Fecal Flora Reconstitution for Recurrent *Clostridium difficile* Infection: Results and Methodology

Faith Rohlke, BA, Christina M. Surawicz, MD, MACG,† and Neil Stollman, MD, FACP, FACG, AGAF**

Goals: Recurrent *Clostridium difficile* infection (RCDI) is an increasingly common clinical problem without ideal treatment options. Our aim was to evaluate our results using Fecal Flora Reconstitution (FFR), and promulgate our methodology to the GI community to foster its more widespread use in appropriate candidates.

Background: FFR, sometimes termed "fecal transplantion" has been shown in numerous reports to be an effective treatment of RCDI, however, most of these studies have small sample sizes and few focus specifically on the methodology used in colonoscopic preparation and delivery of donated stool.

Study: Nineteen patients with confirmed multiply recurrent CDI were treated by infusing donor stool through a colonoscope.

Results: Out of 19 patients, 18 initially responded to treatment with a single FFR treatment, 1 patient responded after a second FFR infusion. All 19 patients maintained prolonged cured status followed until submission, ranging from 6 months to 5 years. Three patients were presumed reinfected after remaining symptom free for a period spanning from 6 months to 4 years. These patients tested positive for *C. difficile* after prescription of additional antibiotics for unrelated infections.

Conclusions: Fecal Flora Reconstitution is an effective, viable, and simple method of treatment for the difficult to treat patients with RCDI who fail standard therapy.

Key Words: *Clostridium difficile*, fecal bacteriotherapy, Fecal Flora Reconstitution, stool transplantation

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When the normal colonic flora are altered by antibiotic use, diarrhea is a common sequela. Rates of antibiotic-associated diarrhea (AAD) in outpatient children and adults range up to 30%,[1–6] and up to 39% for inpatients, depending on the antibiotic administered.[4] Although many cases of AAD are mild and resolve when antibiotics are discontinued, a more severe type of AAD is caused by *Clostridium difficile* infection (CDI). Overgrowth of pathogenic *C. difficile* accounts for 26% to 50% of cases of AAD,[4] with rates highest in patients in hospitals and long-term care facilities, in which there is a 10% risk of being colonized if hospitalized for 2 days or more.[7] *C. difficile* is a leading cause of enteric nosocomial infection, with increasing rates and higher morbidity and mortality reported recently associated with the epidemic strain Nap 1Bi/027.[8]

Treatment of initial CDI with oral metronidazole or vancomycin is usually effective, but in some individuals CDI recurs shortly after therapy is stopped; this is commonly called recurrent CDI (RCDI). The risk for first recurrence has traditionally been reported as approximately 20%,[9–12] but a recurrence rate of 33% after the initial episode was recently reported in Quebec after the outbreak of a particularly virulent strain.[13] Once patients have had 2 or more episodes, recurrence rates increase from 33% to 65%.[14] Treatment requires repeated courses of antibiotics, often with a pulse or tapering dose schedule, but there is no uniformly effective therapy. Additional potential treatments for RCDI include alternative antibiotics, probiotics, bile salt binding resins, intravenous immunoglobulin, and the infusion of stool from a "healthy" donor into the patient's colon, which we term Fecal Flora Reconstitution (FFR). Although anecdotal reports of FFR have reported promising results, few of these studies report in any detail on the specific methodology used, and none that we are aware of focus specifically on the methods for colonoscopic delivery of fecal flora. This article describes our experience with colonosopic FFR in 19 patients with multiply recurrent CDI, focusing on detailed methodology and reporting our uncontrolled results to date.

PATIENTS AND METHODS

Patients

Nineteen patients received FFR over a 5-year period at 2 medical centers under the direction of 2 senior gastroenterologists (C.M.S., N.S.). All recipients had *C. difficile* toxin positivity and consistently recurring symptoms over a span of at least 6 months, despite at least 3 courses of traditional treatments, including pulsed and tapered vancomycin. All patients gave informed consent to colonoscopy with the FFR procedure, recognizing the somewhat experimental nature of the intervention. No prospective Institutional Review Board was obtained, but the Institutional Review Board of the University of Washington approved the retrospective record review for the patients treated at that site, and those patients gave informed consent to use of their data in this study. Patient demographics are summarized in Table 1.

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From the *Northern California Gastroenterology Consultants (NCGC), Oakland, CA; and †Department of Medicine Division of Gastroenterology, University of Washington School of Medicine, Seattle, WA.
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Reprints: Neil Stollman, MD, FACP, FACG, AGAF, Northern California Gastroenterology Consultants (NCGC), 3300 Webster St, Suite 312, Oakland, CA, 94609 (e-mail: Neil@Stollman.com).

TABLE 1. Patient Demographics and Results

Patient	1st Procedure	Sex	Age, Years	#FFR	Donor	Primary Outcome	Follow-up Interval, Months
1	Sep-04	F	42	1	Partner	+	65.0
2	Dec-04	F	33	1	Mother	+	49.0*
3	Oct-05	F	50	1	Partner	+	52.0
4	Dec-05	F	50	2	Partner	—	46.0
5	Aug-06	F	60	1	Partner	+	38.0*
6	Mar-07	F	49	1	Partner	+	35.0
7	Apr-07	F	81	1	Housemate	+	34.0
8	Aug-07	F	46	1	Partner	+	30.0
9	Oct-07	F	36	1	Partner	+	28.0
10	Jan-08	M	32	1	Partner	+	25.0
11	Feb-08	F	29	1	Mother	+	24.0
12	Nov-08	F	37	1	Partner	+	15.0
13	Nov-08	F	55	1	Partner	+	15.0
14	Nov-08	M	42	1	Partner	+	15.0
15	Dec-08	F	54	1	Partner	+	6.0*
16	Dec-08	F	82	1	Son	+	14.0
17	Apr-09	F	47	1	Partner	+	10.0
18	May-09	F	45	1	Sibling	+	9.0
19	Jul-09	F	67	1	Partner	+	7.0
			Mean = 49				Mean = 27.2

Follow-up interval determined from most recent FFR until submission or until symptom return.
*Reinfection.
+ indicates infection resolved; —, FFR unsuccessful.

Donor Identification and Screening

Our preference for donors was first for intimate domestic partners, followed by family members and those living in the same household, and finally close friends. No strangers, anonymous donors, nor staff members were used. Donors located in a close living arrangement with the recipient were viewed favorably owing to the conjectured likelihood that pathogens and flora would have likely already been extensively shared by both participants. Exclusionary factors for donors included recent antibiotic use, current or recent diarrheal illness, hospital or health care worker, and at-risk sexual behaviors. Donor screening was selective, based on the recipient's discretion and desires. Donor stool was variably screened for *C. difficile*, enteric pathogens, and some donors were screened serologically for HIV and viral hepatitis. The donors were also queried about the regularity of their bowel function, and for those who were not reliably able to produce stool in the morning with some predictability, gentle laxation was provided with small doses of citrate of magnesium. The dosing and timing was manipulated in the predonation period attempting to bring the donor into a predictable schedule.

Stool Transplantation

The patients' prior treatment regimens (generally vancomycin) were stopped 1 to 3 days before the procedure. Patients prepped for the FFR with a standard 4.0 liter polyethelyne glycol purge taken the evening before their procedure, as per our normal prior colonoscopy protocols. Full quantity of all donor stool provided the morning of procedure was conserved (NS) or limited to several ounces at the other site (CS). The donor stool was prepared in a room separate from the procedure area by suspending it in nonbacteriostatic saline, with manual shaking in a large suction canister. The aim was to liquefy the stool adequately to be able to pass through a large syringe into the colonoscope's biopsy channel, but at the same time attempting to minimize the volume (and maximize the bacterial concentration) as much as possible. The volume was limited to a maximum of approximately 350 cc, and was generally in the 200 to 300 cc range. No formal assessment of viscosity was used, but the resulting suspension generally produced a liquid of fairly thick consistency, which was then poured once or twice through a filter, depending on the sample's composition. The filter was made of multiple 4×4 gauze sheets opened up, and draped over another suction canister, and then held in place by rubber bands. This process served to remove particulate matter that early experience showed could clog the syringe or scope, but presumably did not significantly affect the bacterial content.

Patients underwent standard colonoscopy with an adult or pediatric colonoscope, using moderate sedation with midazolam and fentanyl (although 1 patient requested an unsedated procedure, which was successfully accomplished). Colonoscopy to the right colon was carried out in most cases, with the intent of ileal intubation, although in 1 patient there was a stricture at an anastamotic site in the left colon and stool was infused at this point (this patient responded successfully). Initially, stool was infused in a graduated fashion during withdrawal, but most recently; all donor material has been instilled at the proximal most extent of the examination, which was often the ileum, but occasionally the cecum. Multiple 60 cc large tip syringes were used for infusion through the working channel of the colonoscope.

At 1 site (NS), patients were instructed to take 2 tablets of over the counter diphenoxylate and atropine (Immodium, McNeil PPC Inc) immediately after the procedure and 2 more tablets again that afternoon or evening, approximately 6 hours later, in an attempt to diminish colonic motility and maximize contact time of the infused stool. Recipients were also instructed to remain at

bed rest for several hours after the colonoscopy, and sometimes for the remainder of the day of infusion, as much as possible, with ad lib activity the next day. Standard postprocedure dietary instructions were given, through which patients were encouraged to reintroduce oral food slowly, and to consume a bland diet. No formal or scheduled postprocedure *C. difficile* testing was scheduled, with repeat stool testing done only for suggestive recurrent symptoms. Patients were asked to contact the office directly if *C. difficile* infection symptoms returned. However, additional follow-up phone calls were made for purposes of this study.

RESULTS

As shown in Table 1, the mean age of the FFR recipients was 49 years, and 17 of the 19 were women. All recipients were outpatients, none were being treated from inpatient facilities. All but 1 patient initially responded to the treatment, remaining symptom free for a range of 6 months to 5 years. The patient who did not respond initially presented with a rapid relapse with confirmed *C. difficile* positivity. Five months after the initial infusion, the patient received a second FFR treatment using the same donor and similar methodology, which was immediately successful. In follow-up, all 19 of the patients in this study maintained prolonged periods free of symptoms and are considered "cured" after treatment with Fecal Flora Reconstitution. Three patients had recurrent symptoms with confirmed positive *C. difficile* lab tests ranging 6 months to 4 years from initial FFR treatment. However, as all 3 patients were treated with antibiotics for an unrelated infection directly before the onset of symptoms, these cases were considered reinfections rather than relapses.

DISCUSSION

Recurrent *C. difficile* infection is an increasingly common clinical challenge, with a number of patients unable to "clear" their infection, despite multiple courses of conventional or standard therapies. Other options for these patients include alternate antibiotics (such as rifaximin),[15,16] probiotics, intravenous immunoglobulin,[17] and now increasingly, Fecal Flora Reconstitution, with an increasing body of uncontrolled evidence supporting its safety and efficacy.

A number of studies have shown promising results after donor feces is used to repopulate the affected patient's colon.[18–32] The assumption is that donated healthy fecal flora repopulates the colon, restoring colonization resistance, which presumably protects against *C. difficile*, a so-called "Fecal Flora Reconstitution." Case reports of FFR in patients with ulcerative colitis,[33,34] inflammatory bowel disease,[35] irritable bowel syndrome,[35,36] and chronic constipation[36,37] have suggested efficacy but sample sizes are small. Routes of administration of FFR have included fecal enemas,[19,22–23,25–26,28,30–32] nasogastric tubes,[18,27] and through a colonoscope,[29] with generally good success in case reports and small case series. A 2004 review of 17 reports suggests that in treating recurrent *C. difficile* cases fecal bacteriotherapy is the most reliable of choices, surveying a collective 92% success rate for the patients in the included studies,[21] quite similar to our results reported here. Although colonoscopic delivery is invasive, we personally favor the colonoscopic route of administration over enemas, in that it seems logical that it provides a more proximal delivery of the "new" flora and perhaps better retention, and over nasal-enteric tubes, owing to both our perception of better comfort and tolerability, and the direct colonic delivery. In addition, patients uniformly preferred this route to enemas or the nasogastric method.

Although we offered patients a choice to opt out of some aspects of donor screening, specifically HIV and viral hepatitis testing, we suggest that the full battery of available tests be conducted to maximize safety of the procedure, including *C. difficile* detection, HIV, viral hepatitis, ova and parasites, and routine culture. It may also be beneficial to have the recipients lay supine for a period of time after fecal transplantation to allow the flora adequate time to repopulate the recipient's colon.

All patients in this case series had undergone multiple courses of treatment with conventional methods that failed to cure the infection before they were considered for this procedure as a fourth line intervention. Our results, whereas uncontrolled, are quite impressive in this difficult and refractory population, in which a placebo effect would seem quite unlikely. After FFR treatment 16 of the 19 patients are now symptom free, off vancomycin or metronidazole, and quite importantly, have resumed a more normal quality of life, an unquantified but very significant morbidity in these patients.

There are many unanswered questions related to this nascent procedure. Ideally, randomized trials would evaluate various aspects of donor stool preparation, instillation techniques, postprocedure interventions, and perhaps other adjuvant interventions, such as using probiotics or prebiotics such as fructooligosaccharides. Patient characteristics predicting success or failure would also be valuable to explore, such as *C. difficile* antibody titers, or infecting strain genotyping. In addition, the preponderance of female patients was interesting in this series. This unequal gender spread was seemingly random; all appropriate patients referred to us were offered FFR, and all offered accepted. However, a large majority of female participants was also recently reported in a similar study,[27] and in the experience of another physician (L Brandt MD, Personal Communication), a female predominance was also seen. In an earlier report of 2 RCDI trials, a female preponderance of 74% was reported,[12] and findings have also reported that being female may be a risk factor for CDI[38,39]; this, therefore could potentially explain why our available sample consisted mainly of females.

As we do not know which bacterial components of our flora are most important in *C. difficile* "defense," we favor reintroducing all flora. In the future, refining the bacterial strains introduced would be ideal. Longer term follow-up to identify risk for subsequent *C. difficile* infections with subsequent antibiotic exposure would also be of interest. Our experience suggests that FFR does not give life long stability to the fecal flora once antibiotics prescribed for unrelated infections have been reintroduced.

In summary, *C. difficile* infection is increasing in frequency and morbidity. Most patients can be effectively treated using the typical antibiotic regimens approved for CDI,[40] which should remain the first line interventions for most patients. For the subpopulation of patients with recurrent disease, despite appropriate initial treatment courses, using pulse or tapering schedules and adjunctive probiotics, Fecal Flora Reconstitution is a rational intervention, with underlying biologic plausibility. With some trial and error, we have found the technique to be fairly

simple and safe, and also quite effective in our admittedly uncontrolled retrospective case series. Our aim with this publication is largely to propagate the methodology we have further refined, and to facilitate its introduction and use by other practitioners treating patients with this difficult disorder.

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