Treatment of COVID-19 patients suspected with gut microbiota dysbiosis with washed microbiota transplantation: study protocol for a randomized controlled trial

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Study protocol

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Abstract

BACKGROUND: Coronavirus disease 2019 (COVID-19) is often accompanied by gastrointestinal symptoms, which is related to gut microbiota dysbiosis (GMD). Whether washed microbiota transplantation (WMT) is effective in COVID-19 patients suspected with GMD by restoring gut microbiota is unknown. This study is designed to explore the efficacy and safety of WMT in COVID-19 patients suspected with GMD.

METHODS: COVID-19 patients suspected with GMD will be randomly divided to receive routine treatment (group A) or receive routine treatment and WMT (group B). The frequency of WMT will be once a day for three consecutive days. Nucleic acid test, imaging examination, and tests related to organ functions, homeostasis, inflammatory response, intestinal barrier function and immunity will be performed at admission, 1, and 2 weeks after treatment and on the day of discharge. The clinical efficacy and safety of WMT in COVID-19 suspected with GMD and the effects of WMT on the organ function, homeostasis, inflammatory response, intestinal barrier function and immunity of the patients will be evaluated. The primary outcome will be the clinical efficacy, as reflected by the SARS-Cov-2 infection status, gastrointestinal symptoms and the recovery of the disease. The secondary outcomes will be the effects of WMT on the organ function, homeostasis, inflammatory response, intestinal barrier function and immunity of the patients, as well as occurrence of adverse events during WMT.

DISCUSSION: In the proposed protocol, WMT is expected to be efficacious and safe for the treatment of COVID-19 patients suspected with GMD, and the therapeutic effect is expected to be associated with the improvement of intestinal barrier function, inflammatory response and immunity. Findings from this study may open up a new way for the prevention and treatment of COVID-19 suspected with GMD.

TRIAL REGISTRATION: Chinese Clinical Trial Registry – URL: <http://www.chictr.org.cn/index.aspx>. Registration number: ChiCTR2000032737. Registered 9 May 2020.

1. Background

Coronavirus disease 2019 (COVID-19), caused by SARS-Cov-2 virus, has become a pandemic worldwide. On January 30, 2020, the World Health Organization announced the outbreak of COVID-19 as a public health emergency of international concern.¹ By Aug 3, 2020, SARS-Cov-2 has infected more than ten million people with more than six hundred thousand deaths worldwide. (<https://covid19.who.int/>) A meta-analysis showed that 17.6% of patients with SARS-Cov-2 infection developed gastrointestinal symptoms.² In addition, Zuo *et al.* found that the gut microbiota changed significantly in COVID-19 patients, which manifested by the increased abundance of opportunistic pathogens such as *Coprobacillus*, *Clostridium ramosum*, and *Clostridium hathewayi*, and the decreased abundance of *Faecalibacterium prausnitzii* (an anti-inflammatory bacterium) and other bacteria that downregulated the expression level of angiotensin converting enzyme 2 (the receptor of SARS-Cov-2), such as *Bacteroides dorei*, *Bacteroides thetaiotaomicron*, *Bacteroides massiliensis* and *Bacteroides ovatus*.³

It has been reported that the imbalance of gut microbiota resulted in the release of lipopolysaccharide (LPS) into blood, leading to the upregulation of toll-like receptors 4 (TLR4) which is activated by influenza virus. The excess activated TLR4 induced cytokine storm through TLR4-NF- κ B pathway,⁴ ultimately the uncontrolled inflammatory response will lead to multiple organ failure in COVID-19 patients.⁵ A recent animal study found that normal gut microbiota composition could effectively reduce the early replication of influenza virus in lung epithelial cells.⁶ Yitbarek *et al* further reported that restoration of gut microbiota balance with the treatments of probiotics or fecal microbiota transplantation (FMT) reduced influenza virus shedding significantly and restored the expression level of interleukin (IL)-22, and thus improved the survival of cases with lethal influenza.⁷

However, whether restoration of gut microbiota has therapeutic effects on viral shedding in patients with COVID-19 is unknown. Washed microbiota transplantation (WMT) is an updated version of FMT developed on the basis of washed bacteria preparation, which has significant effects on the improvement of microbiota dysbiosis.⁸ In this clinical trial protocol, WMT will be employed to treat GMD. We design this clinical trial protocol to explore the clinical efficacy and safety of WMT on COVID-19 suspected with GMD.

2. Methods

2.1 Clinical trial design

2.1.1 Participants

COVID-19 patients suspected with GMD (*i.e.* accompanied with gastrointestinal symptoms or antibiotic-associated diarrhea) will be recruited for this clinical trial.

Patients with one of the following criteria will be excluded: 1) Critical type of COVID-19 according to **COVID-19 diagnosis and treatment guidelines in China (7th edition)**;⁹ 2) Gastrointestinal perforation; 3) Gastrointestinal obstruction; 4) Recent history of intestinal fistula; 5) Disturbance of consciousness; 6) Gastrointestinal hemorrhage; 7) Malignant tumors; and 8) Pregnant or lactating women.

In addition, the criteria for case rejection, dropout and discontinuation in this study include:

1) Patients with poor compliance and unable to perform WMT; 2) Patients who participate in other trials or could not follow the routine treatment during the present trial; 3) Patients with WMT-related adverse events that need to be treated; and 4) Patients with aggravated illness or severe complications during the trial that must be immediately managed.

2.1.2 Interventions

Eligible patients will be randomly divided into group A, who will receive routine treatment, and group B, who will receive WMT with continuing the routine treatment. The frequency of WMT will be once a day for three consecutive days.

2.1.3 Laboratory and clinical evaluations

The laboratory and clinical indicators will be tested or recorded at admission, 1 and 2 weeks after treatment and on the day of discharge.

The laboratory indicators will include COVID-19 nucleic acid test, fasting blood glucose, muscle enzyme, D-dimer, electrolytes, myoglobin, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), procalcitonin(PCT), peripheral blood lymphocyte subsets, oxygenation index, blood gas analysis, blood routine tests, liver function indicators, renal function indicators, inflammatory cytokines such as IL-2, IL-6, tumor necrosis factor α (TNF- α), interferon-gamma (IFN- γ) and intestinal barrier function related factors, such as LPS, D-lactate and diamine oxidase (**Table 1**). Venous and arterial blood samples will be collected by clinical physician. Arterial blood samples will be used for the detection of blood gas analysis and oxygenation indexes with a blood gas analyzer. The venous blood samples will be used for the detection of the remaining indicators, using Sysmex 1000i blood cell analyzer, Cobas501 automatic biochemical analyzer and JYDLT intestinal barrier function analysis system, respectively.

The clinical indicators will include fever, pulmonary shadow absorption, gastrointestinal symptoms, *etc.* (**Table 2**) In addition, all known WMT-related adverse events such as abdominal pain, abdominal distention, diarrhea, *etc.* will be monitored. We will also grade all patients with pneumonia severity index (PSI) (**Supplementary Table 1**) and acute physiology and chronic health evaluation II (APACHE II) (**Supplementary Table 2**).

2.2 Study unit

This trial will be completed jointly by First Affiliated Hospital of Guangdong Pharmaceutical College and Guangzhou Eighth People's Hospital. First Affiliated Hospital of Guangdong Pharmaceutical College will be responsible for the preparation of stool suspension and the operation of WMT, and Guangzhou Eighth People's Hospital will be responsible for collecting all the data listed above. All the data will be analyzed mutually by the biostatisticians participated in this study.

2.3 Randomization

The enrolled patients will be allocated into group A and group B by the block randomization method. Taking the admission time as the compatibility factor, two patients adjacent to the admission time will be taken as a block group. The first patient in each block group will get a random number by querying the random number table. If the random number is odd, it will be included in group A, and the remaining patient in the block group will be included in group B.

Blind methods are not considered because this trial involves invasive procedures such as endoscopic catheterization, which patients have the right to know.

2.4 WMT

2.4.1 Stool donors: The donors' age will be between 18-25 years old, and their body mass index is between 18.5-23.9. All donors need to pass the questionnaire firstly, and those who meet the requirements will receive further interview, psychological examination and physical examination. Donors with infectious diseases, digestive diseases, metabolic diseases, chronic fatigue syndrome, autoimmune diseases, allergic disease, and neuropsychiatric diseases will be excluded. All qualified donors of the above projects will receive the training required by healthy diet, and then donate stool in consultation with researchers.

2.4.2 Stool suspension: The stool samples provided by the donor will be collected, weighed, added with sterile saline according to the ratio of feces to saline 1:5, and then mixed evenly. The mixture will be filtered through the intelligent microbial separation system (GenFMter; FMT Medical, Nanjing, China), and then five-stages of filtration will be carried out. The obtained suspension will be immediately centrifuged at a speed of 2500 rpm for three minutes, and this step will be repeated three times. The final sediment will be suspended again with sterile saline in accordance with the ratio of sediment to saline 1:1.

2.4.3 WMT preoperative preparation:

Metoclopramide will be injected intramuscularly 30 minutes before WMT, and a proton pump inhibitor will be injected intravenously one hour before WMT.

2.4.4 WMT procedure: The nasojejun tube will be placed in the middle digestive tract. Normal saline will be used to flushed the tube first, and followed by the infusion of the stool suspension with a standard volume of 200 ml per person. After WMT, the patient will be asked to stay in the lying position for 30 minutes, with restriction of strenuous activities.

2.5 Participant rights and confidentiality

Participants have the right to withdraw from the study freely at any stage of the study without discrimination or retaliation, and to have their medical rights unaffected. All the information collected in this study belongs to the private information of patients, and none of researchers can disclose the information unless they obtain the permission from patients. Code will be used to identify the participants in all research-related documents. Only research teams and sponsors, ethics committees and regulatory agencies can access code information and other research materials, except that participants' personal information and related information will not be disclosed to any other person. Research results may be published in relevant scientific journals, but participants' name will not appear in any research reports or public publications.

2.6 Outcomes

2.6.1 Primary outcome

According to COVID-19 diagnosis and treatment guidelines in China (7th edition), COVID-19 patients can be classified as mild, common, severe and critical types.⁹ Mild type was defined when the clinical

symptoms were mild, without any imaging change. Common type was defined when patients had clinical symptoms such as fever, respiratory tract infection, etc., with pulmonary imaging changes. Severe type was defined when patients met any of the following: 1) the respiratory rate > 30 rate/min, 2) the pulse oxygen saturation $\leq$ 93%, and 3) the ratio of arterial oxygen partial pressure to fractional inspired oxygen $\leq$ 300 mmHg. Critical type was defined when patients met any of the following: 1) respiratory failure and requiring mechanical ventilation, 2) shock, and 3) comorbid with other organ failure and requiring ICU care. In the present study, patients diagnosed with mild and common types will be defined as non-severe and those diagnosed with severe and critical types will be defined as severe.

The primary outcome of this study will be the clinical efficacy of WMT on the COVID-19 patients suspected with GMD. The clinical efficacy will be reflected by the SARS-Cov-2 infection status and the recovery of the disease, which is defined as "improvement"(from severe to non-severe). In addition, the improvement of gastrointestinal symptoms will be co-primary outcome.

2.6.2 Secondary outcomes

Several secondary outcomes are as following:

- 1) Advantages of WMT over routine treatment, as reflected by the recovery time, PSI grade and APACHE II score.
- 2) Effects of WMT on organ functions and homeostasis, as reflected by fasting blood glucose, muscle enzyme, D-dimer, electrolytes, myoglobin, liver function and renal function.
- 3) Effects of WMT on the inflammatory response, as reflected by blood routine tests, CRP, ESR, PCT, inflammatory cytokines such as IL-2, IL-6, TNF- α , and IFN- γ .
- 4) Effects of WMT on intestinal barrier function as reflected by the factors including LPS, D-lactate, and diamine oxidase.
- 5) Effects on cellular immune functions, as reflected by the peripheral blood lymphocyte subsets.
- 6) The safety of WMT, as reflected by WMT-related adverse events.

2.7 Follow-up visit

Telephone follow-up will be conducted at one week, two weeks, and six months after discharge, and the contents included the clinical indicators listed above (**Table 3**).

The schedule of enrollment, interventions, assessments and follow-up is shown as below. (**Figure 1**)

2.8 Data collection and statistical analysis

Quantitative data are expressed as the mean $\pm$ standard deviation, minimum and maximum, and qualitative data are expressed as the rate and constituent ratio. When the total variance of quantitative

data is equal, student's *t* test will be used to determine the difference between groups. When the total variance of quantitative data is not equal, the rank sum test will be used to determine the difference. Qualitative data will be tested by Chi-square test or Fisher's exact test, where appropriate. $P < 0.05$ will be considered significant. All data will be analyzed by SPSS software version 19.0 (IBM, Armonk, NY, USA).

3. Expected Results

3.1 Primary outcomes

It is expected that, compared with group A, group B will get better improvement of COVID-19, with the negative conversion of SARS-Cov-2 virus and alleviated or disappeared gastrointestinal symptoms (Fig. 2). These expected findings will suggest that WMT is effective in the treatment of COVID-19 suspected with GMD.

3.2 Secondary results

The secondary outcomes are expected as following:

- 1), Compared with group A, group B will have shorter recovery time, and lower PSI grade and APACHE II scores (Fig. 2). These expected findings will suggest that the addition of WMT to routine treatment is beneficial for the recovery of COVID-19 patients suspected with GMD.
- 2), The values of fasting blood glucose, muscle enzyme, D-dimer, electrolytes, myoglobin, liver function, renal function will be restored to the levels closer to normal values and in a faster way in group B than in group A. The difference is statistically significant. These expected findings will suggest that WMT contribute to maintaining the organ function and homeostasis.
- 3) The values of inflammatory indicators including blood routine tests, CRP, ESR, PCT, IL-2, IL-6, TNF- α , and IFN- γ will be restored to the levels closer to normal values and in a faster way in group B than in group A. The difference is statistically significant. These expected findings will indicate that WMT can effectively alleviate systemic inflammation and avoid the lung injury caused by cytokine storm.
- 4) The values of intestinal barrier function-related factors such as LPS, D-lactate, and diamine oxidase will be restored to the levels closer to normal values and in a faster way in group B than in group A. The difference is statistically significant. These expected findings will imply that the intestinal barrier function in COVID-19 patients could be repaired after WMT.
- 5) The peripheral lymphocyte subsets of COVID-19 patients gradually tend to normal in both groups, but faster in group A than in group B. These expected findings will indicate that WMT can effectively improve the abnormal immune activation and immune damage caused by SARS-Cov-2 infection.
- 6) The frequency of adverse events is either similar between the two groups, or is higher in group B than in group A, but the adverse events are all mild, and with no or rare serious adverse events in group B.

These expected findings will show that WMT is safe for COVID-19 patients suspected with GMD (Fig. 2).

4. Discussion

In the proposed clinical trial, WMT plus routine treatment is expected to be more efficacious than the routine treatment, and safe for COVID-19 patients suspected with GMD. The clinical efficacy may be associated with the improvement of intestinal barrier function, inflammatory response and immunity in patients.

SARS-Cov-2 virus is so closely related to the digestive system that it can easily survive in the patient's digestive tract and cause gastrointestinal symptoms.^{10,11} It has been revealed that there are antagonistic or coexisting relationships among various gut microorganisms,¹² and SARS-Cov-2 virus, as a new member, is bound to disrupted the balance of gut microbiota.³ Therefore, the correction of GMD may play a special role in the recovery of COVID-19 patients. WMT has been proved to be a safe method with considerable clinical efficacy and negligible adverse events, particularly in the treatment of gastrointestinal disorders caused by GMD.¹³ A previous study showed that probiotics or WMT interventions for highly pathogenic influenza virus infection can restore IL-22 to normal levels and improve the ability to resist influenza virus in animal⁷. However, there are no clinical trials to confirm the observation.

After SARS-Cov-2 infection, the abundance of anti-inflammatory bacteria and bacteria that downregulate ACE2 expression levels decreased, which makes the colonization of SARS-Cov-2 in the gastrointestinal tract easier.³ Then the GMD caused by COVID-19 destroys intestinal barrier function severely, which leads to the LPS and bacterial metabolites produced by the gut microbiota easily enter the circulatory system and induce systemic inflammatory response.^{14–16} When the host immunity declines, the viral replication will further accelerate, and form a “vicious circle”, which eventually leads to multiple organ failure.^{17,18} WMT not only corrects the imbalance of gut microbiota effectively, but also contributes to improving intestinal barrier function and avoiding the formation of the “vicious cycle”, and thus achieving the efficaciousness in the treatment of highly pathogenic influenza virus infection.⁷ Whether WMT exhibits its clinical efficacy in patients with COVID-19 through this proposed mechanism needs further investigation.

It should be stated that this trial is single-blinded. If all patients are required to be blinded to the treatment, they had to receive endoscopic catheterization, which is an invasive procedure that may damage the patients' health and aggravate the disease.¹⁹ Therefore, we have to abandon the blindness for the patients. However, we will implement a blinding method for researchers in this study. That is, to select one or more staff members to be responsible for recording clinical data and testing the experimental samples for all enrolled patients, but they cannot access the information on which treatment the patients have received and which group the patients belong to.

In conclusion, WMT is efficacious and safe for the treatment of COVID-19 patients suspected with GMD. The therapeutic effects of WMT are related to the improvement of intestinal barrier function,

inflammatory response and immunity.

5. Trial Status

The trial version number is 1.0. The trial began recruitment on May 8, 2020, and is expected to complete recruitment by June 2021.

Abbreviations

GMD: gut microbiota dysbiosis

WMT: washed microbiota transplantation

COVID-19: Coronavirus disease 2019

LPS: lipopolysaccharide

TLR4: toll-like receptors 4

IL: interleukin

CRP: C-reactive protein

ESR: erythrocyte sedimentation rate

PCT: procalcitonin

TNF- α : tumor necrosis factor α

IFN- γ : interferon-gamma

PSI: pneumonia severity index

APACHE II: acute physiology and chronic health evaluation II

Declarations

7.1 Ethics approval and consent to participate

The clinical trial protocol was evaluated and approved by the ethics review committee of the First Affiliated Hospital of Guangdong Pharmaceutical University.

Written informed consent form will be obtained from participants. This trial will be conducted based on the principles of the Helsinki Declaration (Fortaleza, 2013) and the International Ethical Guidelines for Research Involving Human Health (Geneva, 2016).

7.2 Consent for publication

Not applicable.

7.3 Availability of data and materials

Not applicable.

7.4 Competing interests

Not applicable.

7.5 Funding

This trial will be supported by the Special Research Project on Prevention and Control of COVID-19 in Universities of Guangdong Provincial Education Department (No. 2020KZDZX1132). The funders have no role in study design, data collection and management, interpretation of data, writing of the report, and the decision to submit the report for publication.

7.6 Authors' contributions

LHW and ZNY were responsible for the design and writing of this protocol. PP was responsible for providing relevant information of COVID-19. WRX, JTX and XYZ were responsible for providing relevant information of WMT. HHXX and XXH were responsible for amending this protocol. XXH was responsible for the funding application of this study. All authors read and approved the manuscript.

7.7 Acknowledgements

Not applicable.

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Tables

Table 1: Record of laboratory indicators

Visit	1	2	3	4	5	6 or PPW
Day						
COVID-19 nucleic acid test						
Fasting blood glucose						
Muscle enzyme						
D-dimer						
Myoglobin						
C-reactive protein						
Erythrocyte sedimentation rate						
Procalcitonin						
Interleukin-2						
Interleukin-6						
Tumor necrosis factor α						
Interferon γ						
Red blood cell count						
Hematocrit						
Mean corpuscular volume						
Red blood cell distribution width						
Hemoglobin concentration						
Mean corpuscular hemoglobin content						
Mean corpuscular hemoglobin concentration						
White blood cell count						
Monocyte count						
Monocyte ratio						
Neutrophil count						
Neutrophil ratio						
Lymphocyte count						
Lymphocyte ratio						
Platelet count						

Platelet volume distribution width
Mean platelet volume
Platelet larger cell ratio
Platelet crit
Serum potassium
Serum sodium
Serum chlorine
Serum calcium
Serum inorganic phosphorus
Serum magnesium
Serum iron
Serum zinc
Serum copper
Serum mercury
Serum Selenium
Alanine aminotransferase
Glutamic oxaloacetylase
Alkaline phosphatase
Glutamyl transpeptidase
Total protein
Albumin
Globulin
Total bilirubin
Indirect bilirubin
Direct bilirubin
Alpha fetoprotein
Blood urea
Blood creatinine
Blood β 2-microglobulin

Blood uric acid
Lipopolysaccharide
D-lactate
Diamine oxidase
Oxygenation index
pH degree
Partial pressure of carbon dioxide
Total carbon dioxide
Partial pressure of oxygen
Arterial oxygen content
Oxygen saturation
Actual carbonate
Base excess
Alveolar-arterial oxygen partial pressure difference
Hematocrit
Carboxyhemoglobin
Methemoglobin
Anion gap

PPW = Premature patient withdrawal

Table 2: Record of clinical indicators

Visit	1	2	3	4	5	6 or PPW
Day						
Temperature						
Lung shadow absorption						
Cough						
Fatigue						
Nasal congestion						
Pharyngodynia						
WMT						
Diarrhea						
Abdominal pain						
Abdominal distention						
Nausea						
Vomiting						
Gastrointestinal hemorrhage (upper or lower)						

PPW = Premature patient withdrawal

Table 3: Record of telephone follow-up

Follow-up	1	2	3
Day (after discharge)			
Temperature			
Cough			
Expectoration			
Fatigue			
Nasal congestion			
Pharyngodynia			
Diarrhea			
Abdominal pain			
Abdominal distention			
Nausea			
Vomiting			
Gastrointestinal hemorrhage (upper or lower)			

Figures

TIMEPOINT**	STUDY PERIOD									
	Enrolment	Allocation	Post-allocation						Close-out	
	0	0	1D	2D	3D	1W	2W	1W after discharge	2W after discharge	6M after discharge
ENROLMENT:										
Eligibility screen	X									
Informed consent	X									
Allocation		X								
INTERVENTIONS:										
[Routine treatment]
[WMT]
ASSESSMENTS:										
[Laboratory indicators (COVID-19 nucleic acid test, fasting blood glucose, muscle enzyme, D-dimer, electrolytes, myoglobin, C-reactive protein, erythrocyte sedimentation rate, procalcitonin, peripheral blood lymphocyte subsets, oxygenation index, blood gas analysis, blood routine tests, liver function indicators, renal function indicators, inflammatory cytokines such as	X		X			X	X			
[Clinical indicators (fever, gastrointestinal symptoms, etc.)]	X		X			X	X	X	X	X
[WMT-related adverse events (abdominal pain, abdominal distention, diarrhea, etc.)]

Figure 1

Schedule of enrollment, interventions, assessments and follow-up.

Figure 2

Primary and secondary outcomes, expected results and their clinical implications in the proposed study. Group A will be treated with routine treatment, while group B will be treated with WMT on the basis of routine treatment. “ $A = B$ ” means that the value of group A is not different from that of group B in the same observation indicators. “ $A < B$ ” means that the values of the same observation indicators in group B are closer to normal values than those in group A, and the recovery time is less than that in group A.

Supplementary Files

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