Trials

STUDY PROTOCOL

Open Access

Comparisons of efficacy and safety of 400 or 800 ml bacterial count fecal microbiota transplantation in the treatment of recurrent hepatic encephalopathy: a multicenter prospective randomized controlled trial in China

Pengfei Zou[1,2†], Yunjiao Bi[3†], Zhaowei Tong[4†], Tao Wu[5], Qiang Li[6], Kai Wang[7], Yuchen Fan[7], Dan Zhao[8], Xin Wang[9], Hui Shao[10], Haijun Huang[11], Suping Ma[12], Yunsong Qian[13], Guoqiang Zhang[14], Xiao Liu[15], Qiaofei Jin[16], Qingjing Ru[17], Zhiping Qian[18], Wei Sun[19], Qiang Chen[20], Liying You[21], Fang Wang[22], Xiaoting Zhang[23], ZhenXiong Qiu[24], Qing Lin[25], Jiaojian Lv[26], Yongping Zhang[27], Jiawei Geng[28], Richeng Mao[29], Jinfeng Liu[30], Yubao Zheng[31], Feng Ding[32], Hui Wang[33] and Hainv Gao[1,2*]

Abstract

Background Hepatic encephalopathy (HE) represents a critical complications of end-stage liver disease, serving as an independent predictor of mortality among patients with cirrhosis. Despite effective treatment with rifaximin, some patients with HE still progress to recurrent episodes, posing a significant therapeutic challenge. Recurrent HE is defined as experiencing two or more episodes within a 6-month period. Previous research has suggested that FMT may emerge as a promising treatment for recurrent HE. However, there remains a critical need to explore the optimal dosage. This trial aims to abscess the efficacy and safety of two FMT dosages: 800 ml or 400 ml total bacterial count, including mortality and quality of life.

Methods This multicenter, prospective, randomized controlled trial will enroll 100 eligible patients from 31 hospitals in China. Participants will be randomly assigned in a 1:1 ratio to either the high-dose group (800 ml total bacterial count) or the low-dose group (400 ml total bacterial count). The primary objective is to assess the efficacy and safety of both dosages on outcomes at 24 and 48 weeks, including mortality and quality of life.

Discussion If either or both dosages of FMT demonstrate safe and effective treatment of recurrent HE, leading to improve quality of life and survival at 24 and 48 weeks, this trial would address a significant gap in the management of recurrent HE, carrying innovative and clinically significant implications.

Trial registration NCT05669651 on ClinicalTrials.gov. Registered on 29 December 2022. CHiCTR2200067135 on China Registered Clinical Trial Registration Center. Registered on 27 December 2022.

Keywords Fecal microbiota, Transplantation, Recurrent hepatic encephalopathy

†Pengfei Zou, Yunjiao Bi and Zhaowei Tong contributed equally to this work.

*Correspondence:
Hainv Gao
hainv.gao@shulan.com
Full list of author information is available at the end of the article

Administrative information

Note: the numbers in curly brackets in this protocol refer to SPIRIT checklist item numbers. The order of the items has been modified to group similar items (see http://www.equator-network.org/reporting-guidelines/spirit-2013-statement-defining-standard-protocol-items-for-clinical-trials/).

Title {1}	Comparisons of Efficacy and Safety of 400 or 800 ml bacterial count Fecal Microbiota Transplantation in the Treatment of Recurrent Hepatic Encephalopathy: A Multicenter Prospective Randomized Controlled trial in China
Trial registration {2a and 2b}.	NCT05669651 on ClinicalTrials.gov. Registered on 29 December 2022. CHiCTR2200067135 on China Registered Clinical Trial Registration Center. Registered on 27 December 2022.
Protocol version {3}	Version 2.0, dated on 17 September 2022
Funding {4}	National Key Research and Development Program of China(2021YFA1301104) Research Project of Jinan Microecological Biomedicine Shandong Laborator
Author details {5a}	Pengfei Zou[1,2a],Yunjiao Bi[3a], Zhaowei Tong[4a], Tao Wu[5], Qiang Li[6], Kai Wang[7], Yuchen Fan[7], Dan Zhao[8], Xin Wang[9], Hui Shao[10], Haijun Huang[11], Suping Ma[12], Yunsong Qian[13], Guoqiang Zhang[14], Xiao Liu[15], Qiaofei Jin[16], Qingjing Ru[17], Zhiping Qian[18], Wei Sun[19], Qiang Chen[20], Liying You[21], Fang Wang[22], Xiaoting Zhang[23], ZhenXiong Qiu[24], Qing Lin[25], Jiaojian Lv[26], Yongping Zhang[27], Jiawei Geng[28], Richeng Mao[29], Jinfeng Liu[30], Yubao Zheng[31], Feng Ding[32], Hui Wang[33], Hainv Gao[1,2*] Pengfei Zou, Yunjiao Bi and Zhaowei Tong contributed equally to this work. [1]Shulan (Hangzhou) Hospital Affiliated to Zhejiang Shuren University Shulan International Medical Collegey, [2]Jinan Microecological Biomedicine Shandong Laboratory, [3]Zhejiang Chinese Medical University, Hangzhou, China, [4]Huzhou Central Hospital, [5]Hainan General Hospital Branch—Ding'an Hospita, [6]Shandong Public Health Clinical Center, [7]*Department of Hepatology, Qilu Hospital of Shandong University*, [8]Zhengzhou Third People's Hospital, [9]Shulan (Quzhou) Hospital, [10]Taizhou Hospital of Zhejiang, [11]Zhejiang Provincal People's Hospital, [12]The First Affiliated Hospital of Henan University of CM, [13]Ningbo No.2 Hospital, [14]Luoyang Central Hospital, [15]Ningbo Zhenhai District People's Hospital, [16]Hangzhou Xixi Hospital Affiliated to Zhejiang University School of Medicine, [17]The Second Affiliated Hospital of Zhejiang Chinese Medical University, [18]Shanghai Public Health Clinical Center, [19]*The First Affiliated Hospital of Soochow University*, [20]*Zouping People's Hospital*, [21]*ALMETTE Hospital &The first hospital of kunming*, [22]*Zoucheng People's Hospital*, [23]*Shenzhen Bao'an District Songgang People's Hospital*, [24]*Baoan Central Hospital of Shenzhen*, [25]*The people's hospital of Jiulongpo district,Chongqing,China*, [26]*Lishui People's Hospital*, [27]*People's Hospital of Xinjiang Uygur Autonomous Region*, [28]*Department of Infectious Disease and Hepatic Disease, First People's Hospital of Yunnan Province, Affiliated Hospital of Kunming University of Science and Technology, Kunming, Yunnan, China*, [29]*Department Of Infectious Diseases, Huashan Hospital, Fudan University*, [30]*The First Affiliated Hospital of Xi'an Jiaotong University*, [31]*Department of Infectious Diseases, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China*, [32]*Affiliated Hospital of Shaoxing University*, [33]*Department of Infectious Diseases, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine.*
Name and contact information for the trial sponsor {5b}	Hainv Gao. Shulan (Hangzhou) Hospital Affiliated to Zhejiang Shuren University Shulan International Medical College, Jinan Microecological Biomedicine Shandong Laboratory. E-mail: gaohainv@163.com
Role of sponsor {5c}	Hainv Gao all contributed to the design and management of this study. Funders do not participate in this study.

Introduction

Background and rationale {6a}

Cirrhosis is a significant cause of morbidity and mortality globally, particularly among individuals with chronic liver disease (CLD), thus posing a substantial burden on the world's health systems. Hepatic encephalopathy (HE) is one of the most debilitating complications of CLD [1], and its occurrence is associated with decreased survival rates among cirrhotic patients [2]. HE is characterized by a spectrum of potentially reversible neuropsychiatric abnormalities that lead to relapsing–remitting mental status changes [3, 4]. Although the combination of rifaximin and lactulose is commonly used as a secondary preventive measure for HE, a significant number of patients still experience breakthrough HE attacks. Some cases of HE may even progress to recurrent HE, defined as two or more episodes of HE within a 6-month period [5]. However, there is currently no effective treatment available for recurrent HE except liver transplantation. Therefore, it is imperative to explore additional treatment strategies to address this unmet medical need.

The pathogenesis of HE remains elusive, with ammonia wildly believed to play a central role. Recent studies have indicated that intestinal flora disorder significantly contributes to the development and recurrence of HE [6]. The therapeutic role of microbiota in HE has undergone a transformative shift, driven by several groundbreaking trials and microbiology advancements [7]. Microbiome therapies encompass probiotics, synbiotics, postbiotics, and fecal microbiota transplant (FMT), etc. [7]. FMT involves transferring processed stool from a healthy donor to a recipient, offering a potential novel treatment option for recurrent HE. However, the optimal dosing of FMT for HE treatment remains unclear, and larger trials are necessary. Therefore, we conducted a multicenter, prospective, randomized controlled study to compare the efficacy and safety of 400 and 800 ml bacterial count FMT at 24 and 48 weeks post-treatment, focusing on mortality rates and quality of life.

Objectives {7}

We hypothesize that the utilization of 800 ml total bacterial count FMT will demonstrate superior to 400 ml total bacterial count FMT in the treatment of recurrent HE. The primary objective of this study is to comprehensively evaluate and compare the efficacy and safety of 800 ml and 400 ml total bacterial count FMT on outcomes including mortality rates and quality of life at 24 and 48 weeks post-treatment.

Trial design {8}

This multicenter, prospective, and randomized controlled trial will take place across 31 centers in China. A total of 100 eligible participants with recurrent HE refractory to rifaximin and lactulose treatment will be randomly assigned to either the high-dose group or the low-dose group in a 1:1 ratio. The flowchart of the trial is outlined in Fig. 1.

Methods: participants, interventions, and outcomes

Study setting {9}

A total of 100 individuals with recurrent HE will be recruited from 31 hospitals throughout China: (1) Shulan (Hangzhou) Hospital Affiliated to Zhejiang Shuren University Shulan International Medical College, (2) Huzhou Central Hospital, (3) Hainan General Hospital Branch—Ding'an Hospital, (4) Shandong Public Health Clinical Center, (5) Department of Hepatology, Qilu Hospital of Shandong University, (6) Zhengzhou Third People's Hospital, (7) Shulan (Quzhou) Hospital, (8) Taizhou Hospital of Zhejiang, (9) Zhejiang Provincal People's Hospital, (10) the First Affiliated Hospital of Henan University of CM, (11) Ningbo No. 2 Hospital, (12) Luoyang Central Hospital, (13) Ningbo Zhenhai District People's Hospital, (14) Hangzhou Xixi Hospital Affiliated to Zhejiang University School of Medicine, (15) the Second Affiliated Hospital of Zhejiang Chinese Medical University, (16) Shanghai Public Health Clinical Center, (17) the First Affiliated Hospital of Soochow University, (18) Zouping People's Hospital, (19) ALMETTE Hospital & the First Hospital of Kunming, (20) Zoucheng People's Hospital, (21) Shenzhen Bao'an District Songgang People's Hospital, (22) Baoan Central Hospital of Shenzhen, (23) the People's Hospital of Jiulongpo District, Chongqing, China, (24) Lishui People's Hospital, (25) People's Hospital of Xinjiang Uygur Autonomous Region, (26) Department of Infectious Disease and Hepatic Disease, First People's Hospital of Yunnan Province, Affiliated Hospital of Kunming University of Science and Technology, Kunming, Yunnan, China, (27) Department Of Infectious Diseases, Huashan Hospital, Fudan University, (28) the First Affiliated Hospital of Xi'an Jiaotong University, (29) Department of Infectious Diseases, the Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China, (30) Affiliated Hospital of Shaoxing University, and (31) Department of Infectious Diseases, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine.

Eligibility criteria {10}

Eligible patients for this study must meet the following criteria: (1) age between 18 and 75 years at the time of enrollment; (2) have HE in remission upon enrollment; (3) have experienced at least two episodes of significant HE (West Haven grade 2 or higher) associated with liver cirrhosis within the past 6 months; episodes of HE caused by blood transfusions (at least 2 units), sedative drug use, renal failure requiring dialysis, or central nervous system injury do not count towards this criterion; (4) have a Model for End-Stage Liver Disease (MELD) score of 25 or below; (5) be medically suitable for FMT delivery via the nasal jejunal tube; and (6) provide signed informed consent from the participant (or their legal guardian).

The exclusion criteria are outlined as follows: (1) patients anticipated to undergo liver transplantation within 1 month; (2) patients who have had known causes of HE, including gastrointestinal bleeding and portosystemic shunt or transjugular intrahepatic portosystemic shunt placement within three months; (3) patients with chronic kidney disease (creatinine level > 2.0 mg/dl), respiratory failure, anemia (Hb < 8 g/dl), and electrolyte disorders (serum sodium < 125 μmol/L; serum calcium > 10 mg/dl [2.5 μmol/L]; or serum potassium of < 2.5 mmol/l); (4) patients who are not active drinkers, defined as consuming alcohol in excess of 140 g/week for women and 210 g/week for men; (5) use of drugs that can affect the Psychometric Hepatic Encephalopathy Score (PHES) within the last 4 weeks, such as antidepressants and sedative-hypnotics; (6) patients who are allergic to antibiotics prior to FMT treatment; (7) patients with existing infection before FMT (pathogens acquired through sterile sites); (8) patients diagnosed with chronic gastrointestinal disease, including inflammatory bowel disease (such as ulcerative colitis, Crohn's disease, or microscopic colitis) as well as those with irritable bowel syndrome; (9) patients who have neurological disorders, including stroke, epilepsy, dementia, and Parkinson's disease; (10) patients with uncontrolled malignant liver tumor or malignant tumors of other organs; (11) women who are pregnant or breastfeeding (confirmed through a urinary pregnancy test); (12) patients who refuse to sign the informed consent form; (13) patients who are unwilling or unable to undergo the nasal jejunal tube procedure; (14) patients have other conditions that do not meet the inclusion criteria in the trial.

Who will take informed consent? {26a}

Prior to screening, potentially eligible patients will undergo a thorough explanation of the trial's purpose, procedure, potential risks, and benefits. This ensures that they have a comprehensive understanding of the trial before proceeding. Eligible patients who wish to participate will be required to sign two informed consents forms, one to be retained by the researcher and the other by the patient themselves.

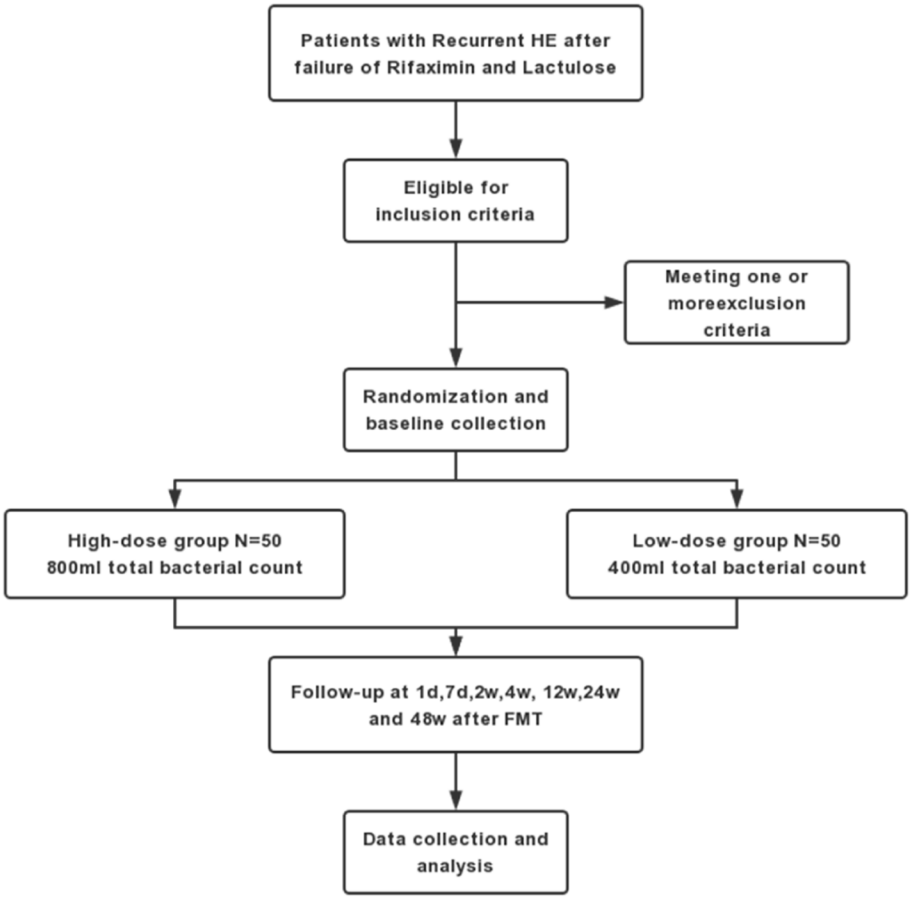

Fig. 1 Flowchart of the clinical trial

Additional consent provisions for collection and use of participant data and biological specimens {26b}

The consent form approved by the patients includes their consent to utilize the data in subsequent, pertinent research studies. Furthermore, participants will be requested to authorize the research team to share pertinent data with individuals affiliated with the universities involved in the research or with regulatory bodies, whenever necessary.

Interventions

Explanation for the choice of comparators {6b}

While receiving rifaximin and lactulose treatment, patients will be allocated to either the high-dose or low-dose FMT group. Specifically, the high-dose group will receive a total bacterial count of 800 ml, whereas the low-dose will receive 400 ml. It is noteworthy that the efficacy of the low-dose was established in our pilot study, which will serve as the control arm in this investigation.

Intervention description {11a}

Single donor via upper gastrointestinal route FMT

Subjects will be randomly assigned to receive either a high-dose (800 ml) or low-dose (400 ml) of total bacterial count FMT. Prior to the transplantation, all patients will undergo *bowel preparation*, consisting of amoxicillin 0.5 g twice daily, metronidazole 0.4 g twice daily, and levofloxacin 0.5 g once daily for three consecutive days. The *high-dose group* will receive 800 ml of total bacterial count, divided into 100 ml aliquots administered twice daily for 4 days. The *low-dose group* will receive 400 ml of total bacterial count, divided into 100 ml aliquots administered once daily for 4 days. Both groups will continue their routine medical treatment, including rifaximin, lactulose, aspartate ornithine, albumin, and diuretics, as necessary.

Follow-up appointments are scheduled at 1 day, 7 days, 2 weeks, 4 weeks, 12 weeks, 24 weeks, and 48 weeks post-FMT. Additionally, telephone follow-ups will be conducted at 24 and 48 weeks to assess patient outcomes.

Criteria for discontinuing or modifying allocated interventions {11b}

The criteria for discontinuing or modifying allocated interventions are outlined below: (1) the participants request withdrawal; (2) the investigators consider it inappropriate to continue for safety concerns. In the event of a participant's withdrawal, the study assistant will conduct a follow-up interview to elicit the reasons for their departure and gather any pertinent safety data. All information collected will be comprehensively documented in the case report forms (CRFs). If a participant experiences an adverse event leading to study discontinuation, the clinician will evaluate the individual's condition and determine if further treatment is warranted.

Strategies to improve adherence to interventions {11c}

To enhance adherence to interventions during the study, the following strategies will be employed: (1) free FMT services—FMT will be offered free of charge to participants; (2) appointment reminders—research assistants will assist patients in scheduling their follow-up visits and remind them of their appointments via phone or WeChat prior to the scheduled date; (3) diary cards for adverse reaction assessment—participants will be provided with diary cards primarily for the purpose of recording any adverse reactions they experience. They will be instructed to fill out the cards daily and present them to the research assistants for review during each visit.

Relevant concomitant care permitted or prohibited during the trial {11d}

During the trial, relevant concomitant care or interventions, apart from routine medical therapy, will be determined by the discretion of physicians based on clinical practice guidelines.

Provisions for post-trial care {30}

We have secured insurance coverage for this study, ensuring that participants will be compensated for any injuries incurred during their participation, in accordance with the pertinent laws of China.

Outcomes {12}

Main outcome

The *primary endpoint* of this study is to assess the effectiveness of FMT in treating recurrent hepatic encephalopathy. Specifically, we define the first breakthrough episode of hepatic encephalopathy as the onset of HE at or above the West Haven grade 2 within 12 weeks following FMT. Patients who experience a breakthrough attack are considered to have responded ineffectively to the treatment, while those who do not experience such an attack are deemed to have responded effectively. *Secondary endpoints* are as follows: (a) serious adverse events related to FMT—the severity of FMT will be graded using *the* Common Terminology Criteria for Adverse Events (*CTCAE*—Version *5.0*); evaluation will occur on days 1, 2, 3, and 4 of FMT as well as on days 1, 7, 14, and 28 post-FMT; serious adverse events refer to the events requiring hospitalization, prolonged hospitalization, result in disability, affect work ability, pose a life-threatening risk, or lead to congenital malformations; (b) non-serious adverse events related to FMT—these events, which do not meet the criteria for serious adverse events, will also be graded using the CTCAE—Version 5.0; (c) time to first episode of hepatic encephalopathy requiring hospitalization post-FMT—this endpoint will be determined based on West Haven grade 2 or higher and the need for hospitalization; (d) Psychometric Hepatic Encephalopathy Score (PHES)—PHES is a validated assessment tool designed specifically for HE trials; it measures cognitive and psychomotor processing speed as well as visuomotor coordination; consisting of three pencil-and-paper tests, it can be completed in 15 to 20 min; following the intervention, comparisons will be drawn at 4 and 12 weeks post-intervention with baseline scores as well as across varying bacterial counts in FMT; (e) changes in microbial ecology—by using metagenome sequencing, comparisons will be made at 1 day, 4 weeks, and 12 weeks post-intervention against baseline and also across varying bacterial counts; these comparisons will assess changes in the abundance and diversity of implanted bacteria before and after FMT; (f) long-term outcome—the study will record the long-term effects on mortality and quality of life at 24 and 48 weeks.

Participant timeline {13}

The participant timeline is shown in Fig. 2.

Sample size {14}

Our previous findings indicated that FMT achieved an impressive 50% effectiveness rate in treating recurrent hepatic encephalopathy. To determine the necessary sample size, we employed the standard formula: $N=(U_\alpha+U_\beta)^2 2P(1-P)/(P_1-P_0)^2$, where U_α and U_β correspond to the U value for α and β respectively. Given $\alpha=0.05$ and $\beta=0.1$, we obtained $U_\alpha(0.05)=1.65$ and $U_\beta(0.1)=1.28$ from the normal distribution fractal table. Here, P_0 represent the original effectiveness of 50%, while P_1 represents the desired effectiveness of 70%. Using these values, we computed a sample size of 46 patients per group. Taking into account a potential shedding rate of 10%, we required 50 cases per group, totaling 100 cases for this study.

Recruitment {15}

Recruitment posters for the study will be displayed at all twenty-five trial centers. Additionally, recruitment advertisements will be circulated on the official WeChat accounts of participating hospitals, ensuring a comprehensive outreach effort to reach our desired target sample size.

Assignment of interventions: allocation

Sequence generation {16a}

Eligible participants will be randomly assigned at a 1:1 ratio to either the high-dose group or the low-dose group. The Statistical Analysis System 9.4(SAS 9.4) "Proc Plan" program has been used to generate the random scheme.

Concealment mechanism {16b}

The randomization process will be performed by a statistical expert independent of the research team. The treatment options will be sequentially placed in light-tight, sealed envelopes based on the random assignments. Group information will not be available until the randomization process is completed, ensuring blinding and minimizing bias.

Implementation {16c}

The randomization list will be administered by a designated randomization manager who will not participate in any other study procedures. This arrangement ensures an unbiased and reliable allocation process, minimizing any potential influence or interference in the randomization procedure.

Assignment of interventions: blinding

Who will be blinded {17a}

The participant, care provider, and outcome assessor are blinded. The trial statistician will be blinded when performing the blinded sample size review but will be unblinded for the final analysis.

Procedure for unblinding if needed {17b}

N/A. Investigators are not blinded; unblinding is not necessary.

Data collection and management

Plans for assessment and collection of outcomes {18a}

Investigators will enter the required data into the case report forms (CRF) according to the protocol. Baseline data, including demographics, clinical diagnosis, and details of HE, will be collected after participants have signed the informed consent form. Post-FMT follow-up will involve phone calls or communication via WeChat.

Plans to promote participant retention and complete follow-up {18b}

To ensure participant retention and complete follow-up, participants will receive free FMT. Additionally, investigators will proactively remind participants of their scheduled follow-up appointments via phone calls or WeChat.

Data management {19}

Data will be collected in CRF, including demographic characteristics, symptoms, signs, laboratory tests, medi-
Data will be collected in CRF, including demographic characteristics, symptoms, signs, laboratory tests, medication, and complications. The principal investigator is responsible for assuring that data entered into the CRF is both comprehensive and precise, ensuring timely data entry. The signature of the investigator serves as confirmation of the data's accuracy on each CRF. All data collected during the study will be kept for 3 years following the conclusion of the study, to ensure its availability for future reference and analysis.

Confidentiality {27}

Participants will be allocated an individual trial identification number by random envelope. The data will be saved on Shulan (Hangzhou) Hospital Research Management System, which will be checked again by the principal investigator at the end. Data access will be strictly limited to the designated study researchers. Any information collected during the trial will be treated with utmost confidentiality. The information will be used exclusively for this study and will not be utilized for any other purpose.

Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in this trial/future use {33}

The collected samples will be securely stored at the Shulan (Hangzhou) Hospital post-intervention and follow-up; the samples will be sent to an analytical institution for detailed analysis.

Statistical methods

Statistical methods for primary and secondary outcomes {20a}

The baseline characteristics and clinical outcomes will be presented as numerical counts (percentages) for categorical variables and mean ± standard deviation for continuous variables. Categorical variables will undergo analysis using the chi-square test or Fisher's exact test, depending on the data's characteristics. Continuous variables will be analyzed using either the independent *t*-test or

TIMEPOINT	STUDY PERIOD												
	Enrolment	Allocation	Post-allocation										
	$-t_1$	0	t_1	t_2	t_3	t_4	t_5	t_6	t_7	t_8	t_9	t_{10}	t_{11}	t_{12}
ENROLMENT:														
Eligibility screen	X													
Informed consent	X													
Allocation		X												
INTERVENTIONS:														
bowel preparation			X											
High-dose FMT				←	—	—	→							
Low-dose FMT				←	—	—	→							
ASSESSMENTS:														
PHES score*												X		
MELD score*												X		
Child-Pugh classification												X		
Fibrosis-4 index												X		
West-Haven criteria												X		
Mortality											X	X	X	X
Adverse events				X	X	X	X	X	X	X	X			
Stool sample	X							X			X	X		
Blood sample	X										X	X		

Fig. 2 Flowchart of the trial for the schedule of enrolment, interventions, and assessments. t_1, day − 28 to day 0, HE in remission at the time of enrollment; t_1, antibiotic pretreatment: amoxicillin, metronidazole, and levofloxacin for 3 days; t_2–t_5, FMT for 4 days; t_6–t_{12}, 1 day, 7 day, 2 weeks, 4 weeks, 12 weeks, 24 weeks, and 48 weeks after FMT. Telephone follow-up would be performed at t_{11}–t_{12}. The asterisk "*" symbol indicates the following: Psychometric Hepatic Encephalopathy Score (PHES), Model for End-Stage Liver Disease (MELD) score

the Wilcoxon signed-rank test, depending on their distribution. The Kaplan–Meier method will be performed to generate survival curves, and the log-rank analysis will be used to compare the differences between survival curves. Multivariate Cox regression, adjusted by centers, will be applied to calculate the hazard ratio risk. A P-value < 0.05 will be considered as statistically significant.

Interim analyses {21b}

This is not applicable. No interim analyses are planned.

Methods for additional analyses (e.g., subgroup analyses) {20b}

After randomization, the patients will be divided in two groups: a high-dose group and a low-dose group. No subgroup analyses are planned.

Methods in analysis to handle protocol non-adherence and any statistical methods to handle missing data {20c}

The analyses will be conducted with intention to treat: meaning that all subjects will be analyzed based on the block they have been randomly assigned to. In cases where

a subject is withdrawn or demonstrates poor compliance, the reasons for the withdrawal or non-compliance must be clearly documented in the CRF. Additionally, it is imperative to obtain the subject's understanding and support through effective communication. If there is missing data, we will use random forest to fill it.

Plans to give access to the full protocol, participant-level data, and statistical code {31c}

Upon reasonable request, the corresponding author will grant access to the complete protocol, participant-level data, and statistical code.

Oversight and monitoring

Composition of the coordinating center and trial steering committee {5d}

The coordinating center for this multi-center clinical trial shall be the Shulan (Hangzhou) Hospital. Monthly evaluations of the study's progress will be conducted. The study management group consists of the principal investigator, the coordinating investigator, two project manager, and a statistician. The data management team consists of a statistician, a data manager, two clinical researchers, and five coordinators. They carry out the day-to-day running of the trial.

Composition of the data monitoring committee, its role and reporting structure {21a}

This study will appoint a data and safety monitoring committee (DSMC) consisting of a hepatologist, ethicist, statistician, and an assistant, all independent of the study. The DSMC will review the trial processes and provide feedback biannually.

Adverse event reporting and harms {22}

The safety of the participants will be monitored by the DSMC throughout the trial. Any adverse events of special interest, as well as serious adverse events, regardless of their relationship to the intervention, will be promptly reported to the sponsor within 24 h. All adverse events will be comprehensively recorded in the CRF, while serious adverse events will be reported in detail to the ethics committee. The investigators will thoroughly evaluate the potential association between the adverse events and the interventions.

Frequency and plans for auditing trial conduct {23}

The ethics committee of Shulan (Hangzhou) Hospital will conduct audits of the study every 12 months to ascertain the integrity and reliability of study data and to ensure that the trial is conducted in strict adherence to ethical standards and the study protocol.

Plans for communicating important protocol amendments to relevant parties (e.g., trial participants, ethical committees) {25}

Any amendments to the study protocol will be promptly reported to the ethics committee of Shulan (Hangzhou) Hospital for re-approval. And the patients will be informed and requested to provide their signed informed consent again.

Dissemination plans {31a}

Upon the completion of the study and finalization of the study report, we intend to publish the findings of this trial in a peer-reviewed journal, ensuring the widespread dissemination of our research outcomes. Several presentations at national and international conferences are planned. If participants wish to receive the results of the study, they will be eligible to receive a summary of the results.

Discussion

Hepatic encephalopathy (HE) represents one of the most serious and potentially fatal complications of end-stage liver disease, manifesting in three distinct temporal patterns: episodic, recurrent, and persistent. The West Haven criteria categorize HE into five grades, ranging from minimal and grade 1(covert HE, CHE) to grades 2–4 (overt HE, OHE) [8, 9]. The prevalence of OHE lies between 10 and 14% [10, 11]. HE has numerous adverse effects, leading to increased hospitalization rates, costs, readmissions rates, and a heightened risk of falls, ultimately resulting in a decline in socioeconomic status [12, 13]. A comprehensive study examining the reasons and rates of readmission in cirrhosis patients revealed a significant association between HE and both 30-day and 90-day readmission rates. The adjusted odds ratios were 3.23 (95% CI 2.97–3.52) and 3.07 (2.86–3.30), respectively [14].

Ammonia has long been recognized as the primary culprit in the development of HE, and the majority of currently approved treatments target this mechanism. Lactulose and lactitol, two nonabsorbable disaccharides (NAD), have traditionally been used as first-line therapies for HE [15]. These substances lower colonic pH, inhibiting colonic bacterial proliferation and subsequently ammonia production. Nonabsorbable antibiotics such as rifaximin also target gut bacteria, leading to reduced ammonia production. Evidence suggests that rifaximin can significantly reduce the risk of recurrent HE and HE-related hospital admissions in patients with a history of recurrent HE [16]. However, despite these treatment options, some patients still experience recurrent OHE. Therefore, there is an urgent need to explore novel treatment options. Fecal

microbiota transplantation (FMT), an innovative therapy, aims to modulate the gut microbiota, aiming to reduce blood ammonia and endotoxin levels while also improving cognitive function. This therapy is expected to alter the composition of the gut microbiota, rebuild intestinal barrier integrity, and minimize ammonia absorption [17, 18]. The primary aim of both trials was to assess the safety of FMT. They did not identify any major safety concerns, except for a minor and reversible elevation in Model for End-Stage Liver Disease (MELD) scores among patients receiving broad-spectrum antibiotic treatment. At the 5-month follow-up, compared to rifaximin combined with lactulose, there was a confirmed improvement in dysbiosis and cognitive function, marked by an increase in beneficial bacteria and diversity [17]. Similar results were reported in phase 1 clinical trials involving FMT oral capsules for patients with recurrent HE [18]. Following FMT, the psychometric HE score (PHES) improved, and the extent of this improvement was donor-dependent. Furthermore, stool samples from FMT responders revealed high levels of Bifidobacterium and other beneficial bacteria. Overall, cognitive improvement in HE was confirmed; however, the effectiveness varied significantly among donors and recipients. A study suggested that the selection of donors and recipients significantly impacts the overall effectiveness of FMT [19].

In conclusion, the previous research on FMT for HE has been somewhat restricted, primarily emphasizing safety without a definitive assessment of its efficacy. Additionally, there are concerns regarding infection risks and safety due to the absence of standardized criteria and tests for donor selection. Despite the promising potential benefits of FMT, large-scale trials are needed to establish optimal dosing strategies. The present trial aims to assess the effectiveness of FMT with total bacterial counts of 400 and 800 ml in patients with recurrent HE. We anticipate that this trial will bridge a crucial research gap and furnish valuable clinical insights to refine the FMT treatment strategy for recurrent HE.

Trial status

The study was registered on clinical trials in China No: ChiCTR2200067135 on December 27, 2022, and on ClinicalTrials.gov under the title: A Multicenter, Prospective, Randomized Controlled Study to Explore the Efficacy and Safety of Fecal Microbiota Transplantation With Different Bacterial Doses in the Treatment of Recurrent Hepatic Encephalopathy, with the Identifier: NCT05669651. The last update for this study was posted on March 22, 2023. Most recent protocol is version 2.0, dated 17 September 2022. The trial began enrolment on 1 January 2023. We project that recruitment will be completed on November 30, 2025.

Abbreviations

HE	Hepatic encephalopathy
CLD	Chronic liver disease
FMT	Fecal microbiota transplantation
CTCAE	Common Terminology Criteria for Adverse Events
PHES	Psychometric Hepatic Encephalopathy Score
MELD	End-stage liver disease
DSMC	Data and safety monitoring committee
CRF	Case report form
NAD	Nonabsorbable disaccharide

Acknowledgements
Not applicable.

Authors' contributions {31b}
GHN and ZPF conceived of, and designed, the study. GHN is grant holder. GHN, ZPF, BYJ, WWH, WT, LQ, WK, FYC, ZD, WX, SH, HHJ, MSP, QYS, ZGQ, LX, JQF, RQJ, QZP, SW, CQ, YLY, WF, ZXT, QZX, LQ, LJJ, and ZYP will execute the study and will collect the data. ZPF and BYJ will manage the data. ZPF and BYJ drafted the initial manuscript. GHN, WWH, FYC, WT, LQ, WK, ZD, WX, SH, HHJ, MSP, QYS, ZGQ, LX, JQF, RQJ, QZP, SW, CQ, YLY, WF, ZXT, QZX, LQ, LJJ, ZYP, GJW, MRC,LJF, ZYB DF, and WH critically revised and approved the manuscript. All authors have read approved the final manuscript. All authors will continue to participate in this study as researchers.
Corresponding authors.
Correspondence to Hainv Gao.

Funding {4}
This study was funded by grants from National Key Research and Development Program of China (2021YFA1301104) and the Research Project of Jinan Microecological Biomedicine Shandong Laboratory. It is important to note that these funding sources had no involvement in the study's design, data collection, analysis, interpretation, or the decision to submit the results.

Data availability {29}
The datasets used and/or analyzed during the current study will be available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate {24}
Ethical approval for this study has been granted by the Ethics Committee of Shulan (Hangzhou) Hospital (Reference Approval No. KY2022083). Informed consent to participate will be obtained from all participants.

Consent for publication {32}
This is not applicable—no identifying images or other personal or clinical details of participants are presented here or will be presented in reports of the trial results. The participant information materials and informed consent form are available from the corresponding author on request.

Competing interests {28}
The authors declare that they have no competing interests.

Author details
[1]Shulan (Hangzhou) Hospital Affiliated to Zhejiang, Shuren University Shulan International Medical College, Hangzhou, China. [2]Jinan Microecological Biomedicine Shandong Laboratory, Jinan, China. [3]Zhejiang Chinese Medical University, Hangzhou, China. [4]Huzhou Central Hospital, Huzhou, China. [5]Hainan General Hospital Branch - Ding'an Hospital, Ding'an, China. [6]Shandong Public Health Clinical Center, Jinan, China. [7]Department of Hepatology, Qilu Hospital of Shandong University, Jinan, China. [8]Zhengzhou Third People's Hospital, Zhengzhou, China. [9]Shulan (Quzhou) Hospital, Quzhou, China. [10]Taizhou Hospital of Zhejiang, Taizhou, China. [11]Zhejiang Provincial People's Hospital, Hangzhou, China. [12]The First Affiliated Hospital of Henan University of CM, Zhengzhou, China. [13]Ningbo No.2 Hospital, Ningbo, China. [14]Luoyang Central Hospital, Luoyang, China. [15]Ningbo Zhenhai District People's Hospital, Ningbo, China. [16]Hangzhou Xixi HospitalAffiliated to, Zhejiang University School of Medicine, Hangzhou, China. [17]The Second Affiliated Hospital of Zhejiang, Chinese Medical University, Hangzhou, China. [18]Shanghai

Public Health Clinical Center, Shanghai, China. [19]The First Affiliated Hospital of Soochow University, Suzhou, China. [20]Zouping People's Hospital, Binzhou, China. [21]ALMETTE Hospital &, The First Hospital of Kunming, Kunming, China. [22]Zoucheng People's Hospital, Jining, China. [23]Shenzhen Bao'an District Songgang People's Hospital, Shenzhen, China. [24]Baoan Central Hospital of Shenzhen, Shenzhen, China. [25]The People's Hospital of Jiulongpo District, Chongqing, China. [26]Lishui People's Hospital, Lishui, China. [27]People's Hospital of Xinjiang Uygur Autonomous Region, Ürümqi, China. [28]Department of Infectious Disease and Hepatic Disease, First People's Hospital of Yunnan Province, Affiliated Hospital of Kunming University of Science and Technology, Kunming, Yunnan, China. [29]Department Of Infectious Diseases, Huashan Hospital, Fudan University, Shanghai, China. [30]The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China. [31]Department of Infectious Diseases, The Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China. [32]Affiliated Hospital of Shaoxing University, Shaoxing, China. [33]Department of Infectious Diseases, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

Received: 28 June 2024 Accepted: 24 October 2024
Published online: 27 November 2024

References

1. Ellul MA, Gholkar SA, Cross TJ. Hepatic encephalopathy due to liver cirrhosis. BMJ. 2015;351: h4187.
2. Stewart CA, et al. Hepatic encephalopathy as a predictor of survival in patients with end-stage liver disease. Liver Transpl. 2007;13(10):1366–71.
3. Tapper EB, et al. Identifying patients with hepatic encephalopathy using administrative data in the ICD-10 era. Clin Gastroenterol Hepatol. 2021;19(3):604-606.e1.
4. Fallahzadeh MA, Rahimi RS. Hepatic encephalopathy and nutrition influences: a narrative review. Nutr Clin Pract. 2020;35(1):36–48.
5. EASL Clinical Practice Guidelines on the management of hepatic encephalopathy. J Hepatol, 2022. 77(3): 807-824.
6. Butterworth RF. Hepatic encephalopathy in cirrhosis: pathology and pathophysiology. Drugs. 2019;79(Suppl 1):17–21.
7. Gao J, et al. A meta-analysis of microbiome therapies for hepatic encephalopathy. Eur J Gastroenterol Hepatol. 2023;35(9):927.
8. Weissenborn K. Hepatic encephalopathy: definition, clinical grading and diagnostic principles. Drugs. 2019;79(Suppl 1):5–9.
9. Patidar KR, Bajaj JS. Covert and overt hepatic encephalopathy: diagnosis and management. Clin Gastroenterol Hepatol. 2015;13(12):2048–61.
10. Tapper EB, et al. Incidence of and risk factors for hepatic encephalopathy in a population-based cohort of Americans with cirrhosis. Hepatol Commun. 2019;3(11):1510–9.
11. Tapper EB, et al. Incidence and bedside predictors of the first episode of overt hepatic encephalopathy in patients with cirrhosis. Am J Gastroenterol. 2020;115(12):2017–25.
12. Elsaid MI, et al. The health care burden of hepatic encephalopathy. Clin Liver Dis. 2020;24(2):263–75.
13. Soriano G, et al. Cognitive dysfunction in cirrhosis is associated with falls: a prospective study. Hepatology. 2012;55(6):1922–30.
14. Tapper EB, Halbert B, Mellinger J. Rates of and reasons for hospital readmissions in patients with cirrhosis: a multistate population-based cohort study. Clin Gastroenterol Hepatol. 2016;14(8):1181-1188.e2.
15. Schiano TD. Treatment options for hepatic encephalopathy. Pharmacotherapy. 2010;30(5 Pt 2):16S-21S.
16. Bass NM, et al. Rifaximin treatment in hepatic encephalopathy. N Engl J Med. 2010;362(12):1071–81.
17. Bajaj JS, et al. Fecal microbiota transplant from a rational stool donor improves hepatic encephalopathy: a randomized clinical trial. Hepatology. 2017;66(6):1727–38.
18. Bajaj JS, et al. Fecal microbial transplant capsules are safe in hepatic encephalopathy: a phase 1, randomized, placebo-controlled trial. Hepatology. 2019;70(5):1690–703.
19. Bloom PP, et al. Fecal microbiota transplant improves cognition in hepatic encephalopathy and its effect varies by donor and recipient. Hepatol Commun. 2022;6(8):2079–89.

Publisher's Note