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CASE REPORT

Fecal Microbiota Transplantation promotes disease remission in a patient with active Crohn's disease: A Case Report

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ABSTRACT

Background: A woman aged 46 years presented with moderate to severe Crohn's disease (CD). Sigmoidoscopy revealed proctitis, with Harvey-Bradshaw Index (HBI)=7. The patient experienced up to six bloody stools per day, stool samples showed increased fecal calprotectin, and low albumin.

Method: Case Report. Therapy with 1 portion fecal microbiota transplantation (FMT) capsules every day as add on treatment for 2 weeks was initiated. Hereafter, FMT therapy was tapered off for 21 weeks and stopped.

Results: The patient achieved disease remission at week 21. From 2020 till 2023, when the patient experienced disease relapses (up till twice a year), the patient was treated with FMT capsules (one portion per day) for 1-2 weeks, which promoted disease remission. At the age of 49, the patient was examined by colonoscopy, blood tests and fecal calprotectin. Endoscopy and biopsies (macroscopic and microscopic) showed mild inflammation around the appendix and anal regions and no inflammation in the remaining part of the colon and ileum. Blood samples and fecal calprotectin collected prior to colonoscopy were normal.

Conclusion: This study suggests that FMT may promote disease remission in CD patients with moderate to severe disease. Microbiome data shows that the patients gut microbiome changed to resemble the donor microbiome. FMT therapy was able to change the intestinal microbiome in a patient with CD accompanied by clinical disease remission.

Keywords: Fecal Microbiota Transplantation, Crohn's disease, Disease relapses, Disease remission, Colonoscopy, biopsy, Microbiome, probiotics

Introduction

Crohn's disease (CD) is a chronic, segmental, localized granulomatous disease that can affect all parts of the gastrointestinal tract, from the mouth to the anus. The clinical presentation depends on the disease location and may include diarrhea, abdominal pain, fever, clinical signs of bowel obstruction and anal passage of blood, mucus, or both[1]. The disease can appear at any age, but most patients are diagnosed in the third decade of their life span[2]. The prevalence of the disease is 30-100 cases per 100,000 inhabitants in Northern Europe and 241.3 cases per 100,000 in the United States[3]. The etiology of CD is still unclear, but studies indicate a pathophysiology that includes genetic[4], immunological[5], nutritional[6], bacterial[7,8], viral[9], and environmental factors[10]. Animal model studies indicate that the gut microbiota plays an essential role in disease relapses in CD cases[11,12] as germfree mice do not develop IBD and antibiotic therapy shows some benefit in short term remission in CD patients[13]. Intestinal dysbiosis has been linked to CD when compared to healthy controls. Among changes in the intestinal microbiome is low densities of bacteria in the proximal and middle small intestine and increased densities in the distal small intestine and the colon is reported[14]. Low redox potential, relatively neutral PH, and slow transit time in the distal colon with high bacteria concentration is also linked to CD[14]. There is currently no curative treatment for CD. Immunosuppressants/ biological treatment such as TNF-α blockers are used to promote disease remission in CD, but biological treatment is expensive with possible serious side-effects[15]. Probiotics have been shown to be partly effective in maintaining disease remission[16,17] in ulcerative colitis. However, effective treatment for CD patients with active disease without serious side effects is urgently needed. FMT capsule therapy has been shown to be effective in the treatment of ulcerative colitis patients[18], but more studies are needed to show FMT capsule efficacy in ulcerative colitis and CD.

The purpose of this case study was to discover the long-term efficacy of FMT capsule therapy to promote disease remission in a CD patient with moderate to severe disease.

Description of the therapeutic procedure, FMT donor and patient sample collection

For this study, a female stool donor aged 34 was recruited. Her stool was tested according to national guidelines[19,20] and was found to be suitable for production of FMT capsules and enema were produced as described by Lauridsen et al 2019[21,22]. Patient colonoscopy/ tissue, stool and blood samples were handled and analyzed at the hospital.

DNA Extraction from fecal sample

Stools from the donor and patient were stored at -80°C until use. DNA extraction of donor and patient stools was performed according to the instruction of the manufacturer (DNA Stool Mini Kit, Qiagen, Hilden, Germany)[23].

Sequencing of Microbial population in fecal samples

Three of the samples were analyzed using the following method (Patient at time zero (year-2019), patient at time 2023 and donor at time 2023): Microbiota diversity analysis relied on sequencing of the ribosomal small subunit (SSU rRNA) genes. Purified genomic DNA was submitted to PCR using a primer set targeting prokaryotes and eukaryotes (one primer set

for 16S, and three different in-house primer sets for 18S (G3-1, G4-3, G6-1)). For Prokaryotes, a modified version of the published universal prokaryotic primers 341F/806R was used[23]. Resulting PCR products were quantified using the Quant-IT^{tm} dsDNA High sensitive Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) and pooled in equimolar amounts (Pooled Amplicon Library (PAL)). Agencourt AMPure XP Beads (Beckman Coulter, Brea, CA, USA) were used to remove DNA fragments shorter than 300 bp and those longer than 1,000 bp, and the purified DNA was sequenced on the Illumina MiSeq system in a 2x250-bp set up (Illumina Inc.). A maximum of 64 samples were sequenced in a single sequencing run[24].

Two of the samples were analyzed using the following method (Patient at time 2020, and donor at time 2020): Purified genomic DNA was submitted to for sequencing to BMR Genomics, DNA Sequencing Service. At the company the samples underwent the library preparation steps as described in the Illumina 16S Metagenomic Sequencing Library Preparation (https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf). The library preparation includes purification with Agencourt AMPure XP beads, ligation of Illumina adapters and indexes (Nextera XT index kit), normalization and multiplexing, Paired End (2x300 bp) sequencing on Illumina MiSeq platform. The sequence output was taxonomically mapped using BION.

Data Analysis

The sequence output was taxonomically mapped using BION (unpublished open-source package for microbiome analyses, that takes sequence machine data as input and produces taxonomic overviews, only analyses 16S/ 18S rRNA), a newly developed k-mer-based mapping software. A k-mer length of 8 was used, with a step size of 4. Query sequences originating from prokaryotes were compared with the 341-806 bp region (rRNA gene position from *Escherichia coli*) in RDP 11.04.

The taxonomic data was analyzed in R version 4.3.0[46] and ggplot2 v. 3.4.4[47] was used for visualizations. The read counts were collapsed on genus level and converted to relative abundances. Genera identified at <1% relative abundance were grouped as "Other".

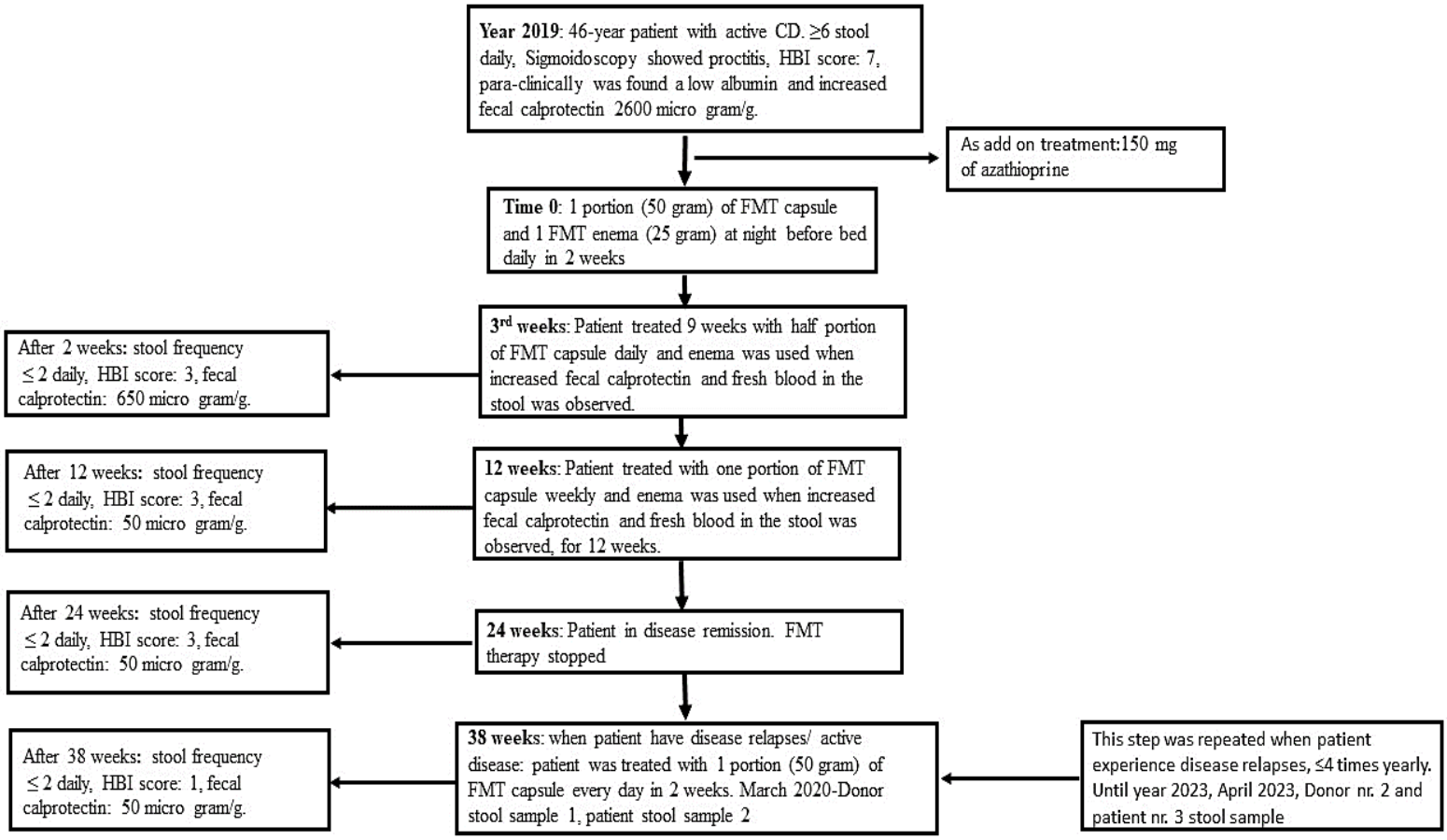
Results

Patient included in the study

A 46-year-old woman presented with moderate to severe CD. She was diagnosed with CD at age of 27. In 2005, the 32-year-old, patient started treatment with 150 mg of azathioprine and 60 mg oral prednisolone, when she had disease relapses. At the age of 34, she started therapy with intravenous infliximab, which promoted disease remission. However, as a side-effect to infliximab, there have been reports of increased patient-infections such as severe pneumococcal infections and flu. Treatments with Infliximab were stopped at the age of 43 since the patient had disease remission for 3 years. By the age of 46, she presented with moderate to severe disease symptoms with bloody stools up to six times a day. At this point, the patient was treated with 150 mg of azathioprine in addition to oral probiotics daily. A sigmoidoscopy was performed and showed proctitis, the HBI score was 7, a low albumin and increased fecal calprotectin 2600 microgram /g was detected. The patient-initiated therapy with FMT. In total 57.5 portions of FMT capsules (50-gram stool each) and 14

portions of FMT enema (25-gram stool each) were used for 23 weeks as therapy.

Figure 1: the figure shows an overview of patient FMT therapy/ follow-up.

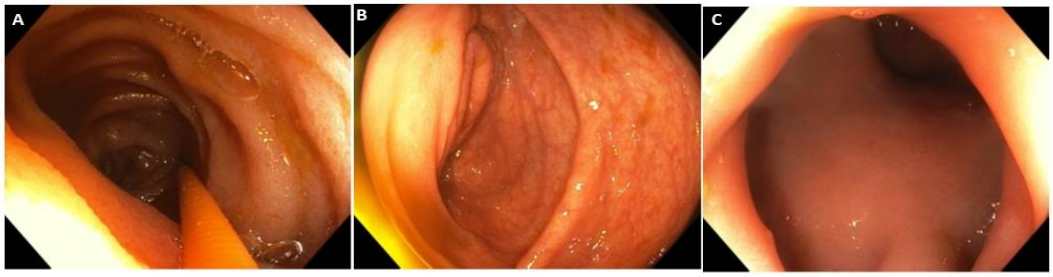


The patient start FMT therapy as described in figure 1. At the end of week 2, the patient experienced relief in symptoms with stool frequency decreasing to only 2-3 times a day without blood, HBI score was reduced to 3 and fecal calprotectin was reduced to 650 microgram/g from 2600 microgram/g. From week 3, the patient was treated for 9 weeks with half a portion of FMT capsules every day and an enema treatment was added, when the patient experienced disease activity in rectum, indicated by increased fecal calprotectin and fresh blood in the stool. By week 12, the patient achieved disease remission, therefore FMT capsule intake was reduced to one portion every week for 12 weeks and then the FMT therapy was finalized. The patient was followed up each 3rd month with blood test for 3 years. When the patient was evaluated at week 22, her blood test, serum albumin and blood cells, were normal.

At week 38, the patient experience disease relapse and her fecal calprotectin was very high, 1,530 microgram/g. Therefore, the patient was treated with one portion of FMT capsules every day and 1 FMT enema every second day for 2 weeks. During this treatment the fecal calprotectin level returned to normal levels; 19 microgram/gram. Hereafter, when the patient experienced disease relapses, she was treated daily with FMT capsules for one or two weeks,

sufficient to promote disease remission. The patient's fecal calprotectin was regularly tested every 3rd month and was found to be within normal levels. In 2023, the patient was examined by colonoscopy. Biopsies were performed from all segments of the colon and rectum. The result of colonoscopy and biopsies (macroscopic and microscopic) showed only mild inflammation around the appendix and the anal 3 cm of rectum as shown in figure 2. There was no inflammation in the remaining part of the colon and ileum (figure 2), blood tests and fecal calprotectin taken before colonoscopy were also normal.

Figure 2: A) normal ileum, B) mild inflammation around appendix. C) mild inflammation 3 cm in the rectum.



Microbiome data

The microbiome data (16S and 18S) showed relevant changes in the patient's microbiome after FMT, reflecting microbiota transferred from the donor to the patient, including increased abundance of Bacteroidetes in the patient and donor at time 2023, 45% and 40% respectively, in comparison to time zero, 10% and 8% respectively, figure 3. When comparing the donor and patient microbiome at time zero, they show increased prevalence of Firmicutes, 67% and 80% respectively, in comparison to microbiome data from 2023, 35% and 42% respectively (figure 3). The microbiome results show increased abundance of Methanobrevibacter and decreased abundance of Actinobacteria. Furthermore, a transfer from the donor to the patient of the presumed beneficial parasites Blastocystis was demonstrated, 17,873 and 5,490 reads in OTU receptively (table 1).

Discussion

Studies indicate that gut microbiota dysbiosis[25] is linked with CD and might play an essential role in causing disease relapses[8]. There is no curative treatment for CD and lasting disease remission in patients with active disease is still a medical challenge. Therefore, more research on this subject is essential to discover a safe, affordable therapy that can promote disease remission, without serious side-effects. In the last decades, biological treatment has been widely used. However, biological medications are expensive and they have possible serious side-effects such as psoriasis, maculopapular rash, exudative-oedematous lesion on the skin, infections etc[15].

In the last decade, many studies have explored the potential of FMT to promote disease remission in patients with gut microbiota dysbiosis[26–29]. Not all studies have been successful[30], presumably caused by errors in administration or manufacturing of FMT products/ components, lacking knowledge on the dosage and duration of the therapy, lack of experience regarding the regulatory and safety of FMT[31].

Figure 3: Relative abundance of bacteria in the patient at time zero, before FMT therapy, 38 weeks after FMT therapy and at March 2023, when FMT therapy stopped, stool samples was taking, and colonoscopy was performed.

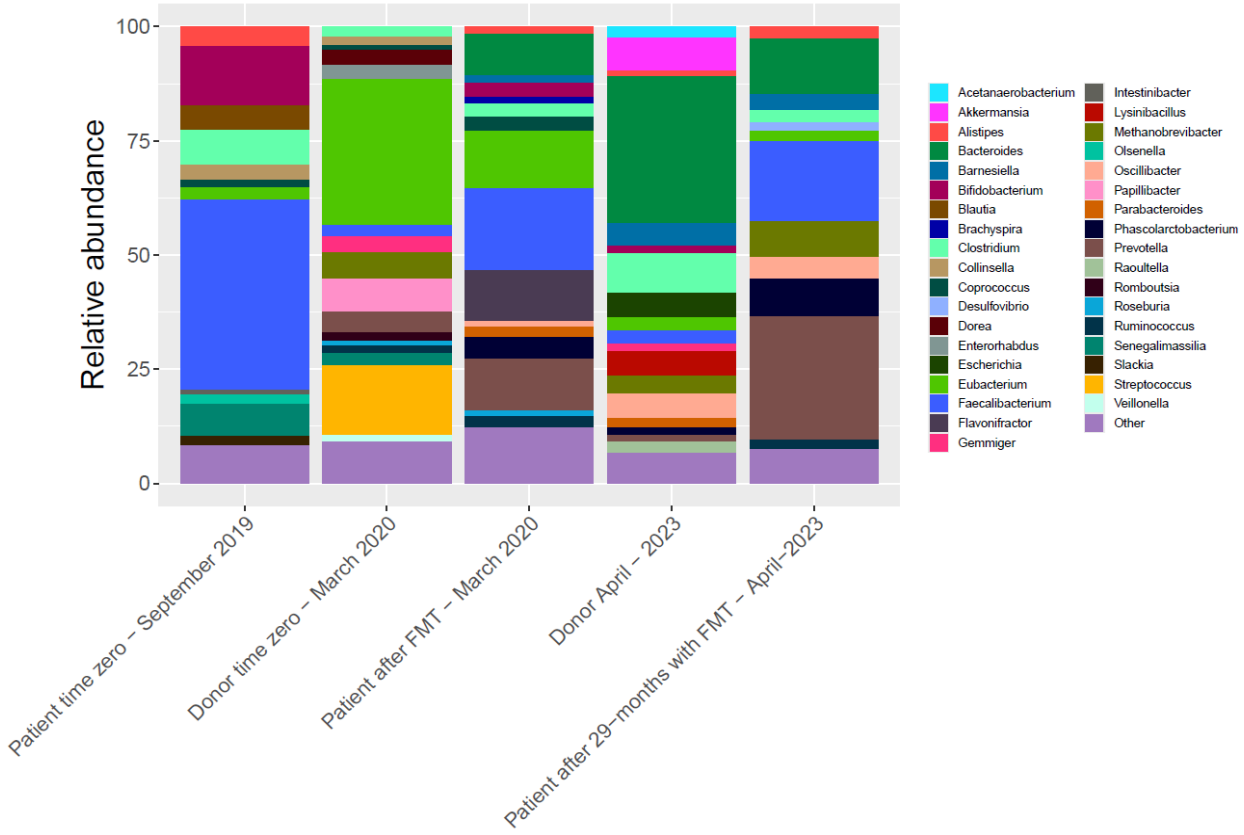


TABLE 1 | Total reads in OTU of selected eukaryotic species with increased abundance in the study group.

Patient- Time zero- 2019			Patient- Time 2023			Donor-Time 2023		
Genus	**Species**	**OTU**	**Genus**	**Species**	**OTU**	**Genus**	**Species**	**OTU**
Cladosporium	cladosporioides	124	**Methanobrevibacter**	**smithii**	**9.34**	**Methanobrevibacter**	**smithii**	**4.936**
Candida	krusei	67.967	Aspergillus	sclerotiorum	2.714	Penicillium	olsonii	20
Candida	tropicalis	355	Aspergillus	unclassified	157	Aspergillus	terreus	408
Saccharomyces	**bayanus**	**156**	Geotrichum	candidum	10.288	Debaryomyces	hansenii	4,370
Saccharomyces	**cerevisiae**	**1.484**	Geotrichum	candidum	53.394	**Saccharomyces**	**bayanus**	**1,587**
Trichocladium	asperum	41	Kazachstania	unispora	34	**Saccharomyces**	**cerevisiae**	**11,270**
Malassezia	globosa	237	**Saccharomyces**	**bayanus**	**593**	Saccharomyces	paradoxus	5
Apium	graveolens	267	**Saccharomyces**	**cerevisiae**	**2.272**	Trichinella	pseudospiralis	257
Brassica	**rapa**	**371**	Hanseniaspora	uvarum	12	**Brassica**	**rapa**	**1,062**
Beta	vulgaris	718	Wickerhamomyces	unclassified	6	Brassica	unclassified	17
Spinacia	oleracea	171	Cryptococcus	antarctica	14	Spinacia	oleracea	62
Alnus	alnobetula	364	Corallocarpus	quinquefida	12	Sassafras	albidum	81
Rhizophagus	irregularis	937	Lagenaria	breviflora	113	Allium	fistulosum	30
Triticum	distachyon	396	Arctium	lappa	14	Allium	sativum	42
Daucus	carota	440	Cicer	arietinum	59	Oryza	meridionalis	566
Beta	vulgaris	363	Phaseoleae	environmental	48	Oryza	unclassified	22
Phaseoleae	environmental	152	Rhoiptelea	chiliantha	58	Cinnamomum	camphora	79
Monarda	fistulosa	200	Rhodotypos	scandens	936	Pyrus	unclassified	301
Anthoxanthum	odoratum	602	Pyrus	persica	150	**Blastocystis**	**unclassified**	**17,873**
Rhodotypos	scandens	254	**Blastocystis**	**unclassified**	**5,490**	Gallus	gallus	5
Theobroma	cacao	281	Lambdavirus	lambda-IPC	19	Lambdavirus	lambda-IPC	17
Urtica	angustifolia	189						
Salmo	salar	147						

However, when the FMT national/international guidelines are met, FMT appears safe[31]. Goyal et al 2018[32] reported FMT to be effective in promoting disease remission in inflammatory bowel disease patients with mild active to moderate disease[32], when 50 gram donor stool was administered using nasojejunal[33], colonoscopy[34] and administered as oral capsules[18]. A few studies based on FMT therapy in children with mild to moderate CD have been performed, and they showed effect in promoting disease remission as first-line treatment followed by increased gut microbiome diversity[32,35].

In the current case study, a CD patient with bloody diarrhea, increased fecal calprotectin and proctitis was included. However, because of the lack of colonoscopy, disease activity in other parts of the colon is unknown. Because of infliximab side-effects, such as infection; the patient was treated with FMT capsules and enema. Combination therapy with FMT capsules and enema showed efficacy to promote and maintain disease remission in this CD patient with active disease. The efficacy of FMT in this case was confirmed, when 2 weeks of therapy reduced fecal calprotectin from 1,530 microgram/g to 19 microgram/g.

Three years of follow up shows no serious side effects using FMT capsules and enemas as therapy in this CD patient, in contrast, colonoscopy results showed remission in the colon and ileum with limited inflammation. Laboratory blood test results and fecal calprotectin were normal. At the endoscopy time point the patient had been treated with 150 mg of azathioprine for 19 years.

The microbiome data (16S and 18S) showed relevant changes in the patient's microbiome after FMT reflecting microbiota transferred from the donor to the patient, including increased abundance of Bacteroidetes, Methanobrevibacter and decreased abundance of Actinobacteria. Furthermore, a transfer from the donor to the patient of the presumed beneficial parasites Blastocystis was demonstrated.

Agata et al 2023[36] showed increased correlation between the prevalence of *Methanobrevibacter* in healthy controls[36] and ulcerative colitis patient with inactive disease. Data shows decreased prevalence of Firmicutes in the patient and the donor in samples collected at time 2023 in comparison to time zero, however, this change was not significant. Spase Stojanov et al. 2021[37] found an increased ratio of Firmicutes and decreased ratio of Bacteroidetes are beneficial for promoting IBD disease remission. Blastocystis is one of the most common human nonfungal eukaryotic enter-parasitic organisms in developing countries[38]. However, the role of Blastocystis in disease and health is still unknown. Some studies link the prevalence of Blastocystis to irritable bowel syndrome (diarrhea), while other studies link high prevalence of Blastocystis with a healthy gut[38–40].This data confirm the results from Petersen et al. (2013)[38], with a significant increase of Blastocystis in the healthy control groups in comparison with the IBD patient.

FMT therapy in CD with active disease, was found to be safe and beneficial in promoting disease remission without side-effects in our patient. This was confirmed by colonoscopy, HBI score and reduced inflammation markers. However, follow-up on the patient´s gut microbiome is needed, since IBD patients are known to have immuno-deficiency, which will provide a beneficial environment for

opportunistic bacteria to overgrow the recipient's intestinal microbiota. These opportunistic bacteria could be transferred from donor stool and could possibly colonize and overgrow the recipient's/ CD patient's intestinal microbiota.

Further research is needed to investigate the donor-dependent healing effect of FMT in patients suffering from CD.

Conclusion

Four years of follow up shows no serious side effects using FMT capsule and enema as therapy, in contrast colonoscopy results showed remission in the colon and ileum, with limited inflammation around the rectum and appendix. Laboratory blood test and fecal calprotectin were normal. This study indicates that FMT can be used to promote disease remission in CD patients with active disease and maintain remission without side effects.

Conflict of Interest Statement:

The authors declare no conflict of interest.

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