 weeks and they also observed only a transient relief of symptoms at three months after FMT[17]. All in all, the result of our primary outcome was negative as we did not observe any significant differences between the groups at any time point.

As secondary endpoints, we evaluated whether the FMT treatment could change the patients´ quality of life or symptoms of depression and anxiety. The overall quality of life as measured with two validated questionnaires, IBS-QoL and 15D total score, remained unchanged after FMT; this might be due to multiple factors affecting the quality of life in addition to IBS symptoms. There were also no differences in the total depression or anxiety scores as measured with BDI and BAI questionnaires as compared to the baseline or between the groups at any time point. We also recorded some adverse events which, in line with previous findings [34], were transient, mild and did not differ between the two groups. Transient fever was even reported more in the placebo group. Hence, fever as well as short term gastrointestinal symptoms seemed to be provoked by colonoscopy or bowel cleansing rather than as a response to allogenic faecal material.

We analysed separately the responders to the therapy and noted that the depression score decreased, and the quality of life score increased among those patients whose IBS symptoms improved after FMT, but not in the patients reporting an IBS symptom reduction after placebo. This observation is intriguing as an altered gut microbiota has been associated with depression [35]

and animal models have shown that microbiota modulation can exert effects on the gut-brain axis [36]. Although the relationship between gut microbiota and depression is unclear, some evidence of causality was demonstrated in a placebo-controlled study in which the administration of the probiotic Bifidobacterium longum strain reduced depression scores in IBS patients [37]. Furthermore, in our previous study, the patients who were treated with FMT for rCDI reported improved mental well-being after the treatment as compared to patients who were treated with antibiotics [14]. Taken together, there is preliminary evidence to suggest that microbiota manipulation may represent a novel means to treat depression.

We demonstrated that a single FMT resulted in a 52-week long lasting change in the microbiota such that it resembled the microbial composition of the donor with an increase in the microbial richness. Thus, the transplanted microbiota seemed to engraft successfully in the patients. This observation is in line with and extends the previous studies which showed successful engraftment of donor´s microbiota in IBS patients for up to six months with FMT capsules [18], for two months with a single FMT administered via colonoscopy [19] and for three months with FMT administered via gastroscopy[21]. Thus, FMT seems to change the microbiota composition in IBS subjects relatively permanently, a phenomenon which has also been observed in FMT-treated rCDI patients [15]. In our study, a similar number of patients responded to the treatment in both groups, although microbiota changes were observed only in the FMT group. High response-rates to placebo are typical in IBS studies [38], but also bowel cleansing may have impacted on the IBS symptoms. Collectively, the FMT trials carried out on IBS patients thus far have shown promising results in terms of the microbiota modulation, although the effects on symptoms have been inconsistent [17-21]. Consequently, gut microbiota remains a potential target for the treatment in IBS. In the future,

405 it may be possible to characterize the microbiota of a patient to choose the most suitable
406 treatment strategy [39] and to identify those subgroups of patients who would be more likely to
407 benefit from microbiota enriching therapies. For example, the selection of optimal donors based
408 on their microbiota profiles may be one way to improve the FMT treatment outcomes in IBS.

409

410 We also evaluated the effect of FMT indirectly on the stool consistency and transit time by
411 performing a stool dry weight analysis and noted that the stool water content decreased in the
412 FMT group as compared to the baseline, and the observed effect on stool water content remained
413 throughout the follow-up. The water content of the stool also decreased in the placebo group
414 although this change did not reach statistical significance. The stool water content correlates with
415 both gut microbiota [40] and bowel transit time [33]. Thus, in our study, FMT resulted in a long-term
416 alteration of the gut microbiota which seems to have contributed to the reduction in the amount
417 of faecal water. Since changes in stool consistency was observed in both groups, this may indicate
418 that also bowel cleansing per se can alter the faecal water content. Nevertheless, IBS-symptoms
419 reduced only transiently, which implies that in addition to microbiota and stool water content,
420 other factors such as diet or socio-psychological factors are important in maintaining IBS
421 symptoms.

422

423 A small sample size is a limitation of our study. We recruited 55 patients and aimed to include
424 minimum of 52 patients based on the power calculation, but finally excluded six due to exclusion
425 criteria and thus 49 patients were left for final analysis. The previously published placebo-
426 controlled trials of FMT in IBS have had patient sizes ranging from 17 to 165 [17-21]. Furthermore, we
427 allowed patients with any IBS subtype or any grade of severity to participate in the study, in

comparison to the study of Johnsen et al. in which constipation predominant IBS patients were excluded [17]. Even though constipation predominant IBS patients were not excluded from our study, none of the participants were classified as constipation predominant IBS according to the Rome III criteria at baseline. Therefore, the clinical results of these two studies were similar i.e. showing a benefit at three months after FMT, but not beyond. The strength of our study was that we also assessed the quality of life, depression and anxiety in the patients as well as conducting a microbiota analysis which revealed that the persistent change in microbiota composition was not reflected in symptom relief over the long term.

The accumulating data in treating rCDI with FMT highlights the good efficacy with a single dose regardless of the route of administration [12]. However, in other indications, defining an adequate FMT protocol, while essential, appears to be rather difficult. The efficacy may depend on the stage of engraftment of the transplanted microbiota or of ensuring the engraftment of some key elements of the microbiota. In ulcerative colitis, repeated treatments [41] or anaerobically prepared FMT [42] have shown promising clinical efficacy, although different protocols have not been compared with each other and trials with a single FMT are lacking. Although, costly and laborious protocols are not ideal for a common and benign disease such as IBS, elevated FMT doses or repeating treatments might increase the response rates of IBS patients [43,44]. It is also possible that, as in the treatment of rCDI, pre-treatment with antibiotics would further enhance the engraftment of the transplanted microbial community or the key microbial elements and could possibly improve the treatment outcomes. Our recent study conducted in mice indicated that antibiotic pre-treatment prior to FMT could improve the engraftment of bifidobacteria [45]; this bacterial group has been associated with reduced abdominal pain in humans and reduced visceral

sensitivity in a mouse model [46-48]. Furthermore, an optimal donor might result in a better response rate also in IBS, as was previously demonstrated for ulcerative colitis in a study where the desired treatment outcome was obtained in only one out of six donors [41]. It may well be that differences in the donor microbiota explain the superior efficacy of FMT in IBS, as demonstrated in the study of El-Salhy et al.[21] as compared to all previous studies[17-20], including ours.

Another possible explanation for the transient relief of IBS symptoms is that the functionality of the transferred microbiota may not be well preserved in the new host, even though the composition did seem to be maintained. The functionality could have been affected by dietary factors, which in this trial were not controlled or recorded in detail and it is well known that IBS patients often reduce their food intake[1] and in particular, they may try to avoid fibrous foods which could have a significant impact on the metabolism and functionality of the microbiota. The role of the microbiota in IBS remains to be clarified, and further FMT studies are warranted to identify possible super-donors or to determine which characteristics of the microbiota could be used to select an optimal donor for a specific indication.

In conclusion, our study did not find a significant difference between the groups and therefore the outcome is negative. However, a single FMT did achieve transient relief of IBS symptoms as compared to the baseline, while the changes in microbial profiles and the decrease of stool water were long-lasting, enduring for at least 52 weeks. Further, we observed a possible link between microbiota and depression, as only in the FMT group, but not in the placebo group, did the patients with reduced IBS symptoms show also lower depression scores. Our results should encourage further studies to define the optimal FMT protocol for IBS patients and to help in donor

and patient selection. Nonetheless, since we did not detect a significant long-term benefit of FMT over placebo in these patients, we conclude that a single FMT infusion via colonoscopy cannot be recommended as a treatment for IBS in clinical practice.

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TABLES:

Table 1. The baseline demographics of the patients included in the analysis. Standard deviation shown in brackets.

	FMT	Placebo	P value

Gender (M/F)	11/12	9/17	0.52
Age	47.3 (16.8)	46.3 (14.3)	0.83
BMI	24.3 (4.5)	24.5 (8.0)	0.94
IBS classification	D=9, M=3, U=9, other=2	D=16, M=4, U=5, other=1	0.03
IBS-SSS	282.5 (85.4)	263.5 (93.2)	0.47
BAI	8.4 (6.1)	13.9 (11.9)	0.05
BD-II	9.7 (7.9)	12.2 (8.3)	0.29
IBS-QoL	56.9 (19.9)	57.2 (20.3)	0.95
15D	0.8 (0.1)	0.9 (0.1)	0.24

609 Abbreviations: D = diarrhoea predominant IBS, M = mixed IBS, U = unsubtyped IBS, other = IBS in remission,
610 (not meeting the Rome III criteria at the baseline).

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613

614 Table 2. IBS symptom severity score (IBS-SSS) in both groups at each time point and the change of the IBS-
615 SSS compared to baseline in both groups at all the time points. The presented values are mean IBS-SSS
616 value or its change in the study groups at each time point; the value in brackets is standard deviation. The
617 statistically significant value as compared to the baseline is bolded (unpaired t-test P = 0.03, 95% CI 3.79 to
618 126.71)

	IBS-SSS		Delta IBS-SSS	
	Placebo	FMT	Placebo	FMT
BL	263.46(93.19)	270.22(102.16)	-	-
4 wk	223.46(100.16)	238.48(119.16)	-40.00(81.13)	-27.14(69.80)
8 wk	238.658(113.18)	219.35(116.27)	-18.60(78.15)	-38.10(84.05)
12 wk	216.15(127.98)	188.91(125.43)	-32.71(94.21)	-62.50(98.54)
26 wk	236.54(125.91)	208.70(125.91)	-20.80(85.16)	-27.50(55.26)
52 wk	244.42(141.91)	231.74(107.64)	-2.08(86.00)	-31.75(76.09)

619

620 Table 3. The number of responders and non-responders in each group and at each time point and their
621 comparative P-values. In brackets the proportion (%) of responders at each time point.

622

	FMT	Placebo	P-value
4wk	RES = 5(24%) / nonRES = 16	12(46%) / 14	0.089
8wk	6(27%) / 16	9(36%) / 16	0.675
12wk	11(55%) / 9	11(46%) / 13	0.689
26wk	5(28%) / 13	6(24%) / 19	0.156
52wk	5(25) / 15	8(33%) / 16	0.690

623

624 RES = responder, nonRES = non-responder.

625

626 Table 4. A list of all adverse events. One patient could have more than one adverse event.

	FMT	Placebo	P-value
All adverse events	7/23	10/25	0.489
Diarrhoea/loose stools	4	1	0.129
Bloating/flatulence	3	2	0.568
Other worsening of GI-symptoms	1	3	0.338
Fever	0	4	0.045
Yeast vulvitis	0	1	0.332

627

628 FIGURE LEGENDS:

629 Figure 1. The IBS symptoms reduced significantly after FMT as compared to the baseline, but the reduction
630 was not significant when compared to placebo. The IBS-symptom severity -score in the placebo group
631 (grey line) and the FMT group (black line) through the 52-week follow up period. Asterisk (*) indicates a
632 significant decline in the IBS symptoms in the FMT group at 12 weeks when compared to the baseline (t-
633 test, P = 0.01).

634

635 Figure 2. The mental health and quality of life parameters at baseline and after intervention showed no
636 significant difference between the groups in the A) the depression (BDI), B) anxiety (BAI), C) general quality
637 of life (15D) and D) IBS related quality of life (IBS-QoL) questionnaires (ANOVA, $P > 0.05$) .

638

639 Figure 3. The responder status had a significant effect on the depression and quality of life measures. A)
640 Depression scores (ANOVA, $P = 0.05$) and B) general quality of life (15D) scores were significantly (ANOVA, P
641 = 0.05) reduced in those FMT -treated patients who responded to the treatment.

642

643 Figure 4. The effect of FMT treatment and placebo on the patient's microbiota composition. A) The FMT
644 treatment significantly increased the microbial richness whereas the placebo group displayed no change. At
645 baseline, both patient groups had lower microbial richness when compared to the donor. B) The microbiota
646 similarity between the donor and patient (measured with Spearman correlation) was significantly higher in
647 the FMT group than in placebo at all time points after the intervention. The effect of the intervention on
648 the microbial composition shown with PCoA (Bray-Curtis dissimilarity) C) in the placebo group did not show
649 difference between baseline and follow-up samples whereas D) the FMT treated group showed significant
650 difference. The microbial composition of the FMT treated patients shifted towards the donor after the
651 intervention whereas there was no significant difference in the placebo group. Asterisk (*) indicates a
652 significant difference ($P < 0.05$) between the baseline and the respective time point.

653

654 Figure 5. The stool water content of the FMT treated and placebo group at baseline (BL) and 12, 26 and 52
655 follow-up time points. There was a significant reduction in the stool water content in the FMT treated
656 patients at 26 weeks (t-test, $P = 0.005$) and 52 weeks ($P = 0.001$) as compared to the baseline Asterisk (*)
657 indicates a significant difference ($P < 0.05$).

658

659

660 SUPPLEMENTARY FIGURE LEGENDS:

661

662 Supplementary Figure 1. The participant flow through the trial according to the Consort guidelines.

663

664 Supplementary Figure 2. The Beck Anxiety Inventory (BAI) scores at the baseline. There were three
665 patients in the placebo group scoring very high values, causing the whole group to be statistically
666 significantly different compared to the treated subjects. If these three outliers should have been excluded,
667 the BAI scores would have been similar between the groups.

668

669 Supplementary Figure 3. The change in the Irritable Bowel Syndrome severity (IBS-SSS, horizontal) and the
670 change in the BDI score (axial). In the whole studied population, the change in IBS-SSS correlated
671 significantly with the change in BDI ($P = 8.0 * 10^{-8}$).

672

673 Supplementary Figure 4. The microbial diversity of the donor (left), placebo group at each time point
674 (middle) and treated group at each time point (right). The diversity did not change significantly after FMT in
675 either of the groups.

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677 Statement of interests:

678 All authors have no conflict of interests to declare.

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CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	3-4
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	6-7
	2b	Specific objectives or hypotheses	8
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	7
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	none
Participants	4a	Eligibility criteria for participants	9 (and 7)
	4b	Settings and locations where the data were collected	9
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	7
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	10
	6b	Any changes to trial outcomes after the trial commenced, with reasons	none
Sample size	7a	How sample size was determined	8
	7b	When applicable, explanation of any interim analyses and stopping guidelines	none
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	8
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	8
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	8
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	8-9

688

Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	8-9
	11b	If relevant, description of the similarity of interventions	7
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	10-11
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	13
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	7
	13b	For each group, losses and exclusions after randomisation, together with reasons	7
Recruitment	14a	Dates defining the periods of recruitment and follow-up	9
	14b	Why the trial ended or was stopped	8
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1.
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	7
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	11-14
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	-
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	13
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	15
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	18
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	15-19
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	15-19
Other information			
Registration	23	Registration number and name of trial registry	4
Protocol	24	Where the full trial protocol can be accessed, if available	Supplement
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	2

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