SUPPLEMENTAL MATERIALS AND METHODS

Patient inclusion

In this prospective pilot study, patients with refractory IBS, aged between 18 and 75 years, were recruited in a tertiary hospital centre. Only patients with symptoms of intermittent diarrhoea and severe bloating were eligible for this trial. Exclusion criteria were the previous diagnosis of coeliac disease, lactose intolerance (unless on a stable diet for more than 3 months), prior gastro-intestinal surgery, antibiotic use within 3 months of inclusion or pregnancy. Patients with serious comorbidity and/or a psychiatric history were also excluded from the trial as per the including physician's discretion. Written informed consent was obtained from all patients and donors prior to study inclusion. The Ethics Committee of the Ghent University Hospital approved this study (EC2013/596).

Study outline

Eligible patients underwent a screening visit at the outpatient clinic. The severity of IBS and quality of life were assessed by means of a daily symptom diary collected over a period of 14 days and by standardized questionnaires, respectively. Following the FMT, patients were monitored for three months with scheduled visits to the outpatient clinic at 4, 8 and 12 weeks post-transplantation. A follow-up consultation 1 year after the FMT was performed.

Donor selection

Healthy donors, who were selected by the patients themselves, provided donor samples. Donors were required to be in good overall condition, to be between 18 and 65 years of age, to have normal, regular bowel movements and to have no gastrointestinal symptoms. Exclusion criteria for donors included body mass index (BMI) > 30, antibiotic use in the past 6 months, chronic disease or abnormal screening results. Following a clinical examination at the outpatient clinic, donors were screened for various transmittable diseases. Serological screening included testing for hepatitis A, B and C, HIV-1 and 2 and *Treponema pallidum*. Donor stools were screened by culturing for the presence of *Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, *Campylobacter* spp., *Clostridium difficile* and *Aeromonas* spp. Additionally, specific screening for antibiotic-resistant strains was performed using the active detection of carbapenemase-producing *Enterobacteriaceae* (CPE) and extended spectrum beta-lactamase (ESBL) producing organisms. Microscopic examination was performed to confirm the absence of

eggs, cysts and/or larvae of parasites, and the presence of Clostridium difficile toxins A and B was screened using an enzyme immuno-assay.

FMT procedure

Fresh donor stools were collected on the day of transplantation, stored at 4°C and processed within 6 hours, as previously described. [1] Briefly, 300 mL of 0.9% NaCl was added to the stool sample and thoroughly mixed using a handheld blender. The resulting suspension was filtered and transferred into 60 mL syringes. All procedures were performed under laminar airflow conditions. Prior to transplantation, patients underwent a bowel preparation using macrogol. The FMT was performed by means of colonoscopy with an injection of 300 mL of the donor faeces suspension into the terminal ileum and caecum. Following the transplantation, patients were kept sober and under close observation in a supine position for four hours. A dose of 2 mg loperamide was administered both prior to and following transplantation.

Primary and secondary endpoints

The primary endpoint of this pilot study was the relief of global IBS symptoms and abdominal bloating at 12 weeks following the FMT. Adequate relief was defined as a positive answer to two key questions: '1) Do you feel improvement in your overall IBS symptoms since transplantation?' and '2) Do you feel improvement in your sensation of bloating since transplantation?'.

The secondary endpoints were as follows: 1) changes in daily assessed IBS symptoms of abdominal discomfort, abdominal pain, abdominal bloating, flatulence, stool consistency, number of defecations and abdominal circumference; 2) changes of quality of life; and 3) changes of faecal microbiota composition.

Evaluation of clinical endpoints

IBS-related symptoms were assessed using a daily symptom diary (supplementary table 1) that evaluated general abdominal discomfort, abdominal bloating, abdominal pain and flatulence on a scale of one to six. In addition, the number of daily bowel movements, the consistency of the stools and the abdominal circumference (measured at the umbilicus by patients themselves at exactly the same time every day) were assessed as well. Quality of life was assessed using a standardized, IBS-specific quality of life questionnaire (IBS-QoL) in the patient's native language.

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67 **Microbiome analysis**

68 Consecutive faecal samples were collected for microbiome analyses. Stool samples of patients
69 and donors were collected before the FMT (at baseline) and at selected time-points after
70 treatment. Samples were immediately stored at -20°C and transferred to -80°C within one week.
71 DNA was extracted from the frozen faecal samples using the PowerMicrobiome RNA Isolation
72 Kit (MOBIO Laboratories Inc.) as previously described. [2] Bacterial and archaeal 16S rRNA
73 genes were amplified using the 515F/806R primer set targeting the V4 hypervariable region
74 according to Caporaso and colleagues. [3] Sequencing was performed using the Illumina MiSeq
75 platform with sequencing kit MiSeq v2, producing 250 bp paired-end reads.

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77 **16S data processing**

78 Sequence analysis was performed according to Geirnaert and colleagues. [4] FLASH[16]-merged
79 sequences were subject to quality filtering using the FASTX-toolkit, and chimaera removal was
80 performed using UCHIME. [5,6] Samples were rarefied to 10,000 reads; then, the taxonomical
81 classifications of sequences were performed using an RDP classifier [7] to generate phylum to
82 genus level composition matrices. Bootstrap values from the RDP classifier were used to identify
83 sequences with high-confidence genus assignments (bootstrap value >0.8), whereas sequences
84 classified with lower confidence were binned at the family level (labelled unclassified family). A
85 species-level matrix was generated using *de novo* OTU clustering with the UPARSE pipeline [8]
86 and a 97% identity threshold.

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88 **Statistical analysis**

89 Statistical analysis was performed using SPSS Statistics version 22 (Chicago, USA). Wilcoxon
90 signed ranks test and the Mann-Whitney U test were used for analysis of paired and non-paired
91 data, respectively. Two-tailed probabilities were calculated and *p*-values of less than 0.05 were
92 considered statistically significant.

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