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The efficacy of fecal microbiota transplantation in the treatment of mild-to-moderate ulcerative colitis: a prospective, randomized, double-blinded, controlled clinical trial

A. Study Purpose and Rationale

Ulcerative colitis (UC) is a type of inflammatory bowel disease (IBD) characterized by recurring episodes of colonic mucosal inflammation leading to attacks of bloody diarrhea often accompanied by abdominal pain, tenesmus, urgency, and incontinence. Chronic colonic inflammation in this disease may lead to significant chronic health issues (such as severe anemia, colonic perforation, colorectal carcinoma, and eventual need for colectomy) and have a significant impact on quality of life.[7] It is estimated that 1.5 million Americans suffer from IBD, and that the disease leads to 2.3 million physician visits and 180,000 hospital admissions, and costs $6.3 billion annually.[14]

The exact etiology of UC remains unclear, but it appears to be multifactorial, involving the interplay of genetics and environment.[10] Multiple laboratory and clinical studies suggest that these patients suffer from a dysregulation of the innate and adaptive immune systems, resulting in an inappropriate immune response to gut microbiota (with possible contribution from inherent or acquired abnormalities in mucosal integrity or a lack of regulatory cells or mediators).[10] There is also increasing evidence that UC patients have a different composition of colonic flora in comparison to healthy controls, which may contribute to this inappropriate immune response. For example, patients with IBD have a reduced diversity of the fecal microbiota with 25 percent fewer microbial genes than healthy controls.[11] Additionally, in comparison with healthy controls, patients with active UC have been found to have significantly reduced amounts of bifidobacteria and lactobacilli, both bacteria that are believed to play important roles in promoting intestinal health[9] by improving intestinal barrier function and modulating the mucosal immune system.[13]

There are several case reports and case series of fecal microbiota transplantation (FMT) in UC that suggest a beneficial effect. For example, a 2003 report documented resolution of disease in 6 patients with severe, refractory UC treated with FMT; the patients remained asymptomatic off UC medications without any clinical, colonoscopic, or histologic evidence of UC at 1 to 13 years of follow-up.[4] Additionally, in a 2012 meta-analysis of 17 case studies of FMT in IBD, the majority of patients experienced a reduction of symptoms (19/25), cessation of IBD medications, (13/17) and complete disease remission (15/24).[1]

While recent decades have led to the discovery and development of effective medical therapies for UC (including anti-inflammatory and immunomodulating treatments), some patients are either refractory to standard medical therapy, are unable to tolerate medical therapy, or continue to experience mild active symptoms despite treatment.[14] Additionally, FMT has recently been shown in a small randomized, controlled clinical trial to be an effective treatment in recurrent clostridium dificile colitis, presumably by restoring natural bowel flora,[16] and mat be beneficial in UC by a similar mechanism. FMT has also been shown to have few if any adverse reactions.

UC is a disease of the colon, generally causing rectal inflammation that extends proximally in a contiguous manner; approximately 20 percent of patients experience pancolitis with disease extending to the cecum and occasionally the terminal ileus (“backwash ileitis”). While enemas have been used in many past case series evaluating FMT in UC,[2, 4] they are generally only able to reach the splenic flexure, while colonoscopy allows for administration of bacteria throughout the entire colon. Given the ease of procedure completion in both the outpatient and inpatient settings as well as the delivery of bacterial flora throughout the colon, colonoscopy has been proposed as the preferential route of FMT for clostridium difficile infection,[5] and the same logic would apply to FMT in the treatment of UC. This will thus be the route of FMT administration utilized in this study, although further research into the most beneficial method of FMT is needed.

B. Study Design and Statistical Analysis
This study is a prospective, single-center, randomized, double-blinded, controlled clinical trial to establish the efficacy of FMT in improving clinical symptoms and colonic inflammation in patients with mild-to-moderate ulcerative colitis, defined as a Mayo score of 4 to 10 in previous studies.[8] Upon enrollment in the study, informed consent will be obtained, and patients will be randomized to either a control group or a treatment group via an online random number generator. Patients in the control group will receive FMT with their own feces, and patients in the treatment group will receive FMT with feces from a healthy donor (as described below in Study Procedure). Patients may continue their current treatment regimen for UC (including mesalamine, 5-ASA derivatives, azathioprine, oral or topical steroids) as long as the treatment regimen has remained stable for 12 weeks prior to randomization. All analyses following data collection will be conducted as intention-to-treat. No cross-over will occur in this study.

The primary outcome for this study will be the mean change in the Mayo score for UC disease severity (see attached table) from baseline at 6 weeks. This initial study will analyze change in Mayo score as a continuous variable. The Mayo score will be calculated for each patient at baseline and at 6 weeks following FMT treatment. In order to achieve 80% power with an alpha of 0.05, a sample size of 10 patients for each group was calculated using the T-test on the group means of change in Mayo score. This calculation assumes a mean change in the treatment group of 3 points (defined by previous studies as a clinically significant response), a mean change in the treatment group of 1 point (representing a placebo effect of approximately 30%), and an effect size of 2. The range of possible change in Mayo score in the control group was estimated to be 6 (representing approximately 4 standard deviations), with a calculated standard deviation of 1.5.

Secondary outcomes will include the proportion of patients who experience a clinical response, defined as a reduction in Mayo score by 3 points or greater; the proportion of patients who experience clinical remission as defined by a Mayo score of 2 or less with no individual subscore of more than 1; and mucosal healing, defined as a score of 0 or 1 on the Mayo endoscopic findings subscore. Chi-square tests will be used to compare the distributions of categorical outcomes.

C. Study Procedure.
The likely duration of the entire study for patients will be approximately 12 weeks.

Donor Selection and Screening:
Stool donors will be healthy adult volunteers less than 60 years of age with normal bowel function (i.e., normal daily stools) without recent antibiotic exposure (within the past 6 months) or a history of inflammatory bowel disease who do not share living quarters with the recipient. A thorough donor interview will be conducted to identify risk factors for transmissible diseases that standard laboratory tests (as described below) may not detect. Donor stool will be screened for enteric pathogens prior to donation via stool culture to screen for enteropathogenic bacteria, light microscopy examination for ova and parasites (including Entamoeba histolytica, Giardia lamblia, Cryptosporidium sp., Dientamoeba fragilis, Blastocystis hominis, Ascaris lumbricoides, trematodes and tape worms), and testing for C. difficile. Donor blood will be screened for antibodies to hepatitis A, B, and C; HIV-1 and HIV-2; HTLV1 and HTLV2; syphilis; CMV; EBV; Strongyloides stercoralis; and Entamoeba histolytica.

Administration of Donor Feces:
Each patient will receive pre-treatment with vancomycin 500mg twice daily for seven days for suppression of clostridial organisms.[4] Colonic lavage will be performed on the evening of the last day of antibiotic therapy prior to endoscopy via oral ingestion of 4 L of a polyethylene glycol solution.

For the treatment group: Donor feces will be collected on the day of the infusion and immediately

transported to the hospital. Donor feces (approximately 200-300mL, or the approximate size of a regular adult bowel movement) will be diluted with 200-300mL of sterile saline and stirred. The solution will be strained to remove particulate matter, poured into a sterile bottle, and infused as described below. The stool will be prepared within 6 hours of donation, and the fecal slurry administered within 10 minutes of preparation. Fecal solutions will be prepared and the colonoscopy will be performed by investigators who do not know the randomization of the patient.

Colonoscopy will be performed by an experienced operator using moderate sedation with midazolam and fentanyl and will likely last for approximately 30 minutes; the physician operator will score the patient based on the endoscopic appearance of the bowel using the Mayo sub-score for endoscopic findings (i.e., normal-appearing mucosa or inactive colitis = 0, mild colitis: mild friability, erythema, and decrease in vascularity = 1, moderate colitis: friability, marked erythema, vascular pattern absent, erosions visualized = 2, severe colitis: ulcerations and spontaneous bleeding = 3). The operator will then instill the fecal solution into the cecum via the colonoscope. Patients will be monitored for 2 hours following endoscopic instillation of the feces to monitor for adverse effects and allow for resolution of sedation. Following the procedure, patients may experience mild bloating and abdominal cramps or minimal bleeding if a biopsy is taken. Although there is always a risk of colonic perforation with colonoscopy, it is a very rare occurrence with a perforation rate of approximately 0.01 to 0.1 percent. Performing endoscopic examinations 6 weeks apart is likely more frequent than would be required in standard clinical practice; however, this is necessary to evaluate the effect of the treatment on disease severity.

An identical protocol will be followed for the control group, but using the patient's own feces. Every patient in the study will be required to provide a stool sample prior to colonoscopy to ensure that they are blinded to their group assignment.

Standard post-procedure dietary instructions will be given to all patients, to reintroduce oral food slowly, and to consume a bland diet. The patients will be instructed to contact the investigators or go to the emergency room if significant bleeding or pain occurs.

Assessment of Disease Severity:
There is no single standardized, validated disease activity index for ulcerative colitis; indeed, there are at least seven different symptom-based activity scores, two composite scores and four patient evaluation scoring systems.[15] The Mayo Clinic score, or the Disease Activity Index, has been most widely used in large clinical trials of UC,[12, 15, 17] and is currently favored by the FDA for trial design[6]; it is a composite index that attempts to include objective indices of disease severity, including frequency of bowel movements, severity of rectal bleeding, endoscopic findings, and global assessment by a physician. It will be used in this study to score the severity of ulcerative colitis in each patient based on the above-mentioned indices before and after FMT. A scoring outline is attached, as previously described in the literature.[18]

The severity of ulcerative colitis at baseline and 6 weeks following FMT will be assessed via the Mayo score for ulcerative colitis activity (see attached table). Patients will be asked to record daily the frequency of their stools, any rectal bleeding, their subjective sense of well-being, and the degree of abdominal discomfort over the 6-week period prior to FMT and after FMT. Endoscopic evaluation will be performed at baseline and at 6-week follow-up with the endoscopic sub-score calculated each time (as described above).

One of the investigators will calculate the physician's global-assessment score for the patient at pre- and post-procedure patient visits. This score will reflect the patient's recorded symptoms, the endoscopic appearance of the colonic mucosa, physical and laboratory findings, and the patient's performance status. A score of zero will indicate no symptoms of colitis, the patient feels well, and the endoscopic score was zero. A score of 1 will indicate mild symptoms and endoscopic findings that were mildly abnormal. A score of 2 will reflect more serious abnormalities, and endoscopic and symptom scores of 1 to 2. A score of 3 will indicate that endoscopic and symptom scores were 2 to 3.

D. Study Drugs
Vancomycin: This study will use the antibiotic vancomycin for pre-treatment prior to FMT to suppress the growth of clostridial species. This technique has been used and cited extensively in previous studies of FMT in UC in the literature with no adverse events reported.[1] This is an FDA-approved medication. It will be administered as an oral capsule with the dosage of 500mg twice daily for seven days. Side effects may include abdominal pain, bad taste, or nausea in over 10 percent of patients. Additionally, 1 to 10 percent of patients may experience peripheral edema, fatigue, fever, headache, diarrhea, flatulence, vomiting, or back pain. Renal failure, ototoxicity, vasculitis, or thrombocytopenia can occur in fewer than 1 percent of patients.

E. Medical Device
Colonoscope: A colonoscope will be used to instill donor feces into each participant's cecum and to evaluate colonic inflammation at baseline and at the 6-week period. This will allow administration of donor stool throughout the colon in comparison to alternative methods (including retention enema), and has been shown in case series to have a greater overall response rate than FMT through the stomach or small intestine in other disease processes, such as C. difficile colitis[5] although this has not been established for UC. This device is commercially available. The safety and efficacy of colonoscopy is well-established, and no adverse events have been previously reported for FMT in this patient population.[3]

F. Study Questionnaires
There will no be no questionnaires used as part of this study.

G. Study Subjects
Inclusion criteria will be:
-Mild-to-moderate ulcerative colitis, defined as a Mayo score from 4 to 10 (disease can be newly or previously diagnosed)
-Age $\geq$ 18 years
-Continued treatment with topical mesalamine, 5-ASA derivatives, azathioprine, 6-mercaptopurine, anti-TNF therapy (e.g. infliximab), or topical or oral steroids will be permitted if taken at a stable dose for greater than or equal to 12 weeks prior to randomization.

Exclusion criteria will be:
-Severe ulcerative colitis, defined as a Mayo score of > 10
-Severe complications of ulcerative colitis, including severe systemic disease (fever, tachycardia, hypotension) requiring hospitalization, severe symptomatic anemia, hemodynamically significant GI bleeding, fulminant colitis, toxic megacolon, bowel perforation, or colorectal cancer
-Current treatment for any other infection
-Lack of ability to give informed consent

H. Recruitment of Subjects
Subjects will be identified and approached about study enrollment via the gastrointestinal clinics at New York Presbyterian Hospital-Columbia University Medical Center by making all the physicians in the department aware of the research study, inclusion criteria, and possible risks/benefits. The patient's gastroenterologist and/or primary care physician will agree that the patient is suitable for the study and will ascertain that the patient is willing to discuss participation with the research team before any approach by the research team.

I. Confidentiality of Study Data
All study participants will receive a unique study code number. Personal identifying information, including hospital unit numbers, social security numbers, subject names/initials, phone numbers, and addresses will be removed from study material and data. The information used in the study will be stored in a secure database, accessible only to participating investigators.

J. Potential Conflict of Interest

The study investigators do not have a proprietary interest in the drugs, devices, or procedures used in this study and will not benefit financially in any way from the results of the investigation. There is no conflict of interest to disclose.

K. Location of the Study
The study will take place at New York Presbyterian Hospital – Columbia University Medical Center. Endoscopic evaluation will occur in the endoscopy suites, while pre- and post-procedure protocol as described above will occur in waiting areas surrounding the endoscopy suites. Clinical interviews will be conducted in various exam rooms within the hospital.

L. Potential Risks
Potential risks of the study include risks associated with colonoscopy (including adverse reaction to sedative medications, mild cramping/bloating following the procedure, rare colonic perforation), which will be mitigated by the use of experienced operators, as well as risks associated with vancomycin use (as described above in Study Drugs). Additionally, the patient may be randomized to the control arm of the study and thus receive self-FMT; the patient's clinical condition may worsen or remain stable compared to the treatment arm as a result.

M. Potential Benefits
The study participants may or may not benefit as a result of participation in this study. Based on prior observational studies and case series, the severity of the participants' disease may improve. Even if the participants' condition does not improve, their participation will provide new information on the efficacy of FMT for the treatment of UC with the potential to benefit other patients who suffer from this disease and advance medical knowledge.

N. Alternative Therapies
Alternative medical therapies for treatment of mild-to-moderate UC include topical mesalamine, 5-ASA derivatives, azathioprine, 6-mercaptopurine, anti-TNF therapy (e.g. infliximab), or topical or oral steroids. As stated above, this study does not exclude the use of alternative therapies, but requires that the participating patients be taking a stable dose of these medications for greater than or equal to 12 weeks prior to randomization.

0. Compensation to Subjects
The study participants will not be compensated.

P. Costs to Subjects
The subjects will not incur any additional costs as a result of participating in the study.

Q. Minors as Research Subjects
Not Applicable.

R. Radiation or Radioactive Substances
Not Applicable.

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Mayo Score for Assessment of Ulcerative Colitis Severity, as described previously in the literature.[18]

Table 1. Mayo Scoring System for Assessment of Ulcerative Colitis Activity.*

Stool frequency†
- 0 = Normal no. of stools for this patient
- 1 = 1 to 2 stools more than normal
- 2 = 3 to 4 stools more than normal
- 3 = 5 or more stools more than normal
- Subscore, 0 to 3

Rectal bleeding‡
- 0 = No blood seen
- 1 = Streaks of blood with stool less than half the time
- 2 = Obvious blood with stool most of the time
- 3 = Blood alone passes
- Subscore, 0 to 3

Findings on endoscopy
- 0 = Normal or inactive disease
- 1 = Mild disease (erythema, decreased vascular pattern, mild friability)
- 2 = Moderate disease (marked erythema, lack of vascular pattern, friability, erosions)
- 3 = Severe disease (spontaneous bleeding, ulceration)
- Subscore, 0 to 3

Physician's global assessment§
- 0 = Normal
- 1 = Mild disease
- 2 = Moderate disease
- 3 = Severe disease
- Subscore, 0 to 3

* The Mayo score ranges from 0 to 12, with higher scores indicating more severe disease. Data are from Schroeder et al.[24]

† Each patient serves as his or her own control to establish the degree of abnormality of the stool frequency.

‡ The daily bleeding score represents the most severe bleeding of the day.

§ The physician's global assessment acknowledges the three other criteria, the patient's daily recollection of abdominal discomfort and general sense of well-being, and other observations, such as physical findings and the patient's performance status.