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

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

3

4 Short title: Faecal microbiota transplantation in IBS

5

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28

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30 made the data analysis; Arkkila, Satokari and Mattila planned the study; Arkkila and Satokari
31 performed the study and collected most of the data; Arkkila assessed all the colonoscopies, Arkkila
32 and Satokari assessed the FMT treatments, Hartikainen conducted the laboratory analysis, Mattila,
33 Hillilä, Koskenpato and Punkkinen recruited patients to the study; Anttila and Tillonen provided
34 expertise in study design and components of the article; all authors contributed to drafting the
35 article and revised the manuscript for important intellectual content. All authors had access to the
36 study data and have reviewed and approved the final manuscript.

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43 Summary:

44 Background

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

46 Aim

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

48 Methods

49 Forty-nine IBS-patients were randomized to receive autologous or allogenic FMT via colonoscopy.

50 The primary endpoint was a sustained, minimum of 50-point, reduction of the IBS Symptom

51 Severity Score. The secondary outcomes were levels of anxiety and depression, changes in quality

52 of life, gut microbiota and faecal water content as assessed with validated questionnaires,

53 intestinal microbiota composition and stool dry weight.

54 Results

55 The primary endpoint, a sustained reduction of IBS symptoms as compared to the baseline, was

56 not achieved in either group. However, there was a transient reduction in the mean IBS Symptom

57 Severity Score in the FMT group at 12 weeks after the treatment as compared to baseline ($P =$

58 0.01). The groups did not differ in the number of patients achieving clinical response at 12 weeks.

59 In the FMT-treated patients, microbial composition had changed to resemble that of the donor

60 and the stool water content decreased significantly as compared to the baseline. The depression

61 score decreased in patients with a reduction in IBS symptoms after FMT, but not in those placebo-

62 treated patients nonetheless experiencing a reduction in IBS symptoms.

63 Conclusions

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

67 ClinicalTrials.Org, Trial registration number: NCT03561519

68

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

70

71 1 INTRODUCTION

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

77

78 The aetiology of IBS is unknown although many theories have been proposed. Altered gut
79 motility, epithelial hyperpermeability, low grade inflammation, visceral hypersensitivity,
80 epigenetics and genetics, altered gut-brain interaction and psychological stressors have all been
81 reported in patients with IBS¹. In some IBS-patients, psychological factors play a role in the
82 severity and persistence of symptoms; these may also affect the decision to seek health care and
83 alter the response to the provided treatment ³. IBS has been particularly associated with anxiety
84 and depression, for example, IBS-patients have a three-fold risk of suffering from either
85 depression or anxiety in comparison to the general population ⁴.

86

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

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

101

102 FMT has been shown to be highly effective in treating recurrent *Clostridioides difficile* infections
103 (rCDI). Although comparative trials between different administration routes are lacking, in this
104 patient group, colonoscopy is often considered as the optimal route¹². The excellent treatment
105 efficacy in rCDI-patients and its favourable safety profile have encouraged investigators to seek
106 other avenues for FMT research ¹³. Data from rCDI-patients indicates that FMT may also relieve
107 functional GI-symptoms as our recent results revealed that rCDI-patients treated with FMT
108 experienced less severe GI-tract symptoms over the long-term than rCDI-patients treated with
109 antibiotics only ¹⁴. Furthermore, FMT for rCDI alters the microbiota of the recipient to resemble
110 that of the donor, a change lasting for at least 12 months ^{15,16}. Currently, the long-term
111 engraftment of the transplanted microbiota has not been as well documented in patient groups
112 other than rCDI.

113

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

administered via gastroscopy²¹. 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 rate²¹. 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 donor¹⁸, a concurrent decrease in the symptoms was observed only when FMT was administered via colonoscopy¹⁹ or gastroscopy²¹. 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.

126

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.

134

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

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

151

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

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

163

164 This study was approved by the ethical committee of Helsinki University Hospital (registration nro.
165 40/13/03/01/2015) and all participants signed an informed consent. All authors had access to the
166 study data and have reviewed and approved the final manuscript.

167

168 2.2 Sample size

169 The sample size was calculated based on the assumption of a 40 % placebo effect and a clinically
170 significant 40 % treatment effect over placebo. A two group χ^2 test with a 0.05 two-sided
171 significance level with 80 % power to detect this difference yielded a sample size of 26 in each
172 group. The decrease of 50 points in IBS-SSS score was considered as a significant and clinically
173 meaningful change in the severity of the symptoms. The confidence interval was selected to be 95
174 % ($\alpha=0.05$ and $\beta=0.1$).

175

176 2.3 Randomisation

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

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

184

185 2.4 Patients

186 Adult patients (18-73 years old), diagnosed by an experienced gastroenterologist to have IBS and
187 remaining symptomatic despite receiving conventional treatment, were recruited into the study.
188 After the enrolment, the patients completed the Rome III questionnaire. At this stage, there were
189 three patients not fulfilling the Rome III criteria for IBS due to the fluctuating nature of their
190 symptoms. The participating patients were recruited from occupational health care, Porvoo
191 hospital, Helsinki municipal hospital, and University Hospital of Helsinki from August 2015 to July
192 2017. The patients had received conventional treatments according to their predominant
193 symptoms but without obtaining satisfactory relief. Patients were excluded from the trial if they
194 were unable to provide written informed consent, had an organic gastrointestinal diagnosis such
195 as inflammatory bowel disease or if they were pregnant (Consort flow diagram in Supplementary
196 Figure 1).

197

198 2.5 The donor

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

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

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

210

211 2.6 Outcomes

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

216

217 2.7 Microbiota analysis

218 The microbial DNA was extracted with a previously described method²⁹ where the mechanical
219 lysis was followed up by high-throughput DNA extraction using KingFisher Flex 96 (Thermo Fisher
220 Scientific). The microbial composition was analysed by sequencing the V3-V4 region of the 16S
221 ribosomal gene using MiSeq sequencing. The resultant reads were pre-processed using the R-
222 package mare pipeline^{30,31}. After pre-processing, there were on average 47 029 (standard
223 deviation, SD=8 475) reads per sample. The reads were annotated to taxonomic assignments
224 using USEARCH (version 8.1.1756) and SILVA 16S rRNA reference database version 115³².

225

226 2.8 Statistical methods

227 Statistical analyses were performed using either the R software program (R core team, version
 228 3.5.2.) or the SPSS software program (IBM Statistics, version 26). Results are shown as mean
 229 (standard deviation, SD) unless otherwise stated. The statistical difference between the treatment
 230 groups was calculated using t-test or ANOVA with Benjamini-Hochbergs correction for multiple
 231 testing when the data was normally distributed; for nonparametric data, this was calculated using
 232 Wilcoxon signed rank test or Kruscal-Wallis tests. The difference between baseline and follow-up
 233 time points (Δ) was calculated for all questionnaire data. The correlation between recorded
 234 symptoms and microbial similarity between donor and recipient was estimated with Spearman
 235 correlation. Variations in the microbiota composition were assessed with Bray-Curtis
 236 dissimilarities and visualized using Principal co-ordinate analysis (PCoA).

237

238 3 RESULTS

239 3.1 Patient characteristics at baseline

240 A total of 49 IBS patients were included in the final analysis; 23 in the FMT group and 26 in the
 241 placebo (Supplementary Figure 1). Randomisation was conducted prior to the Rome III
 242 classification, therefore there were more diarrhoea predominant patients in the placebo group
 243 (Table 1). The majority of the 49 patients had diarrhoea predominant IBS (n=25), 21 patients had
 244 either mixed or unsubtyped IBS, three patients were in remission at baseline, and none of the
 245 patients had constipation predominant IBS. The severity of IBS symptoms as evaluated by IBS-SSS,
 246 was similar in both groups. On average, the symptom severity was moderate in both groups
 247 ranging from mild to severe. The depression scores (BDI) were similar between the groups and on
 248 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

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

272

273 We noted a significant correlation between the measured change in IBS symptoms (Δ IBS-SSS) and
 274 the change in depression (Δ BDI) and the change in the quality of life (Δ 15D) when examining both
 275 treatment groups together. This association was stronger in both cases in the FMT group. In the
 276 FMT group, the change in IBS-SSS had a significant correlation with the change in BDI ($\rho = 0.354$, P
 277 < 0.001 , Supplementary figure 3), whereas this was not statistically significant in the placebo
 278 group. The Δ IBS-SSS and Δ 15D displayed a strong and significant correlation in the treated group
 279 ($\rho = 0.55$, $P < 0.001$) and weaker, but still significant correlation in the placebo group ($\rho = 0.28$, $P =$
 280 0.002). There was no correlation between the changes in IBS symptoms (IBS-SSS) and anxiety
 281 (BAI).

282

283 3.4 Responders to the therapy

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

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

294

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

306

307 3.5 Microbiota results

308 To characterize FMT-induced microbiota changes, we collected stool samples before the FMT and
309 five times during the 52-week follow-up and performed a microbiota analysis by high-throughput
310 sequencing of the V3-V4 region of the bacterial 16S rRNA gene. First, we analysed changes in the
311 microbiota richness and the level of similarity with the donor's microbiota and finally assessed
312 changes in microbiota profiles within the study groups (Figure 4). At baseline, the microbial
313 richness in both groups was lower than that of the donor (Figure 4A). The microbial richness
314 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 time³³. 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.

337

338 3.7 Adverse events

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

346

347 4 DISCUSSION

348 In this placebo-controlled trial, we evaluated the effect of a single FMT treatment on the
349 symptoms of adult IBS patients. We defined the primary endpoint as a sustained 50-point
350 reduction in the IBS-SSS, which is considered to represent a significant reduction in IBS symptom
351 severity. In the FMT group, the mean of IBS-SSS was reduced significantly three months after the
352 FMT as compared to the baseline, whereas those in the placebo group experienced no marked
353 reduction of the mean IBS-SSS at any time point. Therefore, the results are in line with the first
354 placebo-controlled FMT study on IBS¹⁷, which showed that a single FMT treatment in colonoscopy
355 decreased IBS symptoms at three months after FMT, but not beyond. However, in our study, the
356 number of patients achieving a 50-point reduction of IBS-SSS was not significantly different
357 between the groups and the symptom scores between the two groups did not differ significantly
358 at any time point.

359

360 The primary end point of our study was ambitious i.e. a marked reduction in the IBS symptoms at
361 every time point after the FMT throughout the 52-week trial period. In contrast, the follow-up
362 times of drug trials in IBS have typically been shorter than year-long study¹⁰. The study of Johnsen
363 et al. had an equally long follow-up time of 52 weeks and they also observed only a transient relief
364 of symptoms at three months after FMT¹⁷. All in all, the result of our primary outcome was
365 negative as we did not observe any significant differences between the groups at any time point.

366

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

377

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

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

390

391 We demonstrated that a single FMT resulted in a 52-week long lasting change in the microbiota
392 such that it resembled the microbial composition of the donor with an increase in the microbial
393 richness. Thus, the transplanted microbiota seemed to engraft successfully in the patients. This
394 observation is in line with and extends the previous studies which showed successful engraftment
395 of donor's microbiota in IBS patients for up to six months with FMT capsules ¹⁸, for two months
396 with a single FMT administered via colonoscopy ¹⁹ and for three months with FMT administered
397 via gastroscopy²¹. Thus, FMT seems to change the microbiota composition in IBS subjects
398 relatively permanently, a phenomenon which has also been observed in FMT-treated rCDI patients
399 ¹⁵. In our study, a similar number of patients responded to the treatment in both groups, although
400 microbiota changes were observed only in the FMT group. High response-rates to placebo are
401 typical in IBS studies ³⁸, but also bowel cleansing may have impacted on the IBS symptoms.
402 Collectively, the FMT trials carried out on IBS patients thus far have shown promising results in
403 terms of the microbiota modulation, although the effects on symptoms have been inconsistent ¹⁷⁻
404 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

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

436

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

451 sensitivity in a mouse model ⁴⁶⁻⁴⁸. Furthermore, an optimal donor might result in a better
452 response rate also in IBS, as was previously demonstrated for ulcerative colitis in a study where
453 the desired treatment outcome was obtained in only one out of six donors ⁴¹. It may well be that
454 differences in the donor microbiota explain the superior efficacy of FMT in IBS, as demonstrated in
455 the study of El-Salhy et al.²¹ as compared to all previous studies¹⁷⁻²⁰, including ours.

456

457 Another possible explanation for the transient relief of IBS symptoms is that the functionality of
458 the transferred microbiota may not be well preserved in the new host, even though the
459 composition did seem to be maintained. The functionality could have been affected by dietary
460 factors, which in this trial were not controlled or recorded in detail and it is well known that IBS
461 patients often reduce their food intake¹ and in particular, they may try to avoid fibrous foods
462 which could have a significant impact on the metabolism and functionality of the microbiota. The
463 role of the microbiota in IBS remains to be clarified, and further FMT studies are warranted to
464 identify possible super-donors or to determine which characteristics of the microbiota could be
465 used to select an optimal donor for a specific indication.

466

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

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

477

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603

604

605 TABLES:

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

608

	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

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

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

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

	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 ($P = 8.0 \times 10^{-8}$).

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.

676

677 Statement of interests:

678 All authors have no conflict of interests to declare.

679

680

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

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