Study Protocol and Statistical Analysis Plan

Official title: Fecal Microbiota Transplantation in SAP (Severe Acute Pancreatitis) Patients With Intestinal Barrier Dysfunction

NCT number: NCT02318134

Document date: November 15, 2017

Introduction

Acute pancreatitis (AP) is a acute inflammatory disease of the pancreas. The incidence of AP ranges from 5 to 30 cases per 100,000, with an overall case fatality rate of 5%[1]. Approximately 80% cases of AP are mild and self-limited, and about 20% of patients suffer a severe disease course with persistent organ failure and/or infected pancreatic necrosis (IPN), with a mortality risk as high as 20~30%[1]. Gastrointestinal tract is considered not only as a target organ during systemin inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS), but also a “motor organ” of gut-derived infection[2]. Gastrointestinal dysfunction has been proven to be associated with adverse outcomes in AP[3]. Although the mechanisms underlying gastrointestinal dysfunction in the early phase of AP is complicated, early gastrointestinal barrier dysfunction was considered to be the main cause[4,5]. Gastrointestinal microbiota dysbiosis induces the injury of biologic barrier, which plays a key role in the pathogenic of gut-driven infection. Therefore, the maintenance of gastrointestinal microecology balance may be an effective method in treating gastrointestinal dysfunction, preventing gut-driven infection and improve clinical outcomes in AP.

Probiotics, as an adjunct to enteral nutrition, has long been studied as a measure to improve intestinal barrier dysfunction and prevent secondary infection in AP. Several clinical studies have assessed the effect of probiotics prophylaxis with contradictory results, and significant heterogeneity was noted between the trials with regard to the

type, dose and treatment duration of probiotics[6-11]. A multicenter, double-blind, placebo-controlled trial have failed to show a beneficial effect of probiotic prophylaxis on the occurrence of infectious complications, and mortality in the probiotics group was about twice as high as in the placebo group[8]. The current data are not sufficient to draw a conclusion regarding the effects of probiotics on patients with predicted severe acute pancreatitis (SAP). Consequently, there is a clear need for other innovative strategies.

Worldwide, interest in fecal microbiota transplantation (FMT) as an 'ecological' therapy for several diseases is growing rapidly. FMT presents another and more comprehensive approach to microbiota restoration. FMT consists of administering fecal material from a healthy donor into the intestinal tract of a patient. Unlike the few bacterial strains included in probiotics, FMT stool includes practically all the bacteria, viruses, eukaryotes, and metabolites from the healthy donor. In recurrent Clostridium difficile infections, FMT has shown excellent effects by restoring intestinal microbiota balance[12]. Several cases reported that patients with SIRS and severe diarrhea of unclear etiology persistently unresponsive to broad-spectrum antibiotics rapidly improved with FMT in the Intensive Care Unit (ICU). The improvements in clinical parameters were also associated with shifts in the recipients' microbiota patterns toward that of the donors', further attributing the patients' recovery to FMT[13-15]. FMT could therefore theoretically be a possible treatment for AP patients with gastrointestinal dysfunction. No randomized controlled study evaluating FMT in AP patients has been published to date.

Here, we performed the randomized, single-blind, parallel-group, placebo-controlled study to clarify the effects of FMT on gastrointestinal dysfunction and infectious complications, as well as gut microbiota in patients with AP.

Methods

This is a randomized, single-blind, parallel-group, placebo-control, single-center study in consecutive adults diagnosed with acute pancreatitis complicated with gastrointestinal failure in the setting of ICU. Eligible participants were randomized 1:1 to receive fecal transplant or normal saline via a nasoduodenal tube for twice (once

every two days). The primary end point the recovery of gastrointestinal dysfunction assessed by gastrointestinal failure (GIF) score one week after intervention.

Trial organization, committees and boards

The designer of this trial is the First Affiliated Hospital of Nanchang University, China (http://www.cdyfy.com/). This study was investigator-initiated and investigator-driven and done in accordance with the principles of the Revised Declaration of Helsinki. The study protocol was approved by the institutional review boards of the First Affiliated Hospital of Nanchang University (approval No, 2014 [32]). Written informed consent was obtained from all participants or their legal representatives. All physicians involved in the study will repetitively be asked to report any potential adverse events. All possible adverse events will be listed and reported to the institutional review boards of the First Affiliated Hospital of Nanchang University.

Study population

Adult patients with AP were recruited from the ICU, Department of Gastroenterology, First Affiliated Hospital of Nanchang University. The diagnosis of AP requires two of the following three features: 1) upper abdominal pain; 2) serum lipase or amylase activity at least three times greater than the upper limit of normal; and 3) characteristic findings of AP on contrast-enhanced computed tomography (CECT), magnetic resonance imaging (MRI) or transabdominal ultrasonography[16].

Inclusion criteria

1) Aged 18-70 year;
2) Onset of pancreatitis within 2 weeks;
3) Complicated with gastrointestinal failure, which was defined if the patients were complicated with obvious abdominal distention, abdominal rumbling sound weakening or disappearance, no self-defecation as well as intra-abdominal hypertension (IAH).

Exclusion criteria

1) Complicated by gastrointestinal bleeding or intestinal fistula;
2) Pregnancy and lactation women;
3) Not signed the informed consent;

4) Diabetes and autoimmune disease;
5) Multiple organ failure, which was defined as two or more organ failure including respiratory failure, renal failure or circulatory failure, which was defined as a score of 2 or more using the modified Marshall scoring system[17].

Sample size

To detect a minimal clinically relevant difference of 35% between FMT group and control group in the primary outcome, and estimating that FMT would achieve 85% power and control would achieve 50% power and using a type I error of 0.05 and a type II error of 0.20 (80% power), we calculated that a minimal sample size of 48 with 24 patients in each group was required. To allow for dropouts, we included 60 participants with 30 in FMT group and 30 in control group.

Randomization and masking

A randomization sequence for 60 participants with an allocation ratio of 1:1 was generated using SPSS by an independent statistician who not involved in the clinical performance of the trial. The method of allocation concealment was sequentially numbered sealed opaque envelopes technique. Each participant got a study number at enrolment. An allocator (non-study personnel) then allocated participants to FMT or control group by the randomization sequence. This was done in a closed room and the allocation sequence were immediately disposed of. Investigators, patients and outcome assessors were kept masked to the allocation. The randomization key was revealed to researchers when all participants completed the 6-month follow-up and data analysis was completed.

Study duration

The planned starting data of the study is December 2017, and the planned finishing data is December 2018.

Primary Endpoint

The primary end point was the recovery of gastrointestinal dysfunction assessed by GIF score one week after intervention[18]. The normal gastrointestinal function assessed by GIF score means enteral feeding >50% of calculated needs, without FI and IAH. FI was defined when applied enteral feeding appeared to be unsuccessful

and had to be discontinued because of repeated or profuse vomiting, high gastric residuals, ileus, severe diarrhea, abdominal pain, or distension. FI should not be considered as present if enteral feeding is electively not prescribed or is withheld/interrupted due to procedures. IAP was measured by the standardized methodology (preferably the transvesical method, with a maximal instillation of 25 cc of saline in the supine position, measured at the end of expiration, with zeroing done at the level of the midaxillary line). IAH was present if the IAP was found to be 12 mmHg or higher, as confirmed by at least two measurements taken 1-6 h apart[19].

Secondary endpoints

The secondary end points were any infectious complication, OF, functional assessments of gut barrier and inflammatory indicators, hospital stay and mortality. Infectious complications included IPN, infected ascites, bacteremia, pneumonia, urinary tract infection. Microbiological data for each of the infectious complications were collected. OF was defined as a score of 2 or more using the modified Marshall scoring system, which was recommended by 2012 Atlanta Classification Creation[17]. Persistent organ failure, that is, organ failure >48 hours[16]. Blood samples were collected from all included patients before and one week after intervention. Gut barrier function was assessed by detecting serum D-Amino Acid Oxidase (DAO) and endotoxin using commercial kits (Cloud-Clone Corp, TX, USA for DAO and ELX800 (Bio Tek) for endotoxin). C-reactive protein (CRP) and procalcitonin (PCT) were measured by IMMAGE 800 (Beckman Coulter) and AFIAS-50 (JOINSTAR) separately. Stools were requested for microbiota analysis at baseline, one week and two week after intervention.

Microbiomics analysis

Fecal samples were longitudinally collected from patients at baseline before first FMT, one weeks and two weeks after first FMT. The fresh feces were collected in sterile cryopreservation tube by the nurses and kept at -20 ℃ immediately. Then, the feces were transferred within 6 hour after a bowel movement, and were stored at −80 °C until DNA extraction.

Bacterial DNA was extracted from fecal samples using the E.Z.N.A. Soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer's instructions. The final DNA concentration and purification were determined by NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA), and DNA quality was checked by 1% agarose gel electrophoresis. The V3-V4 hypervariable regions of the bacteria 16S rRNA gene were amplified with primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') by thermocycler PCR system (GeneAmp 9700, ABI, USA). The PCR reactions were conducted using the following program: 3 min of denaturation at 95℃, 27 cycles of 30 s at 95℃, 30 s for annealing at 55℃, and 45 s for elongation at 72℃, and a final extension at 72℃ for 10 min. PCR reactions were performed in triplicate 20 μL mixture containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase and 10 ng of template DNA. The resulted PCR products were extracted from a 2% agarose gel and further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and qualified using QuantiFluro-ST (Promega, USA).

Purified amplicons were pooled in equimolar and paired-end sequenced (2×300) on an Illumina MiSeq platform (Illumina, San Diego, USA). The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: SRP). The raw reads were demultiplexed and quality-filtered by Trimmonmatic and merged by FLASH software. The high-quality sequences were assigned to samples according to barcodes. Operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPAPSE (version 7.1, http://drive5.com/uparse/) and chimeric sequences were identified and removed using UCHIME. The taxonomy of each 16S rRNA gene sequencing was analyzed by RDP Classifier algorithm (http://rdp.cme.msu.edu/) against the Siva (SSU123) 16S rRNA database using confidence threshold of 70%.

Intervention

All participants underwent interventions one day after allocation. In the FMT group,

participants received 200 mL fresh donor feces for twice (once every two days). In the control group, participants received 200 mL normal saline. The fecal transplant or normal saline were drawn on 50 mL sterile sealed syringe and infused into each patients via a nasoduodenal tube, which was inserted into distal duodenum by endoscopy. The procedure was performed within 5 min at the bedside of pancreatic intensive care unit by a researcher who were not involved in the clinical performance of the trial. The patients were blinded to the identity of the intervention that they were receiving because either FMT or placebo were stored on a sealed syringe. Before FMT or normal saline, Laxative was stopped for at least one hour to retain stool. To assure maximum delivery and colonization of these fecal transplant, first we assured a at least 4-hour gap between study medication and antibiotics (if prescribed) or laxative. No probiotics or lactulose was used during hospitalization. Considering the feasibility, we altered the protocol (after registration at ClinicalTrials.gov, but before enrolment of participants) to implement FMT or normal saline via a nasoduodenal tube instead of via retention enema.

General treatment regimen

All participants received routine treatment at admission according to the AP guidelines, including goal-oriented fluid resuscitation, enteral nutrition as early as possible, organ support as needed. Antibiotic prophylaxis was not given routinely. The use of antibiotics was recorded, irrespective of indication. As all the patients enrolled was complicated with gastrointestinal failure, we used traditional Chinese medicine-rhubarb (and/or mirabilite) for all patients. Some other purge measures including gastrointestinal decompression or mannitol via nasoduodenal tube were conducted according to patients' conditions. Abdominal puncture and drainage were for patients with abdominal effusion.

Data collection

Data collection was prospectively entered by a researcher, who completed standardized case report forms. During the study an independent data monitor checked the individual patients' data against the primary source data. After double check of key variables by two researchers, data were exported unedited for statistical

analyses.

Follow up

Patients are followed during their hospital stay. There is one follow-up visit, 6 months after discharge by clinical visit or telephone visit, to assess readmission, mortality and adverse events.

Statistical analysis

Quantitative variables are presented as medians (ranges) or means ± standard deviations and were analysed using the Mann-Whitney U test or t-test as appropriate. Categorical variables are reported as absolute numbers and proportions and were tested by the chi-square test or Fisher's exact test as appropriate. The differences between outcomes for matching data were tested with the paired t test or Wilcoxon signed-rank test as appropriate. We estimated relative risks (RRs) and 95% confidence intervals (CIs) for primary outcomes and secondary outcomes. A two-tailed P value <0.05 was considered statistically significant. Data were analyzed using SPSS software (v17.0; SPSS Inc., Chicago, IL, USA).

References

1 Krishna SG, Kamboj AK, Hart PA, et al. The Changing Epidemiology of Acute Pancreatitis Hospitalizations: A Decade of Trends and the Impact of Chronic Pancreatitis. Pancreas 2017;46:482-8.

2 Van Leeuwen PA, Boermeester MA, Houdijk AP, et al. Clinical significance of translocation. Gut 1994;35:S28-34.

3 Sun JK , Li WQ , Ni HB , et al. Modified gastrointestinal failure score for patients with severe acute pancreatitis. Surgery Today 2013;43:506-13.

4 Huang C, Chen J, Wang J, et al. Dysbiosis of Intestinal Microbiota and Decreased Antimicrobial Peptide Level in Paneth Cells during Hypertriglyceridemia-Related Acute Necrotizing Pancreatitis in Rats. Front Microbiol 2017;8:776.

5 Chen J, Huang C, Wang J, et al. Dysbiosis of intestinal microbiota and decrease in paneth cell antimicrobial peptide level during acute necrotizing pancreatitis in rats. PLoS One 2017;12:e0176583.

6 Tan C, Ling Z, Huang Y, et al. Dysbiosis of Intestinal Microbiota Associated With Inflammation Involved in the Progression of Acute Pancreatitis. Pancreas 2015;44:868-75.

7 Olah A, Belagyi T, Issekutz A, et al. Randomized clinical trial of specific lactobacillus and fibre supplement to early enteral nutrition in patients with acute pancreatitis. Br J Surg 2002;89:1103-07.

8 Besselink MG, van Santvoort HC, Buskens E, et al. Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. Lancet 2008;371:651-9.

9 Oláh A, Belágyi T, Pótó L, et al. Synbiotic control of inflammation and infection in severe acute pancreatitis: a prospective, randomized, double blind study. Hepatogastroenterology 2007;54:590-4.

10 Plaudis H, Pupelis G, Zeiza K, et al. Early low volume oral synbiotic/prebiotic supplemented enteral stimulation of the gut in patients with severe acute pancreatitis: a prospective feasibility study. Acta Chir Belg 2012;112:131–8.

11 Gou S, Yang Z, Liu T, et al. Use of probiotics in the treatment of severe acute pancreatitis: a systematic review and meta-analysis of randomized controlled trials. Crit Care 2014;18:R57.

12 Kelly CR, Khoruts A, Staley C, et al. Effect of Fecal Microbiota Transplantation on Recurrence in Multiply Recurrent Clostridium difficile Infection: A Randomized Trial. Ann Intern Med 2016;165:609-16.

13 Li Q, Wang C, Tang C, et al. Therapeutic modulation and reestablishment of the intestinal microbiota with fecal microbiota transplantation resolves sepsis and diarrhea in a patient. Am J Gastroenterol 2014;109:1832-4.

14 Li Q, Wang C, Tang C, et al. Successful treatment of severe sepsis and diarrhea after vagotomy utilizing fecal microbiota transplantation: a case report Crit Care 2015;19:37.

15 Wei Y, Yang J, Wang J, et al. Successful treatment with fecal microbiota transplantation in patients with multiple organ dysfunction syndrome and diarrhea following severe sepsis. Crit Care 2016;20:332.

16 Banks PA, Bollen TL, Dervenis C, et al. Classification of acute pancreatitis—2012: revision of the Atlanta classification and definitions by international consensus. Gut 2013;62:102-11.

17 Marshall JC, Cook DJ, Christou NV, et al. Multiple organ dysfunction score: a reliable descriptor of a complex clinical outcome. Crit Care Med 1995;23:1638-52.

18 Reintam A, Parm P, Kitus R, et al. Gastrointestinal failure score in critically ill patients: a prospective observational study. Crit Care 2008;12:R90.

19 Malbrain ML, Cheatham ML, Kirkpatrick A, et al. Results from the International Conference of Experts on Intra-abdominal Hypertension and Abdominal Compartment Syndrome. I. Definitions. Intensive Care Med 2006 32:1722-32.