Gastrointestinal disturbance and effect of fecal microbiota transplantation in discharged COVID-19 patients

Zhaoqun Deng (zqdeng2002@163.com)
Jiangsu University https://orcid.org/0000-0003-0037-4697
Fengqiong Liu
Fujian medical university
Shanliang Ye
Guangdong provincial people's hospital
Xin Zhu
Jiangsu University
Xuesong He
Ganzhou shanjian bio-technology
Shengzhou Wang
ganzhou shanjian bio technology
Yinbao Li
Gannan Medical University
Jiang Lin
Jiangsu University
Jingsu Wang
Ganzhou shanjian bio techology
Yonggan Lin
Ganzhou shanjian bio technology
Xin Ren
Ganzhou shanjian bio technolgy
Yong Li
Guangdong provincial people's hospital

Short report

Keywords: FMT, COVID-19, gut microbiota

Posted Date: August 6th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-54135/v1

Gastrointestinal disturbance and effect of fecal microbiota transplantation in discharged COVID-19 patients

Fengqiong Liu[3#], Shanliang Ye[2#], Xin Zhu[1#], Xuesong He[5], Shengzhou Wang[5], Yinbao Li[4], Jiang Lin[1], Jingsu Wang[5], Yonggan Lin[5], Xin Ren[5], Yong Li[2*], Zhaoqun Deng[1,*]

1, Department of Laboratory Center, The Affiliated People's Hospital of Jiangsu University, Zhenjiang, China

2, Guangdong Provincial People's Hospital, Guangzhou,China

3, Department of Epidemiology and Health Statistics, Fujian Medical University, Fuzhou, China

4, School of pharmacrutical sciences, Gannan medical university, Ganzhou.China

5, Ganzhou Shanjian Bio-technology Co.,Ltd. , Ganzhou, China

[#]These authors contributed equally to this article.

[*]Corresponding Author:

Zhaoqun Deng, Laboratory Center, Affiliated People's Hospital of Jiangsu University, 8 Dianlilu, 212000, Zhenjiang, P.R. China.

Tel: +86.0511.85234387; E-mail address: zqdeng2002@163.com

Yong Li, Guangdong Provincial People's Hospital, 106, Zhongshan Road II,510080. Guangzhou. P.R. China. Tel: +86. 020-83827812 E-mail address:liyong@gdph.org.cn

Abstract

Background Gastrointestinal manifestations and gut dysbiosis are prevalent after SARS-CoV2 infection.With the continuously increasing number of infected cases, more attention should be paid to this particular population in post-infection recovery. We aimed to investigate the potential beneficial effect of FMT on gastrointestinal symptoms, gut dysbiosis and immune status in discharged COVID-19 patients.

Results Gastrointestinal and psychological disorder (45.5%) were observed in COVID-19 patients during post-infection recovery, improvement of which were observed after FMT. Most of the lab results including blood routine and blood biochemistry, within the normal range. The general distribution of 69 different types of lymphocytes differed between before and after FMT. FMT exert significant effect on B cells which was characterized as decreased naive B cell (P =0.012) and increased memory B cells (P = 0.001) and non-switched B cells (P= 0.012).The microbial community richness indicated by OTUs number, observed species and Chao1 estimators was marginally increased after FMT, whereas the community diversity estimated by the Shannon and Simpson index showed no significant changes after FMT. Gut microbiome composition of discharged COVID-19 patients differed from that of the general population at both phylum and genera level, which was characterized with a lower proportion of *Firmicutes*(41.0%) and *Actinobacteria*(4.0%), higher proportion of *Bacteroidetes*(42.9%) *and Proteobacteriato*(9.2%). FMT can partially restore the gutdysbiosis by increasing the relative abundance of *Actinobacteria*(15.0%) and reducing *Proteobacteriato* (2.8%) at the phylum level. At the genera level, *Bifidobacterium* and *Faecalibacterium*, which were dominant genera in the human gut microbiota and were beneficial for human health, had significantly increased after FMT.

Conclusions

Gastrointestinal and gut dysbiosis were observed in COVID-19 patients during post-infection recovery. FMT can improve the immune functionality, restore the gut microbiota, alleviate gastrointestinal disorders, and may serve as a potential therapeutic and rehabilitative intervention for the COVID-19.

Key words FMT, COVID-19, gut microbiota.

Background

Fever and cough are the most common clinical manifestations of COVID-19 infection. In addition, the disease can also cause digestive symptoms such as nausea and diarrhea, which may be largely underestimated [1-3]. Apart from these, lymphopenia and hypercytokinemia were also common in COVID-19 patients which suggest that COVID-19 could compromise the immune system [4,5]. The presence of both lymphopenia and hyper-cytokinemia in COVID-19 patients might indicate the severity of pathogen infection, as previously reported in severe influenza patients during the pandemic of coronavirus (SARS-CoV) in 2003 [6,7].

Tens of trillions of microbiota are colonized on the mucosal surfaces of the human body such as intestine and respiratory tract. In the past decades, large amount of evidence emerged to support the beneficial effects of commensal bacteria, especially probiotics. In addition to their crucial role in maintaining immune homeostasis of the intestine, studies also reported that commensal bacteria exerts a marked influence on the immune responses at other mucosal surfaces such as the respiratory tract to protect against respiratory influenza virus [8]. Siew C et al observed persistent alterations in the fecal microbiome of SARS-CoV-2 infected patients during the time of hospitalization, which may suggest that targeting gut microbiota is a new therapeutic option or at least a choice of adjuvant therapy for COVID-19 [9].

Fecal microbiota transplantation (FMT), which is an effective way to restore gut microbiota [10], was reported to enhance immunity and would be a potential therapy for individuals with pathogen infection[11-14]. Bradley et al reported that antibiotic treatment can reduce intestinal microbiota, thus change the interferon signature driven by commensal in lung epithelia and promote early influenza virus replication in the respiratory tract. The effects can be reversed by FMT [15]. Therefore, it is very likely

that FMT can enhance immunity and would be a potential therapy for individuals with virus infection. Given the fact of the prevalence of gastrointestinal symptoms and immunity dysfunction in COVID-19 patients, we speculate that FMT is able to restore the gut microbiota, improve function of the immunity system and alleviate the gastrointestinal disorders after SARS-CoV-2 infection. In this pilot study, we recruited 11 discharged COVID-19 patients in March, 2020 in Jiangxi Province and conducted FMT to investigate the potential benefit effect of FMT on the gut dysbiosis and immune system.

Methods

Study participants

This was a prospective, interventional, single-centered pilot study of FMT performed in the Ganzhou city in Jiangxi province of China. In total, 76 COVID-19 cases were confirmed in Ganzhou city since the disease outbreak. In April 2020, we recruited 11 COVID-19 patients who were initially admitted to the Fifth People's Hospital of Ganzhou City from January 26 to March 4, 2020. The patients were released from hospital from February13 to March 23, 2020. All the patients were laboratory confirmed positive COVID-19 cases. The timeline of patient diagnosis, discharge and recruitment was summarized (Fig. 1**)**. All the subjects were abstinent from antibiotics or anti-inflammatory drug two weeks longer prior to the treatment.

Data collection

General information included age, sex, occupation, origin, diagnosis date of COVID-19, severity assessment on admission, and the discharged date, which were collected by interview. Intestinal symptoms including constipation, diarrhea, abdominal pain, gastralgia, acid reflux, gastrectasia, as well as fatigue, depression, anxiety, insomnia were obtained by questionnaire.

Laboratory findings

Laboratory test values before and after FMT were evaluated and collected. Laboratory tests included blood routine (leucocytes, leutrophils, lymphocytes, platelets, erythrocyte, haemoglobin), Blood biochemistry (albumin, globulin, A/G, alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, serum creatinine).

Lymphocyte subset

Peripheral blood was obtained before and one week after FMT treatment in EDTA tubes for lymphocyte subset detection. All samples were tested within 6 hours after collection. Briefly, 69 indicators were measured by multiple-color flow cytometry with hundreds of flow human monoclonal antibody according to the manufacturer's instructions. The cells were analyzed on a BDFACS Canto II flow cytometry system (BD Biosciences).The finally lymphocyte subset data was presented as percentage.

FMT treatment

Protocol of donor screening, FMT capsule preparation, treatment regimen was summarized (Additional file 1). Oral capsule administrations were performed in a monitored clinical setting. Participants received 10 capsules each day for 4 consecutive days. The oral capsules prepared for each participant were from a single donor to guarantee the procedure is traceable. Potential side effects included fever, headache and gastrointestinal symptoms such as diarrhea, nausea, vomiting, distention, abdominal pain were monitored during FMT.

Gut microbiome assessments by 16s RNA **sequencing**

Fecal samples were collected before and one week after FMT. A detailed description of donor stool sampling, 16s sequencing and data processing were summarized (Additional file 1). Briefly, sampling packages were distributed to participant and fecal samples were collected and stored in a sealed container which was transported

with frozen gel packs to provide a low temperature environment until delivered to the laboratory. Fecal samples were pre-treated and gut microbiome were characterized by 16S sequencing. Original sequencing analysis was performed using QIIME2 and the Silva database was used for taxonomic assignments.

Sequencing data statistics

In total, 22 fecal samples from 11 patients were submitted to 16 S V3-V4 DNA sequences. 974,782 sequencing reads were analyzed after filtering the sequences that did not fit the criteria. The filtered sequencing reads were then aligned to known genes according to the 16S rDNA Silva database at a similarity of 97%. An average of 213 operational taxonomic units (OTUs) for each fecal sample was identified. Detailed information of sequencing reads was listed (Additional file 2).

Statistical analyses

Continuous variable were presented as median and interquartile range. Categorical variable were present as percentage. A paired sample *t*-test was adopted for comparison of variable between pre- and post-FMT for normally distributed data, while Wilcoxon matched-pairs test were performed for data with skewed distribution. The diversity indices evaluating gut microbial community richness (the Chao1 estimator) and alpha diversity (the Shannon and Simpson estimator) were calculated using Mothur.

Results

Basic information of the 11 COVID-19 patients treated with oral encapsulated FMT.

A total of 11 COVID-19 patients who were cured and discharged from the hospital were recruited in the study **(Table 1).** The median age was 49 years with an interquartile range of 47–57, and 6 patients (54.5%) were male. As for the degree of

disease severity on admission, 10 participants were categorized as nonsevere. Nonsevere was defined as no radiographic evidence of pneumonia or pneumonia was present along with fever and respiratory tract symptoms, but without obvious oxygen saturation change or respiratory failure requiring mechanical ventilation, shock, or organ failure requiring intensive care. And one participant was categorized as severe, who suffered from shock and required intensive care.

Gastrointestinal improvement after FMT

In total, 5 out of 11 discharged patients presented gastrointestinal symptoms to some extent which included constipation, diarrhea, abdominal pain, gastralgia, acid reflux and gastrectasia **(Table 2).** In addition to gastrointestinal symptom, 5 out of 11 patients suffered from psychological symptoms such as fatigue, depression and anxiety, insomnia. After FMT, 5 and 4 study subjects reported alleviation in gastrointestinal symptom and psychological disorders respectively.

Lab results and peripheral lymphocyte subset alteration after FMT

Most of the lab results including blood routine and blood biochemistry were within the normal range in discharged COVID-19 patients (**Table 3).** However, we find that 8 out of 11(72.7%) study subjects had mildly decreased Albumin/Globulin ratio, which showed no obvious improvement after FMT.

In addition to blood routine test, we analyzed lymphocyte subsets composition by flowcytometry. We obtained detailed expression information of 69 different types of lymphocyte and all the lymphocytes were classified into five major subsets, CD4+ T cells(n=17), CD8+ T cells(n=18), γδT cells (n=12),B cells(n=12) and NK cells(n=10). The general distribution of 69 different types of lymphocytes differed between pre- and post-FMT was summarized (Additional file 3**)**. FMT exert significant effect on B lymphocytes which was characterized as decreased naive B

cells (*P*=0.012) and increased memory B cells (*P*= 0.001), non-switched B cells (*P*= 0.012). In addition, the proportion of double positive T cells increased after FMT (*P*= 0.012). γδT cells also showed marginal difference after FMT (**Table 4).**

Alterations of gut microbiota in discharged COVID-19 patients after FMT

In 22 fecal samples, 970,334 sequencing reads were obtained, and an average of 213 OTUs was identified for each sample. The microbial community richness indicated by OTUs number, observed species and Chao1 estimator was marginally increased after FMT, whereas the alpha diversity estimated by the Shannon and Simpson index showed no significant changes after FMT **(**Additional file 4**).**

At the phylum level, the top 5 phylum at baseline include *Firmicutes* (41.0%), *Bacteroidetes* (42.9%), *Proteobacteriato* (9.2%), *Actinobacteria* (4.0%), *Fusobacteria* (2.8%). The top5 phylum after FMT included *Firmicutes* (41.5%), *Bacteroidetes* (39.3%), *Actinobacteria* (15%), *Proteobacteriato* (2.8%), *Fusobacteria* (1.3%). The relative abundance of *Proteobacteriato* decreased, while *Actinobacteria* increased after intervention (*P*<0.001) **(Fig. 2a)**. For individual patient, patient No.1, who had moderate constipation but greatly improved after FMT, was characterized with high proportion of *Firmicutes* (67.8%) and *Fusobacteria* (22.7%) and absence of *Bacteroidetes* (3.7%). FMT significantly increased *Bacteroidetes* (62.6%), decreased *Firmicutes* (26.0%) and *Fusobacteria* (9.2%). Patient No.7 was a severe COVID-19 survivor who suffered from diarrhea. The patient presented a microbiome profile of extremely high *Bacteroidetes* (84.4%) and low relative abundance of *Firmicutes* (12.3%). After FMT, the proportion of *Bacteroidetes* (45.7%) decreased and *Firmicutes* increased (48.7%). Patient No.11 suffered from severe constipation, gut microbiota profile of whom showed high proportion of *Actinobacteria* (28.6%) and *Proteobacteria* (37.2%), whereas low abundance of

Bacteroidetes (0.2%). Significant decrease in abundance of *Proteobacteria* (2.5%) was observed after FMT.

At the genera level, the top 5 genera before FMT included *Bacteroides*(28.3%), *Prevotella* (13.0%), *Faecalibacterium* (6.5%), *Lachnospiraceae* (6.2%), *Phascolarctobacterium* (5.7%) at baseline, while after FMT the top 5 genera include *Bacteroides* (31.1%),*Faecalibacterium* (11.7%), *Prevotella* (6.6%), *Bifidobacterium* (10.4%), *Collinsella* (4.5%) **(Fig. 2b,c)**. *Bifidobacterium*, *Faecalibacterium*, *Collinsella* significantly increased after FMT. For individual patient, patient No.1 was characterized with high proportion of *Fusobacterium* (22.7%), *Lachnospiraceae* (16.6%), *Blautia* (16.1%), *Clostridium XlVa* (11.1%). After FMT, *Bacteroides* (61.9%) increased, whereas proportions of *Blautia* (0.25%), *ClostridiumXlVa* (1.0%), *Lachnospiraceae* (1.6%), *Fusobacterium* (9.2%) were decreased. Patient No.7 with severe COVID-19 presented high proportion of *Prevotella* (82.5%) which was decreased (0.01%), while *Bacteroides* (44.4%) and *Phascolarctobacterium* (27.8%) increased after FMT. Patient No.11 had high proportion of *Enterobacteriaceae* (30.7%) and *Collinsella* (20.9 %). After FMT, relative abundance of *Enterobacteriaceae* decreased (1.2%), while *Bifidobacterium* significantly increased (36.7%).

Discussion

We are for the first time followed up COVID-19 patients after they were cured and discharged from hospital and observed that even in the discharged COVID-19 patients, problems such as gastrointestinal and psychological disorder, compromised immunity, gut dysbiosis are prevalent. FMT can improve the immune functionality, restore the gut microbiota and alleviate gastrointestinal disorders.

Early reports showed that 2-14.7% of SARS-CoV-2 infected patients had

symptoms of diarrhea and 1-5% of the case had nausea and vomiting. Recently, Cheung et al published a meta-analysis to report that up to 20% had gastrointestinal symptoms [16-18]. Fecal samples from about 50% of COVID-19 patients were detected as SARS-CoV-2 positive, suggesting that the digestive tract might be another site for virus replication and activity [19,20]. However, report about the after-effect of COVID-19 patients is rare. We are among the first to focus on the rehabilitation patient and report that even in the discharged COVID-19 patients, problems such as gastrointestinal and psychological disorder are not uncommon. However, these problems may be easily to be underestimated and neglected. The latest COVID-19 report from China observed that up to 3 % of discharged patients were tested positive on a retest for SARS-CoV-2 and 35% of them had at least one symptom associated with active COVID-19 [21]. Thus, with the rapidly rising number of recovered patients, more and more attention will be paid to the health conditions of patient after the disease.

It has long been reported that respiratory viral infections can lead to alterations in gut microbiome, and gut microbiome disturbance would predispose patients to secondary bacterial infections [22,23]. For COVID-19, the angiotensin converting enzyme 2 (ACE2) was reported to be a key receptor which facilitate the coronavirus to enter the host. ACE2 is not only expressed in respiratory tract, but also highly expressed in the gastrointestinal tract [24,25], which may partly explained the gastrointestinal symptoms presented in COVID-19 patients. To date, the only direct evidence links COVID-19 to gut microbiota was reported by Siew C et al, who investigated changes of fecal microbiomes of COVID-19 patients during hospitalization. Persistent alterations of fecal microbiome were observed in hospitalized patients. Fecal microbiota alterations were positively associated with

fecal virus load of SARS-CoV-2 and the disease severity of COVID-19(*9*). In the current study, instead of focusing on hospitalized patients, we followed up COVID-19 patients and observed persistent changes in the fecal microbiome composition after they were cured and discharged from hospital. At the phylum level, the relative abundance of *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteriato* were 41.0%, 42.9%, 9.2%, 4.0% respectively, which were different from that of the general population. In health population, the dominant phyla are *Firmicutes* and *Bacteroidetes* with a relative abundance of about 60% and 20% respectively [26-28]. As for *Actinobacteria* and *Proteobacteriato*, data from Asia population reported that the relative abundance was within the range of 0.12%-0.22 % and 0.01%-0.03% respectively [29].

As Siew C et al reported in their study that targeting the intestinal microbiota might reduce disease severity of COVID-19 [9]. Actually, at the beginning of February 2020, the guidance of China's National Health Commission (5th edition) recommended that probiotics can be used to maintain the intestinal microecological balance and prevent secondary bacterial infection when treating patients with severe COVID-19 infection. We further investigate whether FMT could be an effective strategy to improving the residual effect of COVID-19 by modifying the gut microbiome. We observed gut microbiome alteration and symptom alleviation after FMT, especially in patients with severe gastrointestinal symptoms. At the phylum level, the relative abundance of *Actinobacteria* (15%) and *Proteobacteria* (2.8%) were restored to the average level of the general population reported [*29*]. At the genera level, *Bifidobacterium* and *Faecalibacterium* significantly increased after FMT, especially in those COVID-19 patients with diarrhea or constipation. *Bifidobacteriu and Faecalibacterium are* both dominant genera in human gut microbiota and are

closely related to gut health [30-32].

Gut microbiota not only could maintain immune homeostasis and immune responses at local mucosal surfaces, but also has distal protective effects and protect against respiratory influenza virus. Several studies have reported the application of FMT to improve immune functionality, thus exert indirect protective effect on virus influenza infection. Bradleyet al reported that antibiotic treatment can reduce intestinal microbiota, thus change the interferon signature driven by commensal in lung epithelia and promote early influenza virus replication in the respiratory tract. The effects can be reversed by fecal transplantation [15]. Tiffany et al conducted FMT experiments on rhesus monkeys infected with chronic SIV during antiretroviral therapy. After antibiotic treatment, greatest microbiota shift was observed, while the frequencies of Th17 and Th22 in peripheral blood increased and the activation of CD4 T cells in intestinal tract decreased after FMT [33]. The latest evidence from Yongxi Zhang et al reported persistent alterations of peripheral lymphocyte subset in COVID-19 patients, which confirmed the immunity dysfunction after SARS-CoV-2 infection [34]. In the current study, we also observed that the general distribution of 71 different types of lymphocytes differed between pre-FMT and post -FMT especially for B lymphocyte subset, which suggest targeting gut microbiota by FMT have favorable effects on the immunity system after SARS-CoV-2 infection.

Limitations of this study

One major limitation of this exploratory study is the limited sample size. Although the association between SARS-CoV-2 infection and gastrointestinal symptoms, gut dysbiosis in discharged patients requires validation from large scale studies, this pilot study for the time examined the after effect of SARS-CoV2 infection which include gastrointestinal symptoms, psychological problems, peripheral lymphocyte alteration

and gut dysbiosis. Another major limitation is that the study is not randomized designed. Although establishing a causative relationship between FMT and gut microbiota regulation in discharged patients requires a parallel control group, it is the first time to examine the effect of FMT on the residual symptoms of SARS-CoV2 infection, and refer to FMT as a potential therapeutic and rehabilitative intervention for the COVID-19. We also attempted to evaluate the immune status and justify the beneficial effects of FMT from the perspective of immunity improvement. Further large scale studies with a randomized design to delineate the role of FMT and microbiome changes in SARS-CoV-2 infection and post-infection recovery.

Conclusions

Gastrointestinal and gut dysbiosis were observed in COVID-19 patients during post-infection recovery. FMT can improve the immune functionality, restore the gut microbiota and alleviate gastrointestinal disorders. Results support the novel idea that FMT may serve as a potential therapeutic and rehabilitative intervention for the COVID-19.

List of abbreviations

FMT Fecal microbiota transplantation

Declarations

Ethics approval and consent to participate

The local ethics committee approved the study (Approval number: 2020001) and patients provided written informed consent.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files].Source data for analyses are available

on request from the corresponding author.

Competing interests

The authors declare that they have no competing interests.

Funding

This project was supported by National Natural Science foundation of China (81970156, 81970118).

Authors' contributions

Study concept and design: ZD, SY and JW. Drafting of the manuscript: FL, YL and XZ. Analysis and interpretation of data: XH, SW and YL. Technical and material support: JL. Acquisition of data: YL and XR.

Acknowledgments

We thank Yuanfei Liu, Fusheng Liu and Bo Cao for assistance in recruiting patients, and thank Bohong Deng, Jinming Ke and Mingqiang Chu for assistance in preparing the manuscript.

References

1. Zhou J, Li C, Zhao G, et al. Human intestinal tract serves as an alternative infection route for Middle East respiratory syndrome coronavirus. *Science advances.* 2017;3(11):eaao4966.
2. Openshaw PJ. Crossing barriers: infections of the lung and the gut. *Mucosal immunology.* 2009;2(2):100-102.
3. Liang W, Feng Z, Rao S, et al. Diarrhoea may be underestimated: a missing link in 2019 novel coronavirus. *Gut.* 2020;69(6):1141-1143.
4. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* 2020;395(10223):497-506.
5. Yang X, Yu Y, Xu J, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *The Lancet. Respiratory medicine.* 2020;8(5):475-481.
6. Cameron MJ, Ran L, Xu L, et al. Interferon-mediated immunopathological events are associated with atypical innate and adaptive immune responses in patients with severe acute respiratory syndrome. *Journal of virology.*2007;81(16):8692-8706.
7. Lee N, Hui D, Wu A, et al. A major outbreak of severe acute respiratory syndrome in Hong Kong. *The New England journal of medicine.*2003;348(20):1986-1994.
8. Ichinohe T, Pang IK, Kumamoto Y, et al. Microbiota regulates immune defense against respiratory tract influenza A virus infection. *Proceedings of the National Academy of Sciences of the United States of America.* 2011;108(13):5354-5359.
9. Zuo T, Zhang F, Lui GCY, et al. Alterations in Gut Microbiota of Patients With COVID-19 During Time of Hospitalization. *Gastroenterology.* 2020;*19:S0016-5085(20)34701-6.*
10. Ooijevaar RE, Terveer EM, Verspaget HW, Kuijper EJ, Keller JJ. Clinical Application and Potential of Fecal Microbiota Transplantation. *Annual review of medicine.* 2019;70:335-351.
11. Hensley-McBain T, Zevin AS, Manuzak J, et al. Effects of Fecal Microbial Transplantation on Microbiome and Immunity in Simian Immunodeficiency Virus-Infected Macaques. *Journal of virology.* 2016;90(10):4981-4989.
12. Kim SM, DeFazio JR, Hyoju SK, et al. Fecal microbiota transplant rescues mice from human pathogen mediated sepsis by restoring systemic immunity. *Nature communications.* 2020;11(1):2354.
13. Ekmekciu I, von Klitzing E, Neumann C, et al. Fecal Microbiota Transplantation, Commensal Escherichia coli and Lactobacillus johnsonii Strains Differentially Restore Intestinal and Systemic Adaptive Immune Cell Populations Following Broad-spectrum Antibiotic Treatment. *Frontiers in microbiology.* 2017;8:2430.
14. Wardill HR, Secombe KR, Bryant RV, Hazenberg MD, Costello SP. Adjunctive fecal microbiota transplantation in supportive oncology: Emerging indications and considerations in immunocompromised patients. *EBioMedicine.* 2019;44:730-740.

15. Bradley KC, Finsterbusch K, Schnepf D, et al. Microbiota-Driven Tonic Interferon Signals in Lung Stromal Cells Protect from Influenza Virus Infection. *Cell reports.* 2019;28(1):245-256 e244.
16. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet.* 2020;395(10223):507-513.
17. Cheung KS, Hung IFN, Chan PPY, et al. Gastrointestinal Manifestations of SARS-CoV-2 Infection and Virus Load in Fecal Samples From a Hong Kong Cohort: Systematic Review and Meta-analysis. *Gastroenterology.* 2020,159(1):81-95.
18. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *Jama.* 2020,323(11):*1061-1069.*
19. Wolfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. *Nature.*2020;581(7809):465-469.
20. Xu Y, Li X, Zhu B, et al. Characteristics of pediatric SARS-CoV-2 infection and potential evidence for persistent fecal viral shedding. *Nature medicine.* 2020;26(4):502-505.
21. Qi M, Jun L, Ronghui D, et al. Assessment of patients who tested positive for COVID-19 after recovery. Lancet Infectious Disease. 2020; online first.
22. Hanada S, Pirzadeh M, Carver KY, Deng JC. Respiratory Viral Infection-Induced Microbiome Alterations and Secondary Bacterial Pneumonia. *Frontiers in immunology.* 2018;9:2640.
23. Yildiz S, Mazel-Sanchez B, Kandasamy M, Manicassamy B, Schmolke M. Influenza A virus infection impacts systemic microbiota dynamics and causes quantitative enteric dysbiosis. *Microbiome.* 2018;6(1):9.
24. Xiao F, Tang M, Zheng X, Liu Y, Li X, Shan H. Evidence for Gastrointestinal Infection of SARS-CoV-2. *Gastroenterology.* May 2020;158(6):1831-1833 e1833. doi: 10.1053/j.gastro.2020.02.055.
25. Hashimoto T, Perlot T, Rehman A, et al. ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation. *Nature.* 2012;487(7408):477-481.
26. Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell.* 2006;124(4):837-848.
27. Dethlefsen L, McFall-Ngai M, Relman DA. An ecological and evolutionary perspective on human-microbe mutualism and disease. *Nature.* 2007;449(7164):811-818.
28. Stewart CJ, Ajami NJ, O'Brien JL, et al. Temporal development of the gut microbiome in early childhood from the TEDDY study. *Nature.* 2018;562(7728):583-588.
29. Mobeen F, Sharma V, Tulika P. Enterotype Variations of the Healthy Human Gut Microbiome in Different Geographical Regions. *Bioinformation.* 2018;14(9):560-573.
30. Feng Y, Duan Y, Xu Z, et al. An examination of data from the American Gut Project reveals that the dominance of the genus Bifidobacterium is associated with the diversity and robustness of the gut microbiota. *MicrobiologyOpen.* 2019;8(12):e939.

31. Ferreira-Halder CV, Faria AVS, Andrade SS. Action and function of Faecalibacterium prausnitzii in health and disease. *Best practice & research. Clinical gastroenterology.* 2017;31(6):643-648.
32. Lopez-Siles M, Duncan SH, Garcia-Gil LJ, Martinez-Medina M. Faecalibacterium prausnitzii: from microbiology to diagnostics and prognostics. *The ISME journal.*2017;11(4):841-852.
33. Mutlu EA, Keshavarzian A, Losurdo J, et al. A compositional look at the human gastrointestinal microbiome and immune activation parameters in HIV infected subjects. *PLoS pathogens.* 2014;10(2):e1003829.
34. Wang F, Nie J, Wang H, et al. Characteristics of Peripheral Lymphocyte Subset Alteration in COVID-19 Pneumonia. *The Journal of infectious diseases.* 2020;221(11):1762-1769.

Table 1 Basic information of 11 COVID-19 patients

Patient number	Age,years	Sex	Date of diagnosis	Date of discharge	Severity of Covid-19 on admission
1	57	Male	2020/1/28	2020/2/22	nonsevere
2	51	Female	2020/1/28	2020/2/14	nonsevere
3	45	Male	2020/2/7	2020/2/22	nonsevere
4	49	Male	2020/1/26	2020/2/21	nonsevere
5	49	Female	2020/1/26	2020/2/21	nonsevere
6	23	Female	2020/1/28	2020/2/13	nonsevere
7	48	Male	2020/2/2	2020/2/24	severe
8	47	Male	2020/1/30	2020/2/20	nonsevere
9	58	Male	2020/3/4	2020/3/23	nonsevere
10	68	Female	2020/1/29	2020/2/19	nonsevere
11	53	Female	2020/2/28	2020/3/20	nonsevere

Table 2 Symptoms of 11 COVID-19 patients before and after FMT*

Symptoms	Pre-FMT n(%)	Symptoms relieved Post-FMT n(%)
Constipation	3(27.3%)	3(27.3%)
Diarrhea	1(9.1%)	1(9.1%)
Abdominal pain	1(9.1%)	1(9.1%)
Gastralgia	1(9.1%)	1(9.1%)
Acid reflux	2(18.2%)	1(9.1%)
Gastrectasia	1(9.1%)	1(9.1%)
GI Symptoms(In total)	5(45.5%)	5(45.5%)
Fatigue	3(27.3%)	2(18.2%)
Depression and anxiety	2(18.2%)	1(9.1%)
Insomnia	3(27.3%)	3(27.3%)
Psychological Symptoms (In total)	5(45.5%)	4(36.4%)

*Values are expressed as no. (%)

Table 3 Lab results of 11 COVID-19 patients pre- and post-FMT*

Blood routine	Pre-FMT	Post-FMT
Leucocytes (× 10^9 per L; normal range 3.5–9.5)	5.6 (4.8,6.5)	5.9(4.7,6.8)
Neutrophils (× 10^9 per L; normal range 1.8–6.3)	3.2 (2.9,3.6)	3.3 (2.6,4.1)
Lymphocytes (× 10^9 per L; normal range 1.1–3.2)	1.7 (1.6,2.3)	1.8 (1.6,2.2)
Monocytes(× 10^9 per L; normal range 0.1–0.8）	0.4 (0.3,0.4)	0.4(0.3,0.5)
Erythrocyte(× 10^{12} per L; normal range 3.5–5.1）	4.8 (4.3,5.5)	4.8 (4.5,5.3)
Increased	2(18.2%)	2(18.2%)
Haemoglobin (g/L; normal range 120–175)	133(130,146)	133 (130,151）
Increased	1(9.1%)	1(9.1%)
Decreased	0 (0.0%)	1(9.1%)
Platelets (× 10^9 per L; normal range 125–350)	262(239,297)	257(182,295）
Increased	2(18.2%)	2(18.2%)
Blood biochemistry		
ALT (U/L; normal range 9.0–50.0)	18.0 (13.0,23.0)	15.0 (14.8,22.0）
AST(U/L; normal range 15.0–40.0)	19.6(18.0,20.4)	18.0 (17.0,21.0）
AST/ALT(normal range 0-3)	1.1 (0.9,1.5)	1.1(0.9,1.4）
Albumin(34～54g/L)	43.0 (40.1,43.2)	43.0 (42.0,44.0）
Globulin (20-45g/L)	30.8(28.1,32.3)	30.7(28.8,32.4）
A/G (1.5-2.5)	1.4(1.3,1.5)	1.4(1.3,1.5）
Decreased	8(72.7%)	8(72.7%)
Blood urea nitrogen (mmol/L; normal range 3.6–9.5)	4.9 (4.0,5.9)	5 .0(4.5,5.4）
Increased	1(9.1%)	1(9.1%)
Serum creatinine (μmol/L; normal range 57.0–111.0)	77.1(62.9,83.0)	79.2(62.4,84.0)

*Values are expressed in number (percentage) and median (interquartile range).

Table 4 Proportion of lymphocyte subset before and after FMT*

	Pre-FMT	**Post-FMT**	***P*** **value**
T cells (%of lymphocyte **)**	64.0(56.2,70.9)	62.2(54.5,71.6)	0.663
Helper T cells (%of T cells)	56.4(53.8,66.2)	55.0(52.0,62.9)	0.333
Killer T cells (%of T cells)	27.1(22.7,33.8)	28.2(24.1,33.4)	0.062
Double positive T cells (%of T cells)	0.8(0.5,3.5)	1.1(0.7,2.9)	0.012
Th to Tc ratio	2.1(1.6,2.5)	2.0(1.7,2.5)	0.673
γδT cells (%of T cells)	1.4(1.0,5.3)	4.4(2.0,7.6)	0.149
NK cells **(**%of lymphocyte **)**	13.2(9.5,18.7)	13.3(8.9,18.6)	0.938
Immature NK cells (%of NK cells)	5.1(3.8,8.4)	7.4(4.8,8.3)	0.936
Mature NK cells (%of NK cells)	93.3(91.6,96.2)	91.9(91.5,95.3)	0.966
Immature/mature NK cells	0.05(0.04,0.09)	0.08(0.05,0.09)	0.905
B cells (%of lymphocyte **)**	10.9(7.6,14.0)	8.3(3.7,11.5)	0.012
Naïve B cells (%of B cells)	62.2(54.3,69.1)	40.8(32.8,65.2)	0.012
Memory B cells (%of B cells)	25.3(20.2,30.8)	37.4(26.3,48.2)	0.001
Non-switched B cells (%of B cells)	10.2(7.8,15.2)	21.9(13.7,26.0)	0.012
Immature regulatory B cells (%of B cells)	0.5(0.3,0.8)	1.0(0.2,3.0)	0.054

*Values are expressed in number (percentage) and median (interquartile range).

Figure legends

Figure 1 The timeline of patient diagnosis, discharge and recruitment. In total, 11 COVID-19 patients were diagnosed and admitted to the Hospital from January 26 to March 4, 2020. The patients were discharged from hospital from February13 to March 23, 2020. The recruitment was initiated in April 3, 2020.

Figure 2 Socio-psychological status assessed by SCL-90. In total 3 out of 11patients showed mild psychological symptoms after the disease as reflected by the total score of SCL-90 and the general symptomatic index. Symptoms of patients No.7 and No.11 were alleviated after FMT.

Figure 3 Alterations of gut microbiota in discharged COVID-19 patients after FMT.

Alterations of gut microbiota were presented at the phylum and genera level. (A) Changes of relative abundance of individual phylum for both individual patients and at average level were presented. (B) Changes of relative abundance of individual genus for both individual patients and at average level were presented. (C) Heatmap of changes in relative abundance of the top15 genera. $A^{\#}$represent pre-FMT, $B^{\#}$ represent post-FMT.

File name: Additional file 1

File format: .txt

Title of data: Detail methods information

Description of data: Detail information of Donor screening,Preparation of fecal microbiota transplant (FMT) and placebo capsules,Capsule dosing regimen,Fecal sample collection,Microbiome sequencing and 16S Data Processing and Diversity Analyses

File name: Additional file 2

File format: .xls

Title of data: Supplementary table 1. Detailed information of sequencing reads

Description of data: Detail information of sequencing reads. “A” refers to data before FMT. “B” refers to data after FMT.

File name: Additional file 3

File format: .xls and .txt

Title of data: Supplementary table 2 Detailed information of 69 different types of lymphocytes pre- and post-FMT. Supplementary figure 1 Heatmap of 69 different types of lymphocytes pre- and post-FMT

Description of data: . The median and interquartile range of 69 different types of lymphocytes pre- and post-FMT, data are presented as percentage. “A” refers to data before FMT and “B” refers to data after FMT.

File name: Additional file 4

File format: .xls

Title of data: Supplementary table 3.Community richness and diversity of gut microbiota pre- and post-FMT.

Description of data: OTU number, Observed species, Chao1 index ,Shannon index, Simpson index before and after FMT.

Figures

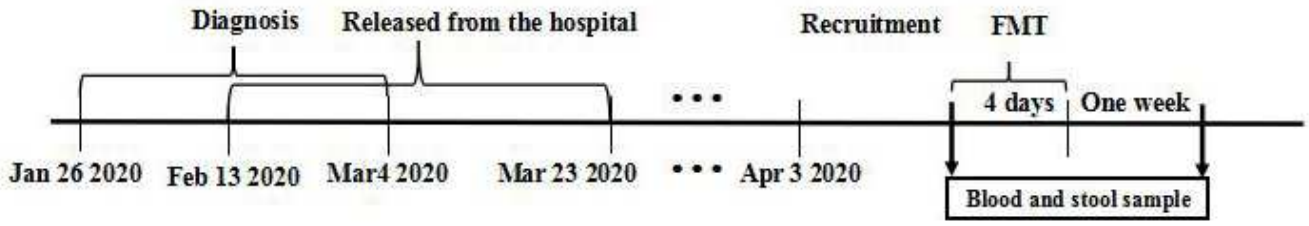

Figure 1

The timeline of patient diagnosis, discharge and recruitment. In total, 11 COVID-19 patients were diagnosed and admitted to the Hospital from January 26 to March 4, 2020. The patients were discharged from hospital from February13 to March 23, 2020. The recruitment was initiated in April 3, 2020.

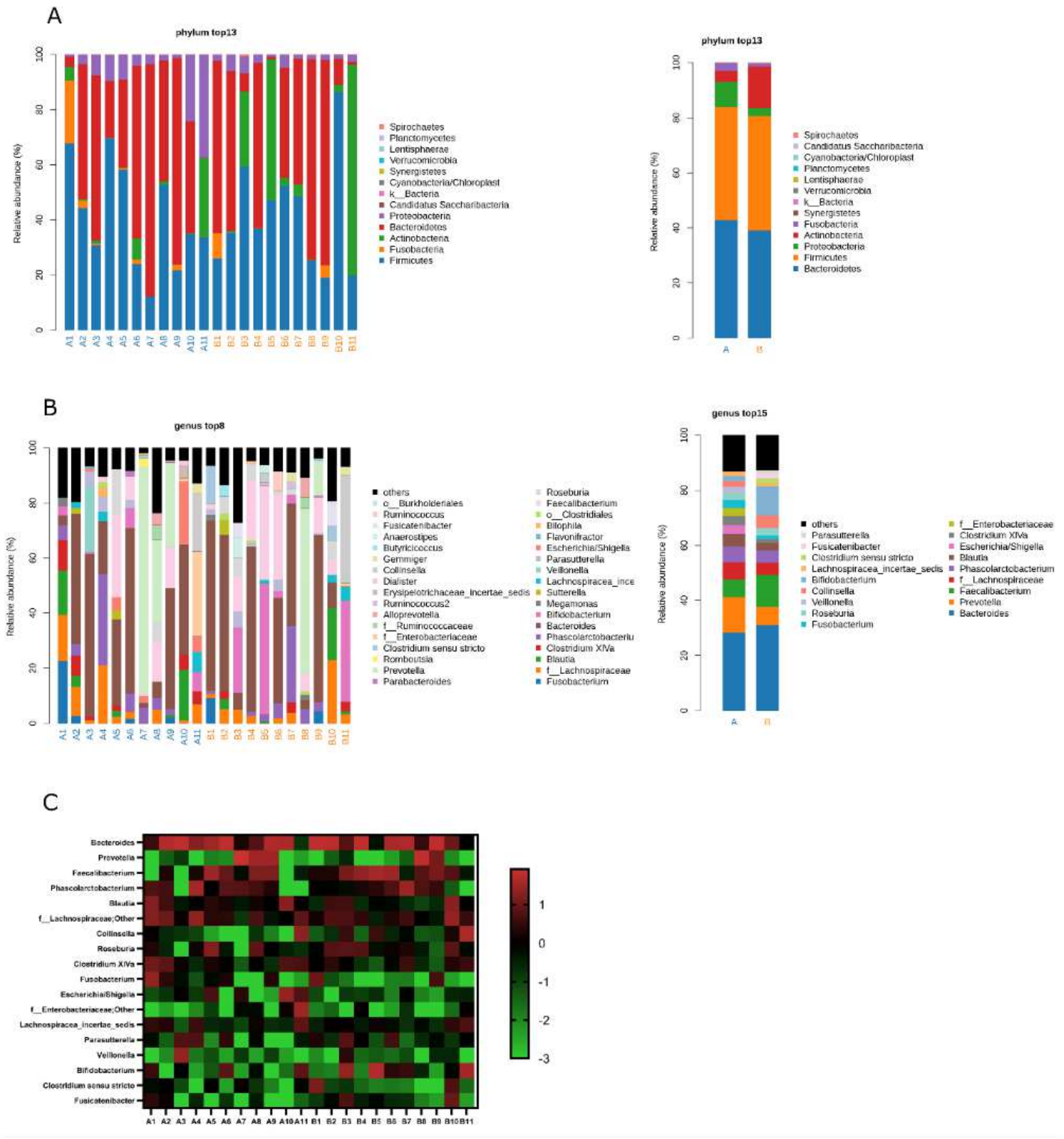

Figure 2

Alterations of gut microbiota in discharged COVID-19 patients after FMT. Alterations of gut microbiota were presented at the phylum and genera level. (A) Changes of relative abundance of individual phylum for both individual patients and at average level were presented. (B) Changes of relative abundance of individual genus for both individual patients and at average level were presented. (C) Heatmap of changes in relative abundance of the top15 genera. A#represent pre-FMT, B# represent post-FMT.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementalmaterial.doc