Fecal transplant for *Clostridium difficile* infection relapses using "pooled" frozen feces from non-related donors

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To the editor,

Clostridium difficile infection (CDI) is associated with high morbidity and mortality (1). About 20-50% of patients develop relapses after antibiotic treatment (2,4). Fecal microbiota transplantation (FMT) is an effective, inexpensive and secure treatment option for these patients (1,3), even in immunosuppressed patients (5). Conventional FMT (from fresh or frozen feces) from a healthy related donor (2), has shown effectiveness in approximately 90% of CDI relapses (1,3,4).

Methods

Donor selection criteria

Included donors were over 18 years old, apparently healthy, non-pregnant, and with a BMI of 20-25 kg/m^2. Any of the followings was considered as exclusion criteria : a first-degree relative with diabetes mellitus, high-risk sexual behavior, abdominal surgery, any gastrointestinal disease or cancer, consumption of antibiotics, proton-pump inhibitors, immunosuppressive medication, hospitalization or diarrhea in the three previous months. They must have tested negative for HAV, HBV, HCV, HIV-1, HIV-2, *Trypanosoma cruzi, Brucella* spp., and *Treponema pallidum*, as well as tested within the normal range in total blood count, liver enzyme levels, and blood chemistry. Parasites or enteropathogenic bacteria were discarded of donor's stool. After the full screening, three donors were recruited in the study.

Three feces collections per donor were obtained and immediately stored at -70°C. All feces collections from all three donors were thawed, pooled and homogenized in 4.5 L of 10% USP glycerol in saline. Afterward, the suspension was filtered to remove coarse particles. The total weight of pooled feces was 881.62 g in a 4.64 L volume (concentration of 0.19 g/mL). Aliquots of 45 mL were frozen at -70°C until used. One hour before use, aliquots were thawed in water submersion at 30°C.

Local ethics committee approved the study (IF14-005). Patients and donors signed an informed consent.

Case description

Patient 1 : 46 yo male undergoing chemotherapy due to acute leukemia presented diarrhea, 5 bowel movements in 24 h Bristol 7, abdominal distension and tenderness ; toxins A/B were positive. An ATLAS score of 5 was recorded. He received metronidazole and vancomycin, after which conventional FMT was performed without improvement. Surgical treatment was indicated, but not considered the cause of the patient's severe refractory thrombocytopenia. He received a pooled-FMT by colonoscopy. After 48 h a decrease of stool movements and improvement in stool consistency were noted, and abdominal tenderness disappeared. At the 15th day after the pooled-FMT, the patient had remained asymptomatic.

Patient 2 : 40 yo male on renal replacement therapy developed SSI and was treated with ciprofloxacin. He developed fever and diarrhea, with pseudomembranes on sigmoidoscopy, 5 bowel movements/day, Bristol 6 an ATLAS score of 3, abdominal distension, tenderness, and WBC 28.9 K/μL. Pooled-FMT by colonoscopy performed. After 48 h, he had 3 bowel movements/day, Bristol 5, the pain and the abdominal distension had also decreased, along with WBC (15.3 K/μL). Although clinical improvement was clear stools persisted Bristol 5 and a second pooled-FMT was performed by enema.

Patient 3 : 60 yo male with a 10-year partial colectomy due to colon cancer. He had a recurrent CDI, diarrhea and abdominal distension 5 days after he was treated with azithromycin for an upper respiratory tract infection, *C. difficile* toxins tested positive. ATLAS score of 7 was recorded, 3-5 bowel movements/day Bristol 6. The patient's condition worsened after 5 days of vancomycin and metronidazole. A pooled-FMT was performed by nasoenteral tube and a second pooled-FMT was performed

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Table 1. — **Demographic an laboratory characteristics of stool donors**

	Donor 1	Donor 2	Donor 3	mean
Gender	Male	female	female	
Age (years)	20	27	24	23.67
Body mass index (kg/m^2)	24.09	24.12	20.26	22.82
Hemoglobin (g/dL)	17.8	15	13.8	15.53
White blood cell (10^3/μL)	7.7	6.89	4.6	6.40
Platelet (10^3/μL)	266	375	252	297
Glucose (mg/dL)	83	87	88	86
Creatinine (mg/dL)	0.8	0.7	0.6	0.7
Albumin (g/dL)	4.5	4.0	4.5	4.33
ALT (UI/L)	19	17	20	18.67
AST (UI/L)	19	10	11	13.33
Total bilirubin (mg/dL)	1.7	0.7	0.9	1.1
Cholesterol (mg/dL)	171	190	74	145
Triglycerides (mg/dL)	72	46	57	58.33

after 12 h. After 48 h, the patient had 4 bowel movements Bristol 5 with less abdominal distension. At day 5 he had normal bowel movements and was eating regularly. He developed central line-associated bloodstream infection (CLABSI) by *Staphylococcus aureus* and was treated with IV teicoplanin without eventualities.

Patient 4 : 60 yo male with diabetes mellitus and an open fracture of the tibia treated with clindamycin and moxifloxacin, he had recurrent CDI, with 10-15 bowel movements/day, Bristol 7 and an ATLAS score of 7. Treatment was started with vancomycin and metronidazole, but no change in symptoms was noticed. An NE tube was placed for a 2-step pool-FMT (as described in patient 3). After 48 h, the patient had less abdominal distension, tolerated po liquids and was feverless. During the next 10 days, diarrhea was intermittent, and he was re-transplanted with a pooled-FMT by colonoscopy after which the patient remitted all of the symptoms at 72 h and persisted asymptomatic at the 4th week after the last intervention.

Discussion

In this report, we describe a group of patients that were treated for CDI with frozen pooled- feces from non-related donors after failing antibacterial therapy and conventional FMTs.

In the screening of donors, some tests are performed to discard the presence of viral, bacterial and parasitic agents and eliminate the possibility of transmission, but at present, there are no tests available for evaluating the quality and composition of the gut microbiota in the selection of donors. There may be variations in composition and, therefore, the quality of gut microbiota between donors and in an attempt to minimize the effect of these variations, in this project we used a pool of microbiota (feces) of three donors carefully evaluated. Several other studies have shown the utility of conventional FMT for the treatment of CDI relapses (1-2,4). In these studies, a single donor, (familiar or not) and fresh or frozen feces were used and a higher diversity of gut microbiota was demonstrated after infusion of FMT comparable to healthy donors (6). Conventional FMT failed in one of our patients after that FMT with pooled feces showed better results than conventional FMT in that case. The main contribution of our work is the use of pooled-feces of carefully evaluated donors to minimize the repercussions of the donors not having an "ideal microbiota" and the results showed promissory results.

Frozen stool feces, although preserved in glycerol at -70 °C will diminish microbiota viability at an unknown rate when compared to a "fresh" stool sample. Also, there is a cell damage associated with freezing and thawing process. In our study, all three patients were this drawback, the main advantage in the use of frozen stools (pooled or from a single donor) is the almost immediate availability of the fecal microbiota for transplant. In our study, the time between the indication of FMT and the actual procedure was about 1.5 h, in comparison to conventional FMT, that when indicated, at least, 3 days are needed for the screening of donors and performance of laboratory tests.

There are concerns about infectious complications in FMT. This concern is specially considered in immunosuppressed patients such as patient 1. There are reports of its use with successful and safe outcomes although the information in neutropenic patients is scarce (5). There were no identifiable infectious complications in all, but in one of the participants (patient 3) ; *S. aureus* CLABSI was not attributable to pooled-FMT since none of the

donor's collections tested positive for *S. aureus* during screening and this microorganism is not an expected complication of FMT.

We conclude that in the current group of patients with persistent CDI, pooled-FMT proved to be efficient and safe. There is the necessity of further controlled studies exploring this topic.

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