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Complete List of Authors:	Pai, Nikhil; McMaster University, Pediatrics, Division of Pediatric Gastroenterology & Nutrition Popov, Jelena; McMaster University, Pediatrics, Division of Pediatric Gastroenterology & Nutrition; McMaster University, McMaster Integrative Neuroscience Discovery and Study Graduate Program
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Randomized, placebo-controlled pilot study for assessing feasibility and efficacy of fecal microbial transplants in a pediatric ulcerative colitis population: PediFETCh Trial

Nikhil Pai[A], Jelena Popov[A,B]

AFFILIATIONS

[A]Department of Pediatrics, Division of Gastroenterology & Nutrition, McMaster Children's Hospital, McMaster University, Hamilton, Ontario, Canada

[B]McMaster Integrative Neuroscience Discovery and Study Graduate Program, McMaster University, Hamilton, Ontario, Canada

CONTRIBUTIONS:

NP: substantial contributions to the conception and design of the trial, acquisition of preliminary trial data; drafting of the protocol; final approval of the protocol to be published; agreement to be accountable for all aspects of the work.

JP: substantial contributions to the design of the trial, acquisition of preliminary trial data; drafting of the protocol; final approval of the protocol to be published; agreement to be accountable for all aspects of the work.

CORRESPONDING AUTHOR:

Nikhil Pai, MD FAAP FRCPC, Assistant Professor, Department of Pediatrics, Division of Pediatric Gastroenterology, McMaster Children's Hospital, McMaster University, Hamilton, 1280 Main Street West, HSC 3A21, Ontario L8N 3Z5, Canada.

pain@mcmaster.ca

Telephone: +1-905-5212100

Fax: +1-905-5212655

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The authors wish to declare no real, or perceived conflicts of interests relevant to the conduct of the trial.

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ABSTRACT

Introduction: Ulcerative colitis (UC) is a chronic, relapsing condition characterized by colonic inflammation. Increasing prevalence in early-age diagnosis provides opportunities for additional complications in later life as a result of prolonged exposure to inflammatory and therapeutic insults, necessitating novel avenues for therapeutics which may result in fewer side effects. Fecal Microbial Transplants (FMT) has previously demonstrated potential therapeutic benefit in an adult randomized-controlled trial, as well as several recurrent *Clostridium difficile* infection (RCDI) studies. This pilot will be the first randomized, placebo-controlled trial to assess feasibility and patient outcomes in a pediatric inflammatory bowel disease (IBD) population.

Methods and analysis: 50 patients will be randomized 1:1 to receive control or active sample. Enema administrations will be performed twice weekly for six weeks, followed at a six-month follow-up period. Feasibility outcomes will include measures of patient eligibility, recruitment, willingness to participate, samples collections, hospitalizations, and drop-out rate. Improvements in disease symptoms will determine efficacy of treatment. Clinical disease scores will be taken throughout the study period using the Pediatric Ulcerative Colitis Activity Index (PUCAI). Monitoring of inflammatory markers in blood and stool will be performed at regular intervals. Microbiome analysis will be conducted on stool samples collected throughout the trials period. Imaging and endoscopic surveillance will be conducted if clinically necessary.

Ethics and dissemination: Ethics was obtained from local hospital research ethics boards across all three sites. Health Canada and FDA approval was obtained for use of an Investigatory New Drug product. Results from this trial will be presented in international conferences and published in peer-review journals.

Trial registration number: NCT02487238; pre-results.

Strengths and limitations of the study:

- This multi-center pilot study is the first randomized, placebo-controlled trial assessing the feasibility and clinical efficacy of a fecal microbial transplant protocol for the treatment of pediatric UC and IBD- unclassified. This pilot trial will provide insight towards the role of FMT in the treatment of pediatric IBD.
- Results from this trial will be valuable for future FMT research involving larger population sizes, through multi-center, double-blinded IBD trials.
- The lack of investigator blinding (single-blinding) is a limitation to the study design.

INTRODUCTION

Ulcerative colitis (UC) is a disease characterized by chronic inflammation of the colonic mucosa. Approximately 500,000 North Americans are affected by ulcerative colitis, and Ontario, Canada has among the highest incidence in the world[1,2]. A diagnosis of ulcerative colitis can be debilitating in childhood. Chronic diseases have significant implications for child and adolescent development, and the effects of ulcerative colitis on growth and development are particularly profound. Long-term immunosuppression is often required, and pediatric patients may face higher lifetime rates of lymphoma and infection given their longer duration of illness. While colectomy may be considered curative, prophylactic removal of the colon in childhood can have longterm impacts on fertility, and may negatively affect psychosocial function[3,4]. For many pediatric ulcerative colitis patients, their quality of life is marred by flares of abdominal pain, bloody diarrhea, and treatments that have significant toxicity[5].

The traditional approach to managing ulcerative colitis involves immunosuppression to dampen an overactive immune system[5]. An alternative strategy involves focusing on those factors that cause upregulation of the immune system in the first place. The gut microbiome is a provocative candidate. The intestinal tract is a rich immunologic organ. Numerous immune receptors (Toll-like receptors, NOD-like receptors) interact with the intestinal lumen to directly respond to bacterial antigens and trigger systemic immune responses[6].

Human biotherapy, or fecal microbial transplant (FMT) involves the administration of a stool and water mixture from a healthy screened donor. By modifying intestinal bacteria through fecal microbial transplant – effectively replacing it with a healthier bacterial milieu – we may be able to prevent the cascade of immune disruption that characterizes ulcerative colitis.

Rationale

Fecal microbial transplant has primarily been used for the treatment of recurrent *Clostridium difficile* colitis. A recent systematic review showed that over 90% of patients with recurrent *Clostridium difficile* infection have been cured with fecal microbial transplant[7]. These results have since been replicated in larger studies worldwide and strongly implicate colonic bacteria as a potential therapy for other gastrointestinal disease conditions[8,9].

Four small case-series have demonstrated success of fecal microbial transplant for pediatric inflammatory bowel disease[10-13]. Protocols and response rates varied, but lower gastrointestinal tract administration yielded clinical response in 67-100% of patients[10,11].

Two single-center pediatric case reports have been recently published showing marked clinical improvement in two patients with severe colitis. A 2015 case report described a 4-month old female presenting with an early-onset colitis with UC-like phenotype, who responded after 7 serial FMT infusions with anonymous donor stool[14]. A 2016 case report described an 11-year old female with corticosteroid-dependent UC who responded after serial FMT infusions every 2 to 4 weeks over a 10 month period. The patient remained in clinical remission at 40 weeks post final FMT, and showed complete endoscopic healing[15].

Significance

Factors that contribute to the success of FMT in the treatment of IBD remain unclear, and need definitive randomized controlled trials to identify benefit.

This pilot study will be the largest randomized, single-blind, placebo-controlled trial evaluating the role of fecal microbial transplant in pediatric ulcerative colitis ever conducted. This pilot study will build upon the fecal microbial transplant in adult ulcerative colitis trial recently completed at our center[15]. We will assess the feasibility of delivering twice-weekly fecal retention enemas from anonymous fecal microbial donors, to patients with pediatric ulcerative colitis. Previous fecal transplant studies in pediatrics have typically used within-family, or within-household donors; previous fecal transplant trials have also used protocols that delivered transplants infrequently. No pediatric study has ever measured response after fecal microbial transplant from an anonymous donor. These differences in donor selection, and transplant frequency may have compromised efficacy. This pilot will help identify factors associated with the success, or failure of fecal microbial transplants in pediatric ulcerative colitis. Our results will provide important preliminary data for the design, and completion of a definitive pediatric trial with a larger sample size, and power calculation to attain statistical significance of our primary clinical outcomes in the future.

This study, and smaller pediatric trials by other investigators, suggest the following: fecal microbial transplant is an effective therapy in adult ulcerative colitis; fecal microbial transplant is an

effective therapy for pediatric ulcerative colitis; donor selection has a significant role in optimizing therapeutic response; there are differences in response between patients with duration of disease ≤1 year versus >1 year. For these reasons, a large randomized, single-blind, placebo-controlled trial in pediatric ulcerative colitis patients, who typically have a shorter disease duration, using an anonymous donor, will be scientifically and clinically valuable.

The aim of this paper is to describe the first randomized controlled protocol for fecal microbial transplant in a pediatric inflammatory bowel disease population.

Study Objectives

The objective of this pilot study is to assess the feasibility of fecal microbial transplants for the therapy of pediatric ulcerative colitis. Specifically, we will test the hypothesis that a protocol of twice-weekly retention enemas over six weeks, using fecal transplant material from an anonymous unrelated donor, will improve the efficacy of fecal microbial transplant in pediatric ulcerative colitis patients, and build a framework for future studies to assess the effectiveness of FMT intervention. We will evaluate key feasibility measures of:

1. Participant recruitment (Sample Size)
2. Participant retention (Sample Size)
3. Participant eligibility criteria (Sample Size)
4. Acceptance of patients to participate in study (Process)
5. Effect of intervention on disease primary and secondary disease outcomes (Clinical)
6. Effect of intervention on fecal bacterial community structure (Clinical)
7. Rate of adverse events in patients receiving fecal microbial transplant (Clinical)

METHODS AND ANALYSIS

Study Design

The Pedi FETCh Trial is a randomized, placebo-controlled multicenter trial. It utilizes a parallel arm approach consisting of a fecal transplant group and a placebo group. In Ontario, CANADA there are two sites involved in this multi-center trial: McMaster Children's Hospital, Hamilton, and Children's Hospital of Western Ontario, London (primary and secondary sites, respectively).

Patients are seen on-site for all scheduled visits, in accordance with the protocol (FIGURE 1). Fecal enemas are screened, prepared, and tested by Rebiotix®, and sent to the sites for patient administration. Enemas are administered at the respective site's pediatric gastroenterology clinics by study team members familiar with the single-blinded fecal microbial transplant retention enema protocol, twice per week for six weeks.

Patient enrollment began in November 2015 at McMaster Children's Hospital, and is tentatively planned for March, 2017 at Children's Hospital of Western Ontario. A third site is projected to receive research ethics board approval in Spring, 2017 (Sainte Justine's Hospital, Montreal). We aim to complete recruitment of 50 patients with a target recruitment end-date of Winter, 2017. No other pediatric Gastroenterology sites in Canada are currently offering fecal microbial transplant trials for pediatric ulcerative colitis; thus, patients referred from outside centers for entry into our study will be considered if they meet eligibility criteria.

	OUTCOME	MEASURE		ANALYSIS
FEASIBILITY OUTCOMES	Participant recruitment	Recruitment/month	CUMULATIVE ACROSS ALL STUDY SITES	≥2 participants/month recruited and retained for duration of study
	Participant retention	Percent dropout post enrolment		
	Participant eligibility	Percent meeting eligibility		
	Adverse events	Hospitalization or ↑PUCAI ≥20 x 2 consecutive meas.		<10% of participants
	Blood specimens	Participant provides all required blood work		>90% of participants
	Stool specimens	Participant provides all required stool samples		>90% of participants
	Microbiome	Microbiome analyses (16s rRNA profile, metagenomics) performed for participant at all required time points		>80% of participants
	PUCAI	Participant provides information to calculate all required PUCAI scores		>90% of participants
	Week 30 MRE	MRE or endoscopy obtained		>10% of participants
CLINICAL OUTCOMES	Clinical remission (6wks)	PUCAI ≤10		Chi-squared test
	Clinical remission (30wks)	PUCAI ≤10		Chi-squared test
	Clinical remission (6-30wks)	Sustained PUCAI <10		Chi-squared test
	Clinical improvement (6wks)	↓ PUCAI ≥15		Chi-squared test
	Clinical improvement (6-30wks)	Sustained ↓PUCAI ≥15		Chi-squared test
	Biological improvement (6wks)	↓C-reactive protein		T-test
	Biological improvement (6wks)	↓fecal calprotectin		T-test
	Biological improvement (30wks)	↓C-reactive protein		T-test
	Biological improvement (30wks)	↓fecal calprotectin		T-test
	Mucosal healing (30wks)	MRE		Chi-squared test
	Change in microbiota (6wks)	Δ16s rRNA profile, metagenomics profiles		T-test, α/β Diversity
	Change in microbiota (30wks)	Δ16s rRNA profile, metagenomics profiles		T-test, α/β Diversity

TABLE 1: TRIAL OUTCOMES (FEASIBILITY, CLINICAL OUTCOMES)
PUCAI: Pediatric Ulcerative Colitis Activity Index; MRE: Magnetic Resonance Enterography

Eligibility Criteria

Pediatric patients (3-17 years old) with ulcerative colitis (UC) and inflammatory bowel disease-unclassified (IBD-U) subtype are eligible for the study. Patients classified as IBD-U are included because their distribution of disease is often limited to the large intestine -- like classical ulcerative colitis. Eligible patients must also demonstrate signs of disease activity, as determined by measures of elevated inflammatory markers (specifically fecal calprotectin and C-reactive protein (CRP)), Pediatric Ulcerative Colitis Activity Index (PUCAI) scores ≥10, or increased disease activity supported by endoscopic findings[16].

Subjects are excluded if they are participating in another clinical trial, are unable to give informed consent or assent, have severe comorbid medical illness, have concomitant *Clostridium difficile* infection, or require hospitalization (at discretion of the treating physician; typically, PUCAI > 65). Continued treatment with 5-ASA, azathioprine, 6-mercaptopurine or anti-TNFα therapy (e.g. infliximab) will be

permitted if taken at stable dose for ≥4 weeks prior to randomization. No new medical therapies (e.g. corticosteroids, antibiotics, probiotics) will be permitted during the study period, unless patients meet criteria for a suspected adverse event.

Patients younger than 3 years old will also be excluded as these patients meet definition for "Infantile Inflammatory Bowel Disease", which may have a different biological and clinical phenotype than other pediatric age presentations of IBD[17,18].

Randomization

All patients seen at McMaster Children's Hospital's Division of Gastroenterology who meet eligibility criteria will be approached for participation in the study. Patient recruitment and consent are performed by the clinical research coordinator. A standardized script is used to describe objectives, risks and benefits, and details of the randomization to eligible participants. The sample size required to carry out this study is 50. This figure is based on the number of potentially eligible ulcerative colitis patients at McMaster Children's Hospital (150) and Children's Hospital of Western Ontario (100) using recent census data from respective centers' IBD clinics. 25 patients will be randomized to receive normal saline enemas (control) and 25 patients to receive a fecal microbial transplant (intervention). This pilot study is not powered for any of the outcomes (TABLE 1). Randomization occurs according to a computer-generated block randomization pattern (block size = 4 participants). Eligible patients will be randomized 1:1 to receive fecal microbial transplant, or normal saline fecal retention enemas containing brown food coloring. Patients randomized to receive the normal saline placebo enema will be given an opportunity to enter an open-label phase of the study at completion, to receive the fecal microbial enema and re-enter the trial through a prospective observational design.

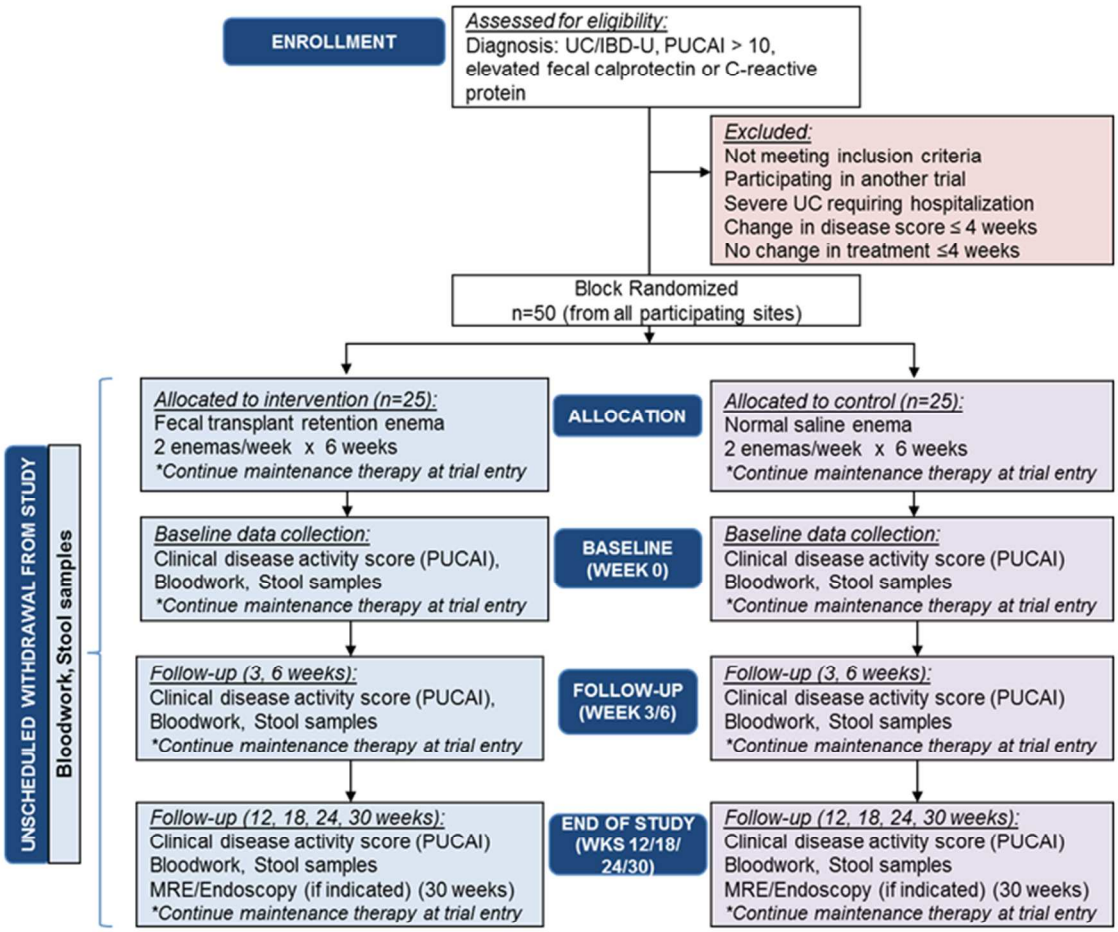

FIGURE 1: STUDY PROTOCOL
PUCAI: Pediatric Ulcerative Colitis Activity Index

Enema Product

Saline and fecal retention enemas are both be obtained from Rebiotix®, a biotechnology company from Minnesota, USA that distributes live, human-derived fecal microbial enemas (RBX2660). RBX2660 has received Health Canada Clinical Trials Application (CTA), and U.S. Food and Drug Administration Investigational New Drug Application (IND) approvals for clinical trials in patients with recurrent Clostridium difficile infection. Enemas contain live, unprocessed human fecal microbiota, or saline with polyethylene glycol preservative (placebo). Fecal microbial donors are screened, stools are tested for infectious pathogens, centrally prepared in opaque enema bags, retested prior to delivery, and sent by Rebiotix® to the investigator through preserved cold-chain delivery. The opaque enema bags, and enema tubing ensure that blinding is preserved to the study patient.

A study team member initially "connects" the enema bag to its tubing. We add 1 ml of commercially available (Club House® brand), food-grade food coloring to the normal saline enema bag (2 drops red, 3 drops green, 7 drops yellow) to confer a brown color to the clear normal saline solution.

Statistical Analysis

Outcomes

Subjects will have outcome measures performed at four time-points (FIGURE 1). In addition to study outcome measures, patients in each randomization arm will receive usual medical care.

Feasibility will be assessed by outcomes of patient eligibility, patient recruitment, patient retention, adherence to blood and stool sample collection times, and adverse events or patient hospitalizations throughout the enema administration and follow-up period.

Bloodwork collection will be performed at the time of the fecal microbial transplant (weeks 0, 3, 6), or at scheduled clinic visits (weeks 18, 30). Standard pediatric ulcerative colitis bloodwork to monitor for systemic inflammation will be ordered: complete blood count (CBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP). Routine laboratory protocols and assays for obtaining, and measuring samples will be followed[5].

Clinical disease activity scores will be determined based on history obtained at each clinical assessment (twice weekly on weeks 0-6; once on weeks 18, 30), or over the telephone on non-clinical assessment days (weeks 12, 24). The internationally validated Pediatric Ulcerative Colitis Activity Index (PUCAI) score will be used to objectively assess clinical disease activity[16].

Stool samples will be collected for microbiome analysis and fecal calprotectin (weeks 0, 3, 6, 12, 18, 24, 30), to assess patterns of bacterial community structure associated with intestinal inflammation. Routine collection of stool specimens will be performed at home independently by patients. Stools unable to be submitted immediately may be kept in a sterile screw-capped sample container in a standard, home freezer until delivered to the laboratory. Microbiome analysis will be conducted through the laboratory of Dr. M. Surette; bacterial community profiling of 16s rRNA genes will be performed on part of each stool sample using 250 nucleotide paired end reads of the V3 (or V3V4) region using the MiSeq Illumina sequencer[19]. Analysis will be performed using an in-house bioinformatics pipeline that generates clusters of operational taxonomic units (OTUs), taxonomic assignment and various measures of alpha and beta-diversity. If required at the bioinformatics stage, additional control microbial data will be obtained through Human Microbiome Project published databanks.

Imaging and endoscopic surveillance may be performed if clinically indicated. Magnetic resonance enterography (MRE) will be done on subjects at week 30 to obtain radiographic assessment of mucosal healing. This will only be ordered if clinically indicated, or would help the primary clinician guide clinical management upon the completion of the trial.

Followup patient outcomes will be measured at Weeks 12, 18, 24, and 30 after the fecal enema intervention (FIGURE 1).

All primary and secondary outcomes will be assessed using both intention-to-treat and per-protocol. The differences in remission rates between the groups will be analysed with support from departmental statistical support personnel. Proportions and percentages will be reported to determine if all feasibility outcomes were reached. Continuous outcomes will be compared using T-tests and categorical outcomes will be compared using Chi-squared tests. 95% confidence intervals and p-values will be reported (TABLE 1).

Safety Monitoring

Study risks include enema delivery, and complications of fecal microbial transplant. Enema administration carries a low risk of proctitis, and fecal microbial transplant has been associated with *C. difficile* colitis, fever, and infection[19].

We will be obtaining fecal microbial transplant materials through a company called Rebiotix®, a biotechnology firm located in Minnesota, USA. The fecal enema (RBX2660) prepared by Rebiotix has received Health Canada Clinical Trials Application (CTA), and U.S. Food and Drug Administration Investigational New Drug Application (IND) approvals for clinical trials in patients with recurrent Clostridium difficile infection.

Donor qualification processes involve: potential donors completing a health and lifestyle questionnaire, providing blood and stool samples for analyses of:

a) Blood - HIV, Hepatitis A, B, and C, syphilis and,
b) Stool - C. difficile toxin B, norovirus, rotavirus, adenovirus, Shigella, Salmonella, Campylobacter, E. coli, Aeromonas, Plesiomonas, Yersinia, Shiga toxins, Giardia antigen, Cryptosporidium antigen, Cyclospora, Isospora, ova and parasites, Vancomycin-resistant enterococci, methicillin-resistant Staphylococcus, Vibrio, and Listeria.

Donors are screened to confirm they are disease-free before donations begin. When donations begin, a sample of each donation from each donor is retained. Retained samples from each donor are pooled with other samples from that same donor and the pooled samples are subjected to repeat stool testing at approximately 45-day intervals to confirm continued donor health. Repeat donor blood testing is also performed at a minimum of 14 days after the last donation and again during the approximately 45-day collection interval. If the donor passes the repeat screening, drug product manufactured from donations collected within that 45-day period is released from quarantine. At the time of each donation, the donor completes a repeat questionnaire to confirm his/her continued health and absence of risky lifestyle behaviors. Rebiotix performs stool and blood testing approximately every 45 days.

Study participants will be monitored for signs of clinical deterioration. PUCAI scores will be measured at defined trial timepoints (FIGURE 1). Any increase in disease activity score ≥20 from previous will be classified as “disease progression”[16]. Reassessment of PUCAI scoring will occur within one week and any further increase ≥20 will remove the patient from the study for implementation of standard inflammatory bowel disease management. Patients who contact study coordinators or clinic nurses reporting fever, or worsening vomiting, abdominal pain, rectal pain, diarrhea, or hematochezia will have PUCAI scores measured to evaluate for progression. All participants who experience an increase in PUCAI scores ≥20 at successive measurements, or admission to hospital for any reason, will be classified as having an adverse event, and unblinding may be performed to the treating clinician and/or study participant at that time.

A Data, Safety and Monitoring Committee (DSMC) has been assembled for the study at the investigators’ discretion. The DSMC will perform an interval assessment of preliminary study data, approximately midway through the trial. The DSMC has been comprised of experts in the fields of pediatric gastroenterology, pediatric inflammatory bowel disease management, and clinical trial statistical analyses, and will operate independently of the investigators and collaborators for the trial, and any sponsors. The terms of the DSMC will be defined at the initial meeting, which has not yet occurred. The DSMC was not assembled earlier, due to the Research Ethics Board at the primary site determining that a DSMC would not be necessary for the trial to commence.

ETHICS AND DISSEMINATION

Ethics

The study will be conducted in accordance with the protocol, the Helsinki declaration, and the Canadian Tri-Council Policy Statement on research ethics. A Health Canada no-objection letter to perform fecal microbial transplants for the purpose of the trial was obtained, and local research ethics board (REB) approval was obtained for McMaster Children’s Hospital, and Children’s Hospital of Western Ontario. All amendments to the trial protocol will be obtained through approval by the local REB, and updated through the clinical trials registry (ClinicalTrials.gov). Research personnel will approach all potentially eligible patients who fulfill eligibility criteria for consent. All patients must sign a consent form to participate in the trial.

Data Sharing

Anonymized data about patient outcomes will be shared with Rebiotix for assessment of RBX2660 (fecal enema) efficacy. The information shared will be limited to:

a) Patient age, height, weight
b) Patient medication history (previous, and ongoing)
c) Date enema was given, volume administered, any reported adverse effects of delivery
d) PUCAI scores, results of bloodwork, and fecal calprotectin

Additionally, a de-identified stool sample will be sent to Rebiotix. These will be collected along with standard stool samples per study protocol (Figure 1).

Follow-up

Results of this pilot will inform several future research goals. Pilot data will be used to inform a definitive multicenter randomized controlled trial using a larger pediatric patient population. Data will be used to support additional research funding for a multicenter trial through a Canadian Institute of Health Research operating grant. Our data may also support further crossover studies between human and mouse models to ascertain host-microbial influences underlying observed changes.

Reporting

Results of the pilot will be reported to key stakeholders. Pilot data will be shared locally with clinical and basic science division members. Results will be shared through presentations at regional hospitals to encourage participation in future multicenter trials. Pilot data will also be submitted to international conferences and published in peer-review journals.

FUTURE DIRECTIONS

Based on the results of this study, future double-blind, randomized, placebo-controlled trials of FMT in pediatric IBD may involve:

Primary outcomes

- Evaluation of efficacy of FMT at inducing clinical remission in new onset UC, or Crohn's Disease
- Evaluation of efficacy of multiple, periodically administered FMT at maintaining clinical remission (prevention of relapse)

Secondary outcomes

- Evaluation of efficacy of FMT at inducing long-term mucosal healing
- Evaluation of change in fecal bacterial community structures of patients receiving FMT

DISCUSSION

Increasing prevalence of UC worldwide, particularly in Canada, and diagnosis in increasingly younger populations necessitates the need for novel treatment approaches[2]. Currently, this trial is open to pediatric UC, and IBD-U patients experiencing symptoms of clinical or mucosal inflammation, who would like to abstain from escalation of therapy, or surgical interventions.

Previous RCT for FMT treatment of adult UC demonstrated a statistically significant benefit of FMT 9/38 (24%) versus 2/37 (5%) at inducing ulcerative colitis remission, with no significant difference in adverse events between the groups[20]. This study will utilize a similar 6-week approach, with the twice-weekly enema administrations instead of once-weekly. Our trial will also involve a greater diversity of anonymous, non-household donors, to decrease the likelihood of observing a donor-dependent outcome. Primary outcomes of this pilot study will be feasibility measures of the reported protocol, and secondary outcomes will include subjective and objective measures of clinical and mucosal healing of UC/IBD-U over the enema administration, and follow-up period.

This trial will provide preliminary evidence for the use of FMT in a pediatric UC population. Our results will be informative for future, larger population size, double-blinded RCT in pediatric UC, which may look into further analyzing the efficacy of FMT in inducing remission and optimize dosage.

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	OUTCOME	MEASURE		ANALYSIS
FEASIBILITY OUTCOMES	Participant recruitment	Recruitment/month	CUMULATIVE ACROSS ALL STUDY SITES	≥2 participants/month recruited and retained for duration of study
	Participant retention	Percent dropout post enrolment		
	Participant eligibility	Percent meeting eligibility		
	Adverse events	Hospitalization or ↑PUCAI ≥20 x 2 consecutive meas.		<10% of participants
	Blood specimens	Participant provides all required blood work		>90% of participants
	Stool specimens	Participant provides all required stool samples		>90% of participants
	Microbiome	Microbiome analyses (16s rRNA profile, metagenomics) performed for participant at all required time points		>80% of participants
	PUCAI	Participant provides information to calculate all required PUCAI scores		>90% of participants
	Week 30 MRE	MRE or endoscopy obtained		>10% of participants
CLINICAL OUTCOMES	Clinical remission (6wks)	PUCAI ≤10		Chi-squared test
	Clinical remission (30wks)	PUCAI ≤10		Chi-squared test
	Clinical remission (6-30wks)	Sustained PUCAI <10		Chi-squared test
	Clinical improvement (6wks)	↓ PUCAI ≥15		Chi-squared test
	Clinical improvement (6-30wks)	Sustained ↓PUCAI ≥15		Chi-squared test
	Biological improvement (6wks)	↓C-reactive protein		T-test
	Biological improvement (6wks)	↓fecal calprotectin		T-test
	Biological improvement (30wks)	↓C-reactive protein		T-test
	Biological improvement (30wks)	↓fecal calprotectin		T-test
	Mucosal healing (30wks)	MRE		Chi-squared test
	Change in microbiota (6wks)	Δ16s rRNA profile, metagenomics profiles		T-test, α/β Diversity
	Change in microbiota (30wks)	Δ16s rRNA profile, metagenomics profiles		T-test, α/β Diversity

TABLE 1: TRIAL OUTCOMES (FEASIBILITY, CLINICAL OUTCOMES)

PUCAI: Pediatric Ulcerative Colitis Activity Index; MRE: Magnetic Resonance Enterography

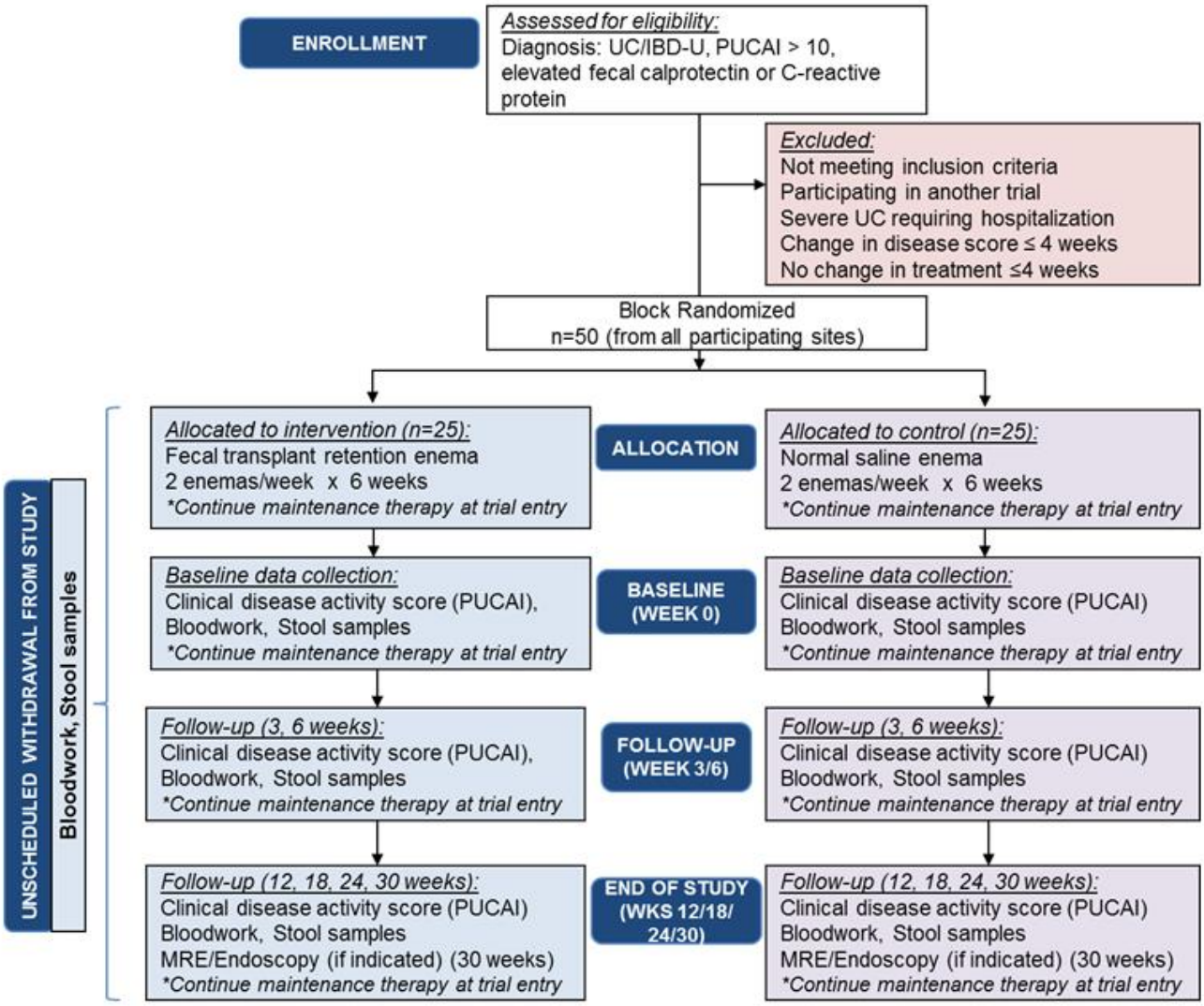

FIGURE 1: STUDY PROTOCOL
PUCAI: Pediatric Ulcerative Colitis Activity Index

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on 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	1
	2b	All items from the World Health Organization Trial Registration Data Set	n/a
Protocol version	3	Date and version identifier	n/a
Funding	4	Sources and types of financial, material, and other support	1
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	1
	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	1
	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)	n/a

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	3
	6b	Explanation for choice of comparators	4
Objectives	7	Specific objectives or hypotheses	4,6
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, noninferiority, exploratory)	4,6
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	5
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)	4,5
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6,7
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	8
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	7
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	5,6,8
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	6,7,8
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)	6,7

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	6
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	6
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 assign interventions	6
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	6,7
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	7
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	7,8
Methods: Data collection, management, and analysis			
Data collection methods	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	9
	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	9

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	9
Statistical methods	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	7
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	8
	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)	7
Methods: Monitoring			
Data monitoring	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	8
	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	n/a
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	n/a
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	n/a
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	8
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	8

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	5
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
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	9
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	1
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	9
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	n/a
Dissemination policy	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	9
	31b	Authorship eligibility guidelines and any intended use of professional writers	n/a
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n/a
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	APPENDIX
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	7

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Randomized, placebo-controlled pilot study for assessing feasibility and efficacy of fecal microbiota transplantation in a pediatric ulcerative colitis population: PediFETCh Trial

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Randomized, placebo-controlled pilot study for assessing feasibility and efficacy of fecal microbiota transplantation in a pediatric ulcerative colitis population: PediFETCh Trial

Nikhil Pai[A], Jelena Popov[A,B]

AFFILIATIONS

[A]Department of Pediatrics, Division of Gastroenterology & Nutrition, McMaster Children's Hospital, McMaster University, Hamilton, Ontario, Canada

[B]McMaster Integrative Neuroscience Discovery and Study Graduate Program, McMaster University, Hamilton, Ontario, Canada

CONTRIBUTIONS:

NP: substantial contributions to the conception and design of the trial, acquisition of preliminary trial data; drafting of the protocol; final approval of the protocol to be published; agreement to be accountable for all aspects of the work.

JP: substantial contributions to the design of the trial, acquisition of preliminary trial data; drafting of the protocol; final approval of the protocol to be published; agreement to be accountable for all aspects of the work.

CORRESPONDING AUTHOR:

Nikhil Pai, MD FAAP FRCPC, Assistant Professor, Department of Pediatrics, Division of Pediatric Gastroenterology, McMaster Children's Hospital, McMaster University, Hamilton, 1280 Main Street West, HSC 3A21, Ontario L8N 3Z5, Canada.

pain@mcmaster.ca

Telephone: +1-905-5212100

Fax: +1-905-5212655

DECLARATION OF INTERESTS:

The authors wish to declare no real, or perceived conflicts of interests relevant to the conduct of the trial.

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ClinicalTrials.gov NCT02487238

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Paul Moayyedi provided substantial contributions to design of the trial.

Michael Surette provided substantial contributions to design of the trial, acquisition and analysis of preliminary trial data.

Waliul Khan provided substantial contributions to acquisition and analysis of preliminary trial data.

KEYWORDS:

Fecal microbiota transplantation, pediatrics, IBD, treatment, microbiome

WORD COUNT:

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ABSTRACT

Introduction: Ulcerative colitis (UC) is a chronic, relapsing condition characterized by colonic inflammation. Increasing prevalence in early-age diagnosis provides opportunities for additional complications in later life as a result of prolonged exposure to inflammatory and therapeutic insults, necessitating novel avenues for therapeutics which may result in fewer side effects. Fecal Microbiota Transplantation (FMT) has previously demonstrated potential therapeutic benefit in an adult randomized-controlled trial, as well as several recurrent *Clostridium difficile* infection studies. This Phase 1b pilot will be the first randomized, single-blinded, placebo-controlled trial to assess feasibility and patient outcomes in a pediatric inflammatory bowel disease (IBD) population.

Methods and analysis: 50 patients will be randomized 1:1 to receive normal saline control or active sample. Enema administrations will be performed twice weekly for six weeks, followed at a six-month follow-up period. Feasibility outcomes will include measures of patient eligibility, recruitment, willingness to participate, samples collections, hospitalizations, and drop-out rate. Improvements in disease symptoms will determine efficacy of treatment. Clinical disease scores will be taken throughout the study period using the Pediatric Ulcerative Colitis Activity Index (PUCAI). Monitoring of inflammatory markers in blood and stool will be performed at regular intervals. Microbiome analysis will be conducted on stool samples collected throughout the trials period. Imaging and endoscopic surveillance will be conducted if clinically necessary.

Ethics and dissemination: Ethics was obtained from local hospital research ethics boards across all three sites. Health Canada and FDA approval was obtained for use of an Investigatory New Drug product. Results from this trial will be presented in international conferences and published in peer-review journals.

Trial registration number: NCT02487238; pre-results.

Strengths and limitations of the study:

- This multi-center pilot study is the first randomized, placebo-controlled trial assessing the feasibility and clinical efficacy of a fecal microbiota transplantation protocol for the treatment of pediatric UC and IBD-unclassified. This pilot trial will provide insight towards the role of FMT in the treatment of pediatric IBD.
- Results from this trial will be valuable for future FMT research involving larger population sizes, through multi-center, double-blinded IBD trials.
- The lack of investigator blinding (single-blinding) is a limitation to the study design.

INTRODUCTION

Ulcerative colitis (UC) is a disease characterized by chronic inflammation of the colonic mucosa. Approximately 104,000 Canadians are affected by UC, and Canada has among the highest incidence in the world[1,2,3]. A diagnosis of UC can be debilitating in childhood. Chronic diseases can have significant impacts on children, and UC may particularly affect childhood growth and development[4,5]. Long-term immunosuppression is often required, and pediatric patients may face higher lifetime rates of lymphoma and infection given their longer duration of illness. While colectomy may be considered curative, prophylactic removal of the colon in childhood can have longterm impacts on fertility, and may negatively affect psychosocial function[6,7]. For many pediatric UC patients, their quality of life is marred by flares of abdominal pain, bloody diarrhea, and treatments that have significant toxicity[8].

The traditional approach to managing UC involves immunosuppression to dampen an overactive immune system[8]. An alternative strategy involves focusing on those factors that cause upregulation of the immune system in the first place. The gut microbiome is a provocative candidate. The intestinal tract is a rich immunologic organ. Numerous immune receptors (Toll-like receptors, NOD-like receptors) interact with the intestinal lumen to directly respond to bacterial antigens and trigger systemic immune responses[9].

Human biotherapy, or fecal microbiota transplantation (FMT) involves the administration of a stool and water mixture from a healthy screened donor. By modifying intestinal bacteria through FMT – effectively replacing it with a healthier bacterial milieu – we may be able to prevent the cascade of immune disruption that characterizes UC.

Rationale

FMT has primarily been used for the treatment of recurrent *Clostridium difficile* colitis. A recent systematic review showed that over 90% of patients with recurrent *Clostridium difficile* infection have been cured with FMT[10]. These results have since been replicated in larger studies worldwide and strongly implicate colonic bacteria as a potential therapy for other gastrointestinal disease conditions[11,12].

Four small case-series have demonstrated success of FMT for pediatric inflammatory bowel disease (IBD)[13,14,15,16]. Protocols and response rates varied across each study, but lower gastrointestinal tract administration yielded clinical response rates in 67-100% of patients[13,14].

Two single-center pediatric case reports have been recently published showing marked clinical improvement in two patients with severe colitis. A 2015 case report described a 18-month old female presenting with an early-onset colitis with UC-like phenotype, who responded after 7 serial FMT infusions with donor stool from and age-matched niece and older brother[17]. A 2016 case report described an 11-year old female with corticosteroid-dependent UC who responded after serial FMT infusions every 2 to 4 weeks over a 10 month period. The patient remained in clinical remission at 40 weeks post final FMT, and showed complete endoscopic healing[18]. A further 2016 case report described a 3-year old female with acute severe UC who was refractory to aminosalicylates and all immunosuppressive drugs[19]. She received 6 successive FMT enemas and 4 FMT via nasoduodenal tube over 10 days, but ultimately required colectomy. Donor fecal microbiota was not identified in the patient, and the authors concluded that due to the severely damaged colonic epithelium, paucity of crypts, and overall decrease of mucous in the outer layer, the donor microbiota could not be retained by the recipient. The authors concluded that patients with severe disease might be a better candidate for FMT. However, factors that optimize bacterial transfer from donor to recipient in FMT remain unclear, and mechanistic conclusions from single-center case reports remain speculative at this time[19].

Significance

Factors that contribute to the success of FMT in the treatment of IBD remain unclear, and need definitive randomized controlled trials to identify benefit.

This pilot study will be the largest randomized, single-blind, placebo-controlled trial evaluating the role of FMT in pediatric UC ever conducted. This pilot study will build upon the FMT in adult UC trial recently completed at our center[20]. We will assess the feasibility of delivering twice-weekly fecal enemas from anonymous fecal microbial donors, to patients with pediatric UC. Previous fecal transplant studies in pediatrics have typically used within-family, or within-household donors; previous fecal transplant trials have also used protocols that delivered transplants infrequently. No pediatric study has ever measured response after FMT from an anonymous donor. These differences in donor selection, and transplant frequency may have compromised efficacy. This pilot will help identify factors associated with the

success, or failure of FMT in pediatric UC. Our results will provide important preliminary data for the design, and completion of a definitive pediatric trial with a larger sample size, and power calculation to attain statistical significance of our primary clinical outcomes in the future.

This study, and smaller pediatric trials by other investigators, suggest the following: FMT is an effective therapy in adult UC; FMT is an effective therapy for pediatric UC; donor selection has a significant role in optimizing therapeutic response; there are differences in response between patients with duration of disease ≤1 year versus >1 year[20]. For these reasons, a large randomized, single-blind, placebo-controlled trial in pediatric UC patients, who typically have a shorter disease duration, using an anonymous donor, will be scientifically and clinically valuable.

The aim of this paper is to describe the first randomized controlled protocol for FMT in a pediatric IBD population.

Study Objectives

The objective of this pilot study is to assess the feasibility of FMT for the therapy of pediatric UC. Specifically, we will test the hypothesis that a protocol of twice-weekly enemas over six weeks, using fecal transplant material from an anonymous unrelated donor, will improve the efficacy of FMT in pediatric UC patients, and build a framework for future studies to assess the effectiveness of FMT intervention. We will evaluate key feasibility measures of:

1. Participant recruitment (Sample Size)
2. Participant retention (Sample Size)
3. Participant eligibility criteria (Sample Size)
4. Acceptance of patients to participate in study (Process)
5. Effect of intervention on disease primary and secondary disease outcomes (Clinical)
6. Effect of intervention on fecal bacterial community structure (Clinical)
7. Rate of adverse events in patients receiving FMT (Clinical)

METHODS AND ANALYSIS

Study Design

The Pedi FETCh Trial is a randomized, placebo-controlled multicenter trial. It utilizes a parallel arm approach consisting of a fecal transplantation group and a placebo group. In Ontario, CANADA there are two sites involved in this multi-center trial: McMaster Children's Hospital, Hamilton, and Children's Hospital of Western Ontario, London (primary and secondary sites, respectively).

Patients are seen on-site for all scheduled visits, in accordance with the protocol (FIGURE 1). Fecal enemas are screened, prepared, and tested by Rebiotix®, and sent to the sites for patient administration. Enemas are administered at the respective site's pediatric gastroenterology clinics by study team members familiar with the single-blinded FMT enema protocol, twice per week for six weeks.

Patient enrollment began in November 2015 at McMaster Children's Hospital, and is tentatively planned for March, 2017 at Children's Hospital of Western Ontario. A third site is projected to receive research ethics board approval in Spring, 2017 (Sainte Justine's Hospital, Montreal). We aim to complete recruitment of 50 patients with a target recruitment end-date of Winter, 2017. No other pediatric Gastroenterology sites in Canada are currently offering FMT trials for pediatric UC; thus, patients referred from outside centers for entry into our study will be considered if they meet eligibility criteria.

	OUTCOME	MEASURE		ANALYSIS
FEASIBILITY OUTCOMES	Participant recruitment	Recruitment/month	CUMULATIVE ACROSS ALL STUDY SITES	≥2 participants/month recruited and retained for duration of study
	Participant retention	Percent dropout post enrolment		
	Participant eligibility	Percent meeting eligibility		
	Adverse events	Hospitalization or ↑PUCAI ≥20 x 2 consecutive measures		<10% of participants
	Blood specimens	Participant provides all required blood samples		>90% of participants
	Stool specimens	Participants provides all required stool samples		>90% of participants
	Microbiome	Microbiome analyses (16s rRNA profile, metagenomics) performed for participant at all required time points		>80% of participants
	PUCAI	Participant provides information to calculate all required PUCAI scores		>90% of participants
	Week 30 Endoscopy	Endoscopy obtained		>10% of participants
CLINICAL OUTCOMES	Clinical remission (6wks)	PUCAI ≤10		Chi-squared test
	Clinical remission (30wks)	PUCAI ≤10		Chi-squared test
	Clinical remission (6-30wks)	Sustained PUCAI <10		Chi-squared test
	Clinical improvement (6wks)	↓ PUCAI ≥15		Chi-squared test
	Clinical improvement (6-30wks)	Sustained ↓ PUCAI ≥15		Chi-squared test
	Biological improvement (6wks)	↓ C-reactive protein		T-test
	Biological improvement (6wks)	↓ fecal calprotectin		T-test
	Biological improvement (30wks)	↓ C-reactive protein		T-test
	Biological improvement (30wks)	↓ fecal calprotectin		T-test
	Mucosal healing (30wks)	Endoscopy		T-test
	Change in microbiota (6wks)	Δ 16s rRNA profile, metagenomics profile		T-test, αβ Diversity
	Change in microbiota (30wks)	Δ 16s rRNA profile, metagenomics profile		T-test, αβ Diversity

TABLE 1: TRIAL OUTCOMES (FEASIBILITY, CLINICAL OUTCOMES)
PUCAI: Pediatric Ulcerative Colitis Activity Index; MRE: Magnetic Resonance Enterography

Eligibility Criteria

Pediatric patients (3-17 years old) with UC and inflammatory bowel disease-unclassified (IBD-U) subtype are eligible for the study. Patients classified as IBD-U are included because their distribution of disease is often limited to the large intestine -- like classical UC. Eligible patients must also demonstrate signs of disease activity, as determined by measures of elevated inflammatory markers (specifically fecal calprotectin and C-reactive protein (CRP)), PUCAI scores ≥15, or increased disease activity supported by endoscopic findings[21,22,23,13].

Subjects are excluded if they are participating in another clinical trial, are unable to give informed consent or assent, have severe comorbid medical illness, have concomitant *Clostridium difficile* infection, or require hospitalization (at discretion of the treating physician; typically, PUCAI > 65). Continued treatment with 5-ASA, azathioprine, 6-mercaptopurine or anti-TNFα therapy (e.g. infliximab) will be permitted if taken at stable dose for ≥4 weeks prior to randomization. No new medical therapies (e.g. corticosteroids, antibiotics, probiotics) will be permitted during the study period, unless patients meet criteria for a suspected adverse event.

Patients younger than 3 years old will also be excluded as these patients meet definition for "Infantile Inflammatory Bowel Disease", which may have a different biological and clinical phenotype than other pediatric age presentations of IBD[24,25].

Randomization

All patients seen at McMaster Children's Hospital's Division of Gastroenterology who meet eligibility criteria will be approached for participation in the study. Patient recruitment and consent are performed by the clinical research coordinator. A standardized script is used to describe objectives, risks and benefits, and details of the randomization to eligible participants. The sample size required to carry out this study is 50. This figure is based on the number of potentially eligible UC patients at McMaster Children's Hospital (150) and Children's Hospital of Western Ontario (100) using recent census data from respective centers' IBD clinics. 25 patients will be randomized to receive normal saline enemas (control; saline/polyethylene glycol 3350) and 25 patients to receive a FMT enema (intervention; 50g/150ml, 10^7 microbes/mL of suspension in saline/polyethylene glycol 3350). This pilot study is not powered for any of the outcomes (TABLE 1). Randomization occurs according to a computer-generated block randomization pattern (block size = 4 participants). Eligible patients will be randomized 1:1 to receive FMT, or normal saline fecal enemas containing brown food coloring. Patients randomized to receive the normal saline placebo enema will be given an opportunity to enter an open-label phase of the study at completion, to receive the fecal microbial enema and re-enter the trial through a prospective observational design.

Enema Product

Saline and fecal enemas are both obtained from Rebiotix®, a biotechnology company from Minnesota, USA that distributes live, human-derived fecal microbial enemas (RBX2660). RBX2660 has received Health Canada Clinical Trials Application (CTA), and U.S. Food and Drug Administration Investigational New Drug Application (IND) approvals for clinical trials in patients with recurrent *Clostridium difficile* infection. Enemas contain live, unprocessed human fecal microbiota, or saline with polyethylene glycol preservative (placebo). Fecal microbial donors are screened, stools are tested for infectious pathogens, centrally prepared in opaque enema bags, retested prior to delivery, and sent by Rebiotix® to the investigator through preserved cold-chain delivery. Specifically, the samples are stored in -80°C freezers at Rebiotix®, Minnesota, with next-day, on-site delivery in styrofoam-insulated boxes containing ice packs. Frozen enemas are removed from the boxes and stored in the clinic fridge (4^{o}C) for up to 3 days. The opaque enema bags and enema tubing ensure that blinding is preserved to the study patient.

A study team member initially "connects" the enema bag to its tubing. We add 1 ml of commercially available (Club House® brand), food-grade food coloring to the normal saline enema bag (2 drops red, 3 drops green, 7 drops yellow) to confer a brown color to the clear normal saline solution.

Statistical Analysis

Outcomes

Subjects will have outcome measures performed at four time-points (FIGURE 1). In addition to study outcome measures, patients in each randomization arm will receive usual medical care.

Feasibility will be assessed by outcomes of patient eligibility, patient recruitment, patient retention, adherence to blood and stool sample collection times, and adverse events or patient hospitalizations throughout the enema administration and follow-up period.

Bloodwork collection will be performed at the time of the FMT (weeks 0, 3, 6), or at scheduled clinic visits (weeks 18, 30). Standard pediatric UC bloodwork to monitor for systemic inflammation will be ordered: complete blood count (CBC), erythrocyte sedimentation rate (ESR), CRP, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP). Routine laboratory protocols and assays for obtaining, and measuring samples will be followed[8].

Clinical disease activity scores will be determined based on history obtained at each clinical assessment (twice weekly on weeks 0-6; once on weeks 18, 30), or over the telephone on non-clinical assessment days (weeks 12, 24). The internationally validated PUCAI score will be used to objectively assess clinical disease activity[21].

Stool samples will be collected for microbiome analysis and fecal calprotectin (weeks 0, 3, 6, 12, 18, 24, 30), to assess patterns of bacterial community structure associated with intestinal inflammation. Routine collection of stool specimens will be performed at home independently by patients. Stools unable

to be submitted immediately may be kept in a sterile screw-capped sample container in a standard, home freezer until delivered to the laboratory. Microbiome analysis will be conducted through the laboratory of Dr. M. Surette; bacterial community profiling of 16s rRNA genes will be performed on part of each stool sample using 250 nucleotide paired end reads of the V3 (or V3V4) region using the MiSeq Illumina sequencer[26]. Analysis will be performed using an in-house bioinformatics pipeline that generates clusters of operational taxonomic units (OTUs), taxonomic assignment and various measures of alpha and beta-diversity. If required at the bioinformatics stage, additional control microbial data will be obtained through Human Microbiome Project published databanks.

Imaging and endoscopic surveillance may be performed if clinically indicated.

Followup patient outcomes will be measured at Weeks 12, 18, 24, and 30 after the fecal enema intervention (FIGURE 1).

All primary and secondary outcomes will be assessed using both intention-to-treat and per-protocol. The differences in remission rates between the groups will be analysed with support from departmental statistical support personnel. Proportions and percentages will be reported to determine if all feasibility outcomes were reached. Continuous outcomes will be compared using T-tests and categorical outcomes will be compared using Chi-squared tests. 95% confidence intervals and p-values will be reported (TABLE 1).

Safety Monitoring

Study risks include enema delivery, and complications of FMT. Enema administration carries a low risk of proctitis, and FMT has been associated with *C. difficile* colitis, fever, and infection[26].

We will be obtaining FMT materials through a company called Rebiotix®, a biotechnology firm located in Minnesota, USA. The fecal enema (RBX2660) prepared by Rebiotix has received Health Canada Clinical Trials Application (CTA), and U.S. Food and Drug Administration Investigational New Drug Application (IND) approvals for clinical trials in patients with recurrent *Clostridium difficile* infection.

Donor qualification processes involve: potential donors completing a health and lifestyle questionnaire, providing blood and stool samples for analyses of:

a) Blood - HIV, Hepatitis A, B, and C, syphilis and,
b) Stool - C. difficile toxin B, norovirus, rotavirus, adenovirus, Shigella, Salmonella, Campylobacter, E. coli, Aeromonas, Plesiomonas, Yersinia, Shiga toxins, Giardia antigen, Cryptosporidium antigen, Cyclospora, Isospora, ova and parasites, Vancomycin-resistant enterococci, Methicillin-resistant Staphylococcus, Vibrio, and Listeria.

Donors are screened to confirm they are disease-free before donations begin. When donations begin, a sample of each donation from each donor is retained. Retained samples from each donor are pooled with other samples from that same donor and the pooled samples are subjected to repeat stool testing at approximately 45-day intervals to confirm continued donor health. Repeat donor blood testing is also performed at a minimum of 14 days after the last donation and again during the approximately 45-day collection interval. If the donor passes the repeat screening, drug product manufactured from donations collected within that 45-day period is released from quarantine. At the time of each donation, the donor completes a repeat questionnaire to confirm his/her continued health and absence of risky lifestyle behaviors. Rebiotix performs stool and blood testing approximately every 45 days.

Study participants will be monitored for signs of clinical deterioration. PUCAI scores will be measured at defined trial timepoints (FIGURE 1). Any increase in disease activity score ≥20 from previous will be classified as “disease progression”[21]. Reassessment of PUCAI scoring will occur within one week and any further increase ≥20 will remove the patient from the study for implementation of standard IBD management. Patients who contact study coordinators or clinic nurses reporting fever, or worsening vomiting, abdominal pain, rectal pain, diarrhea, or hematochezia will have PUCAI scores measured to evaluate for progression. All participants who experience an increase in PUCAI scores ≥20 at successive measurements, or admission to hospital for any reason, will be classified as having an adverse event, and unblinding may be performed to the treating clinician and/or study participant at that time.

A Data, Safety and Monitoring Committee (DSMC) has been assembled for the study at the investigators’ discretion. The DSMC will perform an interval assessment of preliminary study data, approximately midway through the trial. The DSMC has been comprised of experts in the fields of pediatric gastroenterology, pediatric IBD management, and clinical trial statistical analyses, and will operate independently of the investigators and collaborators for the trial, and any sponsors. The terms of

the DSMC will be defined at the initial meeting, which has not yet occurred. The DSMC was not assembled earlier, due to the Research Ethics Board at the primary site determining that a DSMC would not be necessary for the trial to commence.

ETHICS AND DISSEMINATION

Ethics

The study will be conducted in accordance with the protocol, the Helsinki declaration, and the Canadian Tri-Council Policy Statement on research ethics. A Health Canada no-objection letter to perform FMT for the purpose of the trial was obtained, and local research ethics board (REB) approval was obtained for McMaster Children's Hospital, and Children's Hospital of Western Ontario. All amendments to the trial protocol will be obtained through approval by the local REB, and updated through the clinical trials registry (ClinicalTrials.gov). Research personnel will approach all potentially eligible patients who fulfill eligibility criteria for consent. All patients must sign a consent form to participate in the trial.

Data Sharing

Anonymized data about patient outcomes will be shared with Rebiotix for assessment of RBX2660 (fecal enema) efficacy. The information shared will be limited to:

a) Patient age, height, weight
b) Patient medication history (previous, and ongoing)
c) Date enema was given, volume administered, any reported adverse effects of delivery
d) PUCAI scores, results of bloodwork, and fecal calprotectin

Additionally, a de-identified stool sample will be sent to Rebiotix. These will be collected along with standard stool samples per study protocol (Figure 1).

Follow-up

Results of this pilot will inform several future research goals. Pilot data will be used to inform a definitive multicenter randomized controlled trial using a larger pediatric patient population. Data will be used to support additional research funding for a multicenter trial through a Canadian Institute of Health Research operating grant. Our data may also support further crossover studies between human and mouse models to ascertain host-microbial influences underlying observed changes.

Reporting

Results of the pilot will be reported to key stakeholders. Pilot data will be shared locally with clinical and basic science division members. Results will be shared through presentations at regional hospitals to encourage participation in future multicenter trials. Pilot data will also be submitted to international conferences and published in peer-review journals.

FUTURE DIRECTIONS

Based on the results of this study, future double-blind, randomized, placebo-controlled trials of FMT in pediatric IBD may involve:

Primary outcomes

- Evaluation of efficacy of FMT at inducing clinical remission in new onset UC, or Crohn's Disease
- Evaluation of efficacy of multiple, periodically administered FMT at maintaining clinical remission (prevention of relapse)

Secondary outcomes

- Evaluation of efficacy of FMT at inducing long-term mucosal healing
- Evaluation of change in fecal bacterial community structures of patients receiving FMT

DISCUSSION

Increasing prevalence of UC worldwide, particularly in Canada, and diagnosis in increasingly younger populations necessitates the need for novel treatment approaches[27]. Currently, this trial is open to pediatric UC, and IBD-U patients experiencing symptoms of clinical or mucosal inflammation, who would like to abstain from escalation of therapy, or surgical interventions.

A previous randomized controlled trial for FMT in adult UC demonstrated a statistically significant benefit of FMT (9/38, 24% versus 2/37, 5%) at inducing UC remission, with no significant difference in adverse events between the groups[20]. This study will utilize a similar 6-week approach, with the twice-weekly enema administrations instead of once-weekly. Our trial will also involve a greater diversity of anonymous, non-household donors, to decrease the likelihood of observing a donor-dependent outcome. Primary outcomes of this pilot study will be feasibility measures of the reported protocol, and secondary outcomes will include subjective and objective measures of clinical and mucosal healing of UC/IBD-U over the enema administration, and follow-up period.

This trial will provide preliminary evidence for the use of FMT in a pediatric UC population. Our results will be informative for future, larger population size, double-blinded randomized controlled trial in pediatric UC, which may look into further analyzing the efficacy of FMT in inducing remission and optimize dosage.

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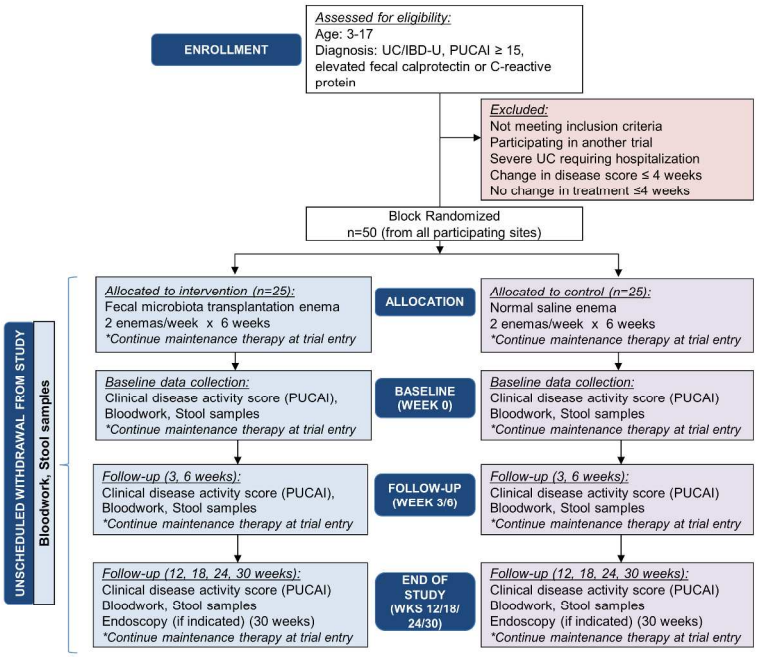

FIGURE 1: STUDY PROTOCOL
PUCAI: Pediatric Ulcerative Colitis Activity Index

279x203mm (300 x 300 DPI)

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on 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	1
	2b	All items from the World Health Organization Trial Registration Data Set	n/a
Protocol version	3	Date and version identifier	n/a
Funding	4	Sources and types of financial, material, and other support	1
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	1
	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	1
	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)	n/a

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	3
	6b	Explanation for choice of comparators	4
Objectives	7	Specific objectives or hypotheses	4,6
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, noninferiority, exploratory)	4,6
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	5
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)	4,5
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6,7
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	8
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	7
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	5,6,8
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	6,7,8
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)	6,7

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	6
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	6
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 assign interventions	6
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	6,7
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	7
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	7,8
Methods: Data collection, management, and analysis			
Data collection methods	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	9
	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	9

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	9
Statistical methods	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	7
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	8
	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)	7
Methods: Monitoring			
Data monitoring	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	8
	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	n/a
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	n/a
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	n/a
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	8
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	8

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	5
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
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	9
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	1
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	9
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	n/a
Dissemination policy	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	9
	31b	Authorship eligibility guidelines and any intended use of professional writers	n/a
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n/a
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	APPENDIX
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	7

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Protocol for a randomized, placebo-controlled pilot study for assessing feasibility and efficacy of fecal microbiota transplantation in a pediatric ulcerative colitis population: PediFETCh Trial

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Primary Subject Heading:	Gastroenterology and hepatology
Secondary Subject Heading:	Medical management, Paediatrics
Keywords:	Fecal Microbial Transplant, Pediatrics, IBD, Treatment, Microbiome

Protocol for a randomized, placebo-controlled pilot study for assessing feasibility and efficacy of fecal microbiota transplantation in a pediatric ulcerative colitis population: PediFETCh Trial

Nikhil Pai[A], Jelena Popov[A,B]

AFFILIATIONS
[A]Department of Pediatrics, Division of Gastroenterology & Nutrition, McMaster Children's Hospital, McMaster University, Hamilton, Ontario, Canada
[B]McMaster Integrative Neuroscience Discovery and Study Graduate Program, McMaster University, Hamilton, Ontario, Canada

CONTRIBUTIONS:
NP: substantial contributions to the conception and design of the trial, acquisition of preliminary trial data; drafting of the protocol; final approval of the protocol to be published; agreement to be accountable for all aspects of the work.

JP: substantial contributions to the design of the trial, acquisition of preliminary trial data; drafting of the protocol; final approval of the protocol to be published; agreement to be accountable for all aspects of the work.

CORRESPONDING AUTHOR:
Nikhil Pai, MD FAAP FRCPC, Assistant Professor, Department of Pediatrics, Division of Pediatric Gastroenterology, McMaster Children's Hospital, McMaster University, Hamilton, 1280 Main Street West, HSC 3A21, Ontario L8N 3Z5, Canada.
pain@mcmaster.ca
Telephone: +1-905-5212100
Fax: +1-905-5212655

DECLARATION OF INTERESTS:
The authors wish to declare no real, or perceived conflicts of interests relevant to the conduct of the trial.

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ClinicalTrials.gov NCT02487238

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Paul Moayyedi provided substantial contributions to design of the trial.
Michael Surette provided substantial contributions to design of the trial, acquisition and analysis of preliminary trial data.
Waliul Khan provided substantial contributions to acquisition and analysis of preliminary trial data.

KEYWORDS:
Fecal microbiota transplantation, pediatrics, IBD, treatment, microbiome

WORD COUNT:
5019 words

ABSTRACT

Introduction: Ulcerative colitis (UC) is a chronic, relapsing condition characterized by colonic inflammation. Increasing prevalence in early-age diagnosis provides opportunities for additional complications in later life as a result of prolonged exposure to inflammatory and therapeutic insults, necessitating novel avenues for therapeutics which may result in fewer side effects. Fecal Microbiota Transplantation (FMT) has previously demonstrated potential therapeutic benefit in an adult randomized-controlled trial, as well as several recurrent *Clostridium difficile* infection studies. This Phase 1b pilot will be the first randomized, single-blinded, placebo-controlled trial to assess feasibility and patient outcomes in a pediatric inflammatory bowel disease (IBD) population.

Methods and analysis: 50 patients will be randomized 1:1 to receive normal saline control or active sample. Enema administrations will be performed twice weekly for six weeks, followed at a six-month follow-up period. Feasibility outcomes will include measures of patient eligibility, recruitment, willingness to participate, samples collections, hospitalizations, and drop-out rate. Improvements in disease symptoms will determine efficacy of treatment. Clinical disease scores will be taken throughout the study period using the Pediatric Ulcerative Colitis Activity Index (PUCAI). Monitoring of inflammatory markers in blood and stool will be performed at regular intervals. Microbiome analysis will be conducted on stool samples collected throughout the trials period. Imaging and endoscopic surveillance will be conducted if clinically necessary.

Ethics and dissemination: Ethics was obtained from local hospital research ethics boards across all three sites. Health Canada and FDA approval was obtained for use of an Investigatory New Drug product. Results from this trial will be presented in international conferences and published in peer-review journals.

Trial registration number: NCT02487238; pre-results.

Strengths and limitations of the study:

- This multi-center pilot study is the first randomized, placebo-controlled trial assessing the feasibility and clinical efficacy of a fecal microbiota transplantation protocol for the treatment of pediatric UC and IBD-unclassified. This pilot trial will provide insight towards the role of FMT in the treatment of pediatric IBD.
- Results from this trial will be valuable for future FMT research involving larger population sizes, through multi-center, double-blinded IBD trials.
- The lack of investigator blinding (single-blinding) is a limitation to the study design.
- The strengths and limitations of normal saline versus autologous stool as placebo are unclear, with previous investigators having used both in studies of FMT for recurrent *Clostridium difficile* colitis, and FMT for the treatment of inflammatory bowel disease.

INTRODUCTION

Ulcerative colitis (UC) is a disease characterized by chronic inflammation of the colonic mucosa. Approximately 104,000 Canadians are affected by UC, and Canada has among the highest incidence in the world[1,2,3]. A diagnosis of UC can be debilitating in childhood. Chronic diseases can have significant impacts on children, and UC may particularly affect childhood growth and development[4,5]. Long-term immunosuppression is often required, and pediatric patients may face higher lifetime rates of lymphoma and infection given their longer duration of illness. While colectomy may be considered curative, prophylactic removal of the colon in childhood can have longterm impacts on fertility, and may negatively affect psychosocial function[6,7]. For many pediatric UC patients, their quality of life is marred by flares of abdominal pain, bloody diarrhea, and treatments that have significant toxicity[8].

The traditional approach to managing UC involves immunosuppression to dampen an overactive immune system[8]. An alternative strategy involves focusing on those factors that cause upregulation of the immune system in the first place. The gut microbiome is a provocative candidate. The intestinal tract is a rich immunologic organ. Numerous immune receptors (Toll-like receptors, NOD-like receptors) interact with the intestinal lumen to directly respond to bacterial antigens and trigger systemic immune responses[9].

Human biotherapy, or fecal microbiota transplantation (FMT) involves the administration of a stool and water mixture from a healthy screened donor. By modifying intestinal bacteria through FMT – effectively replacing it with a healthier bacterial milieu – we may be able to prevent the cascade of immune disruption that characterizes UC.

Rationale

FMT has primarily been used for the treatment of recurrent *Clostridium difficile* colitis. A recent systematic review showed that over 90% of patients with recurrent *Clostridium difficile* infection have been cured with FMT[10]. These results have since been replicated in larger studies worldwide and strongly implicate colonic bacteria as a potential therapy for other gastrointestinal disease conditions[11,12].

Four small case-series have demonstrated success of FMT for pediatric inflammatory bowel disease (IBD)[13,14,15,16]. Protocols and response rates varied across each study, but lower gastrointestinal tract administration yielded clinical response rates in 67-100% of patients[13,14].

Two single-center pediatric case reports have been recently published showing marked clinical improvement in two patients with severe colitis. A 2015 case report described a 18-month old female presenting with an early-onset colitis with UC-like phenotype, who responded after 7 serial FMT infusions with donor stool from and age-matched niece and older brother[17]. A 2016 case report described an 11-year old female with corticosteroid-dependent UC who responded after serial FMT infusions every 2 to 4 weeks over a 10 month period. The patient remained in clinical remission at 40 weeks post final FMT, and showed complete endoscopic healing[18]. A further 2016 case report described a 3-year old female with acute severe UC who was refractory to aminosalicylates and all immunosuppressive drugs[19]. She received 6 successive FMT enemas and 4 FMT via nasoduodenal tube over 10 days, but ultimately required colectomy. Donor fecal microbiota was not identified in the patient, and the authors concluded that due to the severely damaged colonic epithelium, paucity of crypts, and overall decrease of mucous in the outer layer, the donor microbiota could not be retained by the recipient. The authors concluded that patients with mild disease might be a better candidate for FMT. However, factors that optimize bacterial transfer from donor to recipient in FMT remain unclear, and mechanistic conclusions from single-center case reports remain speculative at this time[19].

Significance

Factors that contribute to the success of FMT in the treatment of IBD remain unclear, and need definitive randomized controlled trials to identify benefit.

This pilot study will be the largest randomized, single-blind, placebo-controlled trial evaluating the role of FMT in pediatric UC ever conducted. This pilot study will build upon the FMT in adult UC trial recently completed at our center[20]. We will assess the feasibility of delivering twice-weekly fecal enemas from anonymous fecal microbial donors, to patients with pediatric UC. Previous fecal transplant studies in pediatrics have typically used within-family, or within-household donors with varying frequencies of transplant delivery, most of which were fewer than the frequency proposed in this study. These differences in donor selection, and transplant frequency may have compromised efficacy. This pilot will help identify factors associated with the success, or failure of FMT in pediatric UC. Our results will provide

important preliminary data for the design, and completion of a definitive pediatric trial with a larger sample size, and power calculation to attain statistical significance of our primary clinical outcomes in the future.

This study, and smaller pediatric trials by other investigators, suggest the following: FMT is an effective therapy in adult UC; FMT is an effective therapy for pediatric UC; donor selection has a significant role in optimizing therapeutic response; there may be differences in response between patients with duration of disease ≤1 year versus >1 year[20]. For these reasons, a large randomized, single-blind, placebo-controlled trial in pediatric UC patients, who typically have a shorter disease duration, using an anonymous donor, will be scientifically and clinically valuable.

The aim of this paper is to describe the first randomized controlled protocol for FMT in a pediatric IBD population.

Study Objectives

The objective of this pilot study is to assess the feasibility of FMT for the therapy of pediatric UC. Specifically, we will test the hypothesis that a protocol of twice-weekly enemas over six weeks, using fecal transplant material from an anonymous unrelated donor, will improve the efficacy of FMT in pediatric UC patients, and build a framework for future studies to assess the effectiveness of FMT intervention. We will evaluate key feasibility measures of:

1. Participant recruitment (Sample Size)
2. Participant retention (Sample Size)
3. Participant eligibility criteria (Sample Size)
4. Acceptance of patients to participate in study (Process)
5. Effect of intervention on disease primary and secondary disease outcomes (Clinical)
6. Effect of intervention on fecal bacterial community structure (Clinical)
7. Rate of adverse events in patients receiving FMT (Clinical)

METHODS AND ANALYSIS

Study Design

The Pedi FETCh Trial is a randomized, placebo-controlled multicenter trial. It utilizes a parallel arm approach consisting of a fecal transplantation group and a placebo group. In Ontario, CANADA there are two sites involved in this multi-center trial: McMaster Children's Hospital, Hamilton, and Children's Hospital of Western Ontario, London (primary and secondary sites, respectively).

Patients are seen on-site for all scheduled visits, in accordance with the protocol (FIGURE 1). Fecal enemas are screened, prepared, and tested by Rebiotix®, and sent to the sites for patient administration. Enemas are administered at the respective site's pediatric gastroenterology clinics by study team members familiar with the single-blinded FMT enema protocol, twice per week for six weeks.

Patient enrollment began in November 2015 at McMaster Children's Hospital, and is tentatively planned for March, 2017 at Children's Hospital of Western Ontario. A third site is projected to receive research ethics board approval in Spring, 2017 (Sainte Justine's Hospital, Montreal). We aim to complete recruitment of 50 patients with a target recruitment end-date of Winter, 2017. No other pediatric Gastroenterology sites in Canada are currently offering FMT trials for pediatric UC; thus, patients referred from outside centers for entry into our study will be considered if they meet eligibility criteria.

	OUTCOME	MEASURE		ANALYSIS
FEASIBILITY OUTCOMES	Participant recruitment	Recruitment/month	CUMULATIVE ACROSS ALL STUDY SITES	≥2 participants/month recruited and retained for duration of study
	Participant retention	Percent dropout post enrolment		
	Participant eligibility	Percent meeting eligibility		
	Adverse events	Hospitalization or ↑PUCAI ≥20 x 2 consecutive measures		<10% of participants
	Blood specimens	Participant provides all required blood samples		>90% of participants
	Stool specimens	Participants provides all required stool samples		>90% of participants
	Microbiome	Microbiome analyses (16s rRNA profile, metagenomics) performed for participant at all required time points		>80% of participants
	PUCAI	Participant provides information to calculate all required PUCAI scores		>90% of participants
	Week 30 Endoscopy	Endoscopy obtained		>10% of participants
CLINICAL OUTCOMES	Clinical remission (6wks)	PUCAI ≤10		Chi-squared test
	Clinical remission (30wks)	PUCAI ≤10		Chi-squared test
	Clinical remission (6-30wks)	Sustained PUCAI <10		Chi-squared test
	Clinical improvement (6wks)	↓ PUCAI ≥15		Chi-squared test
	Clinical improvement (6-30wks)	Sustained ↓ PUCAI ≥15		Chi-squared test
	Biological improvement (6wks)	↓ C-reactive protein		T-test
	Biological improvement (6wks)	↓ fecal calprotectin		T-test
	Biological improvement (30wks)	↓ C-reactive protein		T-test
	Biological improvement (30wks)	↓ fecal calprotectin		T-test
	Mucosal healing (30wks)	Endoscopy		T-test
	Change in microbiota (6wks)	Δ 16s rRNA profile, metagenomics profile		T-test, αβ Diversity
	Change in microbiota (30wks)	Δ 16s rRNA profile, metagenomics profile		T-test, αβ Diversity

TABLE 1: TRIAL OUTCOMES (FEASIBILITY, CLINICAL OUTCOMES)
PUCAI: Pediatric Ulcerative Colitis Activity Index; MRE: Magnetic Resonance Enterography

Eligibility Criteria

Pediatric patients (3-17 years old) with UC and inflammatory bowel disease-unclassified (IBD-U) subtype are eligible for the study. Patients classified as IBD-U are included because their distribution of disease is often limited to the large intestine -- like classical UC. Eligible patients must also demonstrate signs of disease activity, as determined by measures of elevated inflammatory markers (specifically fecal calprotectin and C-reactive protein (CRP)), PUCAI scores ≥15, or increased disease activity supported by endoscopic findings[21,22,23,13].

Subjects are excluded if they are participating in another clinical trial, are unable to give informed consent or assent, have severe comorbid medical illness, have concomitant *Clostridium difficile* infection, or require hospitalization (at discretion of the treating physician; typically, PUCAI > 65). Continued treatment with 5-ASA, azathioprine, 6-mercaptopurine or anti-TNFα therapy (e.g. infliximab) will be permitted if taken at stable dose for ≥4 weeks prior to randomization. No new medical therapies (e.g. corticosteroids, antibiotics, probiotics) will be permitted during the study period, unless patients meet criteria for a suspected adverse event.

Patients younger than 3 years old will also be excluded as these patients meet definition for "Infantile Inflammatory Bowel Disease", which may have a different biological and clinical phenotype than other pediatric age presentations of IBD[24,25].

Randomization

All patients seen at McMaster Children's Hospital's Division of Gastroenterology who meet eligibility criteria will be approached for participation in the study. Patient recruitment and consent are performed by the clinical research coordinator. A standardized script is used to describe objectives, risks and benefits, and details of the randomization to eligible participants. The sample size required to carry out this study is 50. This figure is based on the number of potentially eligible UC patients at McMaster Children's Hospital (150) and Children's Hospital of Western Ontario (100) using recent census data from respective centers' IBD clinics. 25 patients will be randomized to receive normal saline enemas (control; saline/polyethylene glycol 3350) and 25 patients to receive a FMT enema (intervention; 50g/150ml, 10^7 microbes/mL of suspension in saline/polyethylene glycol 3350). This pilot study is not powered for any of the outcomes (TABLE 1). Randomization occurs according to a computer-generated block randomization pattern (block size = 4 participants). Eligible patients will be randomized 1:1 to receive FMT, or normal saline fecal enemas containing brown food coloring. Patients randomized to receive the normal saline placebo enema will be given an opportunity to enter an open-label phase of the study at completion, to receive the fecal microbial enema and re-enter the trial through a prospective observational design.

Enema Product

Saline and fecal enemas are both obtained from Rebiotix®, a biotechnology company from Minnesota, USA that distributes live, human-derived fecal microbial enemas (RBX2660). RBX2660 has received Health Canada Clinical Trials Application (CTA), and U.S. Food and Drug Administration Investigational New Drug Application (IND) approvals for clinical trials in patients with recurrent *Clostridium difficile* infection. Enemas contain live, unprocessed human fecal microbiota, or saline with polyethylene glycol preservative (placebo). Fecal microbial donors are screened, stools are tested for infectious pathogens, centrally prepared in opaque enema bags, retested prior to delivery, and sent by Rebiotix® to the investigator through preserved cold-chain delivery. Specifically, the samples are stored in -80°C freezers at Rebiotix®, Minnesota, with next-day, on-site delivery in styrofoam-insulated boxes containing ice packs. Frozen enemas are removed from the boxes and stored in the clinic fridge (4°C) for up to 3 days. The opaque enema bags and enema tubing ensure that blinding is preserved to the study patient.

A study team member initially "connects" the enema bag to its tubing. We add 1 ml of commercially available (Club House® brand), food-grade food coloring to the normal saline enema bag (2 drops red, 3 drops green, 7 drops yellow) to confer a brown color to the clear normal saline solution.

Statistical Analysis

Outcomes

Subjects will have outcome measures performed at four time-points (FIGURE 1). In addition to study outcome measures, patients in each randomization arm will receive usual medical care.

Feasibility will be assessed by outcomes of patient eligibility, patient recruitment, patient retention, adherence to blood and stool sample collection times, and adverse events or patient hospitalizations throughout the enema administration and follow-up period.

Bloodwork collection will be performed at the time of the FMT (weeks 0, 3, 6), or at scheduled clinic visits (weeks 18, 30). Standard pediatric UC bloodwork to monitor for systemic inflammation will be ordered: complete blood count (CBC), erythrocyte sedimentation rate (ESR), CRP, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP). Routine laboratory protocols and assays for obtaining, and measuring samples will be followed[8].

Clinical disease activity scores will be determined based on history obtained at each clinical assessment (twice weekly on weeks 0-6; once on weeks 18, 30), or over the telephone on non-clinical assessment days (weeks 12, 24). The internationally validated PUCAI score will be used to objectively assess clinical disease activity[21].

Stool samples will be collected for microbiome analysis and fecal calprotectin (weeks 0, 3, 6, 12, 18, 24, 30), to assess patterns of bacterial community structure associated with intestinal inflammation. Routine collection of stool specimens will be performed at home independently by patients. Stools unable

to be submitted immediately may be kept in a sterile screw-capped sample container in a standard, home freezer until delivered to the laboratory. Microbiome analysis will be conducted through the laboratory of Dr. M. Surette; bacterial community profiling of 16s rRNA genes will be performed on part of each stool sample using 250 nucleotide paired end reads of the V3 (or V3V4) region using the MiSeq Illumina sequencer[26]. Analysis will be performed using an in-house bioinformatics pipeline that generates clusters of operational taxonomic units (OTUs), taxonomic assignment and various measures of alpha and beta-diversity. If required at the bioinformatics stage, additional control microbial data will be obtained through Human Microbiome Project published databanks.

Imaging and endoscopic surveillance may be performed if clinically indicated.

Followup patient outcomes will be measured at Weeks 12, 18, 24, and 30 after the fecal enema intervention (FIGURE 1).

All primary and secondary outcomes will be assessed using both intention-to-treat and per-protocol. The differences in remission rates between the groups will be analysed with support from departmental statistical support personnel. Proportions and percentages will be reported to determine if all feasibility outcomes were reached. Continuous outcomes will be compared using T-tests and categorical outcomes will be compared using Chi-squared tests. 95% confidence intervals and p-values will be reported (TABLE 1).

Safety Monitoring

Study risks include enema delivery, and complications of FMT. Enema administration carries a low risk of proctitis, and FMT has been associated with *C. difficile* colitis, fever, and infection[26].

We will be obtaining FMT materials through a company called Rebiotix®, a biotechnology firm located in Minnesota, USA. The fecal enema (RBX2660) prepared by Rebiotix has received Health Canada Clinical Trials Application (CTA), and U.S. Food and Drug Administration Investigational New Drug Application (IND) approvals for clinical trials in patients with recurrent *Clostridium difficile* infection.

Donor qualification processes involve: potential donors completing a health and lifestyle questionnaire, providing blood and stool samples for analyses of:

a) Blood - HIV, Hepatitis A, B, and C, syphilis and,
b) Stool - C. difficile toxin B, norovirus, rotavirus, adenovirus, Shigella, Salmonella, Campylobacter, E. coli, Aeromonas, Plesiomonas, Yersinia, Shiga toxins, Giardia antigen, Cryptosporidium antigen, Cyclospora, Isospora, ova and parasites, Vancomycin-resistant enterococci, Methicillin-resistant Staphylococcus, Vibrio, and Listeria.

Donors are screened to confirm they are disease-free before donations begin. When donations begin, a sample of each donation from each donor is retained. Retained samples from each donor are pooled with other samples from that same donor and the pooled samples are subjected to repeat stool testing at approximately 45-day intervals to confirm continued donor health. Repeat donor blood testing is also performed at a minimum of 14 days after the last donation and again during the approximately 45-day collection interval. If the donor passes the repeat screening, drug product manufactured from donations collected within that 45-day period is released from quarantine. At the time of each donation, the donor completes a repeat questionnaire to confirm his/her continued health and absence of risky lifestyle behaviors. Rebiotix performs stool and blood testing approximately every 45 days.

Study participants will be monitored for signs of clinical deterioration. PUCAI scores will be measured at defined trial timepoints (FIGURE 1). Any increase in disease activity score ≥20 from previous will be classified as “disease progression”[21]. Reassessment of PUCAI scoring will occur within one week and any further increase ≥20 will remove the patient from the study for implementation of standard IBD management. Patients who contact study coordinators or clinic nurses reporting fever, or worsening vomiting, abdominal pain, rectal pain, diarrhea, or hematochezia will have PUCAI scores measured to evaluate for progression. All participants who experience an increase in PUCAI scores ≥20 at successive measurements, or admission to hospital for any reason, will be classified as having an adverse event, and unblinding may be performed to the treating clinician and/or study participant at that time.

A Data, Safety and Monitoring Committee (DSMC) has been assembled for the study at the investigators’ discretion. The DSMC will perform an interval assessment of preliminary study data, approximately midway through the trial. The DSMC has been comprised of experts in the fields of pediatric gastroenterology, pediatric IBD management, and clinical trial statistical analyses, and will operate independently of the investigators and collaborators for the trial, and any sponsors. The terms of

the DSMC will be defined at the initial meeting, which has not yet occurred. The DSMC was not assembled earlier, due to the Research Ethics Board at the primary site determining that a DSMC would not be necessary for the trial to commence.

ETHICS AND DISSEMINATION

Ethics

The study will be conducted in accordance with the protocol, the Helsinki declaration, and the Canadian Tri-Council Policy Statement on research ethics. A Health Canada no-objection letter to perform FMT for the purpose of the trial was obtained, and local research ethics board (REB) approval was obtained for McMaster Children's Hospital, and Children's Hospital of Western Ontario. All amendments to the trial protocol will be obtained through approval by the local REB, and updated through the clinical trials registry (ClinicalTrials.gov). Research personnel will approach all potentially eligible patients who fulfill eligibility criteria for consent. All patients must sign a consent form to participate in the trial.

Data Sharing

Anonymized data about patient outcomes will be shared with Rebiotix for assessment of RBX2660 (fecal enema) efficacy. The information shared will be limited to:

a) Patient age, height, weight
b) Patient medication history (previous, and ongoing)
c) Date enema was given, volume administered, any reported adverse effects of delivery
d) PUCAI scores, results of bloodwork, and fecal calprotectin

Additionally, a de-identified stool sample will be sent to Rebiotix. These will be collected along with standard stool samples per study protocol (Figure 1).

Follow-up

Results of this pilot will inform several future research goals. Pilot data will be used to inform a definitive multicenter randomized controlled trial using a larger pediatric patient population. Data will be used to support additional research funding for a multicenter trial through a Canadian Institute of Health Research operating grant. Our data may also support further crossover studies between human and mouse models to ascertain host-microbial influences underlying observed changes.

Reporting

Results of the pilot will be reported to key stakeholders. Pilot data will be shared locally with clinical and basic science division members. Results will be shared through presentations at regional hospitals to encourage participation in future multicenter trials. Pilot data will also be submitted to international conferences and published in peer-review journals.

FUTURE DIRECTIONS

Based on the results of this study, future double-blind, randomized, placebo-controlled trials of FMT in pediatric IBD may involve:

Primary outcomes

- Evaluation of efficacy of FMT at inducing clinical remission in new onset UC, or Crohn's Disease
- Evaluation of efficacy of multiple, periodically administered FMT at maintaining clinical remission (prevention of relapse)

Secondary outcomes

- Evaluation of efficacy of FMT at inducing long-term mucosal healing
- Evaluation of change in fecal bacterial community structures of patients receiving FMT

DISCUSSION

Increasing prevalence of UC worldwide, particularly in Canada, and diagnosis in increasingly younger populations necessitates the need for novel treatment approaches[27]. Currently, this trial is open to pediatric UC, and IBD-U patients experiencing symptoms of clinical or mucosal inflammation, who would like to abstain from escalation of therapy, or surgical interventions.

A previous randomized controlled trial for FMT in adult UC demonstrated a statistically significant benefit of FMT (9/38, 24% versus 2/37, 5%) at inducing UC remission, with no significant difference in adverse events between the groups[20]. A second positive randomized controlled trial for FMT in adult UC was recently published, showing statistically significant benefits of FMT versus normal saline placebo at inducing: steroid-free clinical remission (18/41, 44% versus 8/40, 23%), clinical response (22/41, 54% versus 9/40, 23%), and endoscopic response (13/41, 32% versus 4/40, 10%)[28].

This study will utilize a similar 6-week approach, with the twice-weekly enema administrations instead of once-weekly. We will use a similar normal saline enema placebo. Our trial will also involve a greater diversity of anonymous, non-household donors, to decrease the likelihood of observing a donor-dependent outcome. Primary outcomes of this pilot study will be feasibility measures of the reported protocol, and secondary outcomes will include subjective and objective measures of clinical and mucosal healing of UC/IBD-U over the enema administration, and follow-up period.

This trial will provide preliminary evidence for the use of FMT in a pediatric UC population. Our results will be informative for future, larger population size, double-blinded randomized controlled trial in pediatric UC, which may look into further analyzing the efficacy of FMT in inducing remission and optimize dosage.

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FIGURE 1. STUDY PROTOCOL
PUCAI: Pediatric Ulcerative Colitis Activity Index

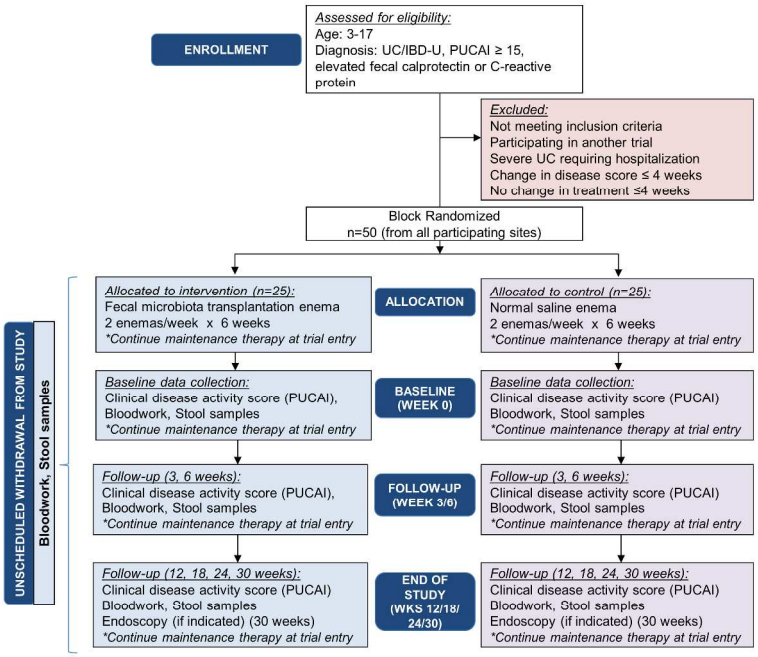

FIGURE 1: STUDY PROTOCOL
PUCAI: Pediatric Ulcerative Colitis Activity Index

279x203mm (300 x 300 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on 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	1
	2b	All items from the World Health Organization Trial Registration Data Set	n/a
Protocol version	3	Date and version identifier	n/a
Funding	4	Sources and types of financial, material, and other support	1
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	1
	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	1
	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)	n/a

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	3
	6b	Explanation for choice of comparators	4
Objectives	7	Specific objectives or hypotheses	4,6
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, noninferiority, exploratory)	4,6
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	5
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)	4,5
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6,7
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	8
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	7
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	5,6,8
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	6,7,8
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)	6,7

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	6
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	6
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 assign interventions	6
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	6,7
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	7
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	7,8
Methods: Data collection, management, and analysis			
Data collection methods	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	9
	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	9

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	9
Statistical methods	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	7
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	8
	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)	7
Methods: Monitoring			
Data monitoring	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	8
	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	n/a
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	n/a
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	n/a
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	8
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	8

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	5
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
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	9
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	1
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	9
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	n/a
Dissemination policy	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	9
	31b	Authorship eligibility guidelines and any intended use of professional writers	n/a
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n/a
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	APPENDIX
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	7

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.