Study Protocol and Statistical Analysis Plan

Title of the project

FECAL MICROBIOTA TRANSPLANTATION IN CHRONIC POUCHITIS: A RANDOMIZED, PARALLEL, DOUBLE-BLINDED CLINICAL TRIAL

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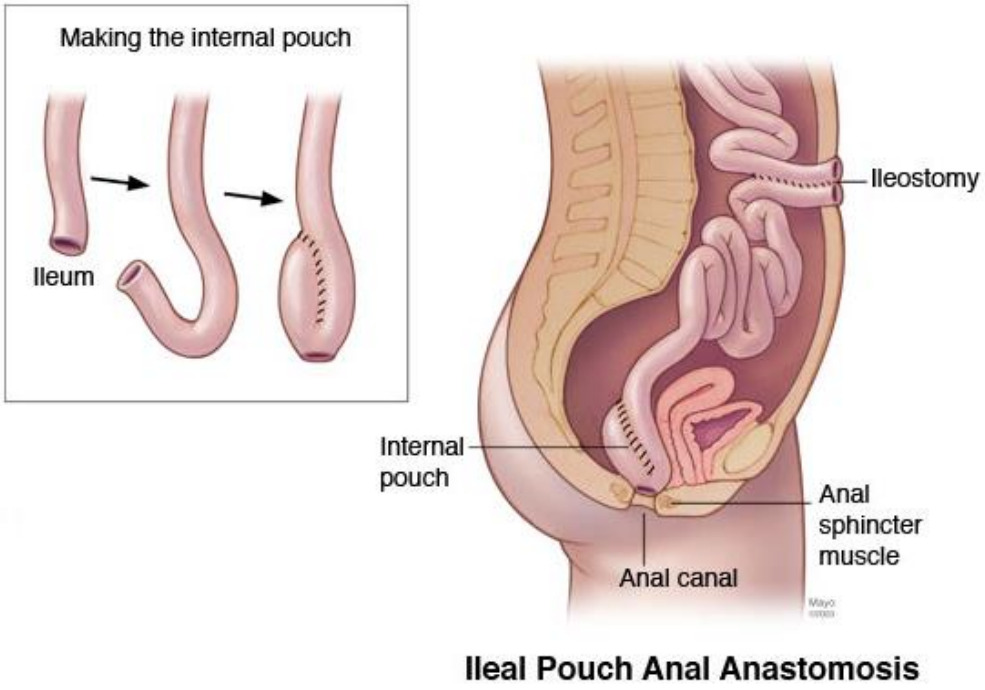
Background

Pouchitis is the most common complication among patients with ulcerative colitis (UC) who have undergone restorative proctocolectomy (Figure 1) with ileal pouch-anal anastomosis. The most common long term complication is pouchitis, which accounts for 10% of pouch failures. It is an idiopathic inflammatory condition that may occur in up to 50% of patients after an ileal-pouch anal anastomosis for UC (Landy 2012).

Figure 1. Ileal pouch anal anastomosis (IPAA)



The etiology of pouchitis remains unclear. There is significant clinical evidence implicating bacteria in the pathogenesis of pouchitis. Studies using culture and molecular methods demonstrate a dysbiosis of the pouch microbiota in pouchitis. The study of ileal pouch mucosal biopsies and fecal samples analyzed with a 16S rDNA-based terminal restriction fragment length polymorphism (TRFLP) approach have revealed statistically significant differences in the mucosal and fecal microbiota between UC and FAP (Familiar adenomatous polyposis) patients who had IPAA (Zella 2011). UC pouches included significantly more TRFLP peaks matching Clostridium and Eubacterium genera compared to FAP pouches and fewer peaks matching Lactobacillus and Streptococcus genera compared to FAP. DNA Sanger sequencing of a subset of luminal samples revealed UC pouches having more identifiable sequences of Firmicutes (51.2% versus 21.2%) and Verrucomicrobia (20.2% versus 3.2%), and fewer Bacteroidetes (17.9% versus 60.5%) and Proteobacteria (9.8% versus 14.7%) compared to FAP. Risk factors, genetic associations, and serological markers of pouchitis suggest that the interactions between the host immune responses and the pouch microbiota underlie the etiology of this idiopathic inflammatory condition. Many factors contribute to the course of refractory pouchitis, such as the use of non-steroidal anti-inflammatory drugs, infection with *Clostridium difficile*, pouch ischemia, or concurrent immune-mediated disorders

Initial acute episodes typically respond to antibiotic therapy such as metronidazole, patients can become dependent on antibiotics or develop refractory disease. Different treatments for the refrac-

tory pouchitis have been tested including different treatments used in the treatment of UC (mesalazine, immunosuppressive or immunomodulative), but long term remission is seldom achieved. Probiotics, especially products containing many strains of probiotics such as VSL3# have been been effective in some studies (Gionchetti 2003). Otherwise a single-strain probiotic bacterium supplement of Lactobacillus GG has been shown to be ineffective (Kuisma 2003).

Treatment of recurrent *Clostridium difficile*-infection (CDI) with antibiotics leads to recurrences in up to 50% of patients. It has been shown that fecal microbiota transplantation (FMT) through colonoscopy is an effective treatment for recurrent CDI in over 90% of CDI patients. Transplantation was done for 70 patients and no significant complications occurred for the transplantation procedure (Mattila 2012). Case reports have also shown promising results of FMT in IBD patients (Anderson 2012). Only one case report about FMT in the treatment of *Clostridium difficile*-associated pouchitis is available (Patel 2013), where a patient with chronic antibiotic-dependent pouchitis contracted refractory CDI. FMT lead to the eradication of CDI, but the baseline chronic pouchitis remained. There are three prospective uncontrolled cohort studies and one case report about FMT in the treatment of chronic pouchitis, reporting on 23 patients in total. Two of the cohort studies used a single FMT infusion with 25% (2/8) achieving clinical response in one study (Landy 2015) and 71% (5/7) in the other study (El-Nachef 2016), but no patients achieved clinical remission. In the third cohort study was used multiple FMT infusions and 4 of 5 patients achieved clinical remission and the other patient achieved clinical response (Stallmach 2016). The only case report used single infusion and the patient achieved clinical remission (Fang 2016).

Currently there is no established effective treatment for chronic antibiotic dependent or refractory pouchitis. The aim of our study is to investigate the efficacy and safety of fecal transplantation in treatment of chronic pouchitis instead of antibiotic therapy. Another aim is to evaluate phylogenetic analysis of the fecal microbiota trying to find microorganisms contributing to good results in fecal transplantation in IPAA patients.

STUDY DESIGN

STUDY POPULATION

Inclusion criteria for study

- Status post of restorative proctocolectomy with ileal pouch-anal anastomosis (IPAA) for ulcerative colitis
- Pouchitis diagnosed by the symptoms and by endoscopy including histology within 6 months prior to FMT.
- Need of frequent or continuous use of antibiotics or probiotics because of the chronic pouchitis
- Availability of consecutive fecal samples during one year
- Compliance to attend FMT and control pouchscopy after 52 weeks
- 18-75 years

Exclusion criteria for study

- Unable to provide informed consent
- Use of immunosuppressive or biological medication
- Pregnancy

METHODS

26 consecutive patients with pouchitis diagnosed by the symptoms and endoscopy. Patients are collected from the Helsinki University Central Hospital. Use of antibiotics or probiotics is not continued during the study. Fecal microbiota transplantation (FMT) is performed by experienced endoscopists using frozen and thawed stool through flexible sigmoideoscopy into the afferent limb. The second FMT is installed via catheter into the pouch 3 weeks after the first FMT. Antibiotic treatment has been stopped 36 hours before the FMT. 13 pouchitis patients receive a fecal transplantation from the healthy tested donor and 13 patients in the control group receive their own feces donated and prepared at the same day. The endoscopists and personnel performing the FMT are blinded for the type of feces. The randomization is done by a doctor not attending the study. Pouchscopy is assessed at 52 weeks to evaluate the endoscopic and mucosal healing. Clinical Pouchitis Disease Activity Index, see table 1 (Sandborn 1994) is assessed at week 4, 12, 26 and 52 and in addition to the endoscopy and histological index at weeks 0 and 52.

Table 1 Pouchitis Disease Activity Index (Sandborn 1994).

Table 1.
The Pouchitis Disease Activity Index[4]

Criteria	Score
Clinical	
Stool frequency	
Usual postoperative stool frequency	0
1–2 stools/day > postoperative usual	1
3 or more stools/day > postoperative usual	2
Rectal bleeding	
None or rare	0
Present daily	1
Fecal urgency or abdominal cramps	
None	0
Occasional	1
Usual	2
Fever (temperature > 37.8° C)	
Absent	0
Present	1
Endoscopic inflammation	
Edema	1
Granularity	1
Friability	1
Loss of vascular pattern	1
Mucous exudates	1
Ulceration	1
Acute histologic inflammation	
Polymorphic nuclear leukocyte infiltration	
Mild	1
Moderate + crypt abscess	2
Severe + crypt abscess	3
Ulceration per low-power field (mean)	
>25%	1
25–50%	2
>50%	3

Donors are excluded if they have taken antibiotics within the preceding 3 months; are on immunosuppressive or chemotherapeutic agents; have known or recent exposure to HIV; hepatitis B or C; have a current communicable disease; are morbidly obese; have IBD, IBS, atopy, chronic diarrhea or constipation; GI malignancy or polyposis; participate in high-risk sexual behaviors; use illicit drugs; have a history of recent incarceration or travel to areas with endemic diarrhea. Donor

blood testing includes tests for HIV, hepatitis A, B and C; donor stool testing includes culture, *C. difficile* toxin, ova and parasites, and *Helicobacter pylori.*

Follow up of the patients include a telephone call after 12 and 26 weeks after the FMT, and a clinical control visit 52 weeks after the transplantation. The clinical part of the Pouchitis Disease Activity Index score is assessed during each call and clinical visit.
A general quality of life measurement (15D-questionnaire, www.15d-instrument.net) is done at the day of stool transplantation and 6 months after the transplantation. Any adverse events are recorded. Changes in the use of medication is recorded.
Fecal stool samples for phylogenetic analysis are collected before FMT and on weeks 4, 12, 26, and 52. Fecal calprotectin indicating level of inflammation is measured before FMT and on weeks 4, 12, 26 and 52.

Endpoint of the study is the remission defined as Pouchitis Disease Activity Index <7 and no need for antibiotic treatment of pouchitis during the 52-week follow-up. Secondary objective for this study include an evaluation of the changes in gut microbiota within the 52 weeks.

STATISTICAL ANALYSIS
The sample size is calculated in according to the estimation that the difference in remaining in remission is 55% (80 vs 25%) over 52 weeks. The calculated sample size is 26 patients and therefore 13 patients are selected for both groups (donor vs own feces). This difference is considered to be clinically meaningful. Confidence interval was selected to be 95% (α=0.05 and β=0.1).

DATE
Patient collection will be made from September 2017 until June 2018. The follow up of the patients will end one year after the last patient included the study. Data analysis and reporting of the results will be during the year 2019.

ETHICAL ASPECTS
The possible ethical risks for this study include the possible transmission of harmful microbiota to the patients causing some infection or other diseases. The risk is most likely minimal, because the feces and the donors are widely tested for many known pathogens and diseases. Risk for the activation of pouchitis is also possible, although this is unlikely according to previous case reports about FMT in IBD. All the patients are informed about the experimental nature of this treatment procedure and about the available results in other previously published reports and possible risks of the procedure. All of the patients are asked for informed consent. The permission for this study will be appealed from the Institutional Review Board of Helsinki University Central Hospital.

WHAT MAKES THIS RESEARCH INNOVATIVE
This is the first placebo controlled study of fecal transplantation in pouchitis patients. The study of microbiota by the repeated fecal samples during the one year follow-up makes it possible to study if the change in the gut microbiota is permanent after the fecal transplantation.

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