Fecal Microbiota Transplantation for Grade IV Steroid Refractory GI-GvHD: Interim Results a Non-randomized, Open-label, Phase 1 Clinical Study

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Research

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Abstract

Background

Gastrointestinal (GI) tract graft-*vs*-host disease (GvHD) is a major cause of post-allo-HCT morbidity and mortality. Patients with steroid-refractory GI-GvHD face a poor prognosis and limited therapeutic options. Here, we report an interim analysis on the safety and efficacy of fecal microbiota transplantation (FMT) in treating steroid-refractory GI-GvHD.

Methods

We did a non-randomized, open-label, phase 1 clinical study on patients with grade IV steroid-refractory GI-GVHD. FMT efficacy was evaluated using indexes of abdominal pain, diarrhea and bloody purulent stool at 14 and 21 days after the diagnosis of steroid-refractory GI-GvHD. The primary outcomes referred to clinical complete remission or partial remission. Secondary outcomes referred to PFS and OS at Day 90 and the end of the research. Safety was evaluated according to adverse events during FMT and the whole follow-up period. The study was registered with ClinicalTrials.gov as #NCT03148743.

Results

A total of 56 patients with steroid-refractory GI-GvHD were enrolled. Of them, 24 patients with grade IV steroid-refractory GI-GvHD were assigned to FMT and 18 to the control group. The characteristics of the two group patients at baseline were similar. At Day 14 after FMT, 13 (54.2%) patients in FMT group and none (0%) of 18 control group achieved clinical remission ($p<0.05$), while 20(83%) patients in FMT group and 7(39%) in control group showed effective response (clinical remission+partial remission) (RR 7.86, 95% CI 1.88–32.9; $p=0.005$). At Day 21, the clinical remission was significantly greater in FMT group than in control group (14 (58.3%) of 24 vs 3(16%) of 18; RR 6.0, 95% CI 1.22–29.45; $p=0.027$). Within a follow up of 90 days, the FMT group showed better OS (HR, 7.0; 95% CI, 1.53-32.08; $p=0.012$). At the end of the research, the median survival time was >600 days in FMT group and 107 days in control group (HR, 4.73; 95% CI, 1.58-14.14; $p=0.005$). Both the PFS (HR, 0.24; 95% CI, 0.06-0.95; $p=0.055$) and OS (HR, 5.97; 95% CI, 1.52-23.43; $p=0.01$) kept increasing during the follow-up in FMT group. Overall, the mortality rate was lower in FMT group (HR, 5.97; 95% CI, 1.52-23.43; $p=0.01$). No difference was observed in the occurrence of other side effects, such as hemorrhagic cystitis, infection of bacteria & fungi, CMV&EBV, septicemia, TMA, cardiac events, thrombocytopenia and epilepsy.

Conclusions

The diversity of intestinal microbiota can be affected by allo-HSCT. FMT is effective and safe in treating grade IV steroid refractory GI–GvHD.

Background

As a treatment for most acute leukemia, the hematopoietic stem cell transplantation (HSCT) can offers a potential cure but may have many complications that include infections, multi-organ failure, and graft-versus-host disease (GvHD)[1-3]. GvHD, especially gut acute GvHD (GI-aGvHD), is a major cause of post-allo-HCT morbidity and mortality[3,4].

Conventionally, glucocorticoids are used in the first-line therapy for GI-GvHD. Unfortunately, almost half of the patients do not respond efficiently[3,4]. The survival of patients developing GI-GvHD treated with standard steroid is in a range of 5–30%[4-7]. By far, no second-line treatments have been established and are urgently needed[7].

Human gut microbiota is associated with many chronic disease which is composed of more than 100 trillion microbes[8]. The influence of intestinal microbiota on immune responses, including post-allo-HCT, has been increasingly recognized[9-11], and became one of the main targets for the treatment of acute GvHD[3,7]. Under normal physiological condition, the diversity of gut microbiota in human GI tract is associated with intestinal inflammation and immune responses[3,12] that may affect the outcome of GvHD treatment. As the intestinal microbial diversity collapses after allo-HSCT[13,14], the abnormal gut microbiota may damage GI mucosa, and consequently influencing the immune response[3].

The fecal microbiota transplant (FMT), a procedure that use the clinical method of infusing a fecal suspension from a healthy donor into recipient's GI tract, can quickly restore the recipient's intestinal microbiota and repair the intestinal mucosal barrier, that may

resolve the inflammatory response and readjust the immune system[15,16]. As a novel therapeutic method, FMT has been proven to be effective for recurrent *clostridium difficile* infection[17,18].

Our pilot study and other available research suggests that FMT can serve as a therapeutic option in treating steroid-refractory GI-GVHD[3,13,14,19]. Here, we assess the safety and efficacy of the FMT in a phase I study involving patients with steroid-refractory gastrointestinal tract GvHD.

Results

Patient Characteristics

A total of 56 patients with steroid-refractory GI-GvHD were enrolled. *Clostridium difficile* infection was not observed among all patients, and were not responders to methylprednisolone (mPSL) at≥2mg/kg per day. Immunosuppressant as the secondary-line therapy was given to all the patients. Eight patients were excluded, including four reluctant to participate in the study and four failing to meet inclusion criteria (one for primary disease recurrence, two for combined TMA, one for combined CMV before FMT). Of 27 patients in FMT group, the data of three patients with < grade IV GI-GVHD were not selected for statistical analysis. Of 21 patients in the control group, the data of three patients (one with missed follow-up and two with < grade IV GI-GVHD) were not used for statistical analysis (Fig.1).

The patients' information were shown in Table 1 and supplement table. For 24 FMT patients, the median age was 29 years (range 13 to 55). The male/female ratio was 16/8. The median stool volume was 865 mL/day (range 360 to 2,100 mL/day). The median stool frequency was 6 times/day (range 3 to 21 times/day). The median abdominal pain score was 3 (range 1 to 4) (Table 1 and supplement table). For the 18 patients in the control group, the median age was 31.5 years old (range 13 to 59) (*vs* FMT group $p>0.05$). The male/female ratio was 7/11(*vs* FMT group $p>0.05$). The median stool volume was 510 mL/day (range 250 to 1,400 mL/day) (*vs* FMT group $p<0.05$). The median stool frequency was 5 times/day (range 3 to 20 times/day) (*vs* FMT group $p>0.05$). The median abdominal pain score was 2 (range 0 to 4) (*vs* FMT group $p>0.05$) (Table 1 and supplement table). No difference was observed in the occurrence of hematologic disease, stem cells donor gender match and stem cells donor relationship in two group. In FMT group, 11 patients received 2 times of FMT, 9 patients received 1 time of FMT, 3 patients received 3 times of FMT and 1 patients received 6 times of FMT (supplement table).

Table 1
Baseline characteristics of patients

	FMT	control	p
median Age(min-max)	29(13-55)	31.5 (13-59)	>0.05
Gender			>0.05
male	16	7	
female	8	11	
Volume ml median(min-max)	865(360-2100)	510(250-1400)	**<0.05***
Frequencies	6(3-21)	5(3-20)	>0.05
Abdominal pain	3(1-4)	2(0-4)	>0.05
Hematologic Disease			
AML	8	9	>0.05
ALL	4	2	>0.05
MDS	5	4	>0.05
AA	4	1	>0.05
CML	2	0	>0.05
Others	1	2	>0.05
Stem cells donor gender match	13	7	>0.05
Stem cells donor relationship			
Haplo-HSCT	20	14	>0.05
SIB-HSCT	2	3	>0.05
URD-HSCT	2	1	>0.05

Clinical Outcomes

With Cox regression model, we first declared that immunosuppressant did not affect the outcomes of the two groups (supplement table).

At Day 14, 13 (27%) patients in FMT group and none in control group achieved clinical remission according to modified intention-to-treat analysis ($p<0.05$). Meanwhile, 20(83%) patients in FMT group and 7(39%) patients in control group showed effective response (clinical remission + partial remission) (RR 7.86, 95%CI 1.88–32.9; $p=0.005$). Three (12.5%) patients died in FMT group and one (5.5%) patient died in control group (RR 0.41, 95%CI 0.04–4.33; $p=0.46$)(Fig.2)(Table 2 and supplement table). No relapse of GI-GvHD was recorded in all patients at this time point.

Table 2

Clinical results of 14th day and 21th day

		FMT (n=24)	Control (n=18)	p
0 day	Stool volume ml median(min-max)	865(360-2100)	510(250-1400)	**<0.05***
	Stool frequencies	6(3-21)	5(3-20)	>0.05
	Abdominal pain score	3(1-4)	2(0-4)	>0.05
14th day	Stool volume ml median(min-max)	200(0-1300)	500(0-1700)	**<0.05***
	Stool frequencies	2(0-9)	5(0-12)	**<0.05***
	Abdominal pain score	0(0-3)	2(0-4)	**<0.05***
	CR	13(54.2%)	0(0%)	**<0.05***
	Efficiency(CR+PR)	20(83.3%)	7(39%)	**<0.05***
	Die	3(12.5%)	1(5.5%)	>0.05
21th day	Stool volume ml median(min-max)	180(0-2365)	450(0-1400)	>0.05
	Stool frequencies	2(0-8)	4(0-15)	>0.05
	Abdominal pain score	0(0-3)	2(0-4)	>0.05
	CR	14(58.3%)	3(16%)	**<0.05***
	Efficiency(CR+PR)	16(66.7%)	9(50%)	>0.05
	Die	5(20.8%)	2(11%)	>0.05
	GI-GVHD Relapse	2(8.3%)	2(11%)	>0.05
CR(clinical remission); PR(partial remission)				

At Day 21, clinical assessments showed that clinical remission was significantly more obvious in FMT group than in control group (14[58.3%] of 24 *vs* 3[16%] of 18; RR 6.0, 95%CI 1.22–29.45; p=0.027), but clinical response did not difference (16 [66.7%] of 24 *vs* 9 [50%] of 18; RR 4.0, 95%CI 0.84–19.16; p=0.083). Five patients died in FMT group and two patients died in control group (5 [20.8%] of 24 *vs* 2 [11%] of 18; RR 0.48, 95%CI 0.08–2.79; p=0.41). Two patients in FMT group and two patients in control group showed disease relapse, but no significant difference (2 [8.3%] of 24 *vs* 2 [11%] of 18; RR 0.73, 95%CI 0.09–5.72; p=0.76). (Fig.2) (Table 2 and supplement table)

Within 90 days of follow-up, PFS showed no significant difference between two groups (HR 0.36, 95%CI, 0.1-1.28; p=0.11) (Fig.3A). The FMT group showed better OS (HR 7.0, 95%CI, 1.53-32.08; p=0.012) (Fig.3B). At the end of the research, the median survival time was >600 days in FMT group and 107 days in control group (HR 4.73, 95%CI, 1.58-14.14; p=0.005). Both the PFS (HR 0.24, 95%CI, 0.06-0.95; p=0.055) and OS (HR 5.97, 95%CI, 1.52-23.43; p=0.01) kept increasing during the follow-up time in FMT group(Fig.3CD).

Safety of FMT

Only one patient experienced thrombocytopenia after FMT and one patient developed cardiac event at day 3 after FMT. Although we were not sure whether there was a possible association between these events and FMT, it cannot be completely exclude. No other severe adverse events were observed in FMT group during seven days of follow-up after FMT. Other common adverse events include

incomplete ileus in one patient，fever in one patient, vomiting and low fever in two patients, and grade-3 rash in two patients, no special treatment only symptomatic treatment need for these patients.

In overall results, the mortality rate was low in FMT group (HR 5.97, 95%CI, 1.52-23.43; p=0.01). No significant difference was observed in the occurrence of hemorrhagic cystitis(p>0.05), bacteria & fungi infection (p>0.05), CMV&EBV infection(p>0.05), septicemia (p>0.05), TMA (p>0.05), cardiac events (p>0.05), thrombocytopenia(p>0.05) and epilepsy (p>0.05). (Table 3 and supplement table)

Table 3
Adverse events during overall follow-up time

	Hemorrhagic cystitis	CMV & EBV	TMA	Infection rate (Bacteria & fungi)	Septicemia	Cardiac event	Thrombocytopenia & cerebral hemorrhage	Epilepsy	Die
FMT group (n=24)	1/24	8/24	6/24	5/24	2/24	2/24	1/24	1/24	5/24(20.8%)
Control group (n=18)	3/18	4/18	6/18	7/18	4/18	1/18	0/18	1/18	10/18(55.6%)
p	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	**<0.05***

Fecal Microbiota and Immunity functions Analysis

Available fecal samples at baseline and weeks 1 after FMT were used for microbiota analyses (N=10). Compared with that of the donors, the diversity of fecal microbiota in fecal samples of the patients was decreased (Fig.4A). *Proteobacteria* increased while *firmicutes* decreased at phylum level in the microbiota of the patients(Fig.4B). After Week 1, the microbiota composition was reconstructed in FMT patients, showing a trend back to normal(Fig.4CD). The bacterial diversity improved at Week 1 after FMT in half patients (5/10) (Figure S1A). Similar to the results of our prior study, the ratio of *firmicutes* to *proteobacteria* was restored (7/10), *proteobacteria* decreased(9/10), and *firmicutes* increased (6/10) after FMT(Figure S1B). *Bacteroidetes* increased (7/10) in the fecal microbiota of patients with steroid-refractory GI-GvHD (Figure S1C) after FMT.

We also recorded the change in the levels of peripheral immunity cells (CD4+ cells, CD8+ cells or CD16+CD56+ cells, CD19+ cells, Treg cells) of four patients during four weeks' follow-up after FMT. Unfortunately, we did not find any obvious trends(Figure S2).

Discussion

Gut-GvHD related complications, especially steroid-refractory GI-GvHD, appeared to be one of cause of post-transplantation death[3,4], and the disruption of gut microbiota is linked to GvHD and transplant-related mortality[20,21].

The FMT may re-structured the gut microbiota of a patient which may consequently reinforce the patient's immune system[18]. The FMT had been proven to be very effective for the treatment of recurrent *Clostridium difficile* infection[17] and other human diseases (inflammatory bowel disease) [3,15,16]. Some case reports and our pilot study suggest that FMT may serve as a therapeutic option for steroid-refractory GI-GVHD[3,13,14,19].

In this research, we enrolled 56 patients with steroid-refractory gastrointestinal tract GvHD. Twenty four patients with grade-IV steroid-refractory GI-GVHD were given FMT(Fig1). As a result, the microbial richness in diversity and abundance were increased after FMT in most patients in comparison with the patient's gut microbiota before treatment. The microbiota composition was also restored. Beneficial bacteria, such as *bacteroidetes* or *firmicutes*, became dominant after FMT in most patients(Fig4).

In this research, the diarrhea and abdominal pain were attenuated after FMT(Fig2). The proportion of patients with clinical remission and effective response was higher at 2 or 3 weeks after FMT(Table 2). During a follow-up of 90 days, although PFS showed no

significant difference between the two groups, the FMT group still showed better OS. In overall results, the median survival was longer in FMT group than that in control group. Furthermore, both PFS and OS kept increasing during the follow-up in FMT group(Fig3).

In our research, only one case of thrombocytopenia, one case of cardiac event, and no cases of other severe adverse events were observed in FMT group during 7 days' follow-up after FMT. In overall results, FMT did not increase the probability of bacteria & fungi infection, CMV&EBV infection and septicemia. The incidences of hemorrhagic cystitis, TMA, cardiac events, thrombocytopenia and epilepsy in two groups were similar(Table 3). Some papers reported that FMT transmitted drug-resistant *E. coli*, leading to patient death[22]. No similar events were observed in our study, which may be attributed to our strict FMT criteria.

Conclusion

Although up to date no reports from any phase I clinical trial of FMT treated GI GvHD were found, our study was also limited in three aspects. It was conducted at a single institution, and thus, our findings may not be applied directly to patients at other institutions[20]. Another, given the severity and emergency of steroid-refractory grade IV GI-GvHD, the trial was not randomized and double blind controlled. And also for this, immunosuppressant was given and its effect could not be completely ruled out. Next, not all patients showed similar responses to FMT, and we did not get enough data to compare responding and non-responding patients, which may be explained by the pathological complexity of steroid-refractory GI-GvHD.

In summary, FMT may be effective and safe in treating GI-GvHD, which should be verified with more studies.

Methods

Study design and participants

We undertook an open-label, non-randomized, phase 1 clinical study at the First Affiliated Hospital of Soochow University. Protocols and other trial related procedures were approved by the Institutional Review Board of the Hospital. All the patients signed written informed consent. FMT group and control group (Without FMT) were set according the patients decision after introducing the possible benefit and disadvantages of fecal bacteria transplantation. FMT was performed after steroid-refractory GI-GvHD was diagnosed. All the patients were given second-line immunosuppressant treatment. No additional treatment was given to the FMT group after receiving FMT. The study was registered with ClinicalTrials.gov as #NCT03148743. The Center for International Blood and Marrow Transplant Research (CIBMTR) criteria were used to assess the grades of GI GvHD[3,23,24]. Criteria for diagnosing steroid-refractory gut GvHD had been described previously[3]. We excluded the patients with uncontrollable infection, irreversible organ failure, and other abnormal conditions that might interfere with the evaluation(Supplement protocol).

Procedures

In FMT, the fecal microbiota collected from four healthy donors (two females aged 23 years, and two males aged 20 years) were conserved in -80℃ with glycerine(Supplement protocol). As these patients couldn't tolerate gastroscopy or enteroscopy, forty to fifty ml of frozen fecal microbiota were suspended in 150-200 ml of warm normal saline and delivered into the intestine of the recipients through a nasojejunal tube or gastric tube after steroid-refractory GI-GvHD was diagnosed[3]. If not got improvement, FMT was repeated in the following week.

Outcomes

The primary outcomes were described with clinical remission or partial remission at Day 14 and Day 21 after steroid-refractory GI-GvHD was diagnosed.

Secondary outcomes were: PFS and OS at Day 90 after steroid-refractory GI-GvHD was diagnosed; PFS and OS after steroid-refractory GI-GvHD was recorded till the end of November 2018

FMT efficacy were evaluated according to the severity of symptoms such as abdominal pain, diarrhea (frequency and volume), and bloody purulent stool within 14 and 21 days after FMT was accomplished. For abdominal pain score, 0.5 was given to occasional pain,

1 to mild pain, 2 to moderate pain, 3 to severe pain without intervention, and 4 to severe pain with intervention. Clinical remission was defined as a condition in which diarrhea and intestinal spasms and/or bleeding disappeared, or stool volume decreased by ≥500mL on average within 3 days. Clinical improvement was defined as a condition in which the stool volume decreased by <500mL, or the abdominal pain value and bleeding relieved. The period during follow-up after first FMT with no progress of GI-GvHD, no death, no GvHD involvement in other organs, no new infection with CMV and EBV were defined as Progression-free survival time (PFS) [3]. OS (overall survival) referred to the period from when steroid-refractory GI-GvHD was diagnosed to November 2018. All deaths, including relapse related or other causes in these period, were included in the statistics.

For each patient, the safety was evaluated according to adverse events (including death or drop-out) during FMT and follow-up time.

Stool Sample Collection and Microbial Community Analysis

Fecal samples were stored at −80° C until DNA extraction. After DNA was extracted, we successfully detected bacterial 16S rDNA in all samples with polymerase chain reaction (PCR) using general bacterial primers (16S V4-V5): 515F:5'-GTGCCAGCMGCCGCGGTAA-3'; 926R: 5'-CCGTCAATTCMTTTGA -GTTT-3'. After purified, the pooling libraries were sequenced by 2*300bp paired-end sequencing on the MiSeq platform (Tiny Gene, Shanghai) using MiSeq v3 Reagent Kit (Illumina). We used a combination of Mothur, UPARSE and R software to analyze the 16S sequences data. Composition of fecal bacteria was analyzed at phylum level. Moreover, the Shannon diversity index was used to depict the diversity of microbiota [3,25].

Statistical Analysis

SPSS16.0 (SPSS, Inc., Chicago, IL, USA) for statistical analyses was used to construct actuarial rate curves and to calculate log-rank hazard ratios (HRs) and significance determinations, Fisher's exact tests, and risk determinations. Cochran's and mantel-haenszel statistical methods examined the differences between the groups. The survival package from “R” statistical software (Vienna, Austria) was used for permutation tests. For the latter, variables were determined for each day of the patient's hospitalization and HRs, confidence intervals, and significance determinations were calculated using Cox proportional hazards models with time varying covariates.

Declarations

Ethics approval and consent to participate

Protocols and other trial related procedures were approved by the Institutional Review Board of the Hospital. All the patients signed written informed consent.

Consent for publication

No applicable

Availability of data and material

Please contact author for data requests

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

XQ, BZ and DW contributed to the study concept and design. YZ, XL, XW, FC, SJ, XH and XM collected the clinical samples. XL and YZ performed the experiments. XQ, JG, and YZ performed bioinformatics analyses. XQ, YZ and BZ wrote the manuscript. XQ, BZ and DW supervised the study. All authors read and approved the final version of the report.

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Figures

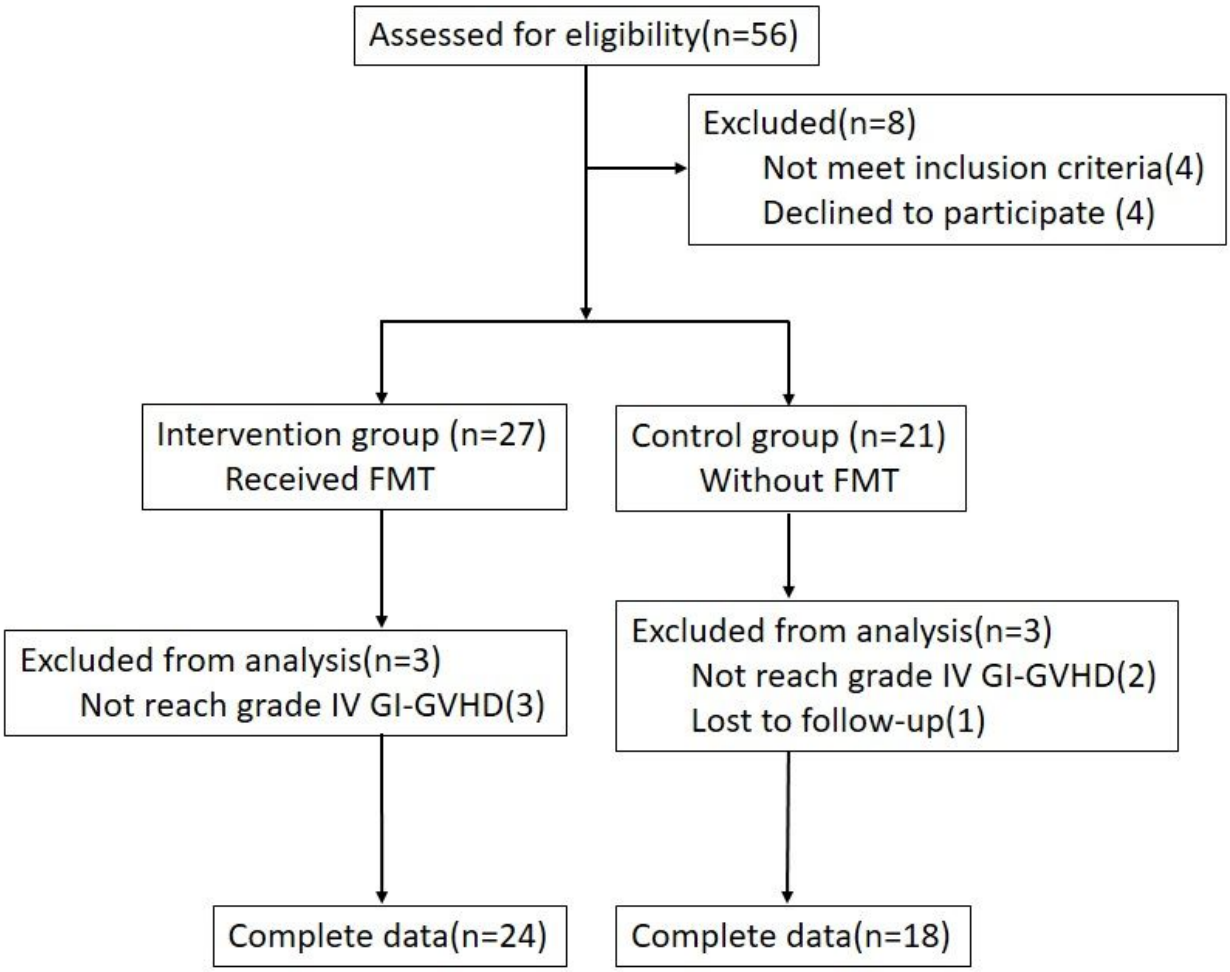

Figure 1

Figure 1

CONSORT flow diagram

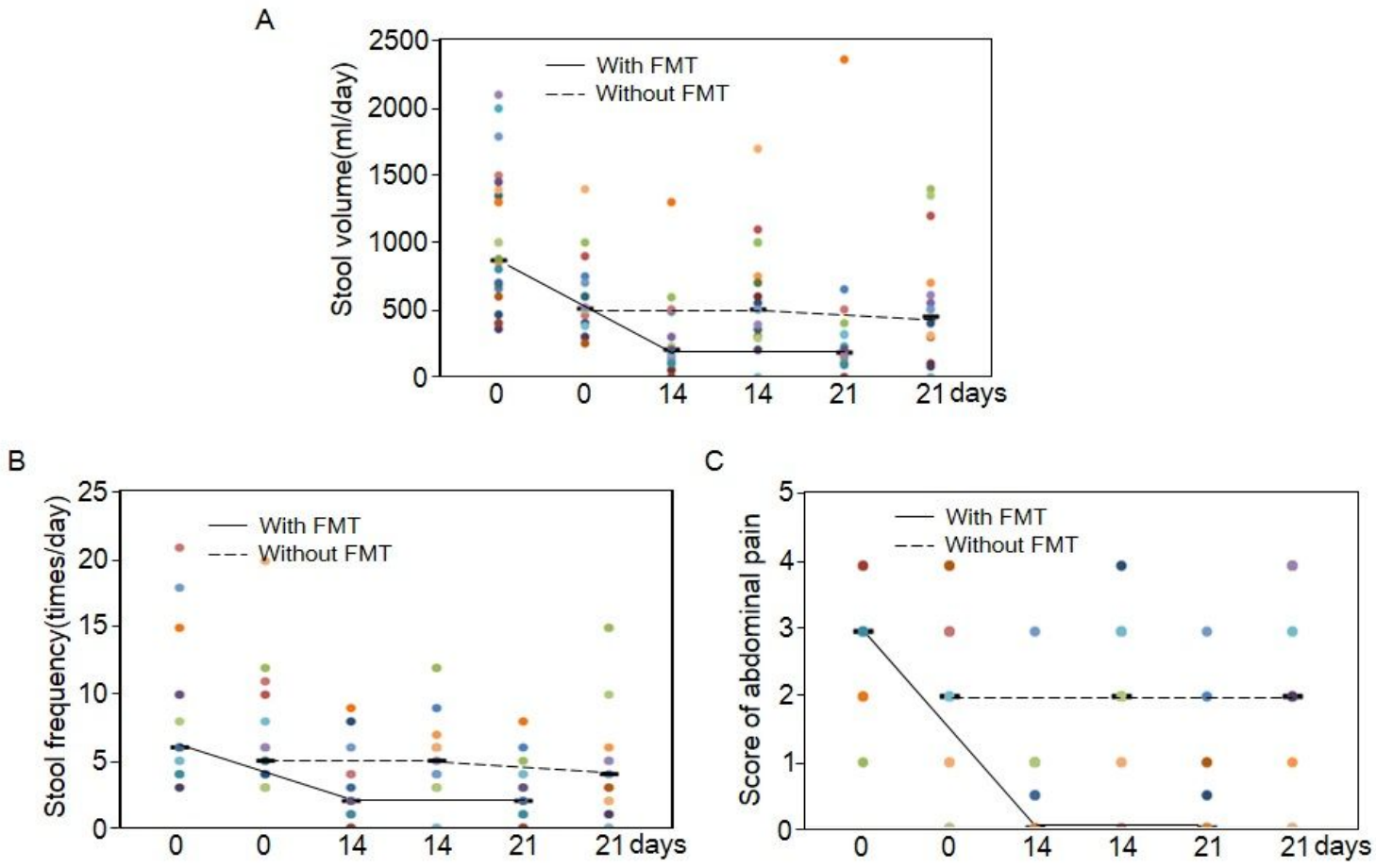

Figure 2

Figure 2

Clinical response to FMT. (A) Stool volumes of all patients at baseline, Day 14 and Day 21 after steroid-refractory GI-GvHD was diagnosed. (B) Stool frequency of all patients at baseline, Day 14 and Day 21 after steroid-refractory GI-GvHD was diagnosed. (C) Abdominal pain score of all patients at baseline, Day 14 and Day 21 after steroid-refractory GI-GvHD was diagnosed

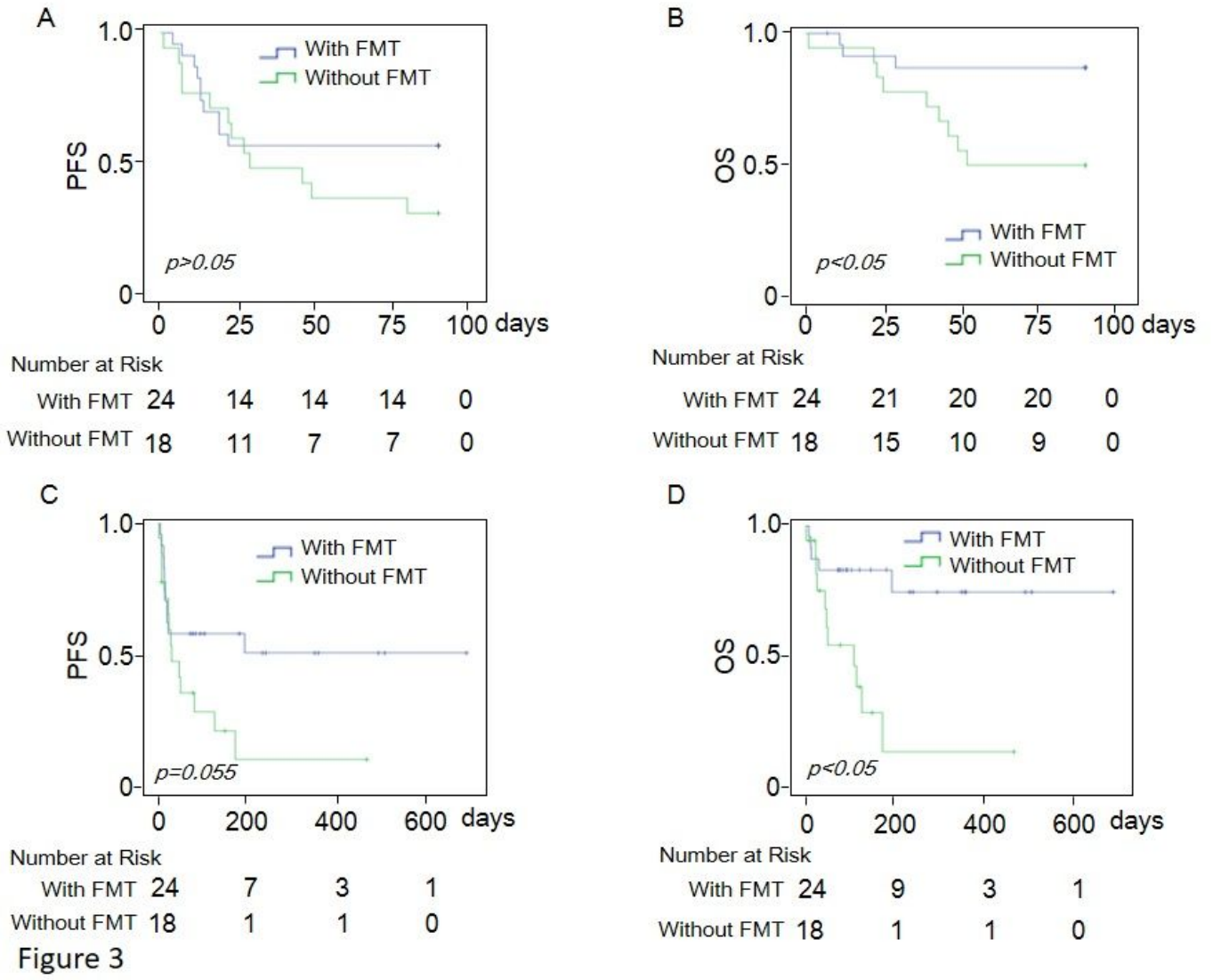

Figure 3

Kaplan-Meier curves demonstrating survival outcomes. PFS (A) and OS (B) of all patients within 90 days of follow-up time; PFS(C) and OS (D) at the end of research

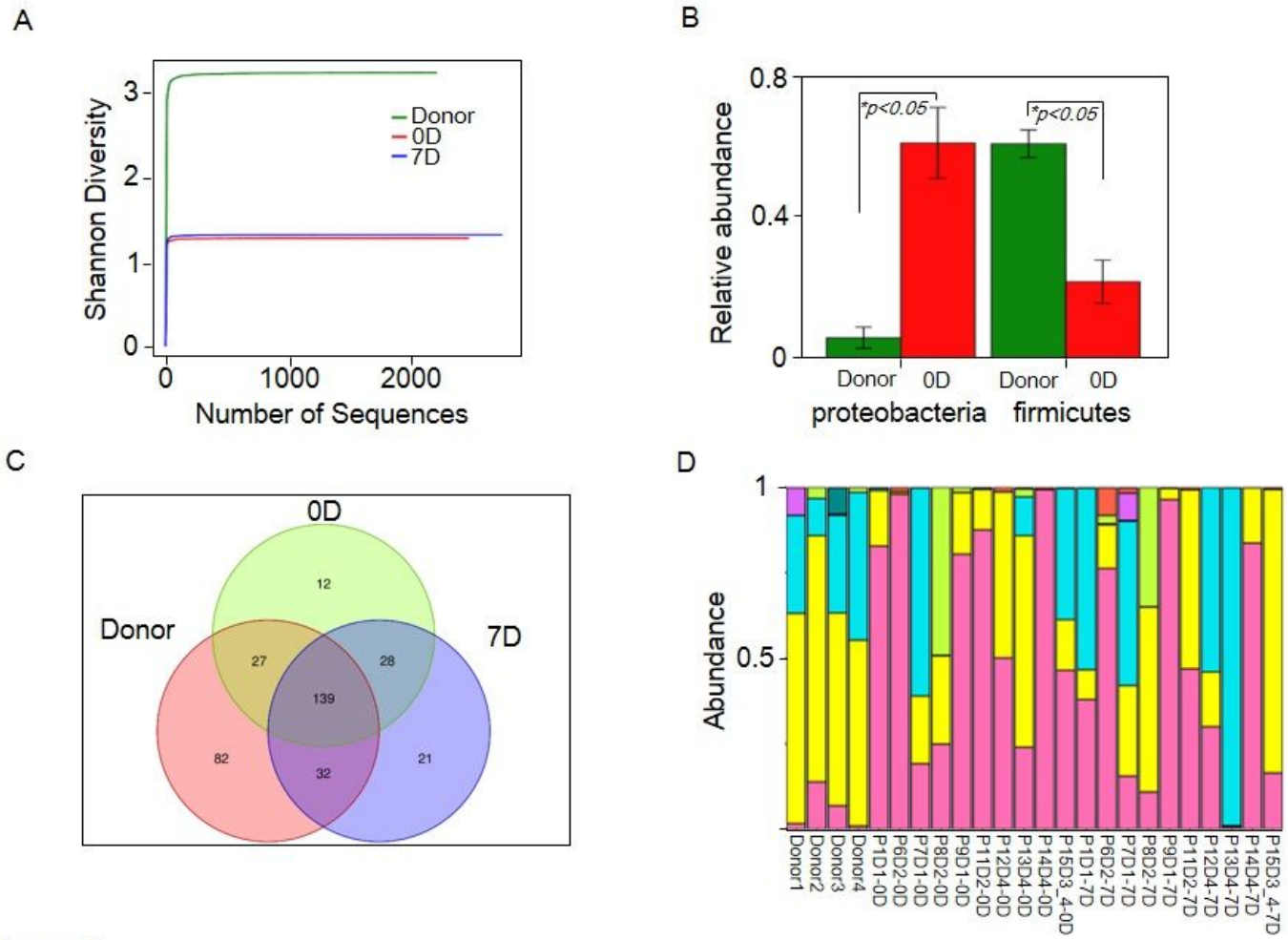

Figure 4

Figure 4

FMT improves gut microbiota diversity and composition in patients. . (A) The diversity of fecal microbiota in all sample (Shannon's diversity index)(ndonor=4, npatient=10).(B) proteobacteria and firmicutes change in donor group, pre-FMT samples. *p < 0.05. (C) OTUs change in donor group, pre-FMT(0D) and post-FMT(7D) samples. (D) Analysis of fecal microbiota composition in all samples at the phylum level(ndonor=4, npatient=10). Each row represents a study subject. Px means patient number, Dx means donor number, xD means day after FMT

Supplementary Files

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- supplementfigure.pptx
- PROTOCOLfinalversionv8.pdf