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Protocol for faecal microbiota transplantation in irritable bowel syndrome – the MISCEAT study: a randomised, double-blind cross-over study utilising mixed microbiota from healthy donors

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Manuscripts

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3 1 **Protocol for faecal microbiota transplantation in irritable bowel syndrome – the MISCEAT**
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5 2 **study: a randomised, double-blind cross-over study utilising mixed microbiota from**
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8 3 **healthy donors**
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46 20
47
48 21 **Word counts:** 3598
49

50
51 22 **Abbreviations:** IBS, Irritable Bowel Syndrome; IBS-D, diarrheal type of irritable bowel syndrome; IBS-
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53 23 M, mixed type of irritable bowel syndrome; IBS-C, constipated type of irritable bowel syndrome; IBS-
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55 24 SSS, Irritable Bowel Syndrome Severity Scale Score
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3 25 **Keywords:** irritable bowel syndrome, faecal microbiota transplantation, irritable bowel syndrome
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5 26 severity scale score, gut microbiome
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ABSTRACT

Introduction. Several studies have demonstrated dysbiosis in irritable bowel syndrome. Therefore, faecal microbiota transplantation, whose effect has been convincingly proven in *Clostridioides difficile* infections, may hold promise in other conditions, including irritable bowel syndrome. Our study will examine the effectiveness of stool transfer with artificially increased microbial diversity in the treatment of irritable bowel syndrome.

Methods and analysis. A three-group, double-blind, randomized, cross-over, placebo-controlled study of two pairs of gut microbiota transfer will be conducted in 99 patients with diarrhoeal or mixed type of irritable bowel syndrome. Patients (males and females aged 18-65) will be randomised into three equally sized groups: group A will first receive two enemas of study microbiota mixture (deep-frozen stored stool microbiota mixed from eight donors), after eight weeks, they will receive two enemas with placebo (autoclaved microbiota mixture), whereas group B will first receive placebo, then study microbiota mixture. Finally, group C will receive placebos only. The irritable bowel syndrome severity symptom score (IBS-SSS) questionnaire scores will be collected at baseline (week -1), and then at weeks 3,5,8,11,13 and 32. Faecal bacteriome will be profiled before and regularly after interventions using 16S rDNA next-generation sequencing. Biochemistry and haematology workup, anthropometry, bioimpedance, dietary questionnaire, and food records data will be obtained at study visits during the follow-up period. The primary outcome is the change in the IBS-SSS between the baseline and four weeks after the intervention for each patient compared to placebo. Secondary outcomes are IBS-SSS at two and 32 weeks compared to placebo; changes in the gut microbiome, urgent defecations frequency, Bristol stool scale, abdominal pain and bloating and anthropometric parameters.

Ethics and dissemination. The study was approved by the Ethics Committee of the Institute for Clinical and Experimental Medicine and Thomayer University Hospital, Czechia (G-18-26). The study

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3 81 results will be published in peer-reviewed journals and presented at international conferences and
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5 82 patient groups meetings.
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8 83 **Study registration number.** NCT04899869
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14 85 **STRENGTHS AND LIMITATIONS OF THIS STUDY**

- 16
17 86 ➤ Usage of mixed microbiota from multiple donors inflates the diversity of transferred microbiota
18
19 87 by enriching it for numerous rare species.
20
21 88 ➤ All interventions will be carried out using the same active mixed microbiota or the same placebo.
22
23 89 ➤ Each intervention consists of two consecutive transfers, which increases the probability that the
24
25 90 transferred microbiota engrafts.
26
27 91 ➤ Microbiome profiling, food records, anthropometry and bioimpedance data allow detailed
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29 92 monitoring of transfer effectiveness.
30
31 93 ➤ Mucosa-associated microbiota will not be assessed because the stool transfer will be performed
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33 94 by enema, not colonoscopy that would allow biopsies.
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95 INTRODUCTION

96 Irritable bowel syndrome (IBS) is characterised as recurrent abdominal pain on average at least one
97 day/week in the last three months, associated with two or more of the following criteria: 1) related
98 to defecation; 2) associated with a change in the frequency of stool; 3) associated with a change in
99 the form (appearance) of stool [1]. It is common among the adult European population (approx. 10%
100 [2]), but its aetiology is still unknown. It may, among other causes, include micro-inflammation,
101 disturbance of the brain-gut axis, inadequate secretion of bile acids, increased permeability of the
102 gut epithelial barrier, or gut dysbiosis. Dysbiosis in IBS has been suggested by several studies
103 (reviewed, e.g. in Rajilic-Stojanovic et al. [3]). There are indications that Firmicutes may be disturbed,
104 with *Dorea*, *Blautia* and *Roseburia* increased, whereas *Veillonella* and *Faecalibacterium* decreased.
105 Among Actinobacteria, a decrease in *Bifidobacterium* was noted, and among Proteobacteria,
106 *Enterobacteriaceae* were increased. Conflicting and heterogeneous results were reported for
107 Bacteroidetes. The major limitation of available studies is their cross-sectional character, which may
108 not be enough in a disease where diarrhoeal episodes alternate with normal stool composition or
109 constipation.

110
111 The faecal microbiota transplantation (FMT) has gained popularity by its remarkable effect in
112 recurrent *Clostridioides difficile* infections, where it has now become a recognised life-saving therapy
113 [4]. The first published randomized, double-blinded study on FMT in IBS used stool intervention from
114 an allogeneic donor or autologous stool. The intervention was centred on a well-defined group of IBS
115 of predominantly diarrhoeal form. The stool was transferred by colonoscopy to the cecum. The
116 primary outcome was an improvement in the *Irritable Bowel Syndrome - Severity Symptom Score*
117 (IBS-SSS). The treatment was associated with a significant effect at three months but not at 12
118 months post-intervention [5]. This study used single donors and did not assess stool microbiota.
119 Thus, the transferred microbiota likely varied between transfers both in their composition and in
120 their diversity.

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5 122 Our study protocol aims to test whether faecal microbiota transplantation of mixed microbiota from
6
7 123 several selected donors can alleviate symptoms of IBS measured by IBS-SSS at four weeks after the
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9 124 intervention, compared to autoclaved placebo. Secondary study aims are to test the acute (after two
10
11 125 weeks) and the long-term effect (after six months) on symptoms relief. We also focus on changes in
12
13 126 the gut microbiome composition, frequency of urgent defecations, Bristol stool scale, abdominal pain
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15 127 and bloating, body weight, fat content and anthropometric measurements (including waist, hip and
16
17 128 limbs circumferences and skinfold thickness.
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23 130 We hypothesise that the transfer of active microbiota of high diversity can lead to changes in the
24
25 131 patient's gut microbiome composition and/or function to alleviate IBS symptoms.
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132 **METHODS AND ANALYSIS**

133 **Study design**

134 This is a three-group, double-blind, placebo-controlled, randomized, cross-over study in adult
135 patients diagnosed with IBS (diarrhoeal or mixed form) according to Rome IV criteria. Each study
136 subject will undergo two pairs of FMT (a total of four enemas for each patient), with the pairs of
137 transfers being eight weeks apart. The active intervention substance is a mixed stool microbiota
138 derived from healthy individuals preselected for high alpha diversity of their microbiome and
139 distance in community ordination from IBS patients microbiota. Placebo is the same mixture,
140 inactivated by autoclaving.

141

142 The study subjects are randomly assigned to one of three groups: A) enema with active substance
143 first and with placebo second or B) enema with placebo first and active substance second or C)
144 enemas of placebo only (detailed scheme in **Figure 1**). Eligible participants will be followed-up for 32
145 weeks after the first intervention to monitor symptom severity scoring of IBS (IBS-SSS), with regular
146 profiling of their gut microbiome and other parameters (frequency of urgent defecations, Bristol
147 stool scale, abdominal pain and bloating, body weight, fat content, and other anthropometric
148 parameters).

149

150 The placebo group is planned because of the unknown onset and duration of the intervention effect:
151 if the beginning of an effect is delayed, or if it persists for a long time, simple cross-over design would
152 not have sufficient power due to the carry-over effect. In case the FMT was associated with
153 significant but not durable amelioration of the status, the control group would still increase the
154 statistical power.

155

156 This study protocol is reported as per the SPIRIT guidelines [6] (for the SPIRIT checklist, see **Appendix**
157 **1**).

158

159 **Study setting**

160 The participants are recruited at a single center, the Department of Internal Medicine, Thomayer
161 University Hospital in Prague, Czech Republic. This hospital has approximately 1,000 beds, including
162 80 in ICU's, serves approximately 50,000 patients per year. The center is experienced in treating
163 patients with IBS and other functional gastrointestinal disorders, with about 200 such patients
164 registered and further subjects coming for consultations from other workplaces to this tertiary
165 referral centre.

166

167 **Recruitment and eligibility criteria**

168 Stool donors

169 Stool donor candidates were recruited among blood donors at Thomayer University Hospital and
170 medical students in their first year of study (i.e. preclinical) from the 2nd Faculty of Medicine, Charles
171 University, Prague. We obtained stool samples from 58 such candidates fulfilling the inclusion criteria
172 (**Table 1**). Based on their high bacterial alpha-diversity and the position on the ordination plot of the
173 weighted Unifrac distance against 46 patients with IBS-D (**Figure 2**), 14 candidates proceeded to the
174 safety screening, whereby eight passed it (for reasons of candidate's exclusion, see **Figure 3**).

175

176 After 14 potential donors were selected based on the microbiota composition, they were screened
177 for infectious diseases and clinically examined as indicated by the *European consensus conference on*
178 *faecal microbiota transplantation in clinical practice guidelines* [7] (**Table 2**). All subjects were also
179 repeatedly tested for SARS-CoV-2 from both nasopharyngeal swab and stool. Six candidates were
180 excluded (for reasons, see **Figure 3**), whereas eight became regular stool donors. These eight donors
181 were regularly investigated as follows:

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3 182 - at every donation: by questionnaire for gastrointestinal symptoms, antibiotic usage, unprotected
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5 183 sex, travelling to exotic countries; clinical signs of COVID-19; the presence of SARS-CoV-2 in the
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7 184 donated stool;
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10 185 - every 4 weeks: for SARS-CoV-2 from nasopharyngeal swab;
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12 186 - every 8-12 weeks: for all other stool tests mentioned in **Table 2**.

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16 188 Prospective study participants

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18 189 Patients diagnosed with IBS-D (diarrheal type) or IBS-M (mixed diarrhoeal and constipation type) who
19
20 190 fulfil the inclusion and exclusion criteria listed in **Table 3** are recruited via regular' patient's check-ups
21
22 191 at the Gastroenterological unit at Thomayer University Hospital, by referrals from their general
23
24 192 practitioners, following our newspaper articles or word of mouth.
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194 **Study microbiota mixture for intervention**

195 The intervention microbiota is a mixture of regular stool donations from the eight regular donors.
196 The collection of stools for this purpose is already completed. The donors were advised to regularly
197 defecate at their home toilet into a clean plastic bag placed in Fecotainer (Excretas Medical, NL) with
198 an Anaerogen bag (Thermo Scientific, USA). This bag generated an anaerobic atmosphere during
199 transport to ensure anaerobes survival. The stool was transported to the laboratory with the
200 maximum allowable time until processing being 6 hours; the actual time was approximately 1.5
201 hours. The stool was weighed upon arrival, inspected for blood admixture, and immediately
202 processed by blending with a solution consisting of sterile 0.9% saline (160 ml per 100 g of stool),
203 sterile phosphate buffer saline at pH 7.4 (20 ml per 100 g of stool) and sterile 99.5% glycerol (20 ml
204 per 100 g stool, which is approximately 10% of solution's volume; therefore, it is unlikely to have
205 laxative properties upon administration). From our experience, ~ 105 ml of the mixture represents
206 ~40 g of stool. The mixture was then filtered through a sterile stainless steel mesh of 0.8 mm pore
207 size into a sterile plastic bottle and immediately frozen at -80°C. Whenever possible (blending or

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3 208 post-filtration), the procedure was performed under a nitrogen atmosphere to protect obligate
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5 209 anaerobes. All stool portions were mixed in a large stainless steel bucket using an electric mortar
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7 210 mixer under anaerobic conditions and low temperatures (on ice).
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12 212 The mixed microbiota substance was divided into aliquots of 13-14 g (which is ~ 35 ml). Two-thirds of
13
14 213 the tubes serve as a placebo: they were immediately autoclaved at 121°C for 30 minutes with slow
15
16 214 cooling. Pre-sterilised tubes were used to ensure that autoclaved placebos will not be visually
17
18 215 distinguishable from tubes with the active substance. Assignment of tubes to the autoclave,
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20 216 numbering, sealing, and labelling was done under the guidance of a statistical unit member (see
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22 217 below).
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28 219 All aliquot tubes are kept frozen at -80°C in the same type of plastic tubes, labelled by codes. Three
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30 220 such aliquots represent one dose for FMT (~40 g of stool, in ~105 ml). Aliquoting into multiple 50 ml
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32 221 tubes instead of one larger volume was decided because of the availability of durable plastic, which
33
34 222 must be both autoclavable and deep frost resistant.
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39 224 Before administering, the study microbiota mixture will be thawed in a warm (37°C) water bath, with
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41 225 intermittent mixing by inverting the tubes.
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44 45 227 **Randomization, allocation and blinding**

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48 228 At Visit 1, each patient is randomised into one of three equally sized groups (Figure 1) as described in
49
50 229 the *Study design*. Randomisation assignments are generated in advance in blocks of nine and stored
51
52 230 in a protected database. For each patient, anonymous codes for tubes containing either active study
53
54 231 microbiota mixture or placebo is received. Thus, the true assignment will remain concealed for the
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56 232 patients and the study staff until the end of the study observation period. The Investigator is
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58 233 encouraged to maintain the blind as far as possible. The actual allocation must not be disclosed to
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3 234 the patient and/or other study personnel, including other site personnel, monitors, corporate
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5 235 sponsors or project office staff; nor should there be any written or verbal disclosure of the code in
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7 236 any of the corresponding patient documents.
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11 238 **Study Intervention**

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14 239 Study substance is administered during Visit 2+3 and then again 7+8 as a retention colon enema and
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16 240 will be held optimally for at least 30 minutes. Bowel preparation is applied the day before the
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18 241 intervention (prior to Visit 2 and Visit 7) (natrii picosulfas 10 milligrams, magnesi oxidum leve 3,5
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20 242 grams, acidum citricum 12 grams). No preparation is performed before the second enema in the pair
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22 243 (visits 3 and 8).
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28 245 A rectal tube is inserted into the rectum, and the enema is applied. Application kit (Irrigator PN
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30 246 0462/E/93, EriLens, Czechia) is used. After the enema is applied, the patient position is changed to
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32 247 enable the study substance to be spread within the colon. The exact time of the enema completion is
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34 248 recorded as well as the enema retention time.
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38 39 250 **Outcomes**

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41 251 Primary outcome

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44 252 The primary outcome is the change in the IBS severity symptom score (IBS-SSS) in the active
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46 253 microbiota group relative to the placebo group. The change will be evaluated as the difference
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48 254 between the score at four weeks after the intervention (study weeks 5 or 13, respectively, see **Figure**
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50 255 **1**) and the baseline score (week -1 in group A or week 8 in group B).
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56 257 Secondary outcomes
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3 258 - The acute change in the IBS severity symptom score (IBS-SSS) between baseline and two weeks
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5 259 after intervention (study weeks 3 and 11, respectively, see **Figure 1**).
- 6
7 260 - The long-term change in the IBS severity symptom score (IBS-SSS) between baseline (week -1)
8
9 261 and week 32 (see **Figure 1**). The long term change will compare group C (placebo only) to
10
11 262 merged groups A+B (active study microbiota mixture).
- 12
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14 263 - Changes due to the intervention in (a) frequency of urgent defecations, (b) Bristol stool scale, (c)
15
16 264 abdominal pain and bloating, (d) body weight, fat content, and other anthropometric parameters
- 17
18 265 - The durability of changes (if any) in the microbial profiles by bacteriome profiling, parasite
19
20 266 screening, and virome sequencing
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22
23 267 - The psychological and well-being effects of the therapy scored by IBS-QoL questionnaires
- 24
25 268 - The long term effects of the therapy on stool frequency and consistency and on the gut
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27 269 microbiome and statistically significant changes in anthropometric measurements.
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31 32 271 **Data collection and follow-up**

33 34 272 Timing of assessments

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37 273 At visit 1 (the randomization), the patient is given detailed instructions and thoroughly instructed by
38
39 274 the study team. The patients are asked to keep the identical type of diet throughout the observation.
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41 275 They are asked to regularly (once a week) fill the study questionnaire. A study team member sends
42
43 276 that via the Survey Monkey smartphone application, an online survey development cloud-based
44
45 277 software. Relevant data are entered in a structured manner (frequency of defecation, Bristol stool
46
47 278 scale, pain measures, other symptoms, dietary records etc.). This member also frequently
48
49 279 communicate with study participants and answer any questions regarding the study to keep the
50
51 280 patient's adherence. An overview of the examinations at each visit and the timing of the study visits
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53 281 could be seen in **Table 4**.

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59 283 Irritable bowel syndrome severity scale score (IBS-SSS).
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3 284 The IBS-SSS is a five-question survey that reflects 1) the severity of abdominal pain, 2) frequency of
4
5 285 abdominal pain, 3) severity of abdominal distention, 4) satisfaction with bowel habits, and 5)
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7 286 interference with quality of life over the past ten days. Subjects respond to each question on a 100-
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9
10 287 point analogue scale ([8]); thus, the score can range from 0 to 500, with higher scores indicating
11
12 288 more severe symptoms.

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16 290 At eligibility screening, the patients are given instructions on how to fill the IBS-SSS questionnaires
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18 291 (via the Survey Monkey application). The questionnaires are filled in at eligibility screening and then
19
20 292 at week -1, 3, 5 (before the first intervention, at the presumed peak of its effect, and after further
21
22 293 two weeks), then at weeks 8, 11, 13 (similarly with the second intervention), and finally at week 32.

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27 295 Weight, height, bioimpedance
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30 296 Bodyweight, height and bioimpedance are examined during Visit 0, 1, 4, 5, 9 and 11. Medical Body
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32 297 Composition Analyzer Seca mBCA 515, (Seca, Germany) is used to measure changes in body
33
34 298 composition (8-point bioelectric impedance analysis at a frequency of 5 - 50 kHz with a current of
35
36 299 100 μ A), scanning performed with three pairs of hand electrodes and two pairs of leg electrodes,
37
38 300 measurements performed with light clothing and without metal objects (jewellery, keys). The weight
39
40 301 is determined in patients wearing underwear using the Seca mBCA 515. The height is determined by
41
42 302 a standardised technique with a metal stadiometer with an accuracy of 1 mm. Seca analytics 115
43
44 303 software is used to analyse the obtained data (Seca, Hamburg, Germany). The measurements is
45
46 304 performed according to the NIHR Southampton Biomedical Research Centre standard protocol (Seca
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48 305 mBCA, NIHR Southampton Biomedical Research Centre, 2014).

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54 307 Detailed anthropometry
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3 308 It is performed by nutritional therapists in Visit 1, 5, 10 and 11. It involves weight, abdominal (waist)
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5 309 circumference, buttocks (hip) circumference, thigh circumference, skinfolds (thigh, triceps,
6
7 310 subscapular, suprailiacal).

9 311

10 312 Serum workup, archiving serum+plasma

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12 313 Blood is sampled at Visits 0, 4, 9, 11 and will include: A) serum+plasma archiving, B) serum workup.

13
14 314 Laboratory panel testing will comprise sodium, potassium, chloride, urea, creatinine, glucose,

15
16 315 calcium, phosphate, total protein and albumin, AST, ALT, ALP, GMT, bilirubin, lipid panel, HS-CRP,

17
18 316 blood cell count with differential count, INR, urine analysis (sediment and biochemistry). One plasma

19
20 317 and one serum aliquot are made at these visits and frozen for forensic reasons.

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24 319 Psychological evaluation

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26 320 It is performed during Visit 0 and Visit 11 using a structured questionnaire evaluated by a qualified

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28 321 psychologist.

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32 323 Dietary questionnaire & advice, evaluation of food records

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34 324 It is performed by nutritional therapists at Visit 4 and 9 and includes: evaluation of food records will

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36 325 include: overall daily energy intake, proteins, carbohydrates and lipids calculations and dietary fibre.

37
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39
40 327 Gut microbiome composition

41
42 328 Faecal samples are collected at home by the subjects in the same way described for donors above

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44 329 and at time points indicated in the sections above. If not immediately brought to the visit, the stool is

45
46 330 frozen in a home freezer and then transported in a frozen tube container. DNA extraction is

47
48 331 performed using the PowerSoil kit (Qiagen), and the bacteriome is characterised by 16S rDNA

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50 332 amplicon profiling using the tagged primers according to Schloss protocol [9], and sequencing on a

51
52 333 MiSeq instrument with the 2x250 bases sequencing kit (both Illumina, USA).

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5 335 The first steps of bioinformatic analysis will be performed in the DADA2 package[10]. Statistical
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7 336 analyses and visualisation will be then performed in R with its Phyloseq package. Finally, the
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9 337 functional potential of the bacteriome will be assessed using the PICRUST software, which predicts
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11 338 functional capabilities based on the 16S rDNA profiles.
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16 340 The virome is assessed in a total of four stool samples per patient at Visit 0, 4, 9 and 11. The aim of
17
18 341 this analysis is to assess the repertoire of major bacteriophages. The virome analysis is based on
19
20 342 metagenomic sequencing of total DNA from a virus-enriched stool sample, according to the
21
22 343 previously published protocol [11].
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26
27 345 Finally, a simple PCR-based semi-quantitative parasite screening aims to identify several mostly
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29 346 benign unicellular parasites (e.g. *Blastocystis*, *Dientamoeba*, *Entamoeba*, *Endolimax*).
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34 348 **Safety monitoring**

36 349 The research team regularly monitors all data for any adverse events, and all potential adverse
37
38 350 events are recorded. Contacts to study coordinators active 24/7 are provided in case adverse effects
39
40 351 occurred. If any concerns are identified during donors or recipients' screening or clinical assessment,
41
42 352 further clinical evaluation and/or examination is immediately realised. All the concerns during the
43
44 353 study are assessed, and the recipient will be withdrawn if this is thought to be in his best interest. A
45
46 354 Data Monitoring and Safety Committee (DMSC) has been established and, based on the data from
47
48 355 the planned interim analysis, has the right to terminate the study if the frequency of severe adverse
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50 356 events crosses the 5% line (for a closer description of DMSC, its responsibilities and premature
51
52 357 termination of the study see **Appendix 2**).
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57 359 **Sample size and power calculation**

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3 360 The study is powered to detect an absolute improvement of 62.5 points in IBS-SSS score over 8
4
5 361 weeks (which is 25% of the expected mean baseline score 250) between the active microbiota
6
7 362 intervention compared to placebo. With a sample size of 33 per group, the probability of detecting
8
9 363 such an improvement is at least 0.9. This calculation assumes 20% dropout rates, variance in IBS-SSS
10
11 364 scores 100 (see the results in [12]), a correlation between the final and baseline IBS-SSS scores 0
12
13 365 (with a positive correlation, the power is higher), and no carry-over or temporal effect.
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17 366

18 367 **Data management**

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21 368 Data from IBS-SSS, frequency of urgent defecations, Bristol stool scale, abdominal pain and bloating
22
23 369 are collected and stored via the application Survey Monkey. All anthropometric data are entered and
24
25 370 stored in password-protected platforms integrated within the hospital information system. Only the
26
27 371 researchers involved in the study have access to the final study dataset (IBS-SSS, frequency of urgent
28
29 372 defecations, Bristol stool scale, abdominal pain and bloating), which will be shared in an anonymised
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31 373 form via the Zenodo repository. The only data in this manuscript are bacteriome data; their
32
33 374 anonymised form will be available on reasonable request.
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37 375

38 376 **Statistical analyses**

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40
41 377 The primary outcome analysis will be based on the difference in IBS-SSS scores over the second
42
43 378 treatment period (week 14 vs week 8) minus the change over the first treatment period (week 5 vs
44
45 379 week -1). This difference will be used as a response in a linear model, with intercept corresponding to
46
47 380 the temporal effect (seen in the placebo group C), an indicator of group A corresponding to the cross-
48
49 381 over effect (resulting from administration of placebo after active microbiota) and differences in
50
51 382 indicators for groups A and B modelling the effect of active microbiota. A robust sandwich estimator
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53 383 of the variance matrix will be used to adjust for potentially unequal variances between the groups.
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55 384 Analyses of secondary outcomes will proceed by similar methodology, comparing absolute or relative
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3 385 differences of the post-intervention measure of each outcome relative to its baseline value. The
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5 386 CONSORT 2010 guidelines will be followed in reporting the main trial results.
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10 388 **Study status**

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13 389 The study was registered at clinicaltrials.gov (NCT04899869) on May 25th 2021. The first patient was
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15 390 recruited on June 17th 2021, and the first intervention was applied on July 29th 2021. As of August
16
17 391 19th 2021, 12 patients have signed the informed consent, and six interventions have been applied. It
18
19 392 is expected that the study will be completed in December 2022.
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22 393
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24 394 **Patient and public involvement**

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26
27 395 Information on the study has been spread at conferences, in newspapers and by local
28
29 396 gastroenterologists contacted by researchers. Everyone interested got information material, which
30
31 397 allowed the potential subjects to read about the study and reach the researchers if they wanted to
32
33 398 participate. Participants were not involved in the development, recruitment of other participants or
34
35 399 conduct of the study. All recipients are asked about any possible adverse effects of treatment at
36
37 400 regular visits planned according to **Figure 1**; a thorough investigation will be conducted if any occurs.
38
39 401 After completing the data analysis, all recipients will receive information about their results and be
40
41 402 offered a roll-over (receiving active study microbiota mixture).
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47 404 **ETHICS AND DISSEMINATION**

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49
50 405 Ethics approval for this study was granted in June 2018 by the Ethics Committee of the Institute for
51
52 406 Clinical and Experimental Medicine and Thomayer Hospital (Václavská 800, 140 59 Prague 4, Czech
53
54 407 Republic). Involvement in this study is completely voluntary; donors and recipients are required to
55
56 408 provide written informed consent prior to participation in the study (see **Appendix 3 and 4**).
57
58 409 Recipients and their caregivers are informed of unexpected findings or unrecognised conditions and
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3 410 by possible future usage of their specimens in ancillary studies by trained physician or nurse; further
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5 411 medical care will be arranged. Study donors received financial compensation to pay for the required
6
7 412 travelling costs when donating the stool. The patient will be offered a roll-over into an observational
8
9 413 study with the administration of active microbiota. The patients are informed of this option at the
10
11
12 414 start of the study and regularly reminded.

13
14
15 415 We aim to publish findings in impact peer-reviewed international journals. Gastroenterologists,
16
17 416 internists and other care providers will be informed through the national conference meetings,
18
19 417 journals and patient groups meetings.

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25
26 419 **Protocol amendment number:** 01. Modification of the study protocol will be communicated with the
27
28 420 Ethics committee.

29
30
31 421 **Registration details** This study is registered with ClinicalTrials.gov (NCT04899869).

32
33
34 422 **Acknowledgement** We thank Peter Holger Johnsen, Linn Skjevling and Hege Hansen from University
35
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38 424 Norway Tromsø, Norway, for valuable advice regarding the study design and study microbiota
39
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41
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43
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45
46 428 and Motol University Hospital, Prague for their laboratory work in the regular microbiological
47
48 429 screening of the study donors.

49
50
51
52
53 430 **Contributors** OC, PK, JH, JV, MK contributed to the conception and design of the study. OC, PK, JH
54
55 431 and JV drafted the protocol with input from all other authors. JV and PK contributed to the patients
56
57 432 recruitment. JH, LV, LK and OC contributed to the microbiome analysis for donor selection. JH, OC
58
59 433 and JV contributed to the donor screening. LV, JH and OC contributed to the study microbiota
60

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3 434 mixture preparation. MK contributed to the power size calculations and statistical analysis. VL
4
5 435 contributed to the randomization. JH and JV contributed equally to this paper, OC and PK contributed
6
7 436 equally either.
8
9

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11
12 438 19-01-00127 . Funding received from this grant support direct research cost. All rights reserved. The
13
14 439 grant agency is responsible for auditing the trial.
15
16

17
18 440 **Competing interests** None declared. No money from commercial sponsors was used.
19
20

21 441 **Patient consent for publication** Not required.
22
23

24 442 **Ethics approval** Ethics approval for this study was granted in June 2018 by the Ethics Committee of
25
26 443 the Institut for Clinical and Experimental Medicine and Thomayer Hospital (Víteňská 800, 140 59
27
28 444 Prague 4, Czech Republic).
29
30

31 445 **Provenance and peer review** Not commissioned; externally peer-reviewed.
32
33

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37
38 448 build upon this work non-commercially, and license their derivative works on different terms,
39
40 449 provided the original work is properly cited, appropriate credit is given, any changes made indicated,
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42 450 and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.
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3 451 **FIGURES AND ILLUSTRATIONS**

4 452 **Figure 1** Per protocol intervention scheme: the visits, questionnaires and samples
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For peer review only

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3 **453** **Figure 2** Ordination plot on the weighted Unifrac distance at the genus level for selection of the
4 **454** donor candidates based on their gut microbiome alpha- and beta-diversity
5
6 **455**

7 **456** These are the results of a comparative microbiome case-control study that helped us to preselect 14 donor candidates.
8 **457** Alpha diversity calculation was based on Chao 1 index. The beta-diversity calculation was based on non-metric dimensional
9 **458** scaling (NMDS) with a weighted UniFrac distance matrix for bacterial Genus. NMDS axis 1 captured 46.8% of variability;
10 **459** NMDS axis 2 represents 14.7% of the variability. Healthy subjects were enriched in negative values of the first ordination
11 **460** axis; therefore, we selected donors among healthy subjects in this half of the graph and based on their microbiome's alpha
12 **461** diversity. The reason for concentrating healthy and enriched subjects in the left part of the plot could be their younger age.
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For peer review only

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3 463 **Figure 3** Process of donor selection and reasons for their excluding
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For peer review only

464 **Table 1.** Inclusion and exclusion criteria for FMT donors

Inclusion	Adults aged 18-65 years
	BMI 18,5-27 kg/m ²
	Lack of restrictive diets (diet discussed with experienced gastroenterologist)
	Bristol stool scale usually between 3 and 4
	High alpha diversity and significant difference in beta-diversity from patients (using 16S rDNA sequencing)
	Expected to donate regularly
	Consented in writing
Exclusion	Any chronic GI disease in patient's history (coeliac disease, inflammatory bowel disease, irritable bowel syndrome, colorectal carcinoma), or active acute GI issues (infectious gastroenteritis or enterocolitis, frequent bloating, diarrhoea or vomiting)
	Chronic disease in ' 'patient's history (cancer, autoimmune conditions, type 2 diabetes mellitus, coronary heart disease, hypertension, hypercholesterolemia, gout)
	<i>Clostridiodes difficile</i> infection in patient's history
	Colorectal carcinoma in family history
	Any restrictive diet habits (raw-vegans, fruitarians, keto or carnivore)
	Any systemic antibiotics in the last 6 months
	Using proton-pump inhibitors in the last 6 months
	Regular unprotected sex with unknown persons

465

466 **Table 2** Laboratory screening of the FMT donors

Blood testing	
Hepatitis A, hepatitis B, hepatitis C and hepatitis E viruses (serology)	
HIV-1 and HIV-2 (p24 antigen)	
Treponema pallidum (serology)	
Strongyloides stercoralis (serology)	
Complete blood cell count with differential	
Creatinine, aminotransferases, bilirubin	
Stool testing	
<i>Clostridioides difficile</i> (cultures, antigen testing)	
Common enteric pathogens, including Salmonella, Shigella, Campylobacter, shiga toxin-producing <i>Escherichia coli</i> , Yersinia and <i>Vibrio cholerae</i> (cultures)	
Antibiotic-resistant bacteria (ARB), including vancomycin-resistant Enterococci, methicillin-resistant <i>Staphylococcus aureus</i>	
Gram-negative ARB including extended-spectrum β -lactamase-producing <i>Enterobacteriaceae</i> , and carbapenem-resistant <i>Enterobacteriaceae</i> /carbapenemase-producing <i>Enterobacteriaceae</i> (cultures)	
Norovirus, rotavirus, adenovirus, sapovirus (PCR)	
SARS-CoV-2 (reverse transcription -PCR)	
Common intestinal parasites, including <i>Giardia intestinalis</i> , <i>Cryptosporidium parvum et hominis</i> (cultures and PCR), <i>Blastocystis hominis</i> *, <i>Dientamoeba fragilis</i> * (both PCR only)	

467

468 *) Based on the literature [13], we decided to test both parasites but do not exclude the donors if they tested
 469 positive and having no gastrointestinal symptoms. *Blastocystis* is believed to be commensal of the gut.
 470 *Dientamoeba's* status is not exact; however, based on our experiment, it does not survive freezing at -80 °C and
 471 thawing to 5°C when mixing the study microbiota mixture. Therefore it can't do any harm.

472

473 The screening strategy is based on [8].

474 **Table 3.** Inclusion and exclusion criteria for recipients of FMT

Inclusion	Adults 18-65 years
	Diagnosed with IBS-D or IBS-M according to the Rome IV criteria
	Expected adherence to following the protocol
	Written consent to the study
Exclusion	The use of antibiotics and probiotics within one month prior to faecal microbiota transplantation
	History of inflammatory bowel disease or gastrointestinal malignancy, systemic autoimmune diseases (ongoing or in history)
	Previous abdominal surgery (other than appendectomy or cholecystectomy or hernioplasty or cesarean section)
	HIV infection or other active infection
	Renal or hepatic disease (both defined by biochemistry workup)
	Diabetes mellitus, abnormal thyroid functions not controlled by thyroid medications
	Bipolar disorder or schizophrenia (ongoing or history thereof), moderately severe depression defined by Patient Health Questionnaire-9 (PHQ-9) score > 15
	Anxiety defined by a Generalised Anxiety Disorder 7 (GAD7) score > 10, with any organic causes that can explain the symptoms of IBS
	Current pregnancy and lactation

475

476 **Table 4.** The study visits with planned activities

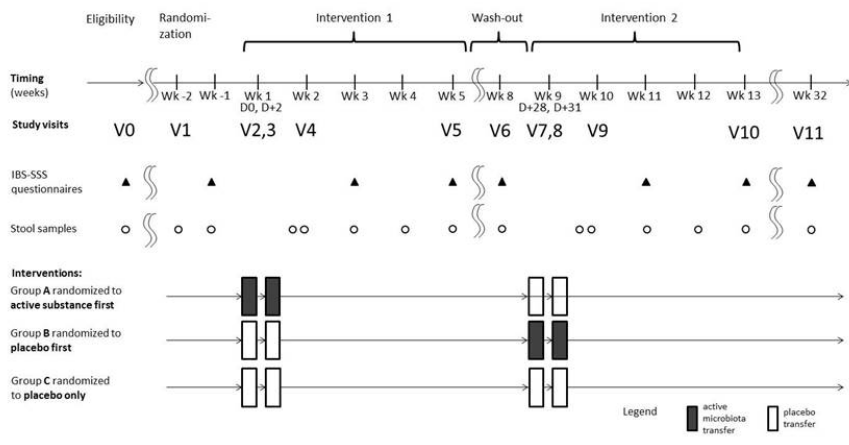
Visit	0	1	X	2+3	4	X	5	6	7+8	9	X	10	11
Study Week	?	-2	-1	1	2	3	5	8	9	10	11	13	32
Eligibility evaluation (E) / Randomization (R) / Wrap-up visit (W) ⁽¹⁾	E	R											W
Colon enema with the study substance (active microbiota or placebo)				XX					XX				
Irritable bowel syndrome severity scale score		X	X			X	X	X			X	X	X
Weight, height, bioimpedance		X			X		X			X		X	X
Detailed anthropometry		X					X					X	X
Serum workup, archiving serum+plasma		X			X					X			X
Psychological evaluation		X											X
Dietary questionnaire & advice, evaluation of food records ⁽²⁾					X					X			
Stool samples for bacteriome profiling using 16S rDNA sequencing	X	X	X		X	X	X	X		X	X	X	X

477 (1) Here, the patient is offered a roll-over into an observational study with active microbiota administration. The patients
478 will be informed of this option at the start of the study and regularly reminded.

479 (2) For IBS-SSS questionnaires assessing the primary outcome, please see the intervention scheme in Figure 2. Their
480 administering is not linked to study visits.

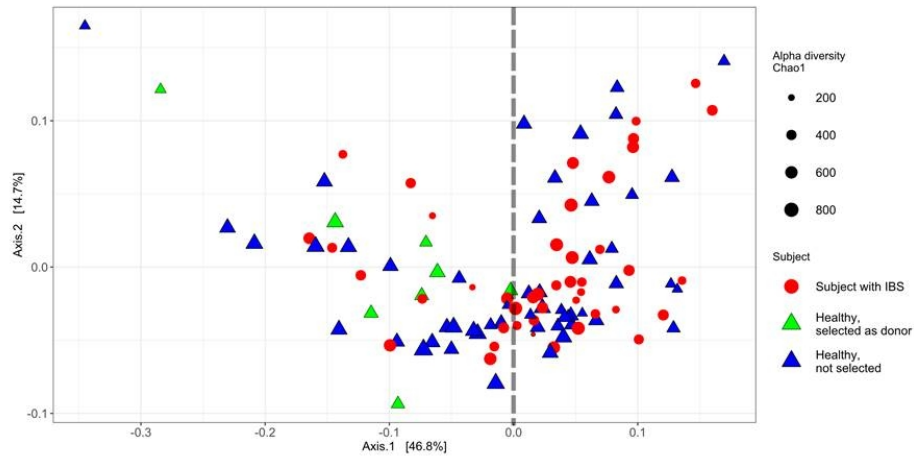
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Per protocol intervention scheme: the visits, questionnaires and samples

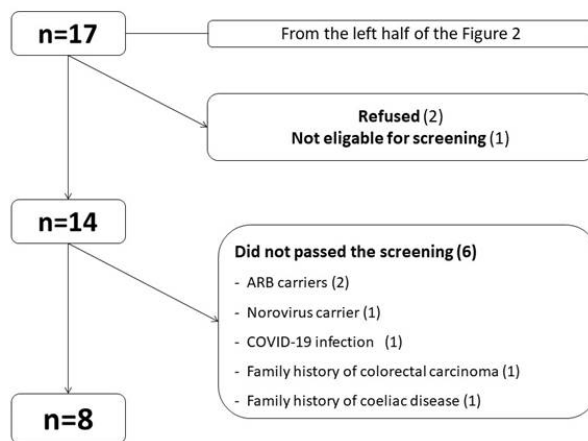
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Ordination plot on the weighted UniFrac distance at the genus level for selection of the donor candidates based on their gut microbiome alpha- and beta-diversity. These are the results of a comparative microbiome case-control study that helped us to preselect 14 donor candidates. Alpha diversity calculation was based on Chao 1 index. The beta-diversity calculation was based on non-metric dimensional scaling (NMDS) with a weighted UniFrac distance matrix for bacterial Genus. NMDS axis 1 captured 46.8% of variability; NMDS axis 2 represents 14.7% of the variability. Healthy subjects were enriched in negative values of the first ordination axis; therefore, we selected donors among healthy subjects in this half of the graph and based on their microbiome's alpha diversity. The reason for concentrating healthy and enriched subjects in the left part of the plot could be their younger age.

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Process of donor selection and reasons for their excluding

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Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Appendix 1 SPIRIT CHECKLIST

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	6 and 19
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	NA – not received yet.
Protocol version	#3	Date and version identifier	19
Funding	#4	Sources and types of financial, material, and other support	20
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	20
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	20
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	20

1	Roles and	#5d	Composition, roles, and responsibilities of the	20
2	responsibilities:		coordinating centre, steering committee, endpoint	
3	committees		adjudication committee, data management team, and	
4			other individuals or groups overseeing the trial, if	
5			applicable (see Item 21a for data monitoring committee)	
6				
7				
8				
9	Introduction			
10				
11	Background and	#6a	Description of research question and justification for	7
12	rationale		undertaking the trial, including summary of relevant	
13			studies (published and unpublished) examining benefits	
14			and harms for each intervention	
15				
16				
17				
18	Background and	#6b	Explanation for choice of comparators	8
19	rationale: choice of			
20	comparators			
21				
22				
23	Objectives	#7	Specific objectives or hypotheses	8
24				
25				
26	Trial design	#8	Description of trial design including type of trial (eg,	9
27			parallel group, crossover, factorial, single group),	
28			allocation ratio, and framework (eg, superiority,	
29			equivalence, non-inferiority, exploratory)	
30				
31				
32				
33	Methods:			
34	Participants,			
35	interventions, and			
36	outcomes			
37				
38				
39	Study setting	#9	Description of study settings (eg, community clinic,	10
40			academic hospital) and list of countries where data will	
41			be collected. Reference to where list of study sites can	
42			be obtained	
43				
44				
45				
46	Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If	10
47			applicable, eligibility criteria for study centres and	
48			individuals who will perform the interventions (eg,	
49			surgeons, psychotherapists)	
50				
51				
52				
53	Interventions:	#11a	Interventions for each group with sufficient detail to allow	13
54	description		replication, including how and when they will be	
55			administered	
56				
57				
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59	Interventions:	#11b	Criteria for discontinuing or modifying allocated	13
60				

1	modifications		interventions for a given trial participant (eg, drug dose	
2			change in response to harms, participant request, or	
3			improving / worsening disease)	
4				
5	Interventions:	#11c	Strategies to improve adherence to intervention	14
6	adherence		protocols, and any procedures for monitoring adherence	
7			(eg, drug tablet return; laboratory tests)	
8				
9				
10	Interventions:	#11d	Relevant concomitant care and interventions that are	14
11	concomitant care		permitted or prohibited during the trial	
12				
13				
14	Outcomes	#12	Primary, secondary, and other outcomes, including the	13
15			specific measurement variable (eg, systolic blood	
16			pressure), analysis metric (eg, change from baseline,	
17			final value, time to event), method of aggregation (eg,	
18			median, proportion), and time point for each outcome.	
19			Explanation of the clinical relevance of chosen efficacy	
20			and harm outcomes is strongly recommended	
21				
22				
23				
24				
25				
26	Participant timeline	#13	Time schedule of enrolment, interventions (including any	See Figure
27			run-ins and washouts), assessments, and visits for	1
28			participants. A schematic diagram is highly	
29			recommended (see Figure)	
30				
31				
32				
33	Sample size	#14	Estimated number of participants needed to achieve	17
34			study objectives and how it was determined, including	
35			clinical and statistical assumptions supporting any	
36			sample size calculations	
37				
38				
39				
40	Recruitment	#15	Strategies for achieving adequate participant enrolment	11
41			to reach target sample size	
42				
43	Methods:			
44	Assignment of			
45	interventions (for			
46	controlled trials)			
47				
48				
49				
50	Allocation: sequence	#16a	Method of generating the allocation sequence (eg,	12
51	generation		computer-generated random numbers), and list of any	
52			factors for stratification. To reduce predictability of a	
53			random sequence, details of any planned restriction (eg,	
54			blocking) should be provided in a separate document	
55			that is unavailable to those who enrol participants or	
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assign interventions

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3	Allocation	#16b	Mechanism of implementing the allocation sequence
4	concealment		(eg, central telephone; sequentially numbered, opaque,
5	mechanism		sealed envelopes), describing any steps to conceal the
6			sequence until interventions are assigned
7			
8			
9	Allocation:	#16c	Who will generate the allocation sequence, who will
10	implementation		enrol participants, and who will assign participants to
11			interventions
12			
13			
14	Blinding (masking)	#17a	Who will be blinded after assignment to interventions
15			(eg, trial participants, care providers, outcome
16			assessors, data analysts), and how
17			
18			
19			
20	Blinding (masking):	#17b	If blinded, circumstances under which unblinding is
21	emergency unblinding		permissible, and procedure for revealing a participant's
22			allocated intervention during the trial
23			
24			
25	Methods: Data		
26	collection,		
27	management, and		
28	analysis		
29			
30			
31			
32	Data collection plan	#18a	Plans for assessment and collection of outcome,
33			baseline, and other trial data, including any related
34			processes to promote data quality (eg, duplicate
35			measurements, training of assessors) and a description
36			of study instruments (eg, questionnaires, laboratory
37			tests) along with their reliability and validity, if known.
38			Reference to where data collection forms can be found,
39			if not in the protocol
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45	Data collection plan:	#18b	Plans to promote participant retention and complete
46	retention		follow-up, including list of any outcome data to be
47			collected for participants who discontinue or deviate
48			from intervention protocols
49			
50			
51			
52	Data management	#19	Plans for data entry, coding, security, and storage,
53			including any related processes to promote data quality
54			(eg, double data entry; range checks for data values).
55			Reference to where details of data management
56			procedures can be found, if not in the protocol
57			
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1	Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary	18
2			outcomes. Reference to where other details of the	
3			statistical analysis plan can be found, if not in the	
4			protocol	
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8	Statistics: additional	#20b	Methods for any additional analyses (eg, subgroup and	18
9	analyses		adjusted analyses)	
10				
11	Statistics: analysis	#20c	Definition of analysis population relating to protocol non-	18
12	population and		adherence (eg, as randomised analysis), and any	
13	missing data		statistical methods to handle missing data (eg, multiple	
14			imputation)	
15				
16				
17				
18	Methods: Monitoring			
19				
20				
21	Data monitoring:	#21a	Composition of data monitoring committee (DMC);	Appendix
22	formal committee		summary of its role and reporting structure; statement of	1
23			whether it is independent from the sponsor and	
24			competing interests; and reference to where further	
25			details about its charter can be found, if not in the	
26			protocol. Alternatively, an explanation of why a DMC is	
27			not needed	
28				
29				
30				
31				
32	Data monitoring:	#21b	Description of any interim analyses and stopping	Appendix
33	interim analysis		guidelines, including who will have Access to these	1
34			interim results and make the final decision to terminate	
35			the trial	
36				
37				
38				
39	Harms	#22	Plans for collecting, assessing, reporting, and managing	17
40			solicited and spontaneously reported adverse events	
41			and other unintended effects of trial interventions or trial	
42			conduct	
43				
44				
45				
46	Auditing	#23	Frequency and procedures for auditing trial conduct, if	20
47			any, and whether the process will be independent from	
48			investigators and the sponsor	
49				
50				
51	Ethics and			
52	dissemination			
53				
54				
55	Research ethics	#24	Plans for seeking research ethics committee /	19
56	approval		institutional review board (REC / IRB) approval	
57				
58				
59	Protocol amendments	#25	Plans for communicating important protocol	20
60				

modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)

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7	Consent or assent	#26a	Who will obtain informed consent or assent from 19
8			potential trial participants or authorised surrogates, and
9			how (see Item 32)
10			
11			
12	Consent or assent:	#26b	Additional consent provisions for collection and use of 19
13	ancillary studies		participant data and biological specimens in ancillary
14			studies, if applicable
15			
16			
17	Confidentiality	#27	How personal information about potential and enrolled 19
18			participants will be collected, shared, and maintained in
19			order to protect confidentiality before, during, and after
20			the trial
21			
22			
23			
24	Declaration of	#28	Financial and other competing interests for principal 20
25	interests		investigators for the overall trial and each study site
26			
27			
28	Data access	#29	Statement of who will have Access to the final trial 18
29			dataset, and disclosure of contractual agreements that
30			limit such Access for investigators
31			
32			
33	Ancillary and post trial	#30	Provisions, if any, for ancillary and post-trial care, and 19
34	care		for compensation to those who suffer harm from trial
35			participation
36			
37			
38			
39	Dissemination policy:	#31a	Plans for investigators and sponsor to communicate trial 19
40	trial results		results to participants, healthcare professionals, the
41			public, and other relevant groups (eg, via publication,
42			reporting in results databases, or other data sharing
43			arrangements), including any publication restrictions
44			
45			
46			
47	Dissemination policy:	#31b	Authorship eligibility guidelines and any intended use of 20
48	authorship		professional writers
49			
50			
51	Dissemination policy:	#31c	Plans, if any, for granting public Access to the full 20
52	reproducible research		protocol, participant-level dataset, and statistical code
53			
54			
55	Appendices		
56			
57	Informed consent	#32	Model consent form and other related documentation Appendix
58	materials		given to participants and authorised surrogates 2
59			
60			

1 Biological specimens [#33](#) Plans for collection, laboratory evaluation, and storage 15-17
2 of biological specimens for genetic or molecular analysis
3 in the current trial and for future use in ancillary studies,
4 if applicable
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10 None The SPIRIT Explanation and Elaboration paper is distributed under the terms of the Creative
11 Commons Attribution License CC-BY-NC. This checklist can be completed online using
12 <https://www.goodreports.org/>, a tool made by the [EQUATOR Network](#) in collaboration with
13 [Penelope.ai](#)
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APPENDIX 2

Charter and responsibilities of the Data Monitoring and Safety Committee

A Data Monitoring and Safety Committee (DMSC) has been established, and its lead by Clinical Study Center at Thomayer University Hospital, Prague. The DMSC is an independent organ from the study investigators. During the period of recruitment to the study, interim analyses will be supplied, in strict confidence, to the DMSC. In the light of these interim analyses, the DMSC will advise the study steering committee (SSC) if, in its view, the active intervention has been proven, beyond reasonable doubt, to be different from the placebo in some or all patients

Based on the reports of DMSC, the Study steering committee (SSC) can then decide whether or not to modify recruitment to the study and its oncoming course. Unless this happens, however, the SSC, will remain ignorant of the interim results.

The frequency of interim analyses will depend on the judgement of the Chair of the DMSC, in consultation with the SSC. However, we anticipate that there might be two to three interim analyses and one final analysis.

The Chair of DSMC is Mr. Jiri Skopek, M.D., Ph.D. who is available on request at jiri.skopek1@ftn.cz

Premature termination of the study

An interim analysis is performed when 50% of patients have already got to Visit 5 (where primary outcome is evaluated.) The interim analysis is performed by a member of the study's statistical unit who is blinded for the allocation of the active study mixture. The statistician will report to the DMSC. The DMSC will have unblinded Access to all data and discuss the interim-analysis results with the SSC. The SSC decides on continuation or termination of the study and will report to the central Ethics committee. The study will be ended if the frequency of severe adverse events crosses the 5% line. Severe adverse event is defined as that one requiring hospitalisation.

Appendix 3: Informed consent for FMT donors



Informovaný souhlas dospělé osoby s účastí na výzkumu změn střevního mikrobiomu u dospělých pacientů se syndromem dráždivého tračníku ve vědeckém projektu týmů Thomayerovy nemocnice a Fakultní nemocnice v Motole.

Vážená paní/vážený pane,

syndrom dráždivého tračníku (irritable bowel syndrome, dále jen IBS) je nejčastější funkční onemocnění trávicího traktu, které pacienta výrazně omezuje v jeho každodenním životě. Může se projevat různě, nejčastěji však jako delší dobu trvající bolest břicha s náhle vzniklým nutkáním na stolici. Léčba této nemoci je zdlouhavá, obtížná a ne vždy úspěšná. Dle recentních studií se však jako účinná léčebná metoda jeví transplantace střevní mikroflóry (faecal microbiota transplantation, dále jen FMT). A právě na její využití se zaměřuje náš projekt v podobě klinické intervenční studie.

Cílem projektu je zjistit, zda je transplantace stolice účinnou léčebnou metodu IBS a jak se po FMT mění složení střevní mikroflóry. K tomu abychom FMT mohli provést je potřeba mít vhodné dárce stolice. A právě zde byste nám mohli pomoci. Znalosti změn složení střevní mikroflóry po FMT bychom pak v budoucnu mohli využít buď k cílené ATB terapii negativně asociovaných bakterií nebo naopak k podávání probiotika prospěšných kmenů.

Proto si Vás dovoluujeme pozvat k účasti na projektu vědeckých týmů Thomayerovy nemocnice a Fakultní nemocnice v Motole. Přečtěte si, prosím, toto poučení. Pokud plně nerozumíte tomuto textu nebo pokud potřebujete doplňující informace, neváhejte se zeptat lékaře na emailu uvedeném níže. **Pokud souhlasíte s Vaší účastí ve studii, vyplňte prosím kontaktní údaje níže dokumentu a podepište prosím prohlášení, které se nachází v závěru tohoto informovaného souhlasu. Vaše účast je dobrovolná.** Tento souhlas můžete kdykoli zrušit, a to i bez udání důvodu.

Získání vzorku stolice by probíhalo ve vašem domácím prostředí. Stolice by bylo potřeba uchovat v běžném domácím mrazáku (teplota -20°C), k odběru byste byli vybaveni jednoduchými odběrovými sety s návodem a poučení o jejich používání. Po domluvě se členy vědeckého týmu (kontakt níže) by vzorky byly převezeny na naše pracoviště a hluboce zamrazeny (-80°C).

Celý proces je dvoufázový. Z prvního vzorku se provede molekulárně-genetická analýza a následně bioinformatické zpracování dat. Na základě výsledků bude vybráno asi 10-20 dárců, které kontaktujeme na základě informací uvedených níže. Splní-li kritéria vhodného dárce (pro vyžádání lze napsat na mail jiri.vejmelka@ftn.cz nebo zavolat na tel.č. 731446619), budou poté znovu požádáni o darování stolice.

Po zpracování pro účely aktuální studie budou vzorky uchovány v hlubokomrazícím boxu v laboratořích Fakultní nemocnice v Motole. Jejich další využití proběhne pouze po přesné specifikaci formou dalšího souhlasu a Vaším podepsáním nového souhlasu.

V tomto projektu řádně dbáme o bezpečnost osobních údajů podle platných zákonů. Zejména je pak zcela zachovaná úplná anonymita pacienta při odesílání vzorků mimo naše pracoviště nebo při

Appendix 3: Informed consent for FMT donors

zveřejňování vědeckých výsledků získaných z naší práce v odborných časopisech. Odebrané vzorky a z nich získané části jsou v našich laboratořích skladovány na dobu neurčitou, odděleně od osobních dat. Pokud byste v budoucnu svůj souhlas odvolali, Vaše jméno a ostatní osobní data budou bez prodlení vymazána z našich databází i papírových záznamů tak, aby se už nikdo nemohl dozvědět, komu vzorek patřil.

Bližší informace o nemoci jako takové můžete získat od členů vědeckého týmu:

MUDr. Jiří Vejmelka (Thomayerova nemocnice), tel: 731446619, email: jiri.vejmelka@ftn.cz

MUDr. Jakub Hurych (Fakultní nemocnice v Motole), tel. 224432089, email: jakub.hurych@lfmotol.cuni.cz

Souhlas se zpracováním osobních údajů (dále jen „Souhlas“)

udělený ve smyslu zákona č. 101/2000 Sb., o ochraně osobních údajů a o změně některých zákonů, ve znění pozdějších předpisů a s Nařízením Evropského parlamentu a Rady (EU) 2016/679

Já, níže podepsaný

Jméno a příjmení:

Datum narození:

Rodné číslo:

Kontaktní email:

Telefonní číslo:

Souhlasím se zpracováním svých osobních údajů/ osobních údajů osoby jejíž jsem zákonným zástupcem Fakultní nemocnicí v Motole a Thomayerově nemocnici v rozsahu těchto údajů:

Jméno, příjmení, titul, datum a místo narození, rodné číslo, národnost, pohlaví, místo trvalého pobytu, telefon, email, výška, hmotnost

Tento projev vůle je platný pouze v případě, že mé osobní údaje budou zpracovávány pouze v rozsahu nezbytném pro dosažení účelu zpracování uvedeného v tomto souhlasném prohlášení a v souladu s příslušnou legislativou v platném znění.

Souhlas je poskytnut za účelem:

Zpracování vzorku stolice pro vědecko-výzkumnou činnost mající za cíl přispět k porozumění změn střevního mikrobiomu u dospělých pacientů se syndromem dráždivého tračníku

Souhlasím se zpracováním svých osobních údajů Fakultní nemocnicí v Motole a Thomayerově nemocnici po dobu:

Do odebrání mého souhlasu

Souhlasím se zpřístupněním svých osobních údajů Fakultní nemocnici v Motole a Thomayerově nemocnici:

Fakultní nemocnice v Motole a Thomayerova nemocnice je oprávněna použít mé osobní údaje pouze v souladu s výše uvedeným účelem a po výše uvedenou dobu, nebo pro legitimní potřebu státních kontrolních

Appendix 3: Informed consent for FMT donors

orgánů a orgánů činných v trestním řízení.

Fakultní nemocnice v Motole a Thomayerova nemocnice je dále oprávněna poskytnout mé osobní údaje pouze subjektům spolupracujícím s **Fakultní nemocnicí v Motole a Thomayerovou nemocnicí** na dosažení primárního účelu, pro který je udělen tento souhlas. S takovými subjekty se **Fakultní nemocnice v Motole a Thomayerova nemocnice** zavazuje uzavřít smlouvu obsahující stejné podmínky pro zpracování mých osobních údajů. Zpracování bude probíhat v souladu s příslušnými právními normami o ochraně osobních údajů a s Nařízením Evropského parlamentu a Rady (EU) 2016/679 ze dne 27. dubna 2016 o ochraně fyzických osob v souvislosti se zpracováním osobních údajů a o volném pohybu těchto údajů a o zrušení směrnice 95/46/ES (obecné nařízení o ochraně osobních údajů).

Byl/a jsem poučen/a o tom, že poskytnutí údajů je dobrovolné.

Dále jsem byl/a v souladu s příslušnou legislativou poučen/a:

- O svém právu tento souhlas odvolat, a to i bez udání důvodu,
- O svém právu přístupu k těmto údajům a právu na jejich opravu,
- O svém právu na vymazání těchto údajů, pokud dochází k jejich zpracování v rozporu s ochranou definovanou příslušnou legislativou nebo v rozporu s tímto souhlasem, nebo byl souhlas odvolán, svém právu podat stížnost u Úřadu pro ochranu osobních údajů.

Byl/a jsem také poučen/a o tom, že tato svá práva mohu uplatnit doručením žádosti na adresu: Fakultní nemocnice v Motole, Samostatné oddělení pověřence pro ochranu osobních údajů, V Úvalu 84, Praha 5.

Beru na vědomí, že odvolání tohoto souhlasu může ovlivnit dosažení účelu, pro který byl tento souhlas vydán, pokud tohoto účelu nelze dosáhnout jinak.

Prohlašuji, že jsem textu poučení porozuměl(a) a byl jsem lékařem srozumitelně informován(a) o povaze daného vyšetření a že jsem měl(a) možnost klást lékaři doplňující dotazy.

Na základě tohoto poučení dále prohlašuji, že souhlasím se zařazením svých vzorků do studie probíhající v **Thomayerově nemocnici a Fakultní nemocnici v Motole**, jejímž cílem je porozumět změnám složení střevního mikrobiomu u dospělých pacientů se syndromem dráždivého tračníku.

V dne

Jméno a příjmení vyšetřované osoby :

Podpis vyšetřované osoby

Prohlašuji, že jsem vysvětlil podstatu, účel a povahu odběrů pacientovi způsobem, který byl podle mého soudu srozumitelný.

Jméno a příjmení lékaře:

Podpis:

Datum:

APPENDIX 4 – INFORMED CONSENT FORM FOR FMT RECIPIENTS (CZECH)**Informovaný souhlas pacienta - studie fekální mikrobiální terapie
u pacientů se syndromem dráždivého tračníku**

Název studie: Fekální mikrobiální terapie u pacientů se syndromem dráždivého tračníku

Jméno pacienta:

Datum narození:

Pacient byl do studie zařazen pod číslem:

Odpovědný lékař:

1. Já, níže podepsaný (á) souhlasím s mou účastí ve studii. Je mi více než 18 let.
2. Byl (a) jsem podrobně informován (a) o cíli studie, o jejích postupech, a o tom, co se ode mě očekává. Lékař pověřený prováděním studie mi vysvětlil očekávané přínosy a případná zdravotní rizika, která by se mohla vyskytnout během mé účasti ve studii, a vysvětlil mi, jak bude postupovat při výskytu jejího nežádoucího průběhu. Beru na vědomí, že prováděná studie je výzkumnou činností. Beru na vědomí pravděpodobnost náhodného zařazení do jednotlivých skupin lišících se léčbou.
3. Informoval (a) jsem lékaře pověřeného studií o všech lécích, které jsem užíval (a) v posledních 3 měsících, i o těch, které v současnosti užívám. Bude-li mi nějaký lék předepsán jiným lékařem, budu ho informovat o své účasti v klinické studii a bez souhlasu lékaře pověřeného touto studií ho nevezmu.
4. Budu při své léčbě se svým lékařem spolupracovat a v případě výskytu jakéhokoliv neobvyklého nebo nečekaného příznaku ho budu ihned informovat.
5. Po celou dobu studie a další 4 týdny po jejím ukončení nebudu dárce krve.
6. Porozuměl (a) jsem tomu, že svou účast ve studii mohu kdykoliv přerušit či odstoupit, aniž by to jakkoliv ovlivnilo průběh mého dalšího léčení. Moje účast ve studii je dobrovolná.
7. Při zařazení do studie budou moje osobní data uchována s plnou ochranou důvěrnosti dle platných zákonů ČR. Do mé původní zdravotní dokumentace budou moci na základě mého uděleného souhlasu nahlédnout za účelem ověření získaných údajů zástupci nezávislých etických komisí a zahraničních nebo místních kompetentních úřadů. Pro tyto případy je zaručena ochrana důvěrnosti mých osobních dat. Při vlastním provádění studie mohou být osobní údaje poskytnuty jiným než výše uvedeným subjektům pouze bez identifikačních údajů, a to jako anonymní data pod číselným kódem. Rovněž pro výzkumné a vědecké účely mohou být moje osobní údaje poskytnuty pouze bez identifikačních údajů (anonymní data) nebo s mým výslovným souhlasem. Při předávání dat po 25. 5. 2018 bude zajištěna ochrana osobních údajů požadovaná „Nařízením Evropského parlamentu a Rady (EU) 2016/679 ze dne 27. dubna 2016 o ochraně fyzických osob v souvislosti se zpracováním osobních údajů“ známé pod označením GDPR.
8. S mou účastí ve studii není spojeno poskytnutí žádné odměny.
9. Porozuměl jsem tomu, že mé jméno se nebude nikdy vyskytovat v referátech o této studii. Já pak naopak nebudu proti použití výsledků z této studie.
10. Převzal/a jsem podepsaný stejnopis tohoto informovaného souhlasu.

Podpis pacienta:

Podpis lékaře pověřeného touto studií:

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Datum:

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For peer review only

BMJ Open

Protocol for faecal microbiota transplantation in irritable bowel syndrome – the MISCEAT study: a randomised, double-blind cross-over study utilising mixed microbiota from healthy donors

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2021-056594.R1
Article Type:	Protocol
Date Submitted by the Author:	09-May-2022
Complete List of Authors:	Hurych, Jakub; Charles University Second Faculty of Medicine, Department of Medical Microbiology; Charles University Second Faculty of Medicine, Department of Paediatrics Vejmelka, Jiri; Charles University Third Faculty of Medicine, Department of Internal Medicine Vodolanova, Lucie; Charles University Second Faculty of Medicine, Department of Paediatrics Kramna, Lenka; Charles University Second Faculty of Medicine, Department of Paediatrics Larionov, Vladyslav; Charles University Second Faculty of Medicine, Department of Paediatrics Kulich, Michal; Charles University, Department of Probability and Statistics Cinek, Ondrej; Charles University Second Faculty of Medicine, Department of Pediatrics; Charles University Second Faculty of Medicine, Department of Medical Microbiology Kohout, Pavel; Charles University Third Faculty of Medicine, Department of Internal Medicine
Primary Subject Heading:	Gastroenterology and hepatology
Secondary Subject Heading:	Evidence based practice, Genetics and genomics, Research methods
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Manuscripts

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6 2 **Protocol for faecal microbiota transplantation in irritable bowel syndrome – the MISCEAT**
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8 3 **study: a randomised, double-blind cross-over study utilising mixed microbiota from**
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10 4 **healthy donors**
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51 22 **Word counts:** 3798
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53

54 23 **Abbreviations:** IBS, Irritable Bowel Syndrome; IBS-D, diarrheal type of irritable bowel syndrome; IBS-
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56 24 M, mixed type of irritable bowel syndrome; IBS-C, constipated type of irritable bowel syndrome; IBS-
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58 25 SSS, Irritable Bowel Syndrome Severity Scale Score
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3 26 **Keywords:** irritable bowel syndrome, faecal microbiota transplantation, irritable bowel syndrome
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5 27 severity scale score, gut microbiome
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ABSTRACT

Introduction. Several studies have demonstrated dysbiosis in irritable bowel syndrome (IBS).

Therefore, faecal microbiota transplantation, whose effect and safety have been proven in *Clostridioides difficile* infections, may hold promise in other conditions, including irritable bowel syndrome. Our study will examine the effectiveness of stool transfer with artificially increased microbial diversity in IBS treatment.

Methods and analysis A three-group, double-blind, randomized, cross-over, placebo-controlled study of two pairs of gut microbiota transfer will be conducted in 99 patients with diarrhoeal or mixed type of IBS. Patients aged 18-65 will be randomised into three equally sized groups: group A will first receive two enemas of study microbiota mixture (deep-frozen stored stool microbiota mixed from eight healthy donors); after eight weeks, they will receive two enemas with placebo (autoclaved microbiota mixture), whereas group B will first receive placebo, then microbiota mixture. Finally, group C will receive placebos only. The irritable bowel syndrome severity symptom score (IBS-SSS) questionnaires will be collected at baseline and then at weeks 3,5,8,11,13,32. Faecal bacteriome will be profiled before and regularly after interventions using 16S rDNA next-generation sequencing. Food records, dietary questionnaires, anthropometry, bioimpedance, biochemistry and haematology workup will be obtained at study visits during the follow-up period. The primary outcome is the change in the IBS-SSS between the baseline and four weeks after the intervention for each patient compared to placebo. Secondary outcomes are IBS-SSS at two weeks after the intervention and 32 weeks compared to placebo and changes in urgent defecations frequency, Bristol stool scale, abdominal pain and bloating, anthropometric parameters, psychological evaluation and the gut microbiome composition.

Ethics and dissemination. The study was approved by the Ethics Committee of Thomayer University Hospital, Czechia (G-18-26). The study results will be published in peer-reviewed journals and presented at international conferences and patient group meetings.

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3 83 **Study registration number.** NTC04899869
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9 85 **STRENGTHS AND LIMITATIONS OF THIS STUDY**
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11 86 ➤ Usage of mixed microbiota from multiple donors inflates the diversity of transferred microbiota
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14 87 by enriching it for numerous rare species.
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16 88 ➤ All interventions will be carried out using the same active mixed microbiota or the same placebo.
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18 89 ➤ Each intervention consists of two consecutive transfers, which increases the probability that the
19
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21 90 transferred microbiota engrafts.
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23 91 ➤ Microbiome profiling, food records, anthropometry and bioimpedance data allow detailed
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25 92 monitoring of transfer effectiveness.
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27 93 ➤ Mucosa-associated microbiota will not be assessed because the stool transfer will be performed
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30 94 by enema, not colonoscopy that would allow biopsies.
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95 INTRODUCTION

96 Irritable bowel syndrome (IBS) is characterised as recurrent abdominal pain on average at least one
97 day/week in the last three months, associated with two or more of the following criteria: 1) related
98 to defecation; 2) associated with a change in the frequency of stool; 3) associated with a change in
99 the form (appearance) of stool [1]. It is common among the adult European population (approx. 10%
100 [2]), but its aetiology is still unknown. It may, among other causes, include micro-inflammation,
101 disturbance of the brain-gut axis, inadequate secretion of bile acids, increased permeability of the
102 gut epithelial barrier, or gut dysbiosis. Dysbiosis in IBS has been suggested by several studies
103 (reviewed, e.g. in Rajilic-Stojanovic et al. [3]). There are indications that Firmicutes may be disturbed,
104 with *Dorea*, *Blautia* and *Roseburia* increased, whereas *Veillonella* and *Faecalibacterium* decreased.
105 Among Actinobacteria, a decrease in *Bifidobacterium* was noted, and among Proteobacteria,
106 *Enterobacteriaceae* were increased. Conflicting and heterogeneous results were reported for
107 Bacteroidetes. The major limitation of available studies is their cross-sectional character, which may
108 not be enough in a disease where diarrhoeal episodes alternate with normal stool composition or
109 constipation.

110
111 The faecal microbiota transplantation (FMT) has gained popularity by its remarkable effect in
112 recurrent *Clostridioides difficile* infections, where it has now become a recognised life-saving therapy
113 [4]. The first published randomized, double-blinded study on FMT in IBS, published in 2018 when
114 starting our study [5], used stool intervention from an allogeneic donor or autologous stool. The
115 intervention was centred on a well-defined group of IBS of predominantly diarrhoeal form. The stool
116 was transferred by colonoscopy to the cecum. The primary outcome was an improvement in the
117 *Irritable Bowel Syndrome - Severity Symptom Score* (IBS-SSS). The treatment was associated with a
118 significant effect at three months but not at 12 months post-intervention [5]. This study used single
119 donors and did not assess stool microbiota. Thus, the transferred microbiota likely varied between
120 transfers both in their composition and in their diversity. Since then, more studies focused on FMT in

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3 121 IBS have been carried out [6, 7, 8, 9, 10, 11]. They differed in design, but none of them used a mixed
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5 122 microbiota from multiple donors as the active substance. Furthermore, a recent meta-analysis of
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7 123 randomized control trials on FMT in IBS (including the above-mentioned articles) pointed out
8
9 124 insufficient evidence quality to support recommending FMT in the treatment of IBS. [12]
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14 126 Our study protocol aims to test whether faecal microbiota transplantation of mixed microbiota from
15
16 127 several selected donors can alleviate symptoms of IBS measured by IBS-SSS four weeks after the
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18 128 intervention, as compared to autoclaved placebo. The secondary study aims to test the acute (after
19
20 129 two weeks) and the long-term effect (after six months) on symptoms relief. We also focus on
21
22 130 changes in frequency of urgent defecations, Bristol stool scale, abdominal pain and bloating, body
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24 131 weight, fat content and anthropometric measurements (including waist, hip and limbs
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26 132 circumferences and skinfold thickness) and the gut microbiome composition.
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32 134 We hypothesise that the transfer of active microbiota of high diversity can lead to changes in the
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34 135 patient's gut microbiome composition and/or function to alleviate IBS symptoms.
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136 **METHODS AND ANALYSIS**

137 **Study design**

138 This is a three-group, double-blind, placebo-controlled, randomized, cross-over study in adult
139 patients diagnosed with IBS (diarrhoeal or mixed form) according to Rome IV criteria. Each study
140 subject will undergo two pairs of FMT (a total of four enemas for each patient), with the pairs of
141 transfers being eight weeks apart. The active intervention substance is a mixed stool microbiota
142 derived from healthy individuals who were preselected for high alpha diversity of their microbiome
143 and distance in community ordination from IBS patient's microbiota. Placebo is the same mixture,
144 inactivated by autoclaving.

145
146 The study subjects are randomly assigned to one of three groups: A) enema with active substance
147 first and with placebo second or B) enema with placebo first and active substance second or C)
148 enemas of placebo only (detailed scheme in **Figure 1**). Eligible participants will be followed-up for 32
149 weeks after the first intervention to monitor symptom severity scoring of IBS (IBS-SSS), with regular
150 profiling of their gut microbiome and other parameters (frequency of urgent defecations, Bristol
151 stool scale, abdominal pain and bloating, body weight, fat content, and other anthropometric
152 parameters).

153
154 The placebo group is planned because of the unknown onset and duration of the intervention effect:
155 if the beginning of an effect is delayed, or if it persists for a long time, simple cross-over design would
156 not have sufficient power due to the carry-over effect. In case the FMT was associated with
157 significant but not durable amelioration of the status, the control group would still increase the
158 statistical power.

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160 This study protocol is reported as per the SPIRIT guidelines [13] (for the SPIRIT checklist see **Appendix**
161 **1**).

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5 163 **Study setting**

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8 164 The participants are recruited at a single center, the Department of Internal Medicine, Thomayer
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10 165 University Hospital in Prague, Czech Republic. This hospital has approximately 1,000 beds, including
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12 166 80 in ICU's, serves approximately 50,000 patients per year. The center is experienced in treating
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14 167 patients with IBS and other functional gastrointestinal disorders, with about 200 such patients
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16 168 registered and further subjects coming for consultations from other workplaces to this tertiary
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18 169 referral centre.
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23 171 **Recruitment and eligibility criteria**24
25 172 Stool donors

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28 173 Stool donor candidates were recruited among blood donors at Thomayer University Hospital and
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30 174 medical students in their first year of study (i.e. preclinical) from the 2nd Faculty of Medicine, Charles
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32 175 University, Prague. We obtained stool samples from 58 such candidates fulfilling the inclusion criteria
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34 176 (**Table 1**). Based on their high bacterial alpha-diversity and by the position on the ordination plot of
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36 177 the weighted Unifrac distance against 46 patients with IBS-D (**Figure 2**), 14 candidates proceeded to
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38 178 the safety screening, whereby eight passed it (for reasons of candidate's exclusion, see **Figure 3**).
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44 180 After 14 potential donors were selected based on the microbiota composition, they were screened
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46 181 for infectious diseases and clinically examined as indicated by the *European consensus conference on*
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48 182 *faecal microbiota transplantation in clinical practice guidelines* [14] (**Table 2**). All subjects were also
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50 183 repeatedly tested for SARS-CoV-2 from both nasopharyngeal swab and stool. Six candidates were
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52 184 excluded (for reasons, see **Figure 3**), whereas eight became regular stool donors. These eight donors
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54 185 were regularly investigated as follows:
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3 186 - at every donation: by questionnaire for gastrointestinal symptoms, antibiotic usage, unprotected
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5 187 sex, travelling to exotic countries; clinical signs of COVID-19; the presence of SARS-CoV-2 in the
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7 188 donated stool;
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10 189 - every 4 weeks: for SARS-CoV-2 from nasopharyngeal swab;
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12 190 - every 8-12 weeks: for all other stool tests mentioned in **Table 2**.

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14 19115
16 192 Prospective study participants

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18 193 Patients diagnosed with IBS-D (diarrheal type) or IBS-M (mixed diarrhoeal and constipation type) who
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20 194 fulfil the inclusion and exclusion criteria listed in **Table 3** are recruited via regular patient's check-ups
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22 195 at the Gastroenterological unit at Thomayer University Hospital, by referrals from their general
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24 196 practitioners, following our newspaper articles or word of mouth.
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29 30 198 **Study microbiota mixture for intervention**

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32 199 The intervention microbiota is a mixture of regular stool donations from the eight regular donors.
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34 200 The collection of stools for this purpose is already completed. The donors were advised to regularly
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36 201 defecate at their home toilet into a clean plastic bag placed in Fecotainer (Excretas Medical, NL) with
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38 202 an Anaerogen bag (Thermo Scientific, USA). This bag generated an anaerobic atmosphere during
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40 203 transport to ensure anaerobes survival. The stool was transported to the laboratory with the
41
42 204 maximum allowable time until processing being 6 hours; the actual time was approximately 1.5
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44 205 hours. The stool was weighed upon arrival, inspected for blood admixture, and immediately
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46 206 processed by blending with a solution consisting of sterile 0.9% saline (160 ml per 100 g of stool),
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48 207 sterile phosphate buffer saline at pH 7.4 (20 ml per 100 g of stool) and sterile 99.5% glycerol (20 ml
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50 208 per 100 g stool, which is approximately 10% of solution's volume; therefore, it is unlikely to have
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52 209 laxative properties upon administration). From our experience, ~ 105 ml of the study mixture
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54 210 represents ~40 g of stool. The mixture was then filtered through a sterile stainless steel mesh of 0.8
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56 211 mm pore size into a sterile plastic bottle, which was then immediately frozen at -80°C. Whenever
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3 212 possible (blending or post-filtration), the procedure was performed under a nitrogen atmosphere to
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5 213 protect obligate anaerobes. All stool portions were mixed together in a large stainless steel bucket
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7 214 using an electric mortar mixer under anaerobic conditions and at low temperature (on ice).
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12 216 Based on the recommendation from the Nanjing consensus [15], the bacterial cell content of the
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14 217 study microbiota mixture was quantified. We performed a real-time PCR of the 16S rRNA gene with a
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16 218 standard curve derived from bacterial culture and controls from previously used stool transplants
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18 219 from another centre. It was estimated that the cell count in the transfer ranged between $2e+12$ and
19
20 220 $1e+13$ (depending on the expected composition of the microbiota as to the 16S gene count per an
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22 221 average bacterial cell). Unfortunately, the Nanjing consensus [15] provides neither reference to the
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24 222 cell counting method (Table 2 therein) nor to control materials. Therefore more exact direct
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26 223 comparison of the requested quantities is not possible.
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32 225 The mixed microbiota substance was divided into aliquots of 13-14 g (which is ~ 35 ml). Two-thirds of
33
34 226 the tubes served as a placebo: they were immediately autoclaved at 121°C for 30 minutes with slow
35
36 227 cooling. Pre-sterilised tubes were used to ensure that autoclaved placebos would not be visually
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38 228 distinguishable from tubes with the active substance. Assignment of tubes to the autoclave,
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40 229 numbering, sealing, and labelling were done under the guidance of a statistical unit member (see
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42 230 below).
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48 232 All aliquot tubes are kept frozen at -80°C in the same type of plastic tubes, labelled by codes. Three
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50 233 such aliquots represent one dose for FMT (~ 40 g of stool, in ~ 105 ml). Aliquoting into multiple 50 ml
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52 234 tubes instead of one larger volume was decided because of the availability of durable plastic, which
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54 235 must be both autoclavable and deep frost resistant.
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3 237 Before administering, the study microbiota mixture will be thawed in a warm (37°C) water bath, with
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5 238 intermittent mixing by inverting the tubes.

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10 240 **Randomization, allocation and blinding**

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12 241 At Visit 1, each patient is randomised into one of three equally sized groups (Figure 1) as described in
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14 242 the *Study design*. Randomisation assignments is generated in advance in blocks of nine and stored in
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16 243 a protected database. For each patient, anonymous codes for tubes containing either active study
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18 244 microbiota mixture or placebo is received. Thus, the true assignment will remain concealed for the
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20 245 patients and the study staff until the end of the study observation period. The Investigator is
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22 246 encouraged to maintain the blind as far as possible. The actual allocation must not be disclosed to
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24 247 the patient and/or other study personnel including other site personnel, monitors, corporate
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26 248 sponsors or project office staff; nor should there be any written or verbal disclosure of the code in
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28 249 any of the corresponding patient documents.

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34 251 **Study Intervention**

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36 252 Study substance is administered during Visit 2+3 and then again 7+8 as a retention colon enema and
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38 253 will be held optimally for at least 30 minutes. Bowel preparation is applied the day before the
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40 254 intervention (prior to Visit 2 and Visit 7) (natrii picosulfas 10 milligrams, magnesi oxidum leve 3,5
41
42 255 grams, acidum citricum 12 grams). No preparation is performed before the second enema in the pair
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44 256 (visits 3 and 8).

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47
48 258 A rectal tube is inserted into the rectum, and the enema is applied. Application kit (Irrigator PN
49
50 259 0462/E/93, EriLens, Czechia) is used. After the enema is applied, the patient position is changed to
51
52 260 enable the study substance to be spread within the colon. The exact time of the enema completion is
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54 261 recorded as well as the enema retention time.

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263 **Outcomes**

264 Primary outcome

265 The primary outcome is the change in the IBS severity symptom score (IBS-SSS) in the active
266 microbiota group relative to the placebo group. The change will be evaluated as the difference
267 between the score at four weeks after the intervention (study weeks 5 or 13, respectively, see **Figure**
268 **1**) and the baseline score (week -1 in group A or week 8 in group B).

269

270 Secondary outcomes

- 271 - The acute change in the IBS severity symptom score (IBS-SSS) between baseline and two weeks
272 after intervention (study weeks 3 and 11, respectively, see **Figure 1**).
- 273 - The long-term change in the IBS severity symptom score (IBS-SSS) between baseline (week -1)
274 and week 32 (see **Figure 1**). The long term change will compare group C (placebo only) to
275 merged groups A+B (active study microbiota mixture).
- 276 - Changes due to the intervention in (a) frequency of urgent defecations, (b) Bristol stool scale, (c)
277 abdominal pain and bloating, (d) body weight, fat content, and other anthropometric parameters
- 278 - The durability of changes (if any) in the microbial profiles by bacteriome profiling, parasite
279 screening, and virome sequencing
- 280 - The psychological and well-being effects of the therapy scored by IBS-QoL questionnaires
- 281 - The long term effects of the therapy on stool frequency and consistency and on the gut
282 microbiome and statistically significant changes in anthropometric measurements.

283

284 **Data collection and follow-up**

285 Timing of assessments

286 At visit 1 (the randomization), the patient is given detailed instructions and thoroughly instructed by
287 the study team. The patients are asked to keep the identical type of diet throughout the observation.
288 They are asked to regularly (once a week) fill the study questionnaire. A study team member sends

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3 289 that via the Survey Monkey smartphone application, an online survey development cloud-based
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5 290 software. Relevant data are entered in a structured manner (frequency of defecation, Bristol stool
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7 291 scale, pain measures, other symptoms, dietary records etc.). This member also frequently
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10 292 communicate with study participants and answer any questions regarding the study to keep the
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12 293 patient's adherence. An overview of the examinations at each visit and the timing of the study visits
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14 294 could be seen in **Table 4**.

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18 296 Irritable bowel syndrome severity scale score (IBS-SSS).
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20 297 The IBS-SSS is a five-question survey that reflects 1) the severity of abdominal pain, 2) frequency of
21
22 298 abdominal pain, 3) severity of abdominal distention, 4) satisfaction with bowel habits, and 5)
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24 299 interference with quality of life over the past ten days. Subjects respond to each question on a 100-
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26 300 point analogue scale ; thus, the score can range from 0 to 500, with higher scores indicating more
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28 301 severe symptoms.[16]

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34 303 At eligibility screening, the patients is given instructions on how to fill the IBS-SSS questionnaires (via
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36 304 the Survey Monkey application). The questionnaires are filled in at eligibility screening and then at
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38 305 week -1, 3, 5 (before the first intervention, at the presumed peak of its effect, and after further 2
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40 306 weeks), then at weeks 8, 11, 13 (similarly with the second intervention), and finally at week 32.

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44 308 Weight, height, bioimpedance

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46 309 Body weight, height and bioimpedance is examined during Visit 0, 1, 4, 5, 9 and 11. Medical Body
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48 310 Composition Analyzer Seca mBCA 515, (Seca, Germany) is used to measure changes in body
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50 311 composition (8-point bioelectric impedance analysis at a frequency of 5 - 50 kHz with a current of
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52 312 100 μ A), scanning performed with three pairs of hand electrodes and two pairs of leg electrodes,
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54 313 measurements performed with light clothing and without metal objects (jewellery, keys). The weight
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56 314 is determined in patients wearing underwear using the Seca mBCA 515. The height is determined by
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3 315 a standardised technique with a metal stadiometer with an accuracy of 1 mm. Seca analytics 115
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5 316 software is used to analyse the obtained data (Seca, Hamburg, Germany). The measurements is
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7 317 performed according to the NIHR Southampton Biomedical Research Centre standard protocol (Seca
8
9 318 mBCA, NIHR Southampton Biomedical Research Centre, 2014).
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12 319

14 320 Detailed anthropometry

16 321 It is performed by nutritional therapists in Visit 1, 5, 10 and 11. It involves weight, abdominal (waist)
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18 322 circumference, buttocks (hip) circumference, thigh circumference, skinfolds (thigh, triceps,
19
20 323 subscapular, suprailiacal).
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25 325 Serum workup, archiving serum+plasma

27 326 Blood is sampled at Visits 0, 4, 9, 11 and will include: A) serum+plasma archiving, B) serum workup.

29 327 Laboratory panel testing will comprise sodium, potassium, chloride, urea, creatinine, glucose,

31 328 calcium, phosphate, total protein and albumin, AST, ALT, ALP, **GGT**, bilirubin, lipid panel, HS-CRP,

33 329 blood cell count with differential count, INR, urine analysis (sediment and biochemistry). One plasma

35 330 and one serum aliquots are made at these visits and frozen for forensic reasons.
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41 332 Psychological evaluation

43 333 It is performed during Visit 0 and Visit 11 using a structured questionnaire evaluated by a qualified

45 334 psychologist.
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50 336 Dietary questionnaire & advice, evaluation of food records

52 337 It is performed by nutritional therapists at Visit 4 and 9 and includes: evaluation of food records will

54 338 include: overall daily energy intake, proteins, carbohydrates and lipids calculations and dietary fibre.
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59 340 Gut microbiome composition

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3 341 Faecal samples are collected at home by the subjects in the same way as described for donors above
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5 342 and at time points indicated in sections above. If not immediately brought to the visit, the stool is
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7 343 frozen in a home freezer and then transported in a frozen tube container. DNA extraction is
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9 344 performed using the PowerSoil kit (Qiagen), and the bacteriome characterised by 16S rDNA amplicon
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11 345 profiling using the tagged primers according to Schloss protocol [17], and sequencing on a MiSeq
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13 346 instrument with the 2x250 bases sequencing kit (both Illumina, USA).
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18 348 The first steps of bioinformatic analysis will be performed in the DADA2 package[18]. Statistical
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20 349 analyses and visualisation will be then performed in R with its Phyloseq package. The functional
21
22 350 potential of the bacteriome will be assessed using the PICRUST software, which predicts functional
23
24 351 capabilities based on the 16S rDNA profiles.
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30 353 The virome is assessed in a total of four stool samples per patient at Visit 0, 4, 9 and 11. The aim of
31
32 354 this analysis is to assess the repertoire of major bacteriophages. The virome analysis is based on
33
34 355 metagenomic sequencing of total DNA from a virus-enriched stool sample, according to the
35
36 356 previously published protocol [[19]].
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41 358 Finally, a simple PCR-based semi-quantitative parasite screening aims to identify several mostly
42
43 359 benign unicellular parasites (e.g. *Blastocystis*, *Dientamoeba*, *Entamoeba*, *Endolimax*).
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47 361 **Safety monitoring**

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49 362 All data are regularly monitored by the research team for any adverse events, and all potential
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51 363 adverse events are recorded. Contacts to study coordinators active 24/7 are provided in case adverse
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53 364 effects occurred. If any concerns are identified during the screening or clinical assessment of donors
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55 365 or recipients, further clinical evaluation and/or examination is immediately realised. All the concerns
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57 366 during the study are assessed, and the recipient will be withdrawn if this is thought to be in his best
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3 367 interest. A Data Monitoring and Safety Committee (DMSC) has been established and based on the
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5 368 data from planned interim analysis has the right to terminate the study if the frequency of severe
6
7 369 adverse events crosses the 5% line (for closer description of DMSC, its responsibilities and premature
8
9
10 370 termination of the study see **Appendix 2**).

11
12 371

14 372 **Sample size and power calculation**

16 373 The study is powered to detect an absolute improvement of 62.5 points in IBS-SSS score over 8
17
18 374 weeks (which is 25% of the expected mean baseline score 250) between the active microbiota
19
20 375 intervention compared to placebo. With a sample size of 33 per group, the probability of detecting
21
22 376 such an improvement is at least 0.9. This calculation assumes 20% dropout rates, variance in IBS-SSS
23
24 377 scores 100 (see the results in [20]), a correlation between the final and baseline IBS-SSS scores 0
25
26 378 (with a positive correlation, the power is higher), and no carry-over or temporal effect.

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32 380 **Data management**

34 381 Data from IBS-SSS, frequency of urgent defecations, Bristol stool scale, abdominal pain and bloating
35
36 382 are collected and stored via the application Survey Monkey. All anthropometric data are entered and
37
38 383 stored in password-protected platforms integrated within the hospital information system. Only the
39
40 384 researchers involved in the study have access to the final study dataset (IBS-SSS, frequency of urgent
41
42 385 defecations, Bristol stool scale, abdominal pain and bloating), which will be shared in an anonymised
43
44 386 form via the Zenodo repository. The only data in this manuscript are bacteriome data; their
45
46 387 anonymised form will be available on reasonable request.

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52 389 **Statistical analyses**

54 390 The primary outcome analysis will be based on the difference in IBS-SSS scores over the second
55
56 391 treatment period (week 14 vs week 8) minus the change over the first treatment period (week 5 vs
57
58 392 week -1). This difference will be used as a response in a linear model, with intercept corresponding to

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2
3 393 the temporal effect (seen in the placebo group C), an indicator of group A corresponding to the cross-
4
5 394 over effect (resulting from administration of placebo after active microbiota) and differences in
6
7 395 indicators for groups A and B modelling the effect of active microbiota. A robust sandwich estimator
8
9
10 396 of the variance matrix will be used to adjust for potentially unequal variances between the groups.
11
12 397 Analyses of secondary outcomes will proceed by similar methodology, comparing absolute or relative
13
14 398 differences of the post-intervention measure of each outcome relative to its baseline value. The
15
16 399 CONSORT 2010 guidelines will be followed in reporting the main trial results.

400

401 **Study status**

402 The study was registered at clinicaltrials.gov (NCT04899869) on May 25th 2021. The first patient was
403 recruited on June 17th 2021, and the first intervention was applied on July 29th 2021. As of August
404 17th 2021, 12 patients have signed the informed consent, and six interventions have been applied. It
405 is expected that the study will be completed in December 2022.

406

407 **Patient and public involvement**

408 Information on the study has been spread at conferences, in newspapers and by local
409 gastroenterologists contacted by researchers. Everyone interested got information material, which
410 allowed the potential subjects to read about the study and reach the researchers if they wanted to
411 participate. Participants were not involved in the development, recruitment of other participants or
412 conduct of the study. All recipients are asked about any possible adverse effects of treatment at
413 regular visits planned according to **Figure 1**; a thorough investigation will be conducted if any occurs.
414 After completing the data analysis, all recipients will receive information about their results and be
415 offered a roll-over (receiving active study microbiota mixture).

416

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3 417 **ETHICS AND DISSEMINATION**
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5 418 Ethics approval for this study was granted in June 2018 by the Ethics Committee of the Institute for
6
7 419 Clinical and Experimental Medicine and Thomayer Hospital (Víteňská 800, 140 59 Prague 4, Czech
8
9 420 Republic). Involvement in this study is completely voluntary; donors and recipients are required to
10
11 421 provide written informed consent prior to participation in the study (see **Appendix 3 and 4**).
12
13 422 Recipients and their caregivers are informed of unexpected findings or unrecognised conditions and
14
15 423 by possible future usage of their specimens in ancillary studies by trained physician or nurse; further
16
17 424 medical care will be arranged. Study donors received financial compensation to pay for the required
18
19 425 travelling costs when donating the stool. The patient will be offered a roll-over into an observational
20
21 426 study with the administration of active microbiota. The patients are informed of this option at the
22
23 427 start of the study and regularly reminded.
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28 428 We aim to publish findings in impact peer-reviewed international journals. Gastroenterologists,
29
30 429 internists and other care providers will be informed through the national conference meetings,
31
32 430 journals and patient groups meetings.
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39 432 **Protocol amendment number:** 01. Modification of the study protocol will be communicated with the
40
41 433 Ethics committee.
42
43

44 434 **Registration details** This study is registered with ClinicalTrials.gov (NCT04899869).
45
46

47 435 **Acknowledgement** We thank Peter Holger Johnsen, Linn Skjevling and Hege Hansen from University
48
49 436 Hospital of North Norway Harstad, Norway and Rasmus Goll from University Hospital of North
50
51 437 Norway Tromsø, Norway, for valuable advice regarding the study design and study microbiota
52
53 438 mixture preparation. We also thank Marcela Krutova, Jan Tkadlec, Daniela Lzicarova, Kamila
54
55 439 Dundrova, Marie Brajerova, Milena Antuskova, Barbora Dravotova, Jana Prasilova, Jana Sumova and
56
57 440 Ales Briksi all from Department of Medical microbiology, 2nd Faculty of Medicine, Charles University
58
59
60

1
2
3 441 and Motol University Hospital, Prague for their laboratory work in the regular microbiological
4
5 442 screening of the study donors.
6
7

8 443 **Contributors** OC, PK, JH, JV, MK contributed to the conception and design of the study. OC, PK, JH
9
10 444 and JV drafted the protocol with input from all other authors. JV and PK contributed to the patients
11
12 445 recruitment. JH, LV, LK and OC contributed to the microbiome analysis for donor selection. JH, OC
13
14 446 and JV contributed to the donor screening. LV, JH and OC contributed to the study microbiota
15
16 447 mixture preparation. MK contributed to the power size calculations and statistical analysis. VL
17
18 448 contributed to the randomization. JH and JV contributed equally to this paper, OC and PK contributed
19
20 449 equally either.
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23

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25
26 451 19-01-00127 . Funding received from this grant support direct research cost. All rights reserved
27
28
29

30 452 **Competing interests** None declared. No money from commercial sponsors was used.
31
32

33 453 **Patient consent for publication** Not required.
34
35

36 454 **Ethics approval** Ethics approval for this study was granted in June 2018 by the Ethics Committee of
37
38 455 the Institut for Clinical and Experimental Medicine and Thomayer Hospital (Víteňská 800, 140 59
39
40 456 Prague 4, Czech Republic).
41
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44 457 **Provenance and peer review** Not commissioned; externally peer-reviewed.
45
46

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49 459 Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt,
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51 460 build upon this work non-commercially, and license their derivative works on different terms,
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53 461 provided the original work is properly cited, appropriate credit is given, any changes made indicated,
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55 462 and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.
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3 463 **FIGURES AND ILLUSTRATIONS**

4 464 **Figure 1** Per protocol intervention scheme: the visits, questionnaires and samples
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For peer review only

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3 465 **Figure 2** Ordination plot on the weighted Unifrac distance at the genus level for selection of the
4 466 donor candidates based on their gut microbiome alpha- and beta-diversity
5 467

6 468 These are the results of a comparative microbiome case-control study which helped us to preselect 14 donor candidates.
7 469 Alpha diversity calculation was based on Chao 1 index. The beta-diversity calculation was based on non-metric dimensional
8 470 scaling (NMDS) with weighted UniFrac distance matrix for bacterial Genus. NMDS axis 1 captured 46.8% of variability;
9 471 NMDS axis 2 represents 14.7% of the variability. Healthy subjects were enriched in negative values of the first ordination
10 472 axis; therefore, we selected donors among healthy subjects in this half of the graph and based on their microbiome's alpha
11 473 diversity. The reason for concentrating healthy and enriched subjects in the left part of the plot could be their younger age.
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For peer review only

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3 475 **Figure 3** Process of donor selection and reasons for their excluding
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476 **Table 1.** Inclusion and exclusion criteria for FMT donors

Inclusion	Adults aged 18-65 years
	BMI 18,5-27 kg/m ²
	Lack of restrictive diets (diet discussed with experienced gastroenterologist)
	Bristol stool scale usually between 3 and 4
	High alpha diversity and significant difference in beta-diversity from patients (using 16S rDNA sequencing)
	Expected to donate regularly
	Consented in writing
Exclusion	Any chronic GI disease in patient's history (coeliac disease, inflammatory bowel disease, irritable bowel syndrome, colorectal carcinoma), or active acute GI issues (infectious gastroenteritis or enterocolitis, frequent bloating, diarrhoea or vomiting)
	Chronic disease in ' 'patient's history (cancer, autoimmune conditions, type 2 diabetes mellitus, coronary heart disease, hypertension, hypercholesterolemia, gout)
	<i>Clostridiodes difficile</i> infection in patient's history
	Colorectal carcinoma in family history
	Any restrictive diet habits (raw-vegans, fruitarians, keto or carnivore)
	Any systemic antibiotics in the last 6 months
	Using proton-pump inhibitors in the last 6 months
	Regular unprotected sex with unknown persons

477

478 **Table 2** Laboratory screening of the FMT donors

Blood testing
Hepatitis A, hepatitis B, hepatitis C and hepatitis E viruses (serology)
HIV-1 and HIV-2 (p24 antigen)
Treponema pallidum (serology)
Strongyloides stercoralis (serology)
Complete blood cell count with differential
Creatinine, aminotransferases, bilirubin
Stool testing
<i>Clostridioides difficile</i> (cultures, antigen testing)
Common enteric pathogens, including Salmonella, Shigella, Campylobacter, shiga toxin-producing <i>Escherichia coli</i> , Yersinia and <i>Vibrio cholerae</i> (cultures)
Antibiotic-resistant bacteria (ARB), including vancomycin-resistant Enterococci, meticillin-resistant <i>Staphylococcus aureus</i>
Gram-negative ARB including extended-spectrum β -lactamase-producing <i>Enterobacteriaceae</i> , and carbapenem-resistant <i>Enterobacteriaceae</i> /carbapenemase-producing <i>Enterobacteriaceae</i> (cultures)
Norovirus, rotavirus, adenovirus, sapovirus (PCR)
SARS-CoV-2 (reverse transcription -PCR)
Common intestinal parasites, including <i>Giardia intestinalis</i> , <i>Cryptosporidium parvum et hominis</i> (cultures and PCR), <i>Blastocystis hominis</i> *, <i>Dientamoeba fragilis</i> * (both PCR only)

479

480 *) Based on the literature [21], we decided to test both parasites but do not exclude the donors if they tested
 481 positive and having no gastrointestinal symptoms. *Blastocystis* is believed to be commensal of the gut.
 482 *Dientamoeba's* status is not exact; however, based on our experiment, it does not survive freezing at -80 °C and
 483 thawing to 5°C when mixing the study microbiota mixture. Therefore it can't do any harm.

484

485 The screening strategy is based on [14].

486 **Table 3.** Inclusion and exclusion criteria for recipients of FMT

Inclusion	Adults 18-65 years
	Diagnosed with IBS-D or IBS-M according to the Rome IV criteria
	Expected adherence to following the protocol
	Written consent to the study
Exclusion	The use of antibiotics and probiotics within one month prior to faecal microbiota transplantation
	History of inflammatory bowel disease or gastrointestinal malignancy, systemic autoimmune diseases (ongoing or in history)
	Previous abdominal surgery (other than appendectomy or cholecystectomy or hernioplasty or cesarean section)
	HIV infection or other active infection
	Renal or hepatic disease (both defined by biochemistry workup)
	Diabetes mellitus, abnormal thyroid functions not controlled by thyroid medications
	Bipolar disorder or schizophrenia (ongoing or history thereof), moderately severe depression defined by Patient Health Questionnaire-9 (PHQ-9) score > 15
	Anxiety defined by a Generalised Anxiety Disorder 7 (GAD7) score > 10, with any organic causes that can explain the symptoms of IBS
	Current pregnancy and lactation

487

488 **Table 4.** The study visits with planned activities

Visit	0	1	X	2+3	4	X	5	6	7+8	9	X	10	11
Study Week	?	-2	-1	1	2	3	5	8	9	10	11	13	32
Eligibility evaluation (E) / Randomization (R) / Wrap-up visit (W) ⁽¹⁾	E	R											W
Colon enema with the study substance (active microbiota or placebo)				XX					XX				
Irritable bowel syndrome severity scale score		X	X			X	X	X			X	X	X
Weight, height, bioimpedance		X			X		X			X		X	X
Detailed anthropometry		X					X					X	X
Serum workup, archiving serum+plasma		X			X					X			X
Psychological evaluation		X											X
Dietary questionnaire & advice, evaluation of food records ⁽²⁾					X					X			
Stool samples for bacteriome profiling using 16S rDNA sequencing	X	X	X		X	X	X	X		X	X	X	X

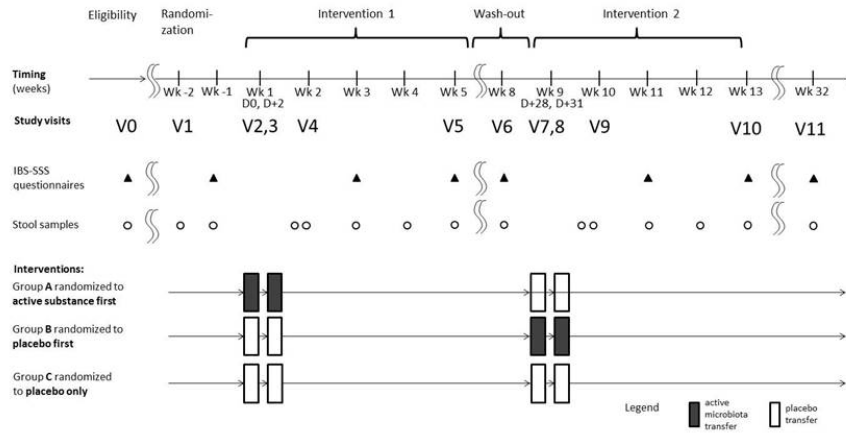
489 (1) Here, the patient is offered a roll-over into an observational study with active microbiota administration. The patients
490 will be informed of this option at the start of the study and regularly reminded.

491 (2) For IBS-SSS questionnaires assessing the primary outcome, please see the intervention scheme in Figure 2. Their
492 administering is not linked to study visits.

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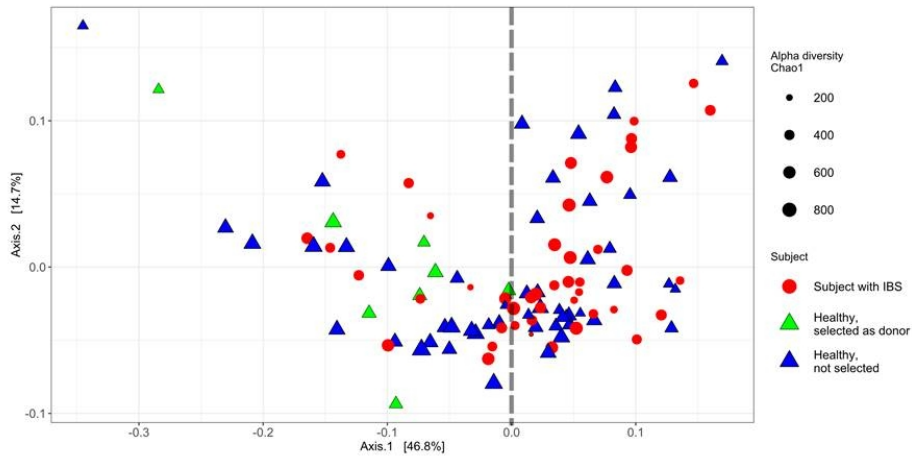
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31 Per protocol intervention scheme: the visits, questionnaires and samples

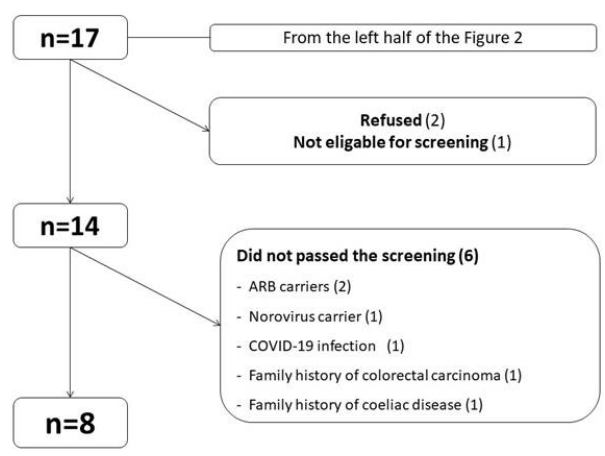
32 254x190mm (96 x 96 DPI)



Ordination plot on the weighted UniFrac distance at the genus level for selection of the donor candidates based on their gut microbiome alpha- and beta-diversity. These are the results of a comparative microbiome case-control study that helped us to preselect 14 donor candidates. Alpha diversity calculation was based on Chao 1 index. The beta-diversity calculation was based on non-metric dimensional scaling (NMDS) with a weighted UniFrac distance matrix for bacterial Genus. NMDS axis 1 captured 46.8% of variability; NMDS axis 2 represents 14.7% of the variability. Healthy subjects were enriched in negative values of the first ordination axis; therefore, we selected donors among healthy subjects in this half of the graph and based on their microbiome's alpha diversity. The reason for concentrating healthy and enriched subjects in the left part of the plot could be their younger age.

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Process of donor selection and reasons for their excluding
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Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Appendix 1 SPIRIT CHECKLIST

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	6 and 19
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	NA – not received yet.
Protocol version	#3	Date and version identifier	19
Funding	#4	Sources and types of financial, material, and other support	20
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	20
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	20
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	20

1	Roles and	#5d	Composition, roles, and responsibilities of the	20
2	responsibilities:		coordinating centre, steering committee, endpoint	
3	committees		adjudication committee, data management team, and	
4			other individuals or groups overseeing the trial, if	
5			applicable (see Item 21a for data monitoring committee)	
6				
7				
8				
9	Introduction			
10				
11	Background and	#6a	Description of research question and justification for	7
12	rationale		undertaking the trial, including summary of relevant	
13			studies (published and unpublished) examining benefits	
14			and harms for each intervention	
15				
16				
17				
18	Background and	#6b	Explanation for choice of comparators	8
19	rationale: choice of			
20	comparators			
21				
22				
23	Objectives	#7	Specific objectives or hypotheses	8
24				
25				
26	Trial design	#8	Description of trial design including type of trial (eg,	9
27			parallel group, crossover, factorial, single group),	
28			allocation ratio, and framework (eg, superiority,	
29			equivalence, non-inferiority, exploratory)	
30				
31				
32				
33	Methods:			
34	Participants,			
35	interventions, and			
36	outcomes			
37				
38				
39	Study setting	#9	Description of study settings (eg, community clinic,	10
40			academic hospital) and list of countries where data will	
41			be collected. Reference to where list of study sites can	
42			be obtained	
43				
44				
45				
46	Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If	10
47			applicable, eligibility criteria for study centres and	
48			individuals who will perform the interventions (eg,	
49			surgeons, psychotherapists)	
50				
51				
52				
53	Interventions:	#11a	Interventions for each group with sufficient detail to allow	13
54	description		replication, including how and when they will be	
55			administered	
56				
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58				
59	Interventions:	#11b	Criteria for discontinuing or modifying allocated	13
60				

1	modifications		interventions for a given trial participant (eg, drug dose	
2			change in response to harms, participant request, or	
3			improving / worsening disease)	
4				
5	Interventions:	#11c	Strategies to improve adherence to intervention	14
6	adherence		protocols, and any procedures for monitoring adherence	
7			(eg, drug tablet return; laboratory tests)	
8				
9				
10	Interventions:	#11d	Relevant concomitant care and interventions that are	14
11	concomitant care		permitted or prohibited during the trial	
12				
13				
14	Outcomes	#12	Primary, secondary, and other outcomes, including the	13
15			specific measurement variable (eg, systolic blood	
16			pressure), analysis metric (eg, change from baseline,	
17			final value, time to event), method of aggregation (eg,	
18			median, proportion), and time point for each outcome.	
19			Explanation of the clinical relevance of chosen efficacy	
20			and harm outcomes is strongly recommended	
21				
22				
23				
24				
25				
26	Participant timeline	#13	Time schedule of enrolment, interventions (including any	See Figure
27			run-ins and washouts), assessments, and visits for	1
28			participants. A schematic diagram is highly	
29			recommended (see Figure)	
30				
31				
32				
33	Sample size	#14	Estimated number of participants needed to achieve	17
34			study objectives and how it was determined, including	
35			clinical and statistical assumptions supporting any	
36			sample size calculations	
37				
38				
39				
40	Recruitment	#15	Strategies for achieving adequate participant enrolment	11
41			to reach target sample size	
42				
43	Methods:			
44	Assignment of			
45	interventions (for			
46	controlled trials)			
47				
48				
49				
50	Allocation: sequence	#16a	Method of generating the allocation sequence (eg,	12
51	generation		computer-generated random numbers), and list of any	
52			factors for stratification. To reduce predictability of a	
53			random sequence, details of any planned restriction (eg,	
54			blocking) should be provided in a separate document	
55			that is unavailable to those who enrol participants or	
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assign interventions

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2			
3	Allocation	#16b	Mechanism of implementing the allocation sequence 12
4	concealment		(eg, central telephone; sequentially numbered, opaque,
5	mechanism		sealed envelopes), describing any steps to conceal the
6			sequence until interventions are assigned
7			
8			
9	Allocation:	#16c	Who will generate the allocation sequence, who will 12
10	implementation		enrol participants, and who will assign participants to
11			interventions
12			
13			
14	Blinding (masking)	#17a	Who will be blinded after assignment to interventions 12
15			(eg, trial participants, care providers, outcome
16			assessors, data analysts), and how
17			
18			
19			
20	Blinding (masking):	#17b	If blinded, circumstances under which unblinding is 12-13
21	emergency unblinding		permissible, and procedure for revealing a participant's
22			allocated intervention during the trial
23			
24			
25	Methods: Data		
26	collection,		
27	management, and		
28	analysis		
29			
30			
31			
32	Data collection plan	#18a	Plans for assessment and collection of outcome, 14-17
33			baseline, and other trial data, including any related
34			processes to promote data quality (eg, duplicate
35			measurements, training of assessors) and a description
36			of study instruments (eg, questionnaires, laboratory
37			tests) along with their reliability and validity, if known.
38			Reference to where data collection forms can be found,
39			if not in the protocol
40			
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45	Data collection plan:	#18b	Plans to promote participant retention and complete 14
46	retention		follow-up, including list of any outcome data to be
47			collected for participants who discontinue or deviate
48			from intervention protocols
49			
50			
51			
52	Data management	#19	Plans for data entry, coding, security, and storage, 18
53			including any related processes to promote data quality
54			(eg, double data entry; range checks for data values).
55			Reference to where details of data management
56			procedures can be found, if not in the protocol
57			
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1	Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary	18
2			outcomes. Reference to where other details of the	
3			statistical analysis plan can be found, if not in the	
4			protocol	
5				
6				
7				
8	Statistics: additional	#20b	Methods for any additional analyses (eg, subgroup and	18
9	analyses		adjusted analyses)	
10				
11	Statistics: analysis	#20c	Definition of analysis population relating to protocol non-	18
12	population and		adherence (eg, as randomised analysis), and any	
13	missing data		statistical methods to handle missing data (eg, multiple	
14			imputation)	
15				
16				
17				
18	Methods: Monitoring			
19				
20				
21	Data monitoring:	#21a	Composition of data monitoring committee (DMC);	Appendix
22	formal committee		summary of its role and reporting structure; statement of	1
23			whether it is independent from the sponsor and	
24			competing interests; and reference to where further	
25			details about its charter can be found, if not in the	
26			protocol. Alternatively, an explanation of why a DMC is	
27			not needed	
28				
29				
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31				
32	Data monitoring:	#21b	Description of any interim analyses and stopping	Appendix
33	interim analysis		guidelines, including who will have Access to these	1
34			interim results and make the final decision to terminate	
35			the trial	
36				
37				
38				
39	Harms	#22	Plans for collecting, assessing, reporting, and managing	17
40			solicited and spontaneously reported adverse events	
41			and other unintended effects of trial interventions or trial	
42			conduct	
43				
44				
45				
46	Auditing	#23	Frequency and procedures for auditing trial conduct, if	20
47			any, and whether the process will be independent from	
48			investigators and the sponsor	
49				
50				
51	Ethics and			
52	dissemination			
53				
54				
55	Research ethics	#24	Plans for seeking research ethics committee /	19
56	approval		institutional review board (REC / IRB) approval	
57				
58				
59	Protocol amendments	#25	Plans for communicating important protocol	20
60				

modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)

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7	Consent or assent	#26a	Who will obtain informed consent or assent from 19
8			potential trial participants or authorised surrogates, and
9			how (see Item 32)
10			
11			
12	Consent or assent:	#26b	Additional consent provisions for collection and use of 19
13	ancillary studies		participant data and biological specimens in ancillary
14			studies, if applicable
15			
16			
17	Confidentiality	#27	How personal information about potential and enrolled 19
18			participants will be collected, shared, and maintained in
19			order to protect confidentiality before, during, and after
20			the trial
21			
22			
23			
24	Declaration of	#28	Financial and other competing interests for principal 20
25	interests		investigators for the overall trial and each study site
26			
27			
28	Data access	#29	Statement of who will have Access to the final trial 18
29			dataset, and disclosure of contractual agreements that
30			limit such Access for investigators
31			
32			
33	Ancillary and post trial	#30	Provisions, if any, for ancillary and post-trial care, and 19
34	care		for compensation to those who suffer harm from trial
35			participation
36			
37			
38			
39	Dissemination policy:	#31a	Plans for investigators and sponsor to communicate trial 19
40	trial results		results to participants, healthcare professionals, the
41			public, and other relevant groups (eg, via publication,
42			reporting in results databases, or other data sharing
43			arrangements), including any publication restrictions
44			
45			
46			
47	Dissemination policy:	#31b	Authorship eligibility guidelines and any intended use of 20
48	authorship		professional writers
49			
50			
51	Dissemination policy:	#31c	Plans, if any, for granting public Access to the full 20
52	reproducible research		protocol, participant-level dataset, and statistical code
53			
54			
55	Appendices		
56			
57	Informed consent	#32	Model consent form and other related documentation Appendix
58	materials		given to participants and authorised surrogates 2
59			
60			

1 Biological specimens [#33](#) Plans for collection, laboratory evaluation, and storage 15-17
2 of biological specimens for genetic or molecular analysis
3 in the current trial and for future use in ancillary studies,
4 if applicable
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10 None The SPIRIT Explanation and Elaboration paper is distributed under the terms of the Creative
11 Commons Attribution License CC-BY-NC. This checklist can be completed online using
12 <https://www.goodreports.org/>, a tool made by the [EQUATOR Network](#) in collaboration with
13 [Penelope.ai](#)
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APPENDIX 2

Charter and responsibilities of the Data Monitoring and Safety Committee

A Data Monitoring and Safety Committee (DMSC) has been established, and its lead by Clinical Study Center at Thomayer University Hospital, Prague. The DMSC is an independent organ from the study investigators. During the period of recruitment to the study, interim analyses will be supplied, in strict confidence, to the DMSC. In the light of these interim analyses, the DMSC will advise the study steering committee (SSC) if, in its view, the active intervention has been proven, beyond reasonable doubt, to be different from the placebo in some or all patients

Based on the reports of DMSC, the Study steering committee (SSC) can then decide whether or not to modify recruitment to the study and its oncoming course. Unless this happens, however, the SSC, will remain ignorant of the interim results.

The frequency of interim analyses will depend on the judgement of the Chair of the DMSC, in consultation with the SSC. However, we anticipate that there might be two to three interim analyses and one final analysis.

The Chair of DSMC is Mr. Jiri Skopek, M.D., Ph.D. who is available on request at jiri.skopek1@ftn.cz

Premature termination of the study

An interim analysis is performed when 50% of patients have already got to Visit 5 (where primary outcome is evaluated.) The interim analysis is performed by a member of the study's statistical unit who is blinded for the allocation of the active study mixture. The statistician will report to the DMSC. The DMSC will have unblinded Access to all data and discuss the interim-analysis results with the SSC. The SSC decides on continuation or termination of the study and will report to the central Ethics committee. The study will be ended if the frequency of severe adverse events crosses the 5% line. Severe adverse event is defined as that one requiring hospitalisation.

Appendix 3: Informed consent for FMT donors



Informovaný souhlas dospělé osoby s účastí na výzkumu změn střevního mikrobiomu u dospělých pacientů se syndromem dráždivého tračníku ve vědeckém projektu týmů Thomayerovy nemocnice a Fakultní nemocnice v Motole.

Vážená paní/vážený pane,

syndrom dráždivého tračníku (irritable bowel syndrome, dále jen IBS) je nejčastější funkční onemocnění trávicího traktu, které pacienta výrazně omezuje v jeho každodenním životě. Může se projevovat různě, nejčastěji však jako delší dobu trvající bolest břicha s náhle vzniklým nutkáním na stolici. Léčba této nemoci je zdlouhavá, obtížná a ne vždy úspěšná. Dle recentních studií se však jako účinná léčebná metoda jeví transplantace střevní mikroflóry (faecal microbiota transplantation, dále jen FMT). A právě na její využití se zaměřuje náš projekt v podobě klinické intervenční studie.

Cílem projektu je zjistit, zda je transplantace stolice účinnou léčebnou metodu IBS a jak se po FMT mění složení střevní mikroflóry. K tomu abychom FMT mohli provést je potřeba mít vhodné dárce stolice. A právě zde byste nám mohli pomoci. Znalosti změn složení střevní mikroflóry po FMT bychom pak v budoucnu mohli využít buď k cílené ATB terapii negativně asociovaných bakterií nebo naopak k podávání probiotika prospěšných kmenů.

Proto si Vás dovoluujeme pozvat k účasti na projektu vědeckých týmů Thomayerovy nemocnice a Fakultní nemocnice v Motole. Přečtěte si, prosím, toto poučení. Pokud plně nerozumíte tomuto textu nebo pokud potřebujete doplňující informace, neváhejte se zeptat lékaře na emailu uvedeném níže. **Pokud souhlasíte s Vaší účastí ve studii, vyplňte prosím kontaktní údaje níže dokumentu a podepište prosím prohlášení, které se nachází v závěru tohoto informovaného souhlasu. Vaše účast je dobrovolná.** Tento souhlas můžete kdykoli zrušit, a to i bez udání důvodu.

Získání vzorku stolice by probíhalo ve vašem domácím prostředí. Stolice by bylo potřeba uchovat v běžném domácím mrazáku (teplota -20°C), k odběru byste byli vybaveni jednoduchými odběrovými sety s návodem a poučení o jejich používání. Po domluvě se členy vědeckého týmu (kontakt níže) by vzorky byly převezeny na naše pracoviště a hluboce zamrazeny (-80°C).

Celý proces je dvoufázový. Z prvního vzorku se provede molekulárně-genetická analýza a následně bioinformatické zpracování dat. Na základě výsledků bude vybráno asi 10-20 dárců, které kontaktujeme na základě informací uvedených níže. Splní-li kritéria vhodného dárce (pro vyžádání lze napsat na mail jiri.vejmelka@ftn.cz nebo zavolat na tel.č. 731446619), budou poté znovu požádáni o darování stolice.

Po zpracování pro účely aktuální studie budou vzorky uchovány v hlubokomrazícím boxu v laboratořích Fakultní nemocnice v Motole. Jejich další využití proběhne pouze po přesné specifikaci formou dalšího souhlasu a Vaším podepsáním nového souhlasu.

V tomto projektu řádně dbáme o bezpečnost osobních údajů podle platných zákonů. Zejména je pak zcela zachována úplná anonymita pacienta při odesílání vzorků mimo naše pracoviště nebo při

Appendix 3: Informed consent for FMT donors

zveřejňování vědeckých výsledků získaných z naší práce v odborných časopisech. Odebrané vzorky a z nich získané části jsou v našich laboratořích skladovány na dobu neurčitou, odděleně od osobních dat. Pokud byste v budoucnu svůj souhlas odvolali, Vaše jméno a ostatní osobní data budou bez prodlení vymazána z našich databází i papírových záznamů tak, aby se už nikdo nemohl dozvědět, komu vzorek patřil.

Bližší informace o nemoci jako takové můžete získat od členů vědeckého týmu:

MUDr. Jiří Vejmelka (Thomayerova nemocnice), tel: 731446619, email: jiri.vejmelka@ftn.cz

MUDr. Jakub Hurych (Fakultní nemocnice v Motole), tel. 224432089, email: jakub.hurych@lfmotol.cuni.cz

Souhlas se zpracováním osobních údajů (dále jen „Souhlas“)

udělený ve smyslu zákona č. 101/2000 Sb., o ochraně osobních údajů a o změně některých zákonů, ve znění pozdějších předpisů a s Nařízením Evropského parlamentu a Rady (EU) 2016/679

Já, níže podepsaný

Jméno a příjmení:

Datum narození:

Rodné číslo:

Kontaktní email:

Telefonní číslo:

Souhlasím se zpracováním svých osobních údajů/ osobních údajů osoby jejíž jsem zákonným zástupcem Fakultní nemocnicí v Motole a Thomayerově nemocnici v rozsahu těchto údajů:

Jméno, příjmení, titul, datum a místo narození, rodné číslo, národnost, pohlaví, místo trvalého pobytu, telefon, email, výška, hmotnost

Tento projev vůle je platný pouze v případě, že mé osobní údaje budou zpracovávány pouze v rozsahu nezbytném pro dosažení účelu zpracování uvedeného v tomto souhlasném prohlášení a v souladu s příslušnou legislativou v platném znění.

Souhlas je poskytnut za účelem:

Zpracování vzorku stolice pro vědecko-výzkumnou činnost mající za cíl přispět k porozumění změn střevního mikrobiomu u dospělých pacientů se syndromem dráždivého tračníku

Souhlasím se zpracováním svých osobních údajů Fakultní nemocnicí v Motole a Thomayerově nemocnici po dobu:

Do odebrání mého souhlasu

Souhlasím se zpřístupněním svých osobních údajů Fakultní nemocnici v Motole a Thomayerově nemocnici:

Fakultní nemocnice v Motole a Thomayerova nemocnice je oprávněna použít mé osobní údaje pouze v souladu s výše uvedeným účelem a po výše uvedenou dobu, nebo pro legitimní potřebu státních kontrolních

Appendix 3: Informed consent for FMT donors

orgánů a orgánů činných v trestním řízení.

Fakultní nemocnice v Motole a Thomayerova nemocnice je dále oprávněna poskytnout mé osobní údaje pouze subjektům spolupracujícím s **Fakultní nemocnicí v Motole a Thomayerovou nemocnicí** na dosažení primárního účelu, pro který je udělen tento souhlas. S takovými subjekty se **Fakultní nemocnice v Motole a Thomayerova nemocnice** zavazuje uzavřít smlouvu obsahující stejné podmínky pro zpracování mých osobních údajů. Zpracování bude probíhat v souladu s příslušnými právními normami o ochraně osobních údajů a s Nařízením Evropského parlamentu a Rady (EU) 2016/679 ze dne 27. dubna 2016 o ochraně fyzických osob v souvislosti se zpracováním osobních údajů a o volném pohybu těchto údajů a o zrušení směrnice 95/46/ES (obecné nařízení o ochraně osobních údajů).

Byl/a jsem poučen/a o tom, že poskytnutí údajů je dobrovolné.

Dále jsem byl/a v souladu s příslušnou legislativou poučen/a:

- O svém právu tento souhlas odvolat, a to i bez udání důvodu,
- O svém právu přístupu k těmto údajům a právu na jejich opravu,
- O svém právu na vymazání těchto údajů, pokud dochází k jejich zpracování v rozporu s ochranou definovanou příslušnou legislativou nebo v rozporu s tímto souhlasem, nebo byl souhlas odvolán, svém právu podat stížnost u Úřadu pro ochranu osobních údajů.

Byl/a jsem také poučen/a o tom, že tato svá práva mohu uplatnit doručením žádosti na adresu: Fakultní nemocnice v Motole, Samostatné oddělení pověřence pro ochranu osobních údajů, V Úvalu 84, Praha 5.

Beru na vědomí, že odvolání tohoto souhlasu může ovlivnit dosažení účelu, pro který byl tento souhlas vydán, pokud tohoto účelu nelze dosáhnout jinak.

Prohlašuji, že jsem textu poučení porozuměl(a) a byl jsem lékařem srozumitelně informován(a) o povaze daného vyšetření a že jsem měl(a) možnost klást lékařovi doplňující dotazy.

Na základě tohoto poučení dále prohlašuji, že souhlasím se zařazením svých vzorků do studie probíhající v **Thomayerově nemocnici a Fakultní nemocnici v Motole**, jejímž cílem je porozumět změnám složení střevního mikrobiomu u dospělých pacientů se syndromem dráždivého tračníku.

V dne

Jméno a příjmení vyšetřované osoby :

Podpis vyšetřované osoby

Prohlašuji, že jsem vysvětlil podstatu, účel a povahu odběrů pacientovi způsobem, který byl podle mého soudu srozumitelný.

Jméno a příjmení lékaře:

Podpis:

Datum:

APPENDIX 4 – INFORMED CONSENT FORM FOR FMT RECIPIENTS (CZECH)**Informovaný souhlas pacienta - studie fekální mikrobiální terapie
u pacientů se syndromem dráždivého tračníku**

Název studie: Fekální mikrobiální terapie u pacientů se syndromem dráždivého tračníku

Jméno pacienta:

Datum narození:

Pacient byl do studie zařazen pod číslem:

Odpovědný lékař:

1. Já, níže podepsaný (á) souhlasím s mou účastí ve studii. Je mi více než 18 let.
2. Byl (a) jsem podrobně informován (a) o cíli studie, o jejích postupech, a o tom, co se ode mě očekává. Lékař pověřený prováděním studie mi vysvětlil očekávané přínosy a případná zdravotní rizika, která by se mohla vyskytnout během mé účasti ve studii, a vysvětlil mi, jak bude postupovat při výskytu jejího nežádoucího průběhu. Beru na vědomí, že prováděná studie je výzkumnou činností. Beru na vědomí pravděpodobnost náhodného zařazení do jednotlivých skupin lišících se léčbou.
3. Informoval (a) jsem lékaře pověřeného studií o všech lécích, které jsem užíval (a) v posledních 3 měsících, i o těch, které v současnosti užívám. Bude-li mi nějaký lék předepsán jiným lékařem, budu ho informovat o své účasti v klinické studii a bez souhlasu lékaře pověřeného touto studií ho nevezmu.
4. Budu při své léčbě se svým lékařem spolupracovat a v případě výskytu jakéhokoliv neobvyklého nebo nečekaného příznaku ho budu ihned informovat.
5. Po celou dobu studie a další 4 týdny po jejím ukončení nebudu dárce krve.
6. Porozuměl (a) jsem tomu, že svou účast ve studii mohu kdykoliv přerušit či odstoupit, aniž by to jakkoliv ovlivnilo průběh mého dalšího léčení. Moje účast ve studii je dobrovolná.
7. Při zařazení do studie budou moje osobní data uchována s plnou ochranou důvěrnosti dle platných zákonů ČR. Do mé původní zdravotní dokumentace budou moci na základě mého uděleného souhlasu nahlédnout za účelem ověření získaných údajů zástupci nezávislých etických komisí a zahraničních nebo místních kompetentních úřadů. Pro tyto případy je zaručena ochrana důvěrnosti mých osobních dat. Při vlastním provádění studie mohou být osobní údaje poskytnuty jiným než výše uvedeným subjektům pouze bez identifikačních údajů, a to jako anonymní data pod číselným kódem. Rovněž pro výzkumné a vědecké účely mohou být moje osobní údaje poskytnuty pouze bez identifikačních údajů (anonymní data) nebo s mým výslovným souhlasem. Při předávání dat po 25. 5. 2018 bude zajištěna ochrana osobních údajů požadovaná „Nařízením Evropského parlamentu a Rady (EU) 2016/679 ze dne 27. dubna 2016 o ochraně fyzických osob v souvislosti se zpracováním osobních údajů“ známé pod označením GDPR.
8. S mou účastí ve studii není spojeno poskytnutí žádné odměny.
9. Porozuměl jsem tomu, že mé jméno se nebude nikdy vyskytovat v referátech o této studii. Já pak naopak nebudu proti použití výsledků z této studie.
10. Převzal/a jsem podepsaný stejnopis tohoto informovaného souhlasu.

Podpis pacienta:

Podpis lékaře pověřeného touto studií:

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BMJ Open

Protocol for faecal microbiota transplantation in irritable bowel syndrome – the MISCEAT study: a randomised, double-blind cross-over study utilising mixed microbiota from healthy donors

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3 1 **Protocol for faecal microbiota transplantation in irritable bowel syndrome – the MISCEAT**
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5 2 **study: a randomised, double-blind cross-over study utilising mixed microbiota from**
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8 3 **healthy donors**
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48 21 **Word counts:** 3865
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51 22 **Abbreviations:** IBS, Irritable Bowel Syndrome; IBS-D, diarrheal type of irritable bowel syndrome; IBS-

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53 23 M, mixed type of irritable bowel syndrome; IBS-C, constipated type of irritable bowel syndrome; IBS-

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55 24 SSS, Irritable Bowel Syndrome Severity Scale Score
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3 25 **Keywords:** irritable bowel syndrome, faecal microbiota transplantation, irritable bowel syndrome
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5 26 severity scale score, gut microbiome
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57 **ABSTRACT**

58 **Introduction.** Several studies have demonstrated dysbiosis in irritable bowel syndrome (IBS).

59 Therefore, faecal microbiota transplantation, whose effect and safety have been proven in

60 *Clostridioides difficile* infections, may hold promise in other conditions, including irritable bowel

61 syndrome. Our study will examine the effectiveness of stool transfer with artificially increased

62 microbial diversity in IBS treatment.

63 **Methods and analysis** A three-group, double-blind, randomized, cross-over, placebo-controlled

64 study of two pairs of gut microbiota transfer will be conducted in 99 patients with diarrhoeal or

65 mixed type of IBS. Patients aged 18-65 will be randomised into three equally sized groups: group A

66 will first receive two enemas of study microbiota mixture (deep-frozen stored stool microbiota mixed

67 from eight healthy donors); after eight weeks, they will receive two enemas with placebo (autoclaved

68 microbiota mixture), whereas group B will first receive placebo, then microbiota mixture. Finally,

69 group C will receive placebos only. The irritable bowel syndrome severity symptom score (IBS-SSS)

70 questionnaires will be collected at baseline and then at weeks 3,5,8,11,13,32. Faecal bacteriome will

71 be profiled before and regularly after interventions using 16S rDNA next-generation sequencing.

72 Food records, dietary questionnaires, anthropometry, bioimpedance, biochemistry and haematology

73 workup will be obtained at study visits during the follow-up period. The primary outcome is the

74 change in the IBS-SSS between the baseline and four weeks after the intervention for each patient

75 compared to placebo. Secondary outcomes are IBS-SSS at two weeks after the intervention and 32

76 weeks compared to placebo and **changes in the number of loose stools, Bristol stool scale,**

77 **abdominal pain and bloating, anthropometric parameters, psychological evaluation and the gut**

78 **microbiome composition.**

79 **Ethics and dissemination.** The study was approved by the Ethics Committee of Thomayer University

80 Hospital, Czechia (G-18-26); study results will be published in peer-reviewed journals and presented

81 at international conferences and patient group meetings.

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3 82 **Study registration number. NCT04899869**
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9 84 **STRENGTHS AND LIMITATIONS OF THIS STUDY**
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11 85 ➤ Usage of mixed microbiota from multiple donors inflates the diversity of transferred microbiota
12
13 by enriching it for numerous rare species.
14 86

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16 87 ➤ All interventions will be carried out using the same active mixed microbiota or the same placebo.
17

18 88 ➤ Each intervention consists of two consecutive transfers, which increases the probability that the
19
20 transferred microbiota engrafts.
21 89

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23 90 ➤ Microbiome profiling, food records, anthropometry and bioimpedance data allow detailed
24
25 monitoring of transfer effectiveness.
26 91

27 92 ➤ Mucosa-associated microbiota will not be assessed because the stool transfer will be performed
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29 by enema, not colonoscopy that would allow biopsies.
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94 INTRODUCTION

95 Irritable bowel syndrome (IBS) is characterised as recurrent abdominal pain on average at least one
96 day/week in the last three months, associated with two or more of the following criteria: 1) related
97 to defecation; 2) associated with a change in the frequency of stool; 3) associated with a change in
98 the form (appearance) of stool [1]. It is common among the adult European population (approx. 10%
99 [2]), but its aetiology is still unknown. It may, among other causes, include micro-inflammation,
100 disturbance of the brain-gut axis, inadequate secretion of bile acids, increased permeability of the
101 gut epithelial barrier, or gut dysbiosis. Dysbiosis in IBS has been suggested by several studies
102 (reviewed, e.g. in Rajilic-Stojanovic et al. [3]). There are indications that Firmicutes may be disturbed,
103 with *Dorea*, *Blautia* and *Roseburia* increased, whereas *Veillonella* and *Faecalibacterium* decreased.
104 Among Actinobacteria, a decrease in *Bifidobacterium* was noted, and among Proteobacteria,
105 *Enterobacteriaceae* were increased. Conflicting and heterogeneous results were reported for
106 Bacteroidetes. The major limitation of available studies is their cross-sectional character, which may
107 not be enough in a disease where diarrhoeal episodes alternate with normal stool composition or
108 constipation.

109
110 The faecal microbiota transplantation (FMT) has gained popularity by its remarkable effect in
111 recurrent *Clostridioides difficile* infections, where it has now become a recognised life-saving therapy
112 [4]. The first published randomized, double-blinded study on FMT in IBS, published in 2018 when
113 starting our study [5], used stool intervention from an allogeneic donor or autologous stool. The
114 intervention was centred on a well-defined group of IBS of predominantly diarrhoeal form. The stool
115 was transferred by colonoscopy to the cecum. The primary outcome was an improvement in the
116 *Irritable Bowel Syndrome - Severity Symptom Score* (IBS-SSS). The treatment was associated with a
117 significant effect at three months but not at 12 months post-intervention [5]. This study used single
118 donors and did not assess stool microbiota. Thus, the transferred microbiota likely varied between
119 transfers both in their composition and in their diversity. Since then, more studies focused on FMT in

1
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3 120 IBS have been carried out [6, 7, 8, 9, 10, 11]. They differed in design, but none of them used a mixed
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5 121 microbiota from multiple donors as the active substance. Furthermore, a recent meta-analysis of
6
7 122 randomized control trials on FMT in IBS (including the above-mentioned articles) pointed out
8
9 123 insufficient evidence quality to support recommending FMT in the treatment of IBS. [12]
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14 125 Our study protocol aims to test whether faecal microbiota transplantation of mixed microbiota from
15
16 126 several selected donors can alleviate symptoms of IBS measured by IBS-SSS four weeks after the
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18 127 intervention, as compared to autoclaved placebo. The secondary study aims to test the acute (after
19
20 128 two weeks) and the long-term effect (after six months) on symptoms relief. **We also focus on the**
21
22 129 **number of loose stools, Bristol stool scale, abdominal pain and bloating, BMI, fat content, waist**
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24 130 **circumference, skinfold thickness, psychological evaluation and the gut microbiome composition.**
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30 132 We hypothesise that the transfer of active microbiota of high diversity can lead to changes in the
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32 133 patient's gut microbiome composition and/or function to alleviate IBS symptoms.
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134 **METHODS AND ANALYSIS**

135 **Study design**

136 This is a three-group, double-blind, placebo-controlled, randomized, cross-over study in adult
137 patients diagnosed with IBS (diarrhoeal or mixed form) according to Rome IV criteria. Each study
138 subject will undergo two pairs of FMT (a total of four enemas for each patient), with the pairs of
139 transfers being eight weeks apart. The active intervention substance is a mixed stool microbiota
140 derived from healthy individuals who were preselected for high alpha diversity of their microbiome
141 and distance in community ordination from IBS patient's microbiota. Placebo is the same mixture,
142 inactivated by autoclaving.

143
144 The study subjects are randomly assigned to one of three groups: A) enema with active substance
145 first and with placebo second or B) enema with placebo first and active substance second or C)
146 enemas of placebo only (detailed scheme in **Figure 1**). Eligible participants will be followed-up for 32
147 weeks after the first intervention to monitor symptom severity scoring of IBS (IBS-SSS), with regular
148 profiling of their gut microbiome and **other parameters like the number of loose stools, Bristol stool**
149 **scale, abdominal pain and bloating, BMI, fat content, waist circumference, skinfold thickness, and**
150 **psychological evaluation.**

151 The placebo group is planned because of the unknown onset and duration of the intervention effect:
152 if the beginning of an effect is delayed, or if it persists for a long time, simple cross-over design would
153 not have sufficient power due to the carry-over effect. In case the FMT was associated with
154 significant but not durable amelioration of the status, the control group would still increase the
155 statistical power.

156

157 This study protocol is reported as per the SPIRIT guidelines [13] (for the SPIRIT checklist see **Appendix**
158 **1**).

159

160 **Study setting**

161 The participants are recruited at a single center, the Department of Internal Medicine, Thomayer
162 University Hospital in Prague, Czech Republic. This hospital has approximately 1,000 beds, including
163 80 in ICU's, serves approximately 50,000 patients per year. The center is experienced in treating
164 patients with IBS and other functional gastrointestinal disorders, with about 200 such patients
165 registered and further subjects coming for consultations from other workplaces to this tertiary
166 referral centre.

167

168 **Recruitment and eligibility criteria**

169 Stool donors

170 Stool donor candidates were recruited among blood donors at Thomayer University Hospital and
171 medical students in their first year of study (i.e. preclinical) from the 2nd Faculty of Medicine, Charles
172 University, Prague. We obtained stool samples from 58 such candidates fulfilling the inclusion criteria
173 (**Table 1**). Based on their high bacterial alpha-diversity and by the position on the ordination plot of
174 the weighted Unifrac distance against 46 patients with IBS-D (**Figure 2**), 14 candidates proceeded to
175 the safety screening, whereby eight passed it (for reasons of candidate's exclusion, see **Figure 3**).

176

177 After 14 potential donors were selected based on the microbiota composition, they were screened
178 for infectious diseases and clinically examined as indicated by the *European consensus conference on
179 faecal microbiota transplantation in clinical practice guidelines* [14] (**Table 2**). All subjects were also
180 repeatedly tested for SARS-CoV-2 from both nasopharyngeal swab and stool. Six candidates were
181 excluded (for reasons, see **Figure 3**), whereas eight became regular stool donors. These eight donors
182 were regularly investigated as follows:

- 183 - at every donation: by questionnaire for gastrointestinal symptoms, antibiotic usage, unprotected
184 sex, travelling to exotic countries; clinical signs of COVID-19; the presence of SARS-CoV-2 in the
185 donated stool;

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3 186 - every 4 weeks: for SARS-CoV-2 from nasopharyngeal swab;
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5 187 - every 8-12 weeks: for all other stool tests mentioned in **Table 2**.
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10 189 Prospective study participants

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12 190 Patients diagnosed with IBS-D (diarrheal type) or IBS-M (mixed diarrhoeal and constipation type) who
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14 191 fulfil the inclusion and exclusion criteria listed in **Table 3** are recruited via regular' patient's check-ups
15
16 192 at the Gastroenterological unit at Thomayer University Hospital, by referrals from their general
17
18 193 practitioners, following our newspaper articles or word of mouth.
19
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22 23 195 **Study microbiota mixture for intervention**

24
25 196 The intervention microbiota is a mixture of regular stool donations from the eight regular donors.

26
27 197 The collection of stools for this purpose is already completed. The donors were advised to regularly
28
29 198 defecate at their home toilet into a clean plastic bag placed in Fecotainer (Excretas Medical, NL) with
30
31 199 an Anaerogen bag (Thermo Scientific, USA). This bag generated an anaerobic atmosphere during
32
33 200 transport to ensure anaerobes survival. The stool was transported to the laboratory with the
34
35 201 maximum allowable time until processing being 6 hours; the actual time was approximately 1.5
36
37 202 hours. The stool was weighed upon arrival, inspected for blood admixture, and immediately
38
39 203 processed by blending with a solution consisting of sterile 0.9% saline (160 ml per 100 g of stool),
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41 204 sterile phosphate buffer saline at pH 7.4 (20 ml per 100 g of stool) and sterile 99.5% glycerol (20 ml
42
43 205 per 100 g stool, which is approximately 10% of solution's volume; therefore, it is unlikely to have
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45 206 laxative properties upon administration). From our experience, ~ 105 ml of the study mixture
46
47 207 represents ~40 g of stool. The mixture was then filtered through a sterile stainless steel mesh of 0.8
48
49 208 mm pore size into a sterile plastic bottle, which was then immediately frozen at -80°C. Whenever
50
51 209 possible (blending or post-filtration), the procedure was performed under a nitrogen atmosphere to
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53 210 protect obligate anaerobes. All stool portions were mixed together in a large stainless steel bucket
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55 211 using an electric mortar mixer under anaerobic conditions and at low temperature (on ice).
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5 213 Based on the recommendation from the Nanjing consensus [15], the bacterial cell content of the
6
7 214 study microbiota mixture was quantified. We performed a real-time PCR of the 16S rRNA gene with a
8
9 215 standard curve derived from bacterial culture and controls from previously used stool transplants
10
11 216 from another centre. It was estimated that the cell count in the transfer ranged between 2×10^{12} and
12
13 217 1×10^{13} (depending on the expected composition of the microbiota as to the 16S gene count per an
14
15 218 average bacterial cell). Unfortunately, the Nanjing consensus [15] provides neither reference to the
16
17 219 cell counting method (Table 2 therein) nor to control materials. Therefore more exact direct
18
19 220 comparison of the requested quantities is not possible.
20
21 221

22
23 222 The mixed microbiota substance was divided into aliquots of 13-14 g (which is ~ 35 ml). Two-thirds of
24
25 223 the tubes served as a placebo: they were immediately autoclaved at 121°C for 30 minutes with slow
26
27 224 cooling. Pre-sterilised tubes were used to ensure that autoclaved placebos would not be visually
28
29 225 distinguishable from tubes with the active substance. Assignment of tubes to the autoclave,
30
31 226 numbering, sealing, and labelling were done under the guidance of a statistical unit member (see
32
33 227 below).
34
35 228

36
37 229 All aliquot tubes are kept frozen at -80°C in the same type of plastic tubes, labelled by codes. Three
38
39 230 such aliquots represent one dose for FMT (~ 40 g of stool, in ~ 105 ml). Aliquoting into multiple 50 ml
40
41 231 tubes instead of one larger volume was decided because of the availability of durable plastic, which
42
43 232 must be both autoclavable and deep frost resistant.
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46
47 234 Before administering, the study microbiota mixture will be thawed in a warm (37°C) water bath, with
48
49 235 intermittent mixing by inverting the tubes.
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54 55 56 57 58 59 237 **Randomization, allocation and blinding**

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3 238 At Visit 1, each patient is randomised into one of three equally sized groups (Figure 1) as described in
4
5 239 the *Study design*. Randomisation assignments is generated in advance in blocks of nine and stored in
6
7 240 a protected database. For each patient, anonymous codes for tubes containing either active study
8
9 241 microbiota mixture or placebo is received. Thus, the true assignment will remain concealed for the
10
11 242 patients and the study staff until the end of the study observation period. The Investigator is
12
13 243 encouraged to maintain the blind as far as possible. The actual allocation must not be disclosed to
14
15 244 the patient and/or other study personnel including other site personnel, monitors, corporate
16
17 245 sponsors or project office staff; nor should there be any written or verbal disclosure of the code in
18
19 246 any of the corresponding patient documents.
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23 247

248 **Study Intervention**

249 Study substance is administered during Visit 2+3 and then again 7+8 as a retention colon enema and
250 will be held optimally for at least 30 minutes. Bowel preparation is applied the day before the
251 intervention (prior to Visit 2 and Visit 7) (natrii picosulfas 10 milligrams, magnesii oxidum leve 3,5
252 grams, acidum citricum 12 grams). No preparation is performed before the second enema in the pair
253 (visits 3 and 8).
254

255 A rectal tube is inserted into the rectum, and the enema is applied. Application kit (Irrigator PN
256 0462/E/93, Erilens, Czechia) is used. After the enema is applied, the patient position is changed to
257 enable the study substance to be spread within the colon. The exact time of the enema completion is
258 recorded as well as the enema retention time.
259

260

260 **Outcomes**

261 Primary outcome

262 The primary outcome is the change in the IBS severity symptom score (IBS-SSS) in the active
263 microbiota group relative to the placebo group. The change will be evaluated as the difference

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3 264 between the score at four weeks after the intervention (study weeks 5 or 13, respectively, see **Figure**
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5 265 **1**) and the baseline score (week -1 in group A or week 8 in group B).

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9
10 267 Secondary outcomes

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13 268 - The acute change in the IBS severity symptom score (IBS-SSS) between baseline and two weeks
14
15 269 after intervention (study weeks 3 and 11, respectively, see **Figure 1**).

16
17 270 - The long-term change in the IBS severity symptom score (IBS-SSS) between baseline (week -1)
18
19 271 and week 32 (see **Figure 1**). The long term change will compare group C (placebo only) to
20
21 272 merged groups A+B (active study microbiota mixture).

22
23
24 273 - **Following outcomes compare changes in the active microbiota group relative to the placebo**
25
26 274 **group between baseline and study week 32:**

27
28 275 • **Quantity of loose stools per day**

29
30 276 • **Stool consistency evaluated by the Bristol stool scale**

31
32 277 • **Abdominal pain measured by the Visual Analogue Scale (VAS)**

33
34 278 • **Frequency of bloating per week**

35
36 279 • **Body Mass Index in kg/m²**

37
38 280 • **Body fat mass estimated by measuring combined skinfold thickness in millimetres at given**
39
40 281 **locations (biceps, triceps, subscapular, suprailiac)**

41
42 282 • **Percentage of body fat mass measured by bioelectrical impedance analysis**

43
44 283 • **Waist circumference in centimetres**

45
46 284 • **The psychological and well-being effects of the therapy scored by IBS-QoL questionnaires**

47
48 285 • **The faecal microbiome's alpha diversity measured by the Chao index**

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50 286 • **The faecal microbiome's beta diversity assessed by the quantitative Bray-Curtis index**
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52 287 **ordinated by non-metric multidimensional scaling (NMDS)**
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3 288 • **Quantity of *Blastocystis* sp. assessed by a specific quantitative PCR assay measured in**
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5 289 **genomic equivalents per microlitre DNA**
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10 291 **Data collection and follow-up**

11
12 292 Timing of assessments

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14 293 At visit 1 (the randomization), the patient is given detailed instructions and thoroughly instructed by
15
16 294 the study team. The patients are asked to keep the identical type of diet throughout the observation.
17
18 295 They are asked to regularly (once a week) fill the study questionnaire. A study team member sends
19
20 296 that via the Survey Monkey smartphone application, an online survey development cloud-based
21
22 297 software. Relevant data are entered in a structured manner (frequency of defecation, Bristol stool
23
24 298 scale, pain measures, other symptoms, dietary records etc.). This member also frequently
25
26 299 communicate with study participants and answer any questions regarding the study to keep the
27
28 300 patient's adherence. An overview of the examinations at each visit and the timing of the study visits
29
30 301 could be seen in **Table 4**.

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36 303 Irritable bowel syndrome severity scale score (IBS-SSS).

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38 304 The IBS-SSS is a five-question survey that reflects 1) the severity of abdominal pain, 2) frequency of
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40 305 abdominal pain, 3) severity of abdominal distention, 4) satisfaction with bowel habits, and 5)
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42 306 interference with quality of life over the past ten days. Subjects respond to each question on a 100-
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44 307 point analogue scale ; thus, the score can range from 0 to 500, with higher scores indicating more
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46 308 severe symptoms.[16]

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52 310 At eligibility screening, the patients is given instructions on how to fill the IBS-SSS questionnaires (via
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54 311 the Survey Monkey application). The questionnaires are filled in at eligibility screening and then at
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56 312 week -1, 3, 5 (before the first intervention, at the presumed peak of its effect, and after further 2
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58 313 weeks), then at weeks 8, 11, 13 (similarly with the second intervention), and finally at week 32.
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5 315 Weight, height, bioimpedance

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7 316 Body weight, height and bioimpedance is examined during Visit 0, 1, 4, 5, 9 and 11. Medical Body

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10 317 Composition Analyzer Seca mBCA 515, (Seca, Germany) is used to measure changes in body

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12 318 composition (8-point bioelectric impedance analysis at a frequency of 5 - 50 kHz with a current of

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14 319 100 μ A), scanning performed with three pairs of hand electrodes and two pairs of leg electrodes,

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16 320 measurements performed with light clothing and without metal objects (jewellery, keys). The weight

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18 321 is determined in patients wearing underwear using the Seca mBCA 515. The height is determined by

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20 322 a standardised technique with a metal stadiometer with an accuracy of 1 mm. Seca analytics 115

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22 323 software is used to analyse the obtained data (Seca, Hamburg, Germany). The measurements is

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24 324 performed according to the NIHR Southampton Biomedical Research Centre standard protocol (Seca

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26 325 mBCA, NIHR Southampton Biomedical Research Centre, 2014).

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32 327 Detailed anthropometry

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34 328 It is performed by nutritional therapists in Visit 1, 5, 10 and 11. It involves weight, abdominal (waist)

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36 329 circumference, buttocks (hip) circumference, thigh circumference, and skinfolds (thigh, triceps,

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38 330 subscapular, suprailiacal).

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43 332 Serum workup, archiving serum+plasma

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45 333 Blood is sampled at Visits 0, 4, 9, 11 and will include: A) serum+plasma archiving, B) serum workup.

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47 334 Laboratory panel testing will comprise sodium, potassium, chloride, urea, creatinine, glucose,

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49 335 calcium, phosphate, total protein and albumin, AST, ALT, ALP, **GGT**, bilirubin, lipid panel, HS-CRP,

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51 336 blood cell count with differential count, INR, urine analysis (sediment and biochemistry). One plasma

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53 337 and one serum aliquots are made at these visits and frozen for forensic reasons.

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59 339 Psychological evaluation

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3 340 It is performed during Visit 0 and Visit 11 using a structured questionnaire evaluated by a qualified
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5 341 psychologist.

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9 343 Dietary questionnaire & advice, evaluation of food records

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11 344 It is performed by nutritional therapists at Visit 4 and 9 and includes: evaluation of food records will
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13 345 include: overall daily energy intake, proteins, carbohydrates and lipids calculations and dietary fibre.

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18 347 Gut microbiome composition

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20 348 Faecal samples are collected at home by the subjects in the same way as described for donors above
21
22 349 and at time points indicated in the sections above. If not immediately brought to the visit, the stool is
23
24 350 frozen in a home freezer and then transported in a frozen tube container. DNA extraction is
25
26 351 performed using the PowerSoil kit (Qiagen), and the bacteriome is characterised by 16S rDNA
27
28 352 amplicon profiling using the tagged primers according to Schloss protocol [17] and sequencing on a
29
30 353 MiSeq instrument with the 2x250 bases sequencing kit (both Illumina, USA).

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36 355 The first steps of bioinformatic analysis will be performed in the DADA2 package[18]. Statistical
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38 356 analyses and visualisation will be then performed in R with its Phyloseq package. The functional
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40 357 potential of the bacteriome will be assessed using the PICRUST software, which predicts functional
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42 358 capabilities based on the 16S rDNA profiles.

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47 360 The virome is assessed in a total of four stool samples per patient at Visit 0, 4, 9 and 11. The aim of
48
49 361 this analysis is to assess the repertoire of major bacteriophages. The virome analysis is based on
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51 362 metagenomic sequencing of total DNA from a virus-enriched stool sample, according to the
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53 363 previously published protocol [[19]].

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3 365 Finally, a simple PCR-based semi-quantitative parasite screening aims to identify several mostly
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5 366 benign unicellular parasites (e.g. *Blastocystis*, *Dientamoeba*, *Entamoeba*, *Endolimax*).
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368 **Safety monitoring**

11 369 All data are regularly monitored by the research team for any adverse events, and all potential
12
13 370 adverse events are recorded. Contacts to study coordinators active 24/7 are provided in case adverse
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15 371 effects occur. If any concerns are identified during the screening or clinical assessment of donors or
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17 372 recipients, further clinical evaluation and/or examination is immediately realised. All the concerns
18
19 373 during the study are assessed, and the recipient will be withdrawn if this is thought to be in his best
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21 374 interest. A Data Monitoring and Safety Committee (DMSC) has been established and based on the
22
23 375 data from the planned interim analysis, has the right to terminate the study if the frequency of
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25 376 severe adverse events crosses the 5% line (for a closer description of DMSC, its responsibilities and
26
27 377 premature termination of the study see **Appendix 2**).
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379 **Sample size and power calculation**

36
37 380 The study is powered to detect an absolute improvement of 62.5 points in IBS-SSS score over 8
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39 381 weeks (which is 25% of the expected mean baseline score 250) between the active microbiota
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41 382 intervention compared to placebo. With a sample size of 33 per group (**99 total**), the probability of
42
43 383 detecting such an improvement is at least 0.9. This calculation assumes 20% dropout rates, variance
44
45 384 in IBS-SSS scores 100 (see the results in [20]), a correlation between the final and baseline IBS-SSS
46
47 385 scores 0 (with a positive correlation, the power is higher), and no carry-over or temporal effect.
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387 **Data management**

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54 388 Data from IBS-SSS, frequency of urgent defecations, Bristol stool scale, abdominal pain and bloating
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56 389 are collected and stored via the application Survey Monkey. All anthropometric data are entered and
57
58 390 stored in password-protected platforms integrated within the hospital information system. Only the
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3 391 researchers involved in the study have access to the final study dataset (IBS-SSS, frequency of urgent
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5 392 defecations, Bristol stool scale, abdominal pain and bloating), which will be shared in an anonymised
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7 393 form via the Zenodo repository. The only data in this manuscript are bacteriome data; their
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10 394 anonymised form will be available on reasonable request.
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14 396 **Statistical analyses**

16 397 The primary outcome analysis will be based on the difference in IBS-SSS scores over the second
17
18 398 treatment period (week 14 vs week 8) minus the change over the first treatment period (week 5 vs
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20 399 week -1). This difference will be used as a response in a linear model, with intercept corresponding to
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22
23 400 the temporal effect (seen in the placebo group C), an indicator of group A corresponding to the cross-
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25 401 over effect (resulting from administration of placebo after active microbiota) and differences in
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27 402 indicators for groups A and B modelling the effect of active microbiota. A robust sandwich estimator
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29 403 of the variance matrix will be used to adjust for potentially unequal variances between the groups.
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32 404 Analyses of secondary outcomes will proceed by a similar methodology, comparing absolute or
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34 405 relative differences of the post-intervention measure of each outcome relative to its baseline value.
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36 406 The CONSORT 2010 guidelines will be followed in reporting the main trial results.
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42 408 **Study status**

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45 409 The study was registered at clinicaltrials.gov (NCT04899869) on May 25th 2021. The first patient was
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47 410 recruited on June 17th 2021, and the first intervention was applied on July 29th 2021. As of August
48
49 411 17th 2021, 12 patients have signed the informed consent, and six interventions have been applied. It
50
51 412 is expected that the study will be completed in December **2023**.
52

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56 414 **Patient and public involvement**

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3 415 Information on the study has been spread at conferences, in newspapers and by local
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5 416 gastroenterologists contacted by researchers. Everyone interested got information material, which
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7 417 allowed the potential subjects to read about the study and reach the researchers if they wanted to
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9 418 participate. Participants were not involved in the development, recruitment of other participants or
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11 419 conduct of the study. All recipients are asked about any possible adverse effects of treatment at
12
13 420 regular visits planned according to **Figure 1**; a thorough investigation will be conducted if any occur.
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15 421 After completing the data analysis, all recipients will receive information about their results and be
16
17 422 offered a roll-over (receiving an active study microbiota mixture).
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22 23 424 **ETHICS AND DISSEMINATION**

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25 425 Ethics approval for this study was granted in June 2018 by the Ethics Committee of the Institute for
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27 426 Clinical and Experimental Medicine and Thomayer Hospital (Václavská 800, 140 59 Prague 4, Czech
28
29 427 Republic). Involvement in this study is completely voluntary; donors and recipients are required to
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31 428 provide written informed consent prior to participation in the study (see **Appendix 3 and 4**).
32
33 429 Recipients and their caregivers are informed of unexpected findings or unrecognised conditions and
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35 430 by possible future usage of their specimens in ancillary studies by trained physician or nurse; further
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37 431 medical care will be arranged. Study donors received financial compensation to pay for the required
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39 432 travelling costs when donating the stool. The patient will be offered a roll-over into an observational
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41 433 study with the administration of active microbiota. The patients are informed of this option at the
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43 434 start of the study and regularly reminded.
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48 435 We aim to publish findings in impact peer-reviewed international journals. Gastroenterologists,
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50 436 internists and other care providers will be informed through the national conference meetings,
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52 437 journals and patient groups meetings.
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3 439 **Protocol amendment number:** 01. Modification of the study protocol will be communicated to the
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5 440 Ethics committee.

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8 441 **Registration details** This study is registered with ClinicalTrials.gov (NCT04899869).

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10
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16
17 445 mixture preparation. We also thank Marcela Krutova, Jan Tkadlec, Daniela Lzicarova, Kamila
18
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24
25 449 screening of the study donors.

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30 450 **Contributors** OC, PK, JH, JV, MK contributed to the conception and design of the study. OC, PK, JH
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32 451 and JV drafted the protocol with input from all other authors. JV and PK contributed to the patients
33
34 452 recruitment. JH, LV, LK and OC contributed to the microbiome analysis for donor selection. JH, OC
35
36 453 and JV contributed to the donor screening. LV, JH and OC contributed to the study microbiota
37
38 454 mixture preparation. MK contributed to the power size calculations and statistical analysis. VL
39
40 455 contributed to the randomization. JH and JV contributed equally to this paper, OC and PK contributed
41
42 456 equally either.

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47
48 458 19-01-00127 . Funding received from this grant support direct research cost. All rights reserved

49
50
51 459 **Competing interests** None declared. No money from commercial sponsors was used.

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55 460 **Patient consent for publication** Not required.

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3 461 **Ethics approval** Ethics approval for this study was granted in June 2018 by the Ethics Committee of
4
5 462 the Institut for Clinical and Experimental Medicine and Thomayer Hospital (Videňská 800, 140 59
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7 463 Prague 4, Czech Republic).

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10 464 **Provenance and peer review** Not commissioned; externally peer-reviewed.

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470 **FIGURES AND ILLUSTRATIONS**

471 **Figure 1** Per protocol intervention scheme: the visits, questionnaires and samples

For peer review only

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3 472 **Figure 2** Ordination plot on the weighted Unifrac distance at the genus level for selection of the
4 473 donor candidates based on their gut microbiome alpha- and beta-diversity
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6 474

7 475 These are the results of a comparative microbiome case-control study which helped us to preselect 14 donor candidates.
8 476 Alpha diversity calculation was based on Chao 1 index. The beta-diversity calculation was based on non-metric dimensional
9 477 scaling (NMDS) with weighted UniFrac distance matrix for bacterial Genus. NMDS axis 1 captured 46.8% of variability;
10 478 NMDS axis 2 represents 14.7% of the variability. Healthy subjects were enriched in negative values of the first ordination
11 479 axis; therefore, we selected donors among healthy subjects in this half of the graph and based on their microbiome's alpha
12 480 diversity. The reason for concentrating healthy and enriched subjects in the left part of the plot could be their younger age.
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482 **Figure 3** Process of donor selection and reasons for their excluding

For peer review only

483 **Table 1.** Inclusion and exclusion criteria for FMT donors

Inclusion	Adults aged 18-65 years
	BMI 18,5-27 kg/m ²
	Lack of restrictive diets (diet discussed with experienced gastroenterologist)
	Bristol stool scale usually between 3 and 4
	High alpha diversity and significant difference in beta-diversity from patients (using 16S rDNA sequencing)
	Expected to donate regularly
	Consented in writing
Exclusion	Any chronic GI disease in patient's history (coeliac disease, inflammatory bowel disease, irritable bowel syndrome, colorectal carcinoma), or active acute GI issues (infectious gastroenteritis or enterocolitis, frequent bloating, diarrhoea or vomiting)
	Chronic disease in ' 'patient's history (cancer, autoimmune conditions, type 2 diabetes mellitus, coronary heart disease, hypertension, hypercholesterolemia, gout)
	<i>Clostridiodes difficile</i> infection in patient's history
	Colorectal carcinoma in family history
	Any restrictive diet habits (raw-vegans, fruitarians, keto or carnivore)
	Any systemic antibiotics in the last 6 months
	Using proton-pump inhibitors in the last 6 months
	Regular unprotected sex with unknown persons

484

485 **Table 2** Laboratory screening of the FMT donors

Blood testing
Hepatitis A, hepatitis B, hepatitis C and hepatitis E viruses (serology)
HIV-1 and HIV-2 (p24 antigen)
Treponema pallidum (serology)
Strongyloides stercoralis (serology)
Complete blood cell count with differential
Creatinine, aminotransferases, bilirubin
Stool testing
<i>Clostridioides difficile</i> (cultures, antigen testing)
Common enteric pathogens, including Salmonella, Shigella, Campylobacter, shiga toxin-producing <i>Escherichia coli</i> , Yersinia and <i>Vibrio cholerae</i> (cultures)
Antibiotic-resistant bacteria (ARB), including vancomycin-resistant Enterococci, meticillin-resistant <i>Staphylococcus aureus</i>
Gram-negative ARB including extended-spectrum β -lactamase-producing <i>Enterobacteriaceae</i> , and carbapenem-resistant <i>Enterobacteriaceae</i> /carbapenemase-producing <i>Enterobacteriaceae</i> (cultures)
Norovirus, rotavirus, adenovirus, sapovirus (PCR)
SARS-CoV-2 (reverse transcription -PCR)
Common intestinal parasites, including <i>Giardia intestinalis</i> , <i>Cryptosporidium parvum et hominis</i> (cultures and PCR), <i>Blastocystis hominis</i> *, <i>Dientamoeba fragilis</i> * (both PCR only)

486

487 *) Based on the literature [21], we decided to test both parasites but did not exclude the donors if they were
 488 tested positive and had no gastrointestinal symptoms. *Blastocystis* is believed to be commensal of the gut.
 489 *Dientamoeba's* status is not exact; however, based on our experiment, it does not survive freezing at -80 °C and
 490 thawing to 5°C when mixing the study microbiota mixture [22]. Therefore it can't do any harm.

491

492 The screening strategy is based on [14].

493 **Table 3.** Inclusion and exclusion criteria for recipients of FMT

Inclusion	Adults 18-65 years
	Diagnosed with IBS-D or IBS-M according to the Rome IV criteria
	Expected adherence to following the protocol
	Written consent to the study
Exclusion	The use of antibiotics and probiotics within one month prior to faecal microbiota transplantation
	History of inflammatory bowel disease or gastrointestinal malignancy, systemic autoimmune diseases (ongoing or in history)
	Previous abdominal surgery (other than appendectomy or cholecystectomy or hernioplasty or cesarean section)
	HIV infection or other active infection
	Renal or hepatic disease (both defined by biochemistry workup)
	Diabetes mellitus, abnormal thyroid functions not controlled by thyroid medications
	Bipolar disorder or schizophrenia (ongoing or history thereof), moderately severe depression defined by Patient Health Questionnaire-9 (PHQ-9) score > 15
	Anxiety defined by a Generalised Anxiety Disorder 7 (GAD7) score > 10, with any organic causes that can explain the symptoms of IBS
	Current pregnancy and lactation

494

495 **Table 4.** The study visits with planned activities

Visit	0	1	X	2+3	4	X	5	6	7+8	9	X	10	11
Study Week	?	-2	-1	1	2	3	5	8	9	10	11	13	32
Eligibility evaluation (E) / Randomization (R) / Wrap-up visit (W) ⁽¹⁾	E	R											W
Colon enema with the study substance (active microbiota or placebo)				XX					XX				
Irritable bowel syndrome severity scale score		X	X			X	X	X			X	X	X
Weight, height, bioimpedance		X			X		X			X		X	X
Detailed anthropometry		X					X					X	X
Serum workup, archiving serum+plasma		X			X					X			X
Psychological evaluation		X											X
Dietary questionnaire & advice, evaluation of food records ⁽²⁾					X					X			
Stool samples for microbiome analysis	X	X	X		X	X	X	X		X	X	X	X

496 (1) Here, the patient is offered a roll-over into an observational study with active microbiota administration. The patients
497 will be informed of this option at the start of the study and regularly reminded.

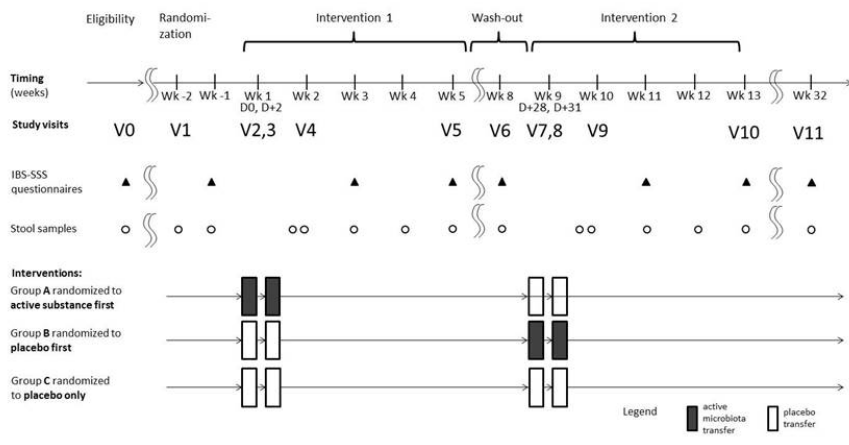
498 (2) For IBS-SSS questionnaires assessing the primary outcome, please see the intervention scheme in Figure 2. Their
499 administering is not linked to study visits.

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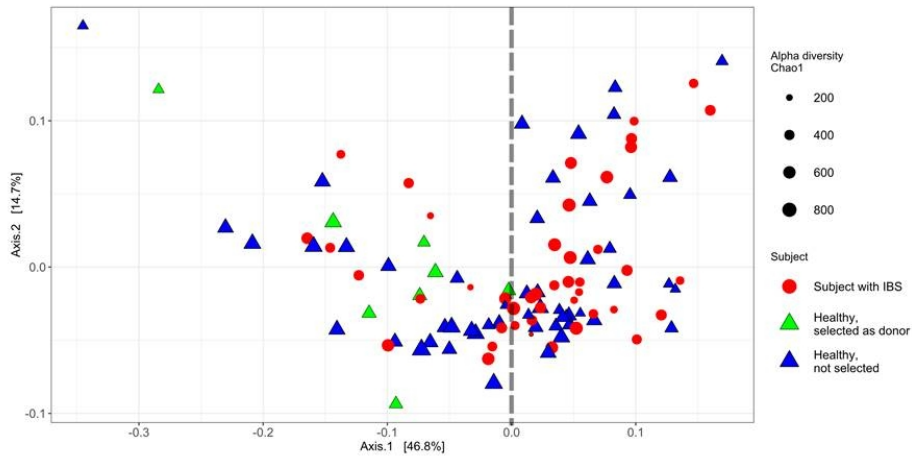
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Per protocol intervention scheme: the visits, questionnaires and samples

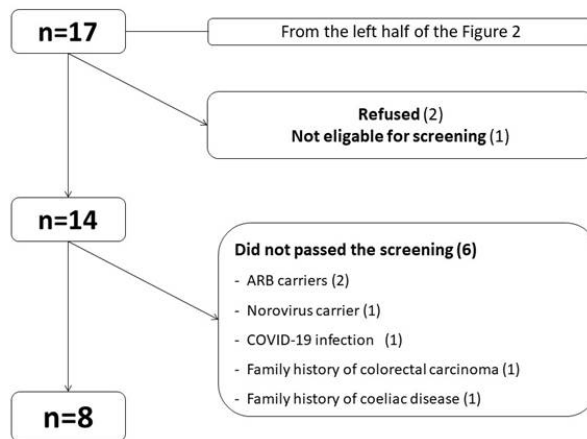
254x190mm (96 x 96 DPI)



Ordination plot on the weighted UniFrac distance at the genus level for selection of the donor candidates based on their gut microbiome alpha- and beta-diversity. These are the results of a comparative microbiome case-control study that helped us to preselect 14 donor candidates. Alpha diversity calculation was based on Chao 1 index. The beta-diversity calculation was based on non-metric dimensional scaling (NMDS) with a weighted UniFrac distance matrix for bacterial Genus. NMDS axis 1 captured 46.8% of variability; NMDS axis 2 represents 14.7% of the variability. Healthy subjects were enriched in negative values of the first ordination axis; therefore, we selected donors among healthy subjects in this half of the graph and based on their microbiome's alpha diversity. The reason for concentrating healthy and enriched subjects in the left part of the plot could be their younger age.

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Process of donor selection and reasons for their excluding

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Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Appendix 1 SPIRIT CHECKLIST

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	6 and 19
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	NA – not received yet.
Protocol version	#3	Date and version identifier	19
Funding	#4	Sources and types of financial, material, and other support	20
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	20
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	20
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	20

1	Roles and	#5d	Composition, roles, and responsibilities of the	20
2	responsibilities:		coordinating centre, steering committee, endpoint	
3	committees		adjudication committee, data management team, and	
4			other individuals or groups overseeing the trial, if	
5			applicable (see Item 21a for data monitoring committee)	
6				
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8				
9	Introduction			
10				
11	Background and	#6a	Description of research question and justification for	7
12	rationale		undertaking the trial, including summary of relevant	
13			studies (published and unpublished) examining benefits	
14			and harms for each intervention	
15				
16				
17				
18	Background and	#6b	Explanation for choice of comparators	8
19	rationale: choice of			
20	comparators			
21				
22				
23	Objectives	#7	Specific objectives or hypotheses	8
24				
25				
26	Trial design	#8	Description of trial design including type of trial (eg,	9
27			parallel group, crossover, factorial, single group),	
28			allocation ratio, and framework (eg, superiority,	
29			equivalence, non-inferiority, exploratory)	
30				
31				
32				
33	Methods:			
34	Participants,			
35	interventions, and			
36	outcomes			
37				
38				
39	Study setting	#9	Description of study settings (eg, community clinic,	10
40			academic hospital) and list of countries where data will	
41			be collected. Reference to where list of study sites can	
42			be obtained	
43				
44				
45				
46	Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If	10
47			applicable, eligibility criteria for study centres and	
48			individuals who will perform the interventions (eg,	
49			surgeons, psychotherapists)	
50				
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53	Interventions:	#11a	Interventions for each group with sufficient detail to allow	13
54	description		replication, including how and when they will be	
55			administered	
56				
57				
58				
59	Interventions:	#11b	Criteria for discontinuing or modifying allocated	13
60				

1	modifications		interventions for a given trial participant (eg, drug dose	
2			change in response to harms, participant request, or	
3			improving / worsening disease)	
4				
5	Interventions:	#11c	Strategies to improve adherence to intervention	14
6	adherence		protocols, and any procedures for monitoring adherence	
7			(eg, drug tablet return; laboratory tests)	
8				
9				
10	Interventions:	#11d	Relevant concomitant care and interventions that are	14
11	concomitant care		permitted or prohibited during the trial	
12				
13				
14	Outcomes	#12	Primary, secondary, and other outcomes, including the	13
15			specific measurement variable (eg, systolic blood	
16			pressure), analysis metric (eg, change from baseline,	
17			final value, time to event), method of aggregation (eg,	
18			median, proportion), and time point for each outcome.	
19			Explanation of the clinical relevance of chosen efficacy	
20			and harm outcomes is strongly recommended	
21				
22				
23				
24				
25				
26	Participant timeline	#13	Time schedule of enrolment, interventions (including any	See Figure
27			run-ins and washouts), assessments, and visits for	1
28			participants. A schematic diagram is highly	
29			recommended (see Figure)	
30				
31				
32				
33	Sample size	#14	Estimated number of participants needed to achieve	17
34			study objectives and how it was determined, including	
35			clinical and statistical assumptions supporting any	
36			sample size calculations	
37				
38				
39				
40	Recruitment	#15	Strategies for achieving adequate participant enrolment	11
41			to reach target sample size	
42				
43	Methods:			
44	Assignment of			
45	interventions (for			
46	controlled trials)			
47				
48				
49				
50	Allocation: sequence	#16a	Method of generating the allocation sequence (eg,	12
51	generation		computer-generated random numbers), and list of any	
52			factors for stratification. To reduce predictability of a	
53			random sequence, details of any planned restriction (eg,	
54			blocking) should be provided in a separate document	
55			that is unavailable to those who enrol participants or	
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assign interventions

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3	Allocation	#16b	Mechanism of implementing the allocation sequence
4	concealment		(eg, central telephone; sequentially numbered, opaque,
5	mechanism		sealed envelopes), describing any steps to conceal the
6			sequence until interventions are assigned
7			
8			
9	Allocation:	#16c	Who will generate the allocation sequence, who will
10	implementation		enrol participants, and who will assign participants to
11			interventions
12			
13			
14	Blinding (masking)	#17a	Who will be blinded after assignment to interventions
15			(eg, trial participants, care providers, outcome
16			assessors, data analysts), and how
17			
18			
19			
20	Blinding (masking):	#17b	If blinded, circumstances under which unblinding is
21	emergency unblinding		permissible, and procedure for revealing a participant's
22			allocated intervention during the trial
23			
24			
25	Methods: Data		
26	collection,		
27	management, and		
28	analysis		
29			
30			
31			
32	Data collection plan	#18a	Plans for assessment and collection of outcome,
33			baseline, and other trial data, including any related
34			processes to promote data quality (eg, duplicate
35			measurements, training of assessors) and a description
36			of study instruments (eg, questionnaires, laboratory
37			tests) along with their reliability and validity, if known.
38			Reference to where data collection forms can be found,
39			if not in the protocol
40			
41			
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44			
45	Data collection plan:	#18b	Plans to promote participant retention and complete
46	retention		follow-up, including list of any outcome data to be
47			collected for participants who discontinue or deviate
48			from intervention protocols
49			
50			
51			
52	Data management	#19	Plans for data entry, coding, security, and storage,
53			including any related processes to promote data quality
54			(eg, double data entry; range checks for data values).
55			Reference to where details of data management
56			procedures can be found, if not in the protocol
57			
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1	Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary	18
2			outcomes. Reference to where other details of the	
3			statistical analysis plan can be found, if not in the	
4			protocol	
5				
6				
7				
8	Statistics: additional	#20b	Methods for any additional analyses (eg, subgroup and	18
9	analyses		adjusted analyses)	
10				
11	Statistics: analysis	#20c	Definition of analysis population relating to protocol non-	18
12	population and		adherence (eg, as randomised analysis), and any	
13	missing data		statistical methods to handle missing data (eg, multiple	
14			imputation)	
15				
16				
17				
18	Methods: Monitoring			
19				
20				
21	Data monitoring:	#21a	Composition of data monitoring committee (DMC);	Appendix
22	formal committee		summary of its role and reporting structure; statement of	1
23			whether it is independent from the sponsor and	
24			competing interests; and reference to where further	
25			details about its charter can be found, if not in the	
26			protocol. Alternatively, an explanation of why a DMC is	
27			not needed	
28				
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32	Data monitoring:	#21b	Description of any interim analyses and stopping	Appendix
33	interim analysis		guidelines, including who will have Access to these	1
34			interim results and make the final decision to terminate	
35			the trial	
36				
37				
38				
39	Harms	#22	Plans for collecting, assessing, reporting, and managing	17
40			solicited and spontaneously reported adverse events	
41			and other unintended effects of trial interventions or trial	
42			conduct	
43				
44				
45				
46	Auditing	#23	Frequency and procedures for auditing trial conduct, if	20
47			any, and whether the process will be independent from	
48			investigators and the sponsor	
49				
50				
51	Ethics and			
52	dissemination			
53				
54				
55	Research ethics	#24	Plans for seeking research ethics committee /	19
56	approval		institutional review board (REC / IRB) approval	
57				
58				
59	Protocol amendments	#25	Plans for communicating important protocol	20
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modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)

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7	Consent or assent	#26a Who will obtain informed consent or assent from	19
8		potential trial participants or authorised surrogates, and	
9		how (see Item 32)	
10			
11			
12	Consent or assent:	#26b Additional consent provisions for collection and use of	19
13	ancillary studies	participant data and biological specimens in ancillary	
14		studies, if applicable	
15			
16			
17	Confidentiality	#27 How personal information about potential and enrolled	19
18		participants will be collected, shared, and maintained in	
19		order to protect confidentiality before, during, and after	
20		the trial	
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22			
23			
24	Declaration of	#28 Financial and other competing interests for principal	20
25	interests	investigators for the overall trial and each study site	
26			
27			
28	Data access	#29 Statement of who will have Access to the final trial	18
29		dataset, and disclosure of contractual agreements that	
30		limit such Access for investigators	
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32			
33	Ancillary and post trial	#30 Provisions, if any, for ancillary and post-trial care, and	19
34	care	for compensation to those who suffer harm from trial	
35		participation	
36			
37			
38			
39	Dissemination policy:	#31a Plans for investigators and sponsor to communicate trial	19
40	trial results	results to participants, healthcare professionals, the	
41		public, and other relevant groups (eg, via publication,	
42		reporting in results databases, or other data sharing	
43		arrangements), including any publication restrictions	
44			
45			
46			
47	Dissemination policy:	#31b Authorship eligibility guidelines and any intended use of	20
48	authorship	professional writers	
49			
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51	Dissemination policy:	#31c Plans, if any, for granting public Access to the full	20
52	reproducible research	protocol, participant-level dataset, and statistical code	
53			
54			
55	Appendices		
56			
57	Informed consent	#32 Model consent form and other related documentation	Appendix
58	materials	given to participants and authorised surrogates	2
59			
60			

1 Biological specimens [#33](#) Plans for collection, laboratory evaluation, and storage 15-17
2 of biological specimens for genetic or molecular analysis
3 in the current trial and for future use in ancillary studies,
4 if applicable
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10 None The SPIRIT Explanation and Elaboration paper is distributed under the terms of the Creative
11 Commons Attribution License CC-BY-NC. This checklist can be completed online using
12 <https://www.goodreports.org/>, a tool made by the [EQUATOR Network](#) in collaboration with
13 [Penelope.ai](#)
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APPENDIX 2

Charter and responsibilities of the Data Monitoring and Safety Committee

A Data Monitoring and Safety Committee (DMSC) has been established, and its lead by Clinical Study Center at Thomayer University Hospital, Prague. The DMSC is an independent organ from the study investigators. During the period of recruitment to the study, interim analyses will be supplied, in strict confidence, to the DMSC. In the light of these interim analyses, the DMSC will advise the study steering committee (SSC) if, in its view, the active intervention has been proven, beyond reasonable doubt, to be different from the placebo in some or all patients

Based on the reports of DMSC, the Study steering committee (SSC) can then decide whether or not to modify recruitment to the study and its oncoming course. Unless this happens, however, the SSC, will remain ignorant of the interim results.

The frequency of interim analyses will depend on the judgement of the Chair of the DMSC, in consultation with the SSC. However, we anticipate that there might be two to three interim analyses and one final analysis.

The Chair of DSMC is Mr. Jiri Skopek, M.D., Ph.D. who is available on request at jiri.skopek1@ftn.cz

Premature termination of the study

An interim analysis is performed when 50% of patients have already got to Visit 5 (where primary outcome is evaluated.) The interim analysis is performed by a member of the study's statistical unit who is blinded for the allocation of the active study mixture. The statistician will report to the DMSC. The DMSC will have unblinded Access to all data and discuss the interim-analysis results with the SSC. The SSC decides on continuation or termination of the study and will report to the central Ethics committee. The study will be ended if the frequency of severe adverse events crosses the 5% line. Severe adverse event is defined as that one requiring hospitalisation.

Appendix 3: Informed consent for FMT donors



Informovaný souhlas dospělé osoby s účastí na výzkumu změn střevního mikrobiomu u dospělých pacientů se syndromem dráždivého tračníku ve vědeckém projektu týmů Thomayerovy nemocnice a Fakultní nemocnice v Motole.

Vážená paní/vážený pane,

syndrom dráždivého tračníku (irritable bowel syndrome, dále jen IBS) je nejčastější funkční onemocnění trávicího traktu, které pacienta výrazně omezuje v jeho každodenním životě. Může se projevovat různě, nejčastěji však jako delší dobu trvající bolest břicha s náhle vzniklým nutkáním na stolici. Léčba této nemoci je zdlouhavá, obtížná a ne vždy úspěšná. Dle recentních studií se však jako účinná léčebná metoda jeví transplantace střevní mikroflóry (faecal microbiota transplantation, dále jen FMT). A právě na její využití se zaměřuje náš projekt v podobě klinické intervenční studie.

Cílem projektu je zjistit, zda je transplantace stolice účinnou léčebnou metodou IBS a jak se po FMT mění složení střevní mikroflóry. K tomu abychom FMT mohli provést je potřeba mít vhodné dárce stolice. A právě zde byste nám mohli pomoci. Znalosti změn složení střevní mikroflóry po FMT bychom pak v budoucnu mohli využít buď k cílené ATB terapii negativně asociovaných bakterií nebo naopak k podávání probiotika prospěšných kmenů.

Proto si Vás dovoluujeme pozvat k účasti na projektu vědeckých týmů Thomayerovy nemocnice a Fakultní nemocnice v Motole. Přečtěte si, prosím, toto poučení. Pokud plně nerozumíte tomuto textu nebo pokud potřebujete doplňující informace, neváhejte se zeptat lékaře na emailu uvedeném níže. **Pokud souhlasíte s Vaší účastí ve studii, vyplňte prosím kontaktní údaje níže dokumentu a podepište prosím prohlášení, které se nachází v závěru tohoto informovaného souhlasu. Vaše účast je dobrovolná.** Tento souhlas můžete kdykoli zrušit, a to i bez udání důvodu.

Získání vzorku stolice by probíhalo ve vašem domácím prostředí. Stolice by bylo potřeba uchovat v běžném domácím mrazáku (teplota -20°C), k odběru byste byli vybaveni jednoduchými odběrovými sety s návodem a poučení o jejich používání. Po domluvě se členy vědeckého týmu (kontakt níže) by vzorky byly převezeny na naše pracoviště a hluboce zamrazeny (-80°C).

Celý proces je dvoufázový. Z prvního vzorku se provede molekulárně-genetická analýza a následně bioinformatické zpracování dat. Na základě výsledků bude vybráno asi 10-20 dárců, které kontaktujeme na základě informací uvedených níže. Splní-li kritéria vhodného dárce (pro vyžádání lze napsat na mail jiri.vejmelka@ftn.cz nebo zavolat na tel.č. 731446619), budou poté znovu požádáni o darování stolice.

Po zpracování pro účely aktuální studie budou vzorky uchovány v hlubokomrazícím boxu v laboratořích Fakultní nemocnice v Motole. Jejich další využití proběhne pouze po přesné specifikaci formou dalšího souhlasu a Vaším podepsáním nového souhlasu.

V tomto projektu řádně dbáme o bezpečnost osobních údajů podle platných zákonů. Zejména je pak zcela zachovaná úplná anonymita pacienta při odesílání vzorků mimo naše pracoviště nebo při

Appendix 3: Informed consent for FMT donors

zveřejňování vědeckých výsledků získaných z naší práce v odborných časopisech. Odebrané vzorky a z nich získané části jsou v našich laboratořích skladovány na dobu neurčitou, odděleně od osobních dat. Pokud byste v budoucnu svůj souhlas odvolali, Vaše jméno a ostatní osobní data budou bez prodlení vymazána z našich databází i papírových záznamů tak, aby se už nikdo nemohl dozvědět, komu vzorek patřil.

Bližší informace o nemoci jako takové můžete získat od členů vědeckého týmu:

MUDr. Jiří Vejmelka (Thomayerova nemocnice), tel: 731446619, email: jiri.vejmelka@ftn.cz

MUDr. Jakub Hurych (Fakultní nemocnice v Motole), tel. 224432089, email: jakub.hurych@lfmotol.cuni.cz

Souhlas se zpracováním osobních údajů (dále jen „Souhlas“)

udělený ve smyslu zákona č. 101/2000 Sb., o ochraně osobních údajů a o změně některých zákonů, ve znění pozdějších předpisů a s Nařízením Evropského parlamentu a Rady (EU) 2016/679

Já, níže podepsaný

Jméno a příjmení:

Datum narození:

Rodné číslo:

Kontaktní email:

Telefonní číslo:

Souhlasím se zpracováním svých osobních údajů/ osobních údajů osoby jejíž jsem zákonným zástupcem Fakultní nemocnicí v Motole a Thomayerově nemocnici v rozsahu těchto údajů:

Jméno, příjmení, titul, datum a místo narození, rodné číslo, národnost, pohlaví, místo trvalého pobytu, telefon, email, výška, hmotnost

Tento projev vůle je platný pouze v případě, že mé osobní údaje budou zpracovávány pouze v rozsahu nezbytném pro dosažení účelu zpracování uvedeného v tomto souhlasném prohlášení a v souladu s příslušnou legislativou v platném znění.

Souhlas je poskytnut za účelem:

Zpracování vzorku stolice pro vědecko-výzkumnou činnost mající za cíl přispět k porozumění změn střevního mikrobiomu u dospělých pacientů se syndromem dráždivého tračníku

Souhlasím se zpracováním svých osobních údajů Fakultní nemocnicí v Motole a Thomayerově nemocnici po dobu:

Do odebrání mého souhlasu

Souhlasím se zpřístupněním svých osobních údajů Fakultní nemocnici v Motole a Thomayerově nemocnici:

Fakultní nemocnice v Motole a Thomayerova nemocnice je oprávněna použít mé osobní údaje pouze v souladu s výše uvedeným účelem a po výše uvedenou dobu, nebo pro legitimní potřebu státních kontrolních

Appendix 3: Informed consent for FMT donors

orgánů a orgánů činných v trestním řízení.

Fakultní nemocnice v Motole a Thomayerova nemocnice je dále oprávněna poskytnout mé osobní údaje pouze subjektům spolupracujícím s **Fakultní nemocnicí v Motole a Thomayerovou nemocnicí** na dosažení primárního účelu, pro který je udělen tento souhlas. S takovými subjekty se **Fakultní nemocnice v Motole a Thomayerova nemocnice** zavazuje uzavřít smlouvu obsahující stejné podmínky pro zpracování mých osobních údajů. Zpracování bude probíhat v souladu s příslušnými právními normami o ochraně osobních údajů a s Nařízením Evropského parlamentu a Rady (EU) 2016/679 ze dne 27. dubna 2016 o ochraně fyzických osob v souvislosti se zpracováním osobních údajů a o volném pohybu těchto údajů a o zrušení směrnice 95/46/ES (obecné nařízení o ochraně osobních údajů).

Byl/a jsem poučen/a o tom, že poskytnutí údajů je dobrovolné.

Dále jsem byl/a v souladu s příslušnou legislativou poučen/a:

- O svém právu tento souhlas odvolat, a to i bez udání důvodu,
- O svém právu přístupu k těmto údajům a právu na jejich opravu,
- O svém právu na vymazání těchto údajů, pokud dochází k jejich zpracování v rozporu s ochranou definovanou příslušnou legislativou nebo v rozporu s tímto souhlasem, nebo byl souhlas odvolán, svém právu podat stížnost u Úřadu pro ochranu osobních údajů.

Byl/a jsem také poučen/a o tom, že tato svá práva mohu uplatnit doručením žádosti na adresu: Fakultní nemocnice v Motole, Samostatné oddělení pověřence pro ochranu osobních údajů, V Úvalu 84, Praha 5.

Beru na vědomí, že odvolání tohoto souhlasu může ovlivnit dosažení účelu, pro který byl tento souhlas vydán, pokud tohoto účelu nelze dosáhnout jinak.

Prohlašuji, že jsem textu poučení porozuměl(a) a byl jsem lékařem srozumitelně informován(a) o povaze daného vyšetření a že jsem měl(a) možnost klást lékařovi doplňující dotazy.

Na základě tohoto poučení dále prohlašuji, že souhlasím se zařazením svých vzorků do studie probíhající v **Thomayerově nemocnici a Fakultní nemocnici v Motole**, jejímž cílem je porozumět změnám složení střevního mikrobiomu u dospělých pacientů se syndromem dráždivého tračníku.

V dne

Jméno a příjmení vyšetřované osoby :

Podpis vyšetřované osoby

Prohlašuji, že jsem vysvětlil podstatu, účel a povahu odběrů pacientovi způsobem, který byl podle mého soudu srozumitelný.

Jméno a příjmení lékaře:

Podpis:

Datum:

APPENDIX 4 – INFORMED CONSENT FORM FOR FMT RECIPIENTS (CZECH)**Informovaný souhlas pacienta - studie fekální mikrobiální terapie
u pacientů se syndromem dráždivého tračníku**

Název studie: Fekální mikrobiální terapie u pacientů se syndromem dráždivého tračníku

Jméno pacienta:

Datum narození:

Pacient byl do studie zařazen pod číslem:

Odpovědný lékař:

1. Já, níže podepsaný (á) souhlasím s mou účastí ve studii. Je mi více než 18 let.
2. Byl (a) jsem podrobně informován (a) o cíli studie, o jejích postupech, a o tom, co se ode mě očekává. Lékař pověřený prováděním studie mi vysvětlil očekávané přínosy a případná zdravotní rizika, která by se mohla vyskytnout během mé účasti ve studii, a vysvětlil mi, jak bude postupovat při výskytu jejího nežádoucího průběhu. Beru na vědomí, že prováděná studie je výzkumnou činností. Beru na vědomí pravděpodobnost náhodného zařazení do jednotlivých skupin lišících se léčbou.
3. Informoval (a) jsem lékaře pověřeného studií o všech lécích, které jsem užíval (a) v posledních 3 měsících, i o těch, které v současnosti užívám. Bude-li mi nějaký lék předepsán jiným lékařem, budu ho informovat o své účasti v klinické studii a bez souhlasu lékaře pověřeného touto studií ho nevezmu.
4. Budu při své léčbě se svým lékařem spolupracovat a v případě výskytu jakéhokoliv neobvyklého nebo nečekaného příznaku ho budu ihned informovat.
5. Po celou dobu studie a další 4 týdny po jejím ukončení nebudu dárce krve.
6. Porozuměl (a) jsem tomu, že svou účast ve studii mohu kdykoliv přerušit či odstoupit, aniž by to jakkoliv ovlivnilo průběh mého dalšího léčení. Moje účast ve studii je dobrovolná.
7. Při zařazení do studie budou moje osobní data uchována s plnou ochranou důvěrnosti dle platných zákonů ČR. Do mé původní zdravotní dokumentace budou moci na základě mého uděleného souhlasu nahlédnout za účelem ověření získaných údajů zástupci nezávislých etických komisí a zahraničních nebo místních kompetentních úřadů. Pro tyto případy je zaručena ochrana důvěrnosti mých osobních dat. Při vlastním provádění studie mohou být osobní údaje poskytnuty jiným než výše uvedeným subjektům pouze bez identifikačních údajů, a to jako anonymní data pod číselným kódem. Rovněž pro výzkumné a vědecké účely mohou být moje osobní údaje poskytnuty pouze bez identifikačních údajů (anonymní data) nebo s mým výslovným souhlasem. Při předávání dat po 25. 5. 2018 bude zajištěna ochrana osobních údajů požadovaná „Nařízením Evropského parlamentu a Rady (EU) 2016/679 ze dne 27. dubna 2016 o ochraně fyzických osob v souvislosti se zpracováním osobních údajů“ známé pod označením GDPR.
8. S mou účastí ve studii není spojeno poskytnutí žádné odměny.
9. Porozuměl jsem tomu, že mé jméno se nebude nikdy vyskytovat v referátech o této studii. Já pak naopak nebudu proti použití výsledků z této studie.
10. Převzal/a jsem podepsaný stejnopis tohoto informovaného souhlasu.

Podpis pacienta:

Podpis lékaře pověřeného touto studií:

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For peer review only