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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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Abbreviations: IBS, Irritable Bowel Syndrome; IBS-D, diarrheal type of irritable bowel syndrome; IBS-M, mixed type of irritable bowel syndrome; IBS-C, constipated type of irritable bowel syndrome; IBS-SSS, Irritable Bowel Syndrome Severity Scale Score

Keywords: irritable bowel syndrome, faecal microbiota transplantation, irritable bowel syndrome 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 diarhhoeal 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

results will be published in peer-reviewed journals and presented at international conferences and patient groups meetings.

Study registration number. NCT04899869

STRENGTHS AND LIMITATIONS OF THIS STUDY

- Usage of mixed microbiota from multiple donors inflates the diversity of transferred microbiota by enriching it for numerous rare species.
- All interventions will be carried out using the same active mixed microbiota or the same placebo.
- Each intervention consists of two consecutive transfers, which increases the probability that the transferred microbiota engrafts.
- Microbiome profiling, food records, anthropometry and bioimpedance data allow detailed monitoring of transfer effectiveness.
- Mucosa-associated microbiota will not be assessed because the stool transfer will be performed by enema, not colonoscopy that would allow biopsies.

INTRODUCTION

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

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

Our study protocol aims to test whether faecal microbiota transplantation of mixed microbiota from several selected donors can alleviate symptoms of IBS measured by IBS-SSS at four weeks after the intervention, compared to autoclaved placebo. Secondary study aims are to test the acute (after two weeks) and the long-term effect (after six months) on symptoms relief. We also focus on changes in the gut microbiome composition, frequency of urgent defecations, Bristol stool scale, abdominal pain and bloating, body weight, fat content and anthropometric measurements (including waist, hip and limbs circumferences and skinfold thickness.

We hypothesise that the transfer of active microbiota of high diversity can lead to changes in the patient's gut microbiome composition and/or function to alleviate IBS symptoms.

METHODS AND ANALYSIS

Study design

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

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

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

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

Study setting

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

Recruitment and eligibility criteria

Stool donors

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

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

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

Prospective study participants

Patients diagnosed with IBS-D (diarrheal type) or IBS-M (mixed diarrhoeal and constipation type) who fulfil the inclusion and exclusion criteria listed in **Table 3** are recruited via regular' patient's check-ups at the Gastroenterological unit at Thomayer University Hospital, by referrals from their general practitioners, following our newspaper articles or word of mouth.

Study microbiota mixture for intervention

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

post-filtration), the procedure was performed under a nitrogen atmosphere to protect obligate anaerobes. All stool portions were mixed in a large stainless steel bucket using an electric mortar mixer under anaerobic conditions and low temperatures (on ice).

The mixed microbiota substance was divided into aliquots of 13-14 g (which is ~ 35 ml). Two-thirds of the tubes serve as a placebo: they were immediately autoclaved at 121°C for 30 minutes with slow cooling. Pre-sterilised tubes were used to ensure that autoclaved placebos will not be visually distinguishable from tubes with the active substance. Assignation of tubes to the autoclave, numbering, sealing, and labelling was done under the guidance of a statistical unit member (see below).

All aliquot tubes are kept frozen at -80°C in the same type of plastic tubes, labelled by codes. Three such aliquots represent one dose for FMT (~40 g of stool, in ~105 ml). Aliquoting into multiple 50 ml tubes instead of one larger volume was decided because of the availability of durable plastic, which must be both autoclavable and deep frost resistant.

Before administering, the study microbiota mixture will be thawed in a warm (37°C) water bath, with intermittent mixing by inverting the tubes.

Randomization, allocation and blinding

At Visit 1, each patient is randomised into one of three equally sized groups (Figure 1) as described in the *Study design*. Randomisation assignments are generated in advance in blocks of nine and stored in a protected database. For each patient, anonymous codes for tubes containing either active study microbiota mixture or placebo is received. Thus, the true assignment will remain concealed for the patients and the study staff until the end of the study observation period. The Investigator is encouraged to maintain the blind as far as possible. The actual allocation must not be disclosed to

the patient and/or other study personnel, including other site personnel, monitors, corporate sponsors or project office staff; nor should there be any written or verbal disclosure of the code in any of the corresponding patient documents.

Study Intervention

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

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

Outcomes

Primary outcome

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

Secondary outcomes

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

Data collection and follow-up

Timing of assessments

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

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

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

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

Weight, height, bioimpedance

Bodyweight, height and bioimpedance are examined during Visit 0, 1, 4, 5, 9 and 11. Medical Body Composition Analyzer Seca mBCA 515, (Seca, Germany) is used to measure changes in body composition (8-point bioelectric impedance analysis at a frequency of 5 - 50 kHz with a current of 100 µA), scanning performed with three pairs of hand electrodes and two pairs of leg electrodes, measurements performed with light clothing and without metal objects (jewellery, keys). The weight is determined in patients wearing underwear using the Seca mBCA 515. The height is determined by a standardised technique with a metal stadiometer with an accuracy of 1 mm. Seca analytics 115 software is used to analyse the obtained data (Seca, Hamburg, Germany). The measurements is performed according to the NIHR Southampton Biomedical Research Centre standard protocol (Seca mBCA, NIHR Southampton Biomedical Research Centre, 2014).

Detailed anthropometry

It is performed by nutritional therapists in Visit 1, 5, 10 and 11. It involves weight, abdominal (waist) circumference, buttocks (hip) circumference, thigh circumference, skinfolds (thigh, triceps, subscapular, suprailiacal).

Serum workup, archiving serum+plasma

Blood is sampled at Visits 0, 4, 9, 11 and will include: A) serum+plasma archiving, B) serum workup. Laboratory panel testing will comprise sodium, potassium, chloride, urea, creatinine, glucose, calcium, phosphate, total protein and albumin, AST, ALT, ALP, GMT, bilirubin, lipid panel, HS-CRP, blood cell count with differential count, INR, urine analysis (sediment and biochemistry). One plasma and one serum aliquot are made at these visits and frozen for forensic reasons.

Psychological evaluation

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

Dietary questionnaire & advice, evaluation of food records

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

Gut microbiome composition

Faecal samples are collected at home by the subjects in the same way described for donors above and at time points indicated in the sections above. If not immediately brought to the visit, the stool is frozen in a home freezer and then transported in a frozen tube container. DNA extraction is performed using the PowerSoil kit (Qiagen), and the bacteriome is characterised by 16S rDNA amplicon profiling using the tagged primers according to Schloss protocol [9], and sequencing on a MiSeq instrument with the 2x250 bases sequencing kit (both Illumina, USA).

The first steps of bioinformatic analysis will be performed in the DADA2 package[10]. Statistical analyses and visualisation will be then performed in R with its Phyloseq package. Finally, the functional potential of the bacteriome will be assessed using the PICRUST software, which predicts functional capabilities based on the 16S rDNA profiles.

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

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

Safety monitoring

The research team regularly monitors all data for any adverse events, and all potential adverse events are recorded. Contacts to study coordinators active 24/7 are provided in case adverse effects occurred. If any concerns are identified during donors or recipients' screening or clinical assessment, further clinical evaluation and/or examination is immediately realised. All the concerns during the study are assessed, and the recipient will be withdrawn if this is thought to be in his best interest. A Data Monitoring and Safety Committee (DMSC) has been established and, based on the data from the planned interim analysis, has the right to terminate the study if the frequency of severe adverse events crosses the 5% line (for a closer description of DMSC, its responsibilities and premature termination of the study see **Appendix 2**).

Sample size and power calculation

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

Data management

Data from IBS-SSS, frequency of urgent defecations, Bristol stool scale, abdominal pain and bloating are collected and stored via the application Survey Monkey. All anthropometric data are entered and stored in password-protected platforms integrated within the hospital information system. Only the researchers involved in the study have access to the final study dataset (IBS-SSS, frequency of urgent defecations, Bristol stool scale, abdominal pain and bloating), which will be shared in an anonymised form via the Zenodo repository. The only data in this manuscript are bacteriome data; their anonymised form will be available on reasonable request.

Statistical analyses

The primary outcome analysis will be based on the difference in IBS-SSS scores over the second treatment period (week 14 vs week 8) minus the change over the first treatment period (week 5 vs week -1). This difference will be used as a response in a linear model, with intercept corresponding to the temporal effect (seen in the placebo group C), an indicator of group A corresponding to the cross-over effect (resulting from administration of placebo after active microbiota) and differences in indicators for groups A and B modelling the effect of active microbiota. A robust sandwich estimator of the variance matrix will be used to adjust for potentially unequal variances between the groups. Analyses of secondary outcomes will proceed by similar methodology, comparing absolute or relative

differences of the post-intervention measure of each outcome relative to its baseline value. The CONSORT 2010 guidelines will be followed in reporting the main trial results.

Study status

The study was registered at clinicaltrials.gov (NCT04899869) on May 25th 2021. The first patient was recruited on June 17th 2021, and the first intervention was applied on July 29th 2021. As of August 19th 2021, 12 patients have signed the informed consent, and six interventions have been applied. It is expected that the study will be completed in December 2022.

Patient and public involvement

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

ETHICS AND DISSEMINATION

Ethics approval for this study was granted in June 2018 by the Ethics Committee of the Institute for Clinical and Experimental Medicine and Thomayer Hospital (Vídeňská 800, 140 59 Prague 4, Czech Republic). Involvement in this study is completely voluntary; donors and recipients are required to provide written informed consent prior to participation in the study (see **Appendix 3 and 4**). Recipients and their caregivers are informed of unexpected findings or unrecognised conditions and

by possible future usage of their specimens in ancillary studies by trained physician or nurse; further medical care will be arranged. Study donors received financial compensation to pay for the required travelling costs when donating the stool. The patient will be offered a roll-over into an observational study with the administration of active microbiota. The patients are informed of this option at the start of the study and regularly reminded.

We aim to publish findings in impact peer-reviewed international journals. Gastroenterologists, internists and other care providers will be informed through the national conference meetings, journals and patient groups meetings.

Protocol amendment number: 01. Modification of the study protocol will be communicated with the Ethics committee.

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

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Contributors OC, PK, JH, JV, MK contributed to the conception and design of the study. OC, PK, JH and JV drafted the protocol with input from all other authors. JV and PK contributed to the patients recruitment. JH, LV, LK and OC contributed to the microbiome analysis for donor selection. JH, OC and JV contributed to the donor screening. LV, JH and OC contributed to the study microbiota

mixture preparation. MK contributed to the power size calculations and statistical analysis. VL contributed to the randomization. JH and JV contributed equally to this paper, OC and PK contributed equally either.

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Competing interests None declared. No money from commercial sponsors was used.

Patient consent for publication Not required.

Ethics approval Ethics approval for this study was granted in June 2018 by the Ethics Committee of the Institut for Clinical and Experimental Medicine and Thomayer Hospital (Vídeňská 800, 140 59 Prague 4, Czech Republic).

Provenance and peer review Not commissioned; externally peer-reviewed.

FIGURES AND ILLUSTRATIONS

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

Figure 2 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.

Figure 3 Process of donor selection and reasons for their excluding

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)
	Clostridiodes difficile 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

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
Clostridioides difficile (cultures, antigen testing)
Common enteric pathogens, including Salmonella, Shigella, Campylobacter, shiga toxin-producing *Escherichia coli*, Yersinia and *Vibrio cholerae* (cultures)
Antibiotic-resistant bacteria (ARB), including vancomycin-resistant Enterococci, meticillin-resistant *Staphylococcus aureus* Gram-negative ARB including extended-spectrum β-lactamase-producing *Enterobacteriaceae*, and carbapenem-resistant *Enterobacteriaceae*/carbapenemase-producing *Enterobacteriaceae* (cultures)
Norovirus, rotavirus, adenovirus, sapovirus (PCR)
SARS-CoV-2 (reverse transcription -PCR)
Common intestinal parasites, including *Giardia intestinalis, Cryptosporidium parvum et hominis* (cultures and PCR), *Blastocystis hominis**, *Dientamoeba fragilis** (both PCR only)

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

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

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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

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

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

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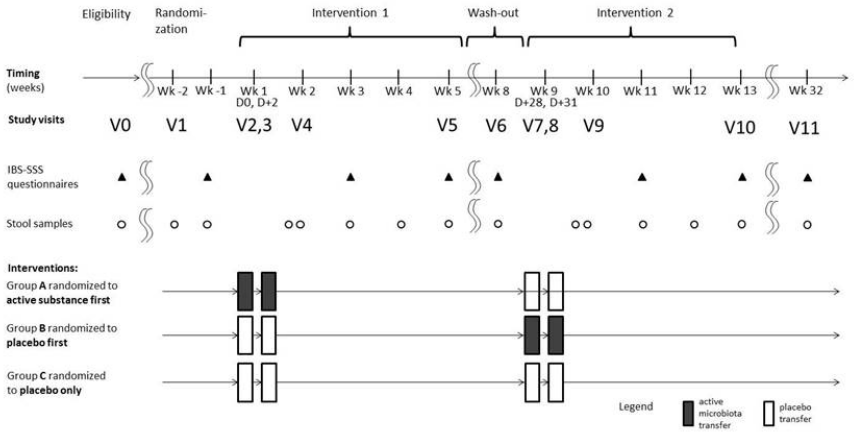
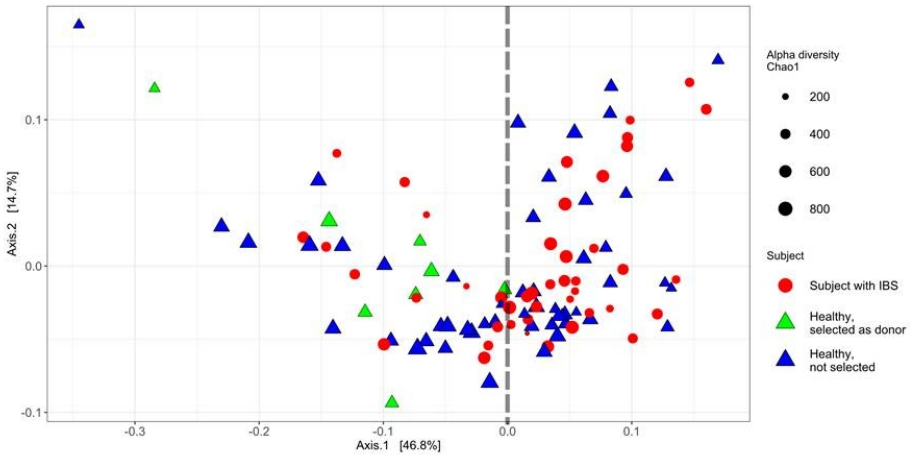


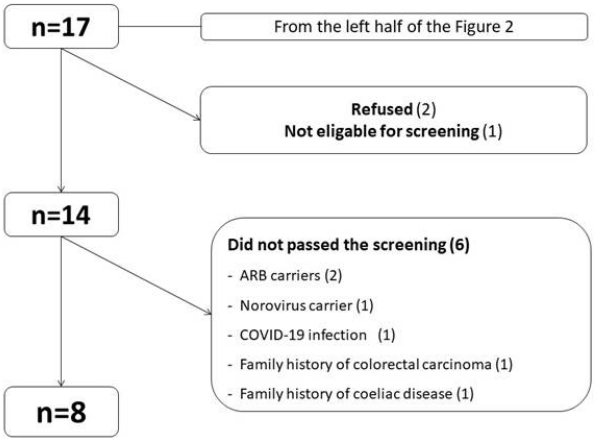
Per protocol intervention scheme: the visits, questionnaires and samples

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-diversityThese 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.

254x190mm (96 x 96 DPI)



Process of donor selection and reasons for their excluding

254x190mm (96 x 96 DPI)

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 recieved 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

Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	20
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	7
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	8
Objectives	#7	Specific objectives or hypotheses	8
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	9
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	10
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	10
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	13
Interventions:	#11b	Criteria for discontinuing or modifying allocated	13

modifications		interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	14
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	14
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	13
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	See Figure 1
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	17
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	11
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or	12

		assign interventions	
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	12
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	12
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	12
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	12-13
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	14-17
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	14
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	18

Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	18
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	18
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	18
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	Appendix 1
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have Access to these interim results and make the final decision to terminate the trial	Appendix 1
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	17
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	20
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	19
Protocol amendments	#25	Plans for communicating important protocol	20

		modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	19
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	19
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	19
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	20
Data access	#29	Statement of who will have Access to the final trial dataset, and disclosure of contractual agreements that limit such Access for investigators	18
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	19
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	19
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	20
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public Access to the full protocol, participant-level dataset, and statistical code	20
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Appendix 2

Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	15-17

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 studí 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 pomoct. 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 dovolujeme 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 poučeni o jejich používání. Po domluvě se členy vědeckého týmu (kontakt níže) by vzorky byly převezeny na naše pracoviště a hluboce zamraženy (-80°C).

Celý proces je dvoufázový. Z prvního vzorku se provede molekulárně-genetická analýza a následné 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 dárcem 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:

Datum: Datum:

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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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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Word counts: 3798

Abbreviations: IBS, Irritable Bowel Syndrome; IBS-D, diarrheal type of irritable bowel syndrome; IBS-M, mixed type of irritable bowel syndrome; IBS-C, constipated type of irritable bowel syndrome; IBS-SSS, Irritable Bowel Syndrome Severity Scale Score

Keywords: irritable bowel syndrome, faecal microbiota transplantation, irritable bowel syndrome 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.

Study registration number. NTC04899869

STRENGTHS AND LIMITATIONS OF THIS STUDY

- Usage of mixed microbiota from multiple donors inflates the diversity of transferred microbiota by enriching it for numerous rare species.
- All interventions will be carried out using the same active mixed microbiota or the same placebo.
- Each intervention consists of two consecutive transfers, which increases the probability that the transferred microbiota engrafts.
- Microbiome profiling, food records, anthropometry and bioimpedance data allow detailed monitoring of transfer effectiveness.
- Mucosa-associated microbiota will not be assessed because the stool transfer will be performed by enema, not colonoscopy that would allow biopsies.

INTRODUCTION

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

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

IBS have been carried out [6, 7, 8, 9, 10, 11]. They differed in design, but none of them used a mixed microbiota from multiple donors as the active substance. Furthermore, a recent meta-analysis of randomized control trials on FMT in IBS (including the above-mentioned articles) pointed out insufficient evidence quality to support recommending FMT in the treatment of IBS. [12]

Our study protocol aims to test whether faecal microbiota transplantation of mixed microbiota from several selected donors can alleviate symptoms of IBS measured by IBS-SSS four weeks after the intervention, as compared to autoclaved placebo. The secondary study aims to test the acute (after two weeks) and the long-term effect (after six months) on symptoms relief. We also focus on changes in frequency of urgent defecations, Bristol stool scale, abdominal pain andbloating, body weight, fat content and anthropometric measurements (including waist, hip and limbs circumferences and skinfold thickness) and the gut microbiome composition.

We hypothesise that the transfer of active microbiota of high diversity can lead to changes in the patient's gut microbiome composition and/or function to alleviate IBS symptoms.

METHODS AND ANALYSIS

Study design

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

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

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

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

Study setting

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

Recruitment and eligibility criteria

Stool donors

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

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

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

Prospective study participants

Patients diagnosed with IBS-D (diarrheal type) or IBS-M (mixed diarrhoeal and constipation type) who fulfil the inclusion and exclusion criteria listed in **Table 3** are recruited via regular' patient's check-ups at the Gastroenterological unit at Thomayer University Hospital, by referrals from their general practitioners, following our newspaper articles or word of mouth.

Study microbiota mixture for intervention

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

possible (blending or post-filtration), the procedure was performed under a nitrogen atmosphere to protect obligate anaerobes. All stool portions were mixed together in a large stainless steel bucket using an electric mortar mixer under anaerobic conditions and at low temperature (on ice).

Based on the recommendation from the Nanjing consensus [15], the bacterial cell content of the study microbiota mixture was quantified. We performed a real-time PCR of the 16S rRNA gene with a standard curve derived from bacterial culture and controls from previously used stool transplants from another centre. It was estimated that the cell count in the transfer ranged between 2e+12 and 1e+13 (depending on the expected composition of the microbiota as to the 16S gene count per an average bacterial cell). Unfortunately, the Nanjing consensus [15] provides neither reference to the cell counting method (Table 2 therein) nor to control materials. Therefore more exact direct comparison of the requested quantities is not possible.

The mixed microbiota substance was divided into aliquots of 13-14 g (which is ~ 35 ml). Two-thirds of the tubes served as a placebo: they were immediately autoclaved at 121°C for 30 minutes with slow cooling. Pre-sterilised tubes were used to ensure that autoclaved placebos would not be visually distinguishable from tubes with the active substance. Assignation of tubes to the autoclave, numbering, sealing, and labelling were done under the guidance of a statistical unit member (see below).

All aliquot tubes are kept frozen at -80°C in the same type of plastic tubes, labelled by codes. Three such aliquots represent one dose for FMT (~40 g of stool, in ~105 ml). Aliquoting into multiple 50 ml tubes instead of one larger volume was decided because of the availability of durable plastic, which must be both autoclavable and deep frost resistant.

Before administering, the study microbiota mixture will be thawed in a warm (37°C) water bath, with intermittent mixing by inverting the tubes.

Randomization, allocation and blinding

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

Study Intervention

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

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

Outcomes

Primary outcome

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

Secondary outcomes

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

Data collection and follow-up

Timing of assessments

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

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

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

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

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

Weight, height, bioimpedance

Body weight, height and bioimpedance is examined during Visit 0, 1, 4, 5, 9 and 11. Medical Body Composition Analyzer Seca mBCA 515, (Seca, Germany) is used to measure changes in body composition (8-point bioelectric impedance analysis at a frequency of 5 - 50 kHz with a current of 100 µA), scanning performed with three pairs of hand electrodes and two pairs of leg electrodes, measurements performed with light clothing and without metal objects (jewellery, keys). The weight is determined in patients wearing underwear using the Seca mBCA 515. The height is determined by

a standardised technique with a metal stadiometer with an accuracy of 1 mm. Seca analytics 115 software is used to analyse the obtained data (Seca, Hamburg, Germany). The measurements is performed according to the NIHR Southampton Biomedical Research Centre standard protocol (Seca mBCA, NIHR Southampton Biomedical Research Centre, 2014).

Detailed anthropometry

It is performed by nutritional therapists in Visit 1, 5, 10 and 11. It involves weight, abdominal (waist) circumference, buttocks (hip) circumference, thigh circumference, skinfolds (thigh, triceps, subscapular, suprailiacal).

Serum workup, archiving serum+plasma

Blood is sampled at Visits 0, 4, 9, 11 and will include: A) serum+plasma archiving, B) serum workup. Laboratory panel testing will comprise sodium, potassium, chloride, urea, creatinine, glucose, calcium, phosphate, total protein and albumin, AST, ALT, ALP, **GGT**, bilirubin, lipid panel, HS-CRP, blood cell count with differential count, INR, urine analysis (sediment and biochemistry). One plasma and one serum aliquots are made at these visits and frozen for forensic reasons.

Psychological evaluation

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

Dietary questionnaire & advice, evaluation of food records

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

Gut microbiome composition

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

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

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

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

Safety monitoring

All data are regularly monitored by the research team for any adverse events, and all potential adverse events are recorded. Contacts to study coordinators active 24/7 are provided in case adverse effects occurred. If any concerns are identified during the screening or clinical assessment of donors or recipients, further clinical evaluation and/or examination is immediately realised. All the concerns during the study are assessed, and the recipient will be withdrawn if this is thought to be in his best

interest. A Data Monitoring and Safety Committee (DMSC) has been established and based on the data from planned interim analysis has the right to terminate the study if the frequency of severe adverse events crosses the 5% line (for closer description of DMSC, its responsibilities and premature termination of the study see **Appendix 2**).

Sample size and power calculation

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

Data management

Data from IBS-SSS, frequency of urgent defecations, Bristol stool scale, abdominal pain and bloating are collected and stored via the application Survey Monkey. All anthropometric data are entered and stored in password-protected platforms integrated within the hospital information system. Only the researchers involved in the study have access to the final study dataset (IBS-SSS, frequency of urgent defecations, Bristol stool scale, abdominal pain and bloating), which will be shared in an anonymised form via the Zenodo repository. The only data in this manuscript are bacteriome data; their anonymised form will be available on reasonable request.

Statistical analyses

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

the temporal effect (seen in the placebo group C), an indicator of group A corresponding to the cross-over effect (resulting from administration of placebo after active microbiota) and differences in indicators for groups A and B modelling the effect of active microbiota. A robust sandwich estimator of the variance matrix will be used to adjust for potentially unequal variances between the groups. Analyses of secondary outcomes will proceed by similar methodology, comparing absolute or relative differences of the post-intervention measure of each outcome relative to its baseline value. The CONSORT 2010 guidelines will be followed in reporting the main trial results.

Study status

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

Patient and public involvement

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

ETHICS AND DISSEMINATION

Ethics approval for this study was granted in June 2018 by the Ethics Committee of the Institute for Clinical and Experimental Medicine and Thomayer Hospital (Vídeňská 800, 140 59 Prague 4, Czech Republic). Involvement in this study is completely voluntary; donors and recipients are required to provide written informed consent prior to participation in the study (see **Appendix 3 and 4**). Recipients and their caregivers are informed of unexpected findings or unrecognised conditions and by possible future usage of their specimens in ancillary studies by trained physician or nurse; further medical care will be arranged. Study donors received financial compensation to pay for the required travelling costs when donating the stool. The patient will be offered a roll-over into an observational study with the administration of active microbiota. The patients are informed of this option at the start of the study and regularly reminded.

We aim to publish findings in impact peer-reviewed international journals. Gastroenterologists, internists and other care providers will be informed through the national conference meetings, journals and patient groups meetings.

Protocol amendment number: 01. Modification of the study protocol will be communicated with the Ethics committee.

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

Acknowledgement We thank Peter Holger Johnsen, Linn Skjevling and Hege Hansen from University Hospital of North Norway Harstad, Norway and Rasmus Goll from University Hospital of North Norway Tromsø, Norway, for valuable advice regarding the study design and study microbiota mixture preparation. We also thank Marcela Krutova, Jan Tkadlec, Daniela Lzicarova, Kamila Dundrova, Marie Brajerova, Milena Antuskova, Barbora Dravotova, Jana Prasilova, Jana Sumova and Ales Briksi all from Department of Medical microbiology, 2nd Faculty of Medicine, Charles University

and Motol University Hospital, Prague for their laboratory work in the regular microbiological screening of the study donors.

Contributors OC, PK, JH, JV, MK contributed to the conception and design of the study. OC, PK, JH and JV drafted the protocol with input from all other authors. JV and PK contributed to the patients recruitment. JH, LV, LK and OC contributed to the microbiome analysis for donor selection. JH, OC and JV contributed to the donor screening. LV, JH and OC contributed to the study microbiota mixture preparation. MK contributed to the power size calculations and statistical analysis. VL contributed to the randomization. JH and JV contributed equally to this paper, OC and PK contributed equally either.

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Competing interests None declared. No money from commercial sponsors was used.

Patient consent for publication Not required.

Ethics approval Ethics approval for this study was granted in June 2018 by the Ethics Committee of the Institut for Clinical and Experimental Medicine and Thomayer Hospital (Vídeňská 800, 140 59 Prague 4, Czech Republic).

Provenance and peer review Not commissioned; externally peer-reviewed.

FIGURES AND ILLUSTRATIONS

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

Figure 2 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 which 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 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.

Figure 3 Process of donor selection and reasons for their excluding

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)
	Clostridiodes difficile 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

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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
Clostridioides difficile (cultures, antigen testing)
Common enteric pathogens, including Salmonella, Shigella, Campylobacter, shiga toxin-producing *Escherichia coli*, Yersinia and *Vibrio cholerae* (cultures)
Antibiotic-resistant bacteria (ARB), including vancomycin-resistant Enterococci, meticillin-resistant *Staphylococcus aureus* Gram-negative ARB including extended-spectrum β-lactamase-producing *Enterobacteriaceae*, and carbapenem-resistant *Enterobacteriaceae*/carbapenemase-producing *Enterobacteriaceae* (cultures)
Norovirus, rotavirus, adenovirus, sapovirus (PCR)
SARS-CoV-2 (reverse transcription -PCR)
Common intestinal parasites, including *Giardia intestinalis, Cryptosporidium parvum et hominis* (cultures and PCR), *Blastocystis hominis**, *Dientamoeba fragilis** (both PCR only)

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

The screening strategy is based on [14].

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

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

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

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

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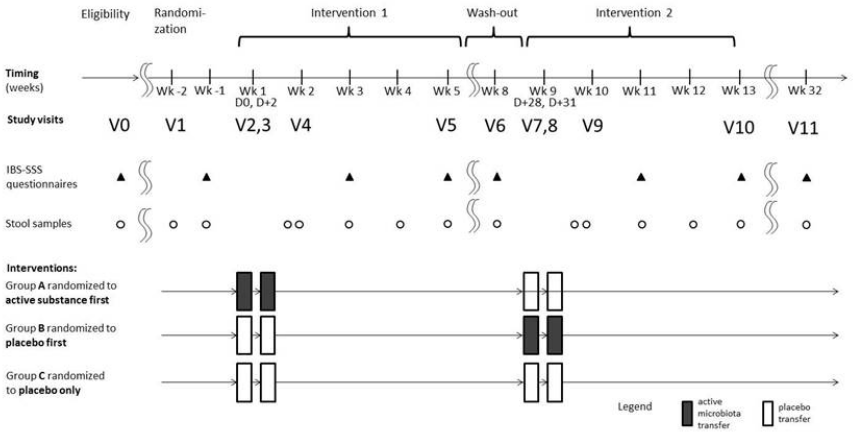

Per protocol intervention scheme: the visits, questionnaires and samples

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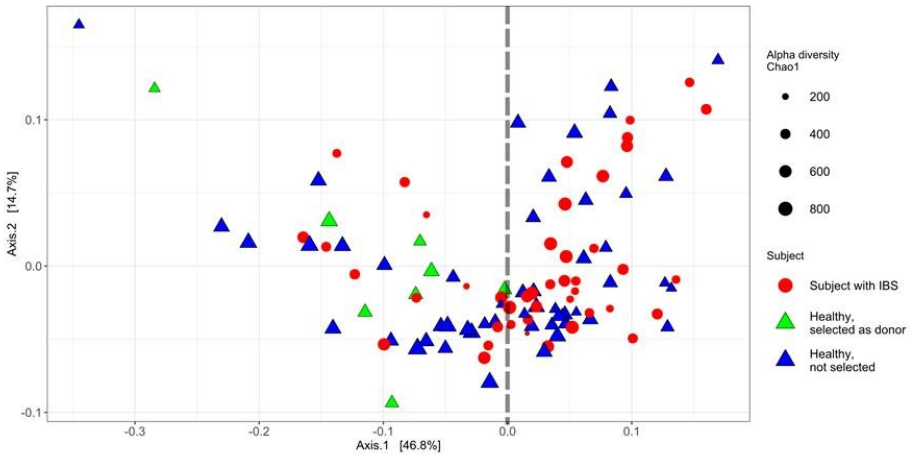
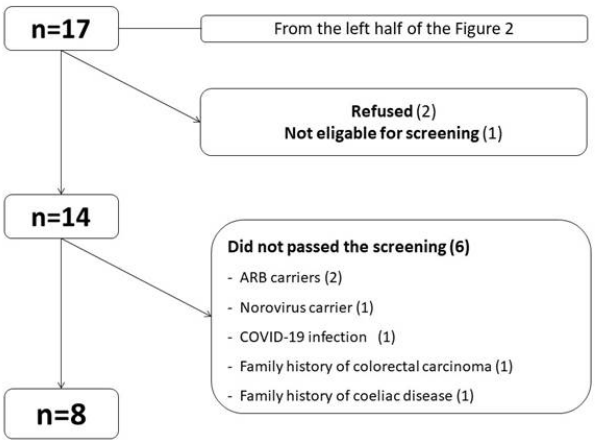


Ordination plot on the weighted Unifrac distance at the genus level for selection of the donor candidates based on their gut microbiome alpha- and beta-diversityThese are the results of a comparative microbiome case-control study that helped us to preselect 14 donor candidates. Alpha diversity calculation 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.

254x190mm (96 x 96 DPI)



Process of donor selection and reasons for their excluding

254x190mm (96 x 96 DPI)

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 recieved 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

Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	20
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	7
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	8
Objectives	#7	Specific objectives or hypotheses	8
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	9
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	10
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	10
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	13
Interventions:	#11b	Criteria for discontinuing or modifying allocated	13

modifications		interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	14
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	14
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	13
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	See Figure 1
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	17
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	11
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or	12

		assign interventions	
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	12
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	12
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	12
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	12-13
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	14-17
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	14
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	18

Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	18
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	18
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	18
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	Appendix 1
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have Access to these interim results and make the final decision to terminate the trial	Appendix 1
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	17
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	20
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	19
Protocol amendments	#25	Plans for communicating important protocol	20

		modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	19
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	19
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	19
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	20
Data access	#29	Statement of who will have Access to the final trial dataset, and disclosure of contractual agreements that limit such Access for investigators	18
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	19
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	19
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	20
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public Access to the full protocol, participant-level dataset, and statistical code	20
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Appendix 2

Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	15-17

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 studí 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 pomoct. 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 dovolujeme 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 poučeni o jejich používání. Po domluvě se členy vědeckého týmu (kontakt níže) by vzorky byly převezeny na naše pracoviště a hluboce zamraženy (-80°C).

Celý proces je dvoufázový. Z prvního vzorku se provede molekulárně-genetická analýza a následné 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 dárcem 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:

Datum:

Datum:

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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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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Word counts: 3865

Abbreviations: IBS, Irritable Bowel Syndrome; IBS-D, diarrheal type of irritable bowel syndrome; IBS-M, mixed type of irritable bowel syndrome; IBS-C, constipated type of irritable bowel syndrome; IBS-SSS, Irritable Bowel Syndrome Severity Scale Score

Keywords: irritable bowel syndrome, faecal microbiota transplantation, irritable bowel syndrome 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 the number of loose stools, 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); study results will be published in peer-reviewed journals and presented at international conferences and patient group meetings.

Study registration number. NCT04899869

STRENGTHS AND LIMITATIONS OF THIS STUDY

- Usage of mixed microbiota from multiple donors inflates the diversity of transferred microbiota by enriching it for numerous rare species.
- All interventions will be carried out using the same active mixed microbiota or the same placebo.
- Each intervention consists of two consecutive transfers, which increases the probability that the transferred microbiota engrafts.
- Microbiome profiling, food records, anthropometry and bioimpedance data allow detailed monitoring of transfer effectiveness.
- Mucosa-associated microbiota will not be assessed because the stool transfer will be performed by enema, not colonoscopy that would allow biopsies.

INTRODUCTION

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

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

IBS have been carried out [6, 7, 8, 9, 10, 11]. They differed in design, but none of them used a mixed microbiota from multiple donors as the active substance. Furthermore, a recent meta-analysis of randomized control trials on FMT in IBS (including the above-mentioned articles) pointed out insufficient evidence quality to support recommending FMT in the treatment of IBS. [12]

Our study protocol aims to test whether faecal microbiota transplantation of mixed microbiota from several selected donors can alleviate symptoms of IBS measured by IBS-SSS four weeks after the intervention, as compared to autoclaved placebo. The secondary study aims to test the acute (after two weeks) and the long-term effect (after six months) on symptoms relief. **We also focus on the number of loose stools, Bristol stool scale, abdominal pain and bloating, BMI, fat content, waist circumference, skinfold thickness, psychological evaluation and the gut microbiome composition.**

We hypothesise that the transfer of active microbiota of high diversity can lead to changes in the patient's gut microbiome composition and/or function to alleviate IBS symptoms.

METHODS AND ANALYSIS

Study design

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

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

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

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

Study setting

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

Recruitment and eligibility criteria

Stool donors

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

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

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

- every 4 weeks: for SARS-CoV-2 from nasopharyngeal swab;
- every 8-12 weeks: for all other stool tests mentioned in **Table 2**.

Prospective study participants

Patients diagnosed with IBS-D (diarrheal type) or IBS-M (mixed diarrhoeal and constipation type) who fulfil the inclusion and exclusion criteria listed in **Table 3** are recruited via regular' patient's check-ups at the Gastroenterological unit at Thomayer University Hospital, by referrals from their general practitioners, following our newspaper articles or word of mouth.

Study microbiota mixture for intervention

The intervention microbiota is a mixture of regular stool donations from the eight regular donors. The collection of stools for this purpose is already completed. The donors were advised to regularly defecate at their home toilet into a clean plastic bag placed in Fecotainer (Excretas Medical, NL) with an Anaerogen bag (Thermo Scientific, USA). This bag generated an anaerobic atmosphere during transport to ensure anaerobes survival. The stool was transported to the laboratory with the maximum allowable time until processing being 6 hours; the actual time was approximately 1.5 hours. The stool was weighed upon arrival, inspected for blood admixture, and immediately processed by blending with a solution consisting of sterile 0.9% saline (160 ml per 100 g of stool), sterile phosphate buffer saline at pH 7.4 (20 ml per 100 g of stool) and sterile 99.5% glycerol (20 ml per 100 g stool, which is approximately 10% of solution's volume; therefore, it is unlikely to have laxative properties upon administration). From our experience, ~ 105 ml of the study mixture represents ~40 g of stool**.** The mixture was then filtered through a sterile stainless steel mesh of 0.8 mm pore size into a sterile plastic bottle, which was then immediately frozen at -80°C. Whenever possible (blending or post-filtration), the procedure was performed under a nitrogen atmosphere to protect obligate anaerobes. All stool portions were mixed together in a large stainless steel bucket using an electric mortar mixer under anaerobic conditions and at low temperature (on ice).

Based on the recommendation from the Nanjing consensus [15], the bacterial cell content of the study microbiota mixture was quantified. We performed a real-time PCR of the 16S rRNA gene with a standard curve derived from bacterial culture and controls from previously used stool transplants from another centre. It was estimated that the cell count in the transfer ranged between 2e+12 and 1e+13 (depending on the expected composition of the microbiota as to the 16S gene count per an average bacterial cell). Unfortunately, the Nanjing consensus [15] provides neither reference to the cell counting method (Table 2 therein) nor to control materials. Therefore more exact direct comparison of the requested quantities is not possible.

The mixed microbiota substance was divided into aliquots of 13-14 g (which is ~ 35 ml). Two-thirds of the tubes served as a placebo: they were immediately autoclaved at 121°C for 30 minutes with slow cooling. Pre-sterilised tubes were used to ensure that autoclaved placebos would not be visually distinguishable from tubes with the active substance. Assignation of tubes to the autoclave, numbering, sealing, and labelling were done under the guidance of a statistical unit member (see below).

All aliquot tubes are kept frozen at -80°C in the same type of plastic tubes, labelled by codes. Three such aliquots represent one dose for FMT (~40 g of stool, in ~105 ml). Aliquoting into multiple 50 ml tubes instead of one larger volume was decided because of the availability of durable plastic, which must be both autoclavable and deep frost resistant.

Before administering, the study microbiota mixture will be thawed in a warm (37°C) water bath, with intermittent mixing by inverting the tubes.

Randomization, allocation and blinding

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

Study Intervention

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

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

Outcomes

Primary outcome

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

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

Secondary outcomes

- The acute change in the IBS severity symptom score (IBS-SSS) between baseline and two weeks after intervention (study weeks 3 and 11, respectively, see **Figure 1).**
- The long-term change in the IBS severity symptom score (IBS-SSS) between baseline (week -1) and week 32 (see **Figure 1**). The long term change will compare group C (placebo only) to merged groups A+B (active study microbiota mixture).
- **Following outcomes compare changes in the active microbiota group relative to the placebo group between baseline and study week 32:**
  - **Quantity of loose stools per day**
  - **Stool consistency evaluated by the Bristol stool scale**
  - **Abdominal pain measured by the Visual Analogue Scale (VAS)**
  - **Frequency of bloating per week**
  - **Body Mass Index in kg/m^2**
  - **Body fat mass estimated by measuring combined skinfold thickness in millimetres at given locations (biceps, triceps, subscapular, suprailiac)**
  - **Percentage of body fat mass measured by bioelectrical impedance analysis**
  - **Waist circumference in centimetres**
  - **The psychological and well-being effects of the therapy scored by IBS-QoL questionnaires**
  - **The faecal microbiome's alpha diversity measured by the Chao index**
  - **The faecal microbiome's beta diversity assessed by the quantitative Bray-Curtis index ordinated by non-metric multidimensional scaling (NMDS)**

- **Quantity of *Blastocystis* sp. assessed by a specific quantitative PCR assay measured in genomic equivalents per microlitre DNA**

Data collection and follow-up

Timing of assessments

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

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

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

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

Weight, height, bioimpedance

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

Detailed anthropometry

It is performed by nutritional therapists in Visit 1, 5, 10 and 11. It involves weight, abdominal (waist) circumference, buttocks (hip) circumference, thigh circumference, and skinfolds (thigh, triceps, subscapular, suprailiacal).

Serum workup, archiving serum+plasma

Blood is sampled at Visits 0, 4, 9, 11 and will include: A) serum+plasma archiving, B) serum workup. Laboratory panel testing will comprise sodium, potassium, chloride, urea, creatinine, glucose, calcium, phosphate, total protein and albumin, AST, ALT, ALP, **GGT**, bilirubin, lipid panel, HS-CRP, blood cell count with differential count, INR, urine analysis (sediment and biochemistry). One plasma and one serum aliquots are made at these visits and frozen for forensic reasons.

Psychological evaluation

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

Dietary questionnaire & advice, evaluation of food records

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

Gut microbiome composition

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

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

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

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

Safety monitoring

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

Sample size and power calculation

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

Data management

Data from IBS-SSS, frequency of urgent defecations, Bristol stool scale, abdominal pain and bloating are collected and stored via the application Survey Monkey. All anthropometric data are entered and stored in password-protected platforms integrated within the hospital information system. Only the

researchers involved in the study have access to the final study dataset (IBS-SSS, frequency of urgent defecations, Bristol stool scale, abdominal pain and bloating), which will be shared in an anonymised form via the Zenodo repository. The only data in this manuscript are bacteriome data; their anonymised form will be available on reasonable request.

Statistical analyses

The primary outcome analysis will be based on the difference in IBS-SSS scores over the second treatment period (week 14 vs week 8) minus the change over the first treatment period (week 5 vs week -1). This difference will be used as a response in a linear model, with intercept corresponding to the temporal effect (seen in the placebo group C), an indicator of group A corresponding to the cross-over effect (resulting from administration of placebo after active microbiota) and differences in indicators for groups A and B modelling the effect of active microbiota. A robust sandwich estimator of the variance matrix will be used to adjust for potentially unequal variances between the groups. Analyses of secondary outcomes will proceed by a similar methodology, comparing absolute or relative differences of the post-intervention measure of each outcome relative to its baseline value. The CONSORT 2010 guidelines will be followed in reporting the main trial results.

Study status

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

Patient and public involvement

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

ETHICS AND DISSEMINATION

Ethics approval for this study was granted in June 2018 by the Ethics Committee of the Institute for Clinical and Experimental Medicine and Thomayer Hospital (Vídeňská 800, 140 59 Prague 4, Czech Republic). Involvement in this study is completely voluntary; donors and recipients are required to provide written informed consent prior to participation in the study (see **Appendix 3 and 4**). Recipients and their caregivers are informed of unexpected findings or unrecognised conditions and by possible future usage of their specimens in ancillary studies by trained physician or nurse; further medical care will be arranged. Study donors received financial compensation to pay for the required travelling costs when donating the stool. The patient will be offered a roll-over into an observational study with the administration of active microbiota. The patients are informed of this option at the start of the study and regularly reminded.

We aim to publish findings in impact peer-reviewed international journals. Gastroenterologists, internists and other care providers will be informed through the national conference meetings, journals and patient groups meetings.

Protocol amendment number: 01. Modification of the study protocol will be communicated to the Ethics committee.

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

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Contributors OC, PK, JH, JV, MK contributed to the conception and design of the study. OC, PK, JH and JV drafted the protocol with input from all other authors. JV and PK contributed to the patients recruitment. JH, LV, LK and OC contributed to the microbiome analysis for donor selection. JH, OC and JV contributed to the donor screening. LV, JH and OC contributed to the study microbiota mixture preparation. MK contributed to the power size calculations and statistical analysis. VL contributed to the randomization. JH and JV contributed equally to this paper, OC and PK contributed equally either.

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Competing interests None declared. No money from commercial sponsors was used.

Patient consent for publication Not required.

Ethics approval Ethics approval for this study was granted in June 2018 by the Ethics Committee of the Institut for Clinical and Experimental Medicine and Thomayer Hospital (Vídeňská 800, 140 59 Prague 4, Czech Republic).

Provenance and peer review Not commissioned; externally peer-reviewed.

FIGURES AND ILLUSTRATIONS

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

Figure 2 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 which 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 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.

Figure 3 Process of donor selection and reasons for their excluding

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)
	Clostridiodes difficile 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

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
Clostridioides difficile (cultures, antigen testing)
Common enteric pathogens, including Salmonella, Shigella, Campylobacter, shiga toxin-producing *Escherichia coli*, Yersinia and *Vibrio cholerae* (cultures)
Antibiotic-resistant bacteria (ARB), including vancomycin-resistant Enterococci, meticillin-resistant *Staphylococcus aureus* Gram-negative ARB including extended-spectrum β-lactamase-producing *Enterobacteriaceae*, and carbapenem-resistant *Enterobacteriaceae*/carbapenemase-producing *Enterobacteriaceae* (cultures)
Norovirus, rotavirus, adenovirus, sapovirus (PCR)
SARS-CoV-2 (reverse transcription -PCR)
Common intestinal parasites, including *Giardia intestinalis, Cryptosporidium parvum et hominis* (cultures and PCR), *Blastocystis hominis**, *Dientamoeba fragilis** (both PCR only)

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

The screening strategy is based on [14].

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

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

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

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

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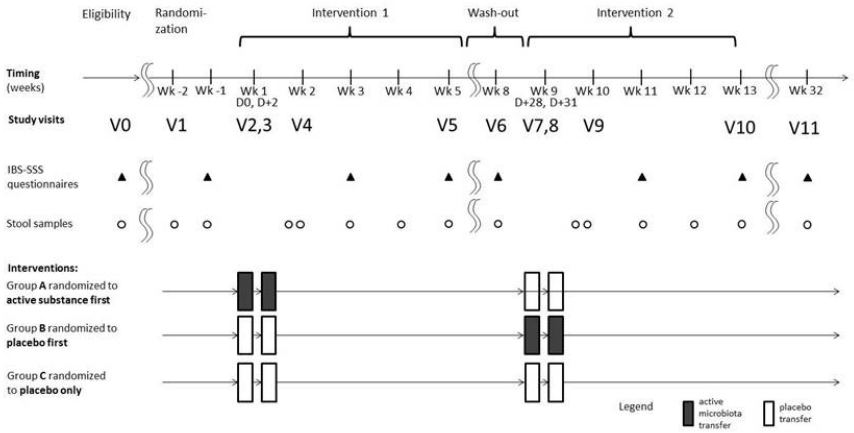
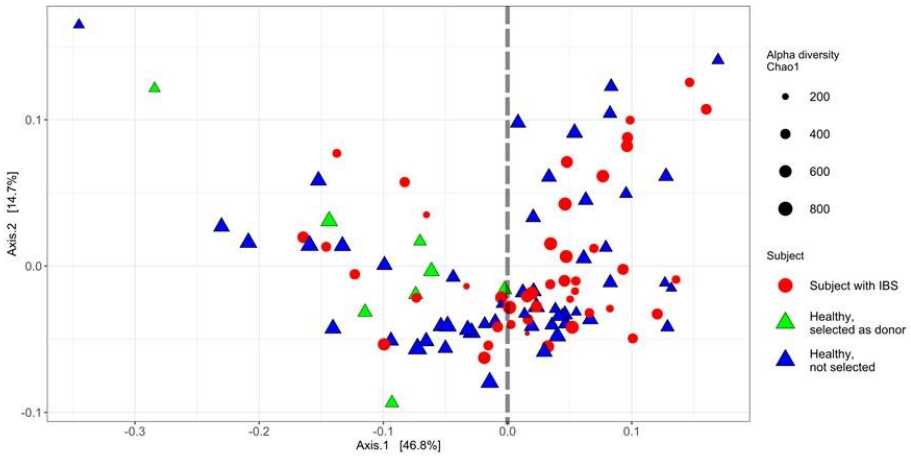


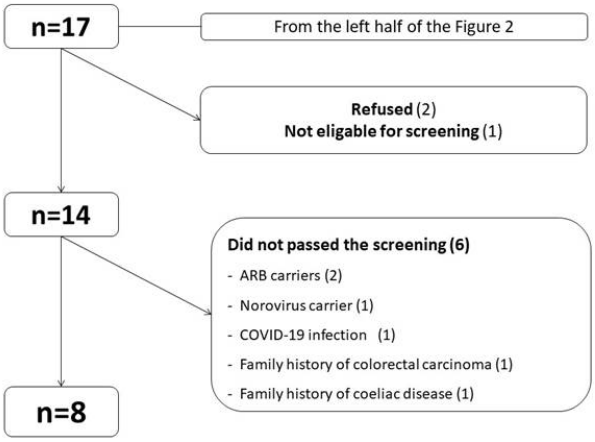
Per protocol intervention scheme: the visits, questionnaires and samples

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-diversityThese 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.

254x190mm (96 x 96 DPI)



Process of donor selection and reasons for their excluding

254x190mm (96 x 96 DPI)

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 recieved 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

Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	20
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	7
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	8
Objectives	#7	Specific objectives or hypotheses	8
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	9
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	10
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	10
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	13
Interventions:	#11b	Criteria for discontinuing or modifying allocated	13

modifications		interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	14
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	14
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	13
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	See Figure 1
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	17
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	11
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or	12

		assign interventions	
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	12
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	12
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	12
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	12-13
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	14-17
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	14
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	18

Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	18
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	18
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	18
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	Appendix 1
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have Access to these interim results and make the final decision to terminate the trial	Appendix 1
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	17
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	20
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	19
Protocol amendments	#25	Plans for communicating important protocol	20

		modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	19
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	19
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	19
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	20
Data access	#29	Statement of who will have Access to the final trial dataset, and disclosure of contractual agreements that limit such Access for investigators	18
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	19
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	19
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	20
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public Access to the full protocol, participant-level dataset, and statistical code	20
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Appendix 2

Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	15-17

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 studí 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 pomoct. 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 dovolujeme 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 poučeni o jejich používání. Po domluvě se členy vědeckého týmu (kontakt níže) by vzorky byly převezeny na naše pracoviště a hluboce zamraženy (-80°C).

Celý proces je dvoufázový. Z prvního vzorku se provede molekulárně-genetická analýza a následné 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 dárcem 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:

Datum: Datum: