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GASTROENTEROLOGY (S GUANDALINI, SECTION EDITOR)

Pediatric Fecal Microbiota Transplantation

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Abstract Given the emerging role of the intestinal microbiota as a key regulator of host physiology in health and disease, therapies that modulate enteric bacterial communities offer promise for a variety of clinical disorders. Fecal microbiota transplantation (FMT) represents a fundamental approach to manipulate the gut microbiota, accomplished by administering stool from a healthy donor into the gastrointestinal tract of a diseased recipient. However, despite the plausible feasibility of FMT, there is a paucity of clinical trials evaluating its efficacy, particularly in the pediatric population. To this end, this review seeks to accomplish three primary objectives. First, the rationale and data supporting the efficacy of FMT will be discussed. Second, practical considerations for the use of FMT in a clinical setting will be reviewed. Finally, future areas of study regarding FMT will be identified. Importantly, key studies addressing the role of FMT in pediatric patients will be highlighted.

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Introduction

The human gastrointestinal (GI) tract contains a remarkably high density of microorganisms, including more than 1,000 bacterial species. These microorganisms are collectively known as the gut microbiota. Emerging evidence suggests that the gut microbiota plays a crucial rule in host metabolism, nutrition, and immune function [1–3]. Furthermore, changes in the gut microbial community, termed dysbiosis, have been implicated in the pathogenesis of a spectrum of human diseases. This includes disorders of the GI tract such as *Clostridium difficile* infection (CDI) [4], irritable bowel syndrome (IBS) [5–9], and inflammatory bowel disease (IBD) [10, 11], as well as non-GI diseases such as obesity [12], autoimmune disorders [13], and autism [14]. Therapies that restore microbial homeostasis may be highly beneficial for these disorders, and also offer the benefit of being potentially safer than the immunosuppressive regimens commonly used to treat many of these ailments.

The concept of altering the gut microbiota as a treatment for human disease has existed for centuries. Indeed, numerous modalities have been developed to modulate gut microbial composition, including prebiotics, probiotics, and antibiotics. However, perhaps the most comprehensive approach to alter the enteric microbiota is via fecal microbiota transplantation (FMT). This technique involves the administration of healthy donor feces to unhealthy individuals. FMT was first described in the fourth century by Ge Hong, a Chinese medicine doctor, as a treatment for

diarrhea [15]. However, it was not until 1958 that FMT gained recognition in modern medicine. At that time, Eiseman et al. [16] successfully treated four patients with pseudomembranous colitis using fecal enemas from healthy donors. Since then, FMT has become an acceptable standard of care for treating adults with refractory CDI. Trials studying the efficacy of FMT for the treatment of additional GI and non-GI conditions are now ongoing. This review will summarize existing data supporting the use of FMT for various clinical disorders, describe the practical aspects involved in implementing FMT in the clinical setting, and identify critical areas for future study that will be necessary to utilize FMT in a safe and efficient manner moving forward.

Mechanism

The central premise underlying FMT postulates that the infusion of stool bacteria from a healthy donor to an unhealthy individual restores bacterial homeostasis in the recipient. However, the mechanisms by which this occurs have not been definitively elucidated. A common theory suggests that the donor microbiota is ultimately established in the host, thereby constituting a "transplant." This is supported by studies that have demonstrated engraftment of the gut microbiota following FMT [17••]. However, work by Petrof et al. [18] has shown that over a period of 6 months, only 25–30 % of transplanted species remained detected in the recipient. This suggests that FMT also promotes a re-expansion of the healthy resident bacteria of the recipient. A recent mouse study of CDI supports this premise by demonstrating that *C. difficile* can be eradicated using a cocktail of only six bacterial strains [19]. This is akin to the "enslapment" theory suggested by Kellermayer, which postulates that the healthy donor bacteria are able to "shock" the recipient microbiota into its former healthier state. Finally, there is also evidence that FMT creates a de novo environment within the recipient gut that is unfavorable for pathogen expansion. For example, a recent study by Ng et al. [20] demonstrates that *C. difficile* flourishes after antibiotic therapy because specific carbohydrates that it requires for growth are released from the dysbiotic microbiota. It is possible, then, that the healthy bacteria provided by FMT may compete for these carbohydrates, thereby depleting the substrate necessary for the expansion of *C. difficile*. Ultimately, it is likely that beneficial effects of FMT are due to multiple mechanisms, each of which may have varying import dependent upon the disease process being treated. Understanding these mechanisms will be critical for optimizing FMT for different disorders, and to develop more efficient modalities to deliver this emerging therapy.

Indications for FMT

Recurrent CDI

With the rising incidence of CDI over the past decades, there has been a parallel increase in disease severity and mortality. In 2010, the incidence of CDI in United States adults was estimated to be 500,000 cases per year, with associated health care costs of approximately $2 billion annually [21–23]. The recurrence rate of CDI after the first infection is 10–12 % and increases to 40–65 % after the initial recurrence [24]. The incidence of CDI is also rising in children [25–27], and it is currently the most common cause of hospital-acquired diarrhea in this population [28, 29]. A recent study demonstrated an increase from 2.2 to 23.5 cases of CDI increase per 100,000 children from 1991 through 2009 [30]. The recurrence rate of CDI in pediatrics is estimated to be 12–24 % [31, 32]. Fortunately, though increased rates of colectomy and mortality from CDI are well reported in adults [33–36], pediatric complications from CDI are not as severe. In a cohort of 299 hospitalized children with CDI, only 2 % were reported to develop a complicated course, and none required colectomy for fulminant CDI [37].

Given the high recurrence rates for CDI, alternatives to standard antibiotic therapies for this pathogen are needed. Numerous case reports and series have reported successful treatment of recurrent CDI using FMT [38–45]. The first randomized control trial comparing vancomycin alone to vancomycin plus FMT for the treatment of CDI was published in 2013 [46•]. This trial was stopped early due to the high-resolution rate (93 %) of the group that received FMT, compared to 30.7 % in those treated with antibiotics alone. A recent systematic review examined the published studies evaluating the efficacy and safety of FMT for CDI and found that 87 % (467/536) of patients had resolution of diarrheal symptoms after FMT [39]. In this review, a single infusion was often sufficient to treat CDI; however, successful treatment of CDI using sequential FMT has been reported when sustained response is not attained [47]. Based on the high success rate of FMT for CDI, the American Gastroenterology Association recommends considering FMT for a 3rd recurrence (after pulsed vancomycin) [48]. In addition, the FMT Workgroup has suggested consideration of FMT for moderate CDI not responding to a week of standard therapy, and severe *C. difficile* colitis not responding to 48 h of therapy [49••].

Due to a paucity of pediatric data, there are currently no guidelines for the use of FMT in children with recurrent CDI. The initial report describing the treatment of recurrent *C. difficile* by FMT in a pediatric patient was of a 2-year-old girl who received FMT via nasogastric administration, using stool donated from her healthy father [45].

Resolution of clinical symptoms was observed by 36 h. Subsequent *C. difficile* toxin was not detected up to 6 months after transplant. A second case reported by Kahn et al. describes a 16-month-old boy who underwent FMT after 6 episodes of CDI. Diarrheal symptoms improved within the first day after transplant, and *C. difficile* toxin PCR was negative at 1 week [41]. Two children aged 6 and 8 years were subjects in a case series of 87 patients (aged 6–94 years) who received FMT recurrent CDI [43]. Of note, one of these patients was reported to have a clinical relapse. Finally, a systematic review by Sha et al. also included 2 abstracts describing pediatric patients treated with FMT. The first was by Garg et al., reporting a 20-month old successfully treated for recurrent CDI with a single FMT infusion delivered colonoscopically. The second by Sing et al. described a 6-year old with UC and CDI that underwent nasogastric stool infusion. *C. difficile* toxin was cleared by 3 weeks. Both these patients received stools from their mothers [50].

Inflammatory Bowel Diseases

Although the precise pathogenesis of IBD remains unclear, evidence suggests that the intestinal microbiota is a key driver of intestinal inflammation in patients with these diseases [51, 52]. IBD is broadly classified into Crohn's disease (CD) and ulcerative colitis (UC), and alterations of the gut microbiota have been described for both of these entities [53, 54]. Hence there is a strong rationale to support the use of FMT to treat IBD. However, as described below, there are limited studies examining the efficacy of FMT for IBD, and further trials are needed to establish its benefit for these patients.

One of the earlier case reports using FMT to treat IBD was published in 1989 by Bennet and Brinkman [55]. In this study, Bennet himself treated his own UC by self-administering large-volume retention enemas using stool from a healthy donor. He remained symptom-free 6 months post-transplant without medication. In 1989, Borody et al. [56] described two IBD patients who remained symptom-free for 3–4 months after FMT. Both patients were adults (a 45-year old with UC, and a 31-year old with CD). This was followed by a report from the same group in 2003, describing six patients with UC (aged 25–53 years) who achieved remission with daily fecal enemas for 5 days [57]. A systematic review examining the role of FMT for the management of IBD has since been published by Anderson et al. [38], reporting 26 patients treated with FMT for management of IBD, ranging in age from 11 to 78 years. 18 individuals had UC, 6 had CD, and 2 carried the diagnosis of indeterminate colitis. 76 % reported improvement of symptoms, and 63 % remained in remission 3–36 months after FMT [38]. Kao et al. [58] recently reported a case of a 26-year-old man with CD who received FMT after failing immunosuppressive therapy. He received a single colonoscopic stool infusion from a universal donor. 48 h after FMT, the consistency of stools improved, correlating with a decreasing trend in C-reactive protein and fecal calprotectin. His post-transplant fecal microbiome composition resembled that of the donor.

Despite the promising results described, not all studies examining the role of FMT for the treatment of IBD have been positive. In a study of five patients with moderate-to-severe UC, none achieved clinical remission after FMT during the 12 weeks of follow up, and only one had clinical response. Fecal microbiota analyses demonstrated differences in the stability of transplanted phylotypes among the recipients. Interestingly, two patients had a similar microbiota as their donors by 12 weeks, yet the disease outcome differed [59]. This raises concerns that inducing changes in the recipient microbiota by FMT may not be as successful for IBD as it is for CDI [60].

Data supporting the use of FMT in children with IBD are limited. In a pediatric pilot study, 10 children aged 7–21 years received FMT for UC. All patients had mild-to-moderate UC and no changes in medical treatment for 2 months prior to FMT. Each patient was administered a 240 mL fecal enema daily for 5 days. 67 % of these children maintained remission at 1 month post-transplant [61••]. A recent study by Vandenplas et al. [62] reported the first case of early-onset colitis treated with FMT. In this report, an 18-month-old girl was successfully treated with 7 stool infusions after failing corticosteroid therapy and azathioprine. Administration was accomplished via both the upper and lower GI tract. Follow up at 6 months post FMT showed no histological evidence of active disease. In addition to these studies, at the time of writing this review, there are 2 FMT trials for pediatric IBD currently in progress (ClinicalTrials.Gov).

In contrast to FMT for recurrent CDI, the available pediatric and adult data on FMT for IBD indicate most patients have received more than one infusion to treat their disease. This has been highlighted by numerous investigators who emphasize that FMT for IBD generally requires multiple infusions [63]. However, in 2013, Zhang et al. treated a 32-year-old male with one FMT for enterocolonic fistulizing CD. After failing multiple medical therapies, this patient received a single 150 mL stool infusion administered endoscopically in the mid-gut. The patient was followed for 9 months and maintained clinical remission during this time [64].

Non-inflammatory Gastrointestinal Disease

In regards to non-inflammatory GI diseases, FMT for IBS has been the most studied [65]. Multiple factors such as

visceral hyperalgesia, dysmotility, aberrant brain–gut responses, and dysbiosis may play a role in the pathogenesis of IBS. Moreover, altered intestinal microbial composition has been observed in patients with IBS as compared to healthy individuals [66]. Anecdotal reports from Borody et al. [56, 67, 68] have shown a beneficial effect of FMT for diarrhea and constipation predominant IBS. FMT in two children aged less than 18 years with chronic constipation achieved long-term restoration of bowel movements [60]. Although these cases suggest a utility of FMT in non-inflammatory GI diseases, randomized control trials remain lacking.

Non-gastrointestinal Diseases

In addition to GI disorders, FMT has also been utilized for various diseases outside of the GI tract. Indeed, the enteric microbiota is now being linked to numerous non-GI diseases, including metabolic, autoimmune, and neurodevelopmental disorders [13, 68]. For example, Vrieze et al. [69] demonstrated improvement of insulin sensitivity in patients with metabolic syndrome after infusion with a healthy intestinal microbiota. Interestingly, in many published cases, FMT has been done for GI complaints but eventually resulted in improvement of the primary extra-intestinal disease. However, to date, no controlled studies have addressed the efficacy of FMT for non-GI disorders. Moreover, to our knowledge, there is no pediatric literature describing the use of FMT for extra-intestinal conditions.

Practical Consideration for the Use of FMT

Donor Selection

FMT requires procurement of stool from a healthy donor for transplantation. However, the definition of "healthy" is not precisely delineated at this time. In general, it is advocated that the donor should engage in "clean living." Per the 2011 FMT workgroup guidelines, this assessment should be made by questioning the potential donor for risk of transmissible disease (sexual behavior, use of illicit drugs), body piercing, and recent travel (within 6 months) to endemic areas with high risk of diarrhea. A useful resource for this type of survey is the American Association of Blood Banks Donor History Questionnaire: http://www.fda.gov/downloads/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/LicensedProductsBLAs/BloodDonorScreening/UCM213552.pdf. Additional screening for transmissible pathogens in donor stool and blood samples is recommended [68, 70, 71]. Specifically, this includes testing donor stool samples for *C. difficile, Giardia, Cryptosprodium, Helicobacter pylori* (for upper GI instillation), ova, parasites, *Cyclospora, Isopora,* and bacterial pathogens. Blood is typically tested for HIV (type 1 and 2), Hepatitis A, B, C, and syphilis. There are no current recommendations on time frame to re-screen, although an interval of 3 months has been suggested [71]. Finally, additional donor exclusion criteria include individuals with preexisting GI comorbidities or conditions that may alter the intestinal microbiota, such as use of antibiotics in the 3 months prior to transplant [49••, 72].

To date, there have been no randomized controlled trials that have addressed the question of whether FMT is safer or more efficacious when utilizing a donor who is related to the recipient. From review articles, reports on success rates using different donor populations vary. In a systematic review, Gough et al. [40] found a 93 % resolution rate of CDI when stool was procured from related donors, as compared to an 84 % resolution rate when obtained from unrelated donors. However, this review was limited, as there was no standardization of the studies involved (i.e., not all studies documented weight, volume, and donor selection). Therefore, based on current evidence, the recommendation to use a related verses unrelated donor for FMT cannot be made. It should be noted that in pediatric FMT studies, the majority of donors reported has been related to the recipients [41, 45, 61••, 62].

Preparation and Processing

Most protocols for FMT utilize fresh stool (preferably passed with 2–6 h of administration), which is processed in a designated lab area with universal precautions. A gentle laxative such as milk of magnesia can be considered for the donor the day before a sample is needed [49••, 71, 72]. Because there can be practical difficulties in regards to collecting fresh stool samples, studies have evaluated the use of frozen stool for FMT. For example, Hamilton et al. [73] described similar success rates using FMT for CDI using frozen or fresh stool samples (90 vs. 92 %, respectively). This suggests that stool banks may eventually play a role in providing samples for FMT. Although interventions such as laxative therapy and freezing samples can affect the composition of the gut microbiota [74], this may be of minimal consequence with CDI. Indeed, high throughput 16S rRNA gene sequencing has supported restoration of gut microbiota with frozen stool in CDI [17••]. For more chronic diseases such as IBD, however, maintaining bacterial diversity of the donor sample may take on more importance, and the utility of processed and stored donor stool samples will need to be evaluated further. In regards to preparing recipients, the FMT workgroup recommends a bowel preparation [49••], and some authors suggest the use of oral vancomycin until the day of FMT [46•]. However, there are no studies comparing the efficacy of FMT in patients who have or have not received

a bowel preparation or antibiotics before transplantation. For patients receiving nasogastric instillation of stool, Bakken et al. [49••] have suggested recipients be administered a proton pump inhibitor starting the day before FMT. After collection of the stool sample, a slurry is prepared for infusion. Stool suspensions are most commonly prepared by diluting with saline or water, though other diluents such as milk and yogurt have been utilized. Interesting, a single study reported a higher resolution of CDI with water (98.5 %) as a diluent compared to normal saline (86 %). However, the relapse rate was two-fold higher when using water as compared to normal saline (8 vs. 3 % for saline) [40]. Hence, most protocols utilize normal saline as the preferred diluent. The stool suspension can be prepared by stirring, shaking, or using a household blender [49••, 72]. The liquefied stool is then filtered, and the final sample is administered immediately or frozen at −80 °C for future use. There has been great variability in reports regarding the quantity of stool and the final volume of stool slurry administered [17••], and both of these factors have been shown to influence outcome. For example, FMT using <50 g of stool has been shown to result in higher recurrence rates of CDI as compared to the administration of larger amounts [40]. Similarly, a final volume of stool suspension >500 ml has resulted in better outcomes than volumes <200 ml [40]. For administration via the upper GI tract, a volume of 25–50 ml is usually recommended [49••]. No guidelines have been published for stool quantities recommended for pediatric FMT. However, when mentioned in pediatric cases, the range of stool utilized was 70–133 g, and the volume instilled varied from 25 to 250 mL.

Route

FMT has been delivered by various routes including upper endoscopy, nasogastric or nasojejunal tube, enema, and colonoscopy. The method of administration is primarily dependent upon physician preference, though the lower GI tract is the most common route for CDI [39, 40, 42]. This appears to have a better response rate than upper tract administration [42]. For example, Cammarota et al. reported CDI resolution rates of 93 % for those who received FMT via the cecum/ascending colon, 86 % when performed via the duodenum/jejunum, 84 % via the distal colon, and 81 % through the stomach [39]. Interestingly, however, a review of 182 patients treated with FMT for CDI showed no significant difference in the efficacy based on delivery route ($P = 0.162$) [75]. From the current data, the most efficacious route of delivery cannot be definitively ascertained.

Regulatory Issues

The Federal Drug Authority (FDA) defines a drug as an "article intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease" [76]. In May 2013, the FDA ruled FMT as an investigational drug and announced that an investigational new drug (IND) application would be required for institutes planning to offer FMT [77]. However, this stance was subsequently changed, and an IND is not currently mandated (although it is encouraged) for treatment of refractory CDI. For research studies, and the use of FMT for conditions other that CDI, an IND is still required. Detailed information for sponsoring a new IND can be found at: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?sia=1. Most recently, in March 2014, the FDA issued a draft guidance for industry entitled "Enforcement Policy Regarding Investigational New Drug Requirements for Use of Fecal Microbiota for Transplantation to Treat *Clostridium difficile* Infection Not Responsive to Standard Therapies" (http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm). This document states that the stool donor should be known either to the health care provider or the recipient. This raises concerns for established stool banks that have screened samples readily available for use. However, the FDA is open to comments on this proposal, and is providing an opportunity for discussion. For all patients receiving FMT, informed consent should be obtained, and includes the potential risks of the procedure, as well as the statement that FMT is an investigational therapy.

Future Directions

For recurrent CDI, substantial evidence supports the use of FMT, particularly when standard medical therapy has not been effective. This has led to clinical practice guidelines suggesting that FMT should be considered for a third recurrence of CDI if pulsed vancomycin has failed [48]. However, despite the compelling success rates of FMT for recurrent CDI, important questions remain unanswered. In particular, there are very few studies examining the efficacy of FMT for recurrent CDI in the pediatric population. Furthermore, safety concerns regarding this therapy mandate larger studies powered to detect low incident adverse outcomes. The concept that there may be long-term consequences of FMT, such as obesity, autoimmune disease, or even neuropsychiatric problems also has not been explored. The potential development of these long-term issues may ultimately impact the definition of the ideal donor for FMT. Although testing donors for standard pathogens is currently advocated, screening for conditions such as obesity, diabetes, and psychiatric disorders is not

routinely performed. Indeed, others have argued that when such rigorous criteria are implemented, more than 90 % of donors would not qualify [78].

An alternative approach to FMT that obviates the need for human donors is the administration of defined microbial communities that recapitulate the beneficial effects of complete fecal transplantation. As few as six bacterial strains have been shown to treat CDI in a mouse model of this infection [19]. These findings are borne out in human studies as well. For example, in 1989, investigators reported that recurrent CDI could be treated using a mixture of only 10 bacterial strains. These organisms included strains of *Clostridia*, *Bacteroides*, *E. coli*, *Streptococcus*, and *Ruminococcus* [79]. More recently, a mixture of 33 stool-derived bacterial strains was used to "RePoopulate" the gut of 2 patients with recurrent CDI [80]. Six months after transplant, both patients were symptom-free, and the administered bacterial species comprised ~25 % bacterial sequences in recipient stool samples. In 2013, the FDA approved a Phase II trial of a similar cocktail of microbial strains, developed by the company Rebiotix [81]. Enrollment has been completed, and plans for a Phase 3 study are in progress. Ultimately, an ideal approach would be to formulate such microbial suspensions into a pill or capsular form to improve ease of administration. Indeed, studies have suggested that this approach may preferred by the majority of patients [82].

For the treatment of diseases other than recurrent CDI, further evidence regarding the safety and efficacy of FMT is clearly required. In disorders such IBD, it is likely that sustained changes to recipient gut microbial communities will be required to impart a clinical benefit. Hence, it is possible that a single FMT will not be effective for managing patients with chronic intestinal disorders. Furthermore, additional measures such as preconditioning the recipient with antibiotics and/or laxative therapy may play a role in establishing long-term "engraftment" of a healthy donor microbiota, and must be evaluated in this context. Similarly, efforts to maintain bacterial diversity in the donor stool may become critical, thus necessitating fresh donor specimens, as opposed to frozen or highly processed materials. Lastly, if long-term alterations of the gut microbiota are achieved in recipient populations, there may be a greater risk of transmission of metabolic, autoimmune, and neurodevelopmental disorders from the donor, as described above.

A final consideration regarding FMT that has not been evaluated extensively is cost. Despite being publicized as "cost-effective," no formal studies have been done to estimate out-of-pocket expenses for patients and families. Currently, the cost for donor screening often falls to the patient. In 2013, the Current Procedural Terminology (CPT) code 44705 was established for the preparation of a FMT, including assessment of donor specimen. The CPT code (44799) also exists for instillation by nasogastric tube or enema, though this is an unlisted code and thus often requires pre-authorization and additional documentation. These issues may become highly relevant for conditions such as IBD that may require multiple fecal infusions.

Conclusions

Existing data support the use of FMT for recurrent CDI when standard medical therapies have failed. Further appropriately powered studies are needed to establish the safety of this procedure, including long-term outcomes in both adults and children. In contrast, there is insufficient evidence to support the routine use of FMT in other GI and non-GI disorders, though active trials are ongoing. Ultimately, FMT may represent a safe and effect treatment modality for a spectrum of clinical disorders that arise from a dysregulated host microbiota.

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Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors

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