BMJ Open

Study protocol for a prospective, single-center, randomized, controlled trial to evaluate the efficacy and safety of ischemia-free liver transplantation (IFLT) in the treatment of end-stage liver disease

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-035374
Article Type:	Protocol
Date Submitted by the Author:	29-Oct-2019
Keywords:	TRANSPLANT SURGERY, Hepatobiliary surgery < SURGERY, Hepatology < INTERNAL MEDICINE

Complete List of Authors:
Huang, Changjun; The First Affiliated Hospital, Sun Yat-sen University,
Huang, Shanzhou
Tang, Yunhua
Zhao, Qiang
Wang, Dongping; The First Affiliated Hospital of Sun Yat-sen University
Ju, Weiqiang; The First Affiliated Hospital, Sun Yat-sen University
Yang, Lu; The First Affiliated Hospital, Sun Yat-sen University
Zhang, Jian
Wu, Linwei; The First Affiliated Hospital, Sun Yat-sen University
Chen, Maogen; The First Affiliated Hospital, Sun Yat-sen University
Zhang, Zhiheng; The First Affiliated Hospital, Sun Yat-sen University
Zhu, Zebin
Wang, Linhe; The First Affiliated Hospital, Sun Yat-sen University
Zhu, Caihui; The First Affiliated Hospital, Sun Yat-sen University
Zhang, Yixi; The First Affiliated Hospital, Sun Yat-sen University
Sun, Chengjun; The First Affiliated Hospital, Sun Yat-sen University
Xiong, Wei; The First Affiliated Hospital, Sun Yat-sen University
Shen, Yuekun; The First Affiliated Hospital, Sun Yat-sen University
Chen, Xiaoxiang; The First Affiliated Hospital, Sun Yat-sen University
Ma, Yi; The First Affiliated Hospital, Sun Yat-sen University
Hu, Anbin; The First Affiliated Hospital, Sun Yat-sen University
Zhu, Xiaofeng; The First Affiliated Hospital, Sun Yat-sen University
Rong, Jian
Cai, Changjie; The First Affiliated Hospital, Sun Yat-sen University
Guo, Zhiyong; The First Affiliated Hospital, Sun Yat-sen University
He, Xiaoshun; The First Affiliated Hospital, Sun Yat-sen University

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Study protocol for a prospective, single-center, randomized, controlled trial to evaluate the efficacy and safety of ischemia-free liver transplantation (IFLT) in the treatment of end-stage liver disease

Changjun Huang[1,2,3,†], Shanzhou Huang[1,2,3,†], Yunhua Tang[1,2,3,†], Qiang Zhao[1,2,3], Dongping Wang[1,2,3], Weiqiang Ju[1,2,3], Lu Yang[4], Jian Zhang[5], Linwei Wu[1,2,3], Maogen Chen[1,2,3], Zhiheng Zhang[1,2,3], Zebin Zhu[1,2,3], Linhe Wang[1,2,3], Caihui Zhu[1,2,3], Yixi Zhang[1,2,3], Chengjun Sun[1,2,3], Wei Xiong[4], Yuekun Shen[4], Xiaoxiang Chen[4], Yi Ma[1,2,3], Anbin Hu[1,2,3], Xiaofeng Zhu[1,2,3], Jian Rong[6], Changjie Cai[7], Zhiyong Guo[1,2,3,*], Xiaoshun He[1,2,3,*]

[1]Organ Transplant Center, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, China

[2]Guangdong Provincial Key Laboratory of Organ Donation and Transplant Immunology, Guangzhou 510080, China

[3]Guangdong Provincial International Cooperation Base of Science and Technology (Organ Transplantation), Guangzhou 510080, China

[4]Department of Anesthesiology, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, China

[5]State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou 510080, China

[6]Department of Cardiopulmonary Bypass, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, China

[7]Surgical Intensive Care Unit, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, China

*Corresponding authors: Zhiyong Guo, and Xiaoshun He, Organ Transplant Center, The First Affiliated Hospital, Sun Yat-sen University, NO.58 Zhongshan Er Road, Guangzhou 510080, China (Tel: 86-20-87306082; Fax: 86-20-87306082; E-mail: rockyucsf1981@126.com and gdtrc@163.com)

[†]The first three authors contributed equally to this work and should be considered as co-first authors.

Strengths and limitations of this study

This study is the first to compare the efficacy and safety of ischemic-free liver transplantation (IFLT) and conventional liver transplantation (CLT) in treatment of end-stage liver disease in a randomized controlled clinical trial.

The study will answer the question: "Can IFLT reduce ischemia-reperfusion injury (IRI)-related unfavorable impacts and achieve better transplant outcomes?"

The inclusion of a series of well-designed endpoints and multiple research parameters will enable an in-depth analysis of the effects of IFLT on human liver transplantation.

The randomization design will allow us to achieve a homogeneous distribution of patients between IFLT and CLT.

Open-label design is considered as a limitation of this trial. Due to the virtue nature of the surgical procedure, it is not possible to blind the surgical team for the group allocation.

ABSTRACT

Introduction During conventional liver transplantation (CLT), ischemia-reperfusion injury (IRI) is inevitable and associated with complications such as early allograft dysfunction (EAD), primary non-function, and ischemic-type biliary lesions. We've established a novel procedure called ischemia-free liver transplantation (IFLT). The results from the pilot study suggest that IFLT might prevent IRI and yield better transplant outcomes in comparison to CLT. The purpose of this study was to further assess the efficacy and safety of IFLT versus CLT in patients with end-stage liver disease.

Methods and analysis This is an investigator initiated, open-label, phase-III, prospective, single-center randomized, controlled trial on the effects of IFLT in patients with end-stage liver disease. Adult patients (18-75 years) eligible for liver transplantation are screened for participating in this trial and will be randomized between IFLT group (n=34) and CLT group (n=34). In the IFLT group, donor liver will be procured, preserved and implanted with continuous normothermic machine perfusion (NMP). In the CLT group, donor liver will be procured after a fast cold flush, preserved in 0-4°C solution, and implanted under hypothermic and hypoxia conditions. Patients in both groups are managed according to the standard protocol

of our center. The primary endpoint is the incidence of EAD after liver transplantation. Intraoperative and postoperative parameters of donor livers and recipients will be observed and recorded, and postoperative liver graft function, complications, recipient and graft survival will be evaluated. After 12-month follow-up of the last enrolled recipient, the outcomes will be analyzed to evaluate the safety and efficacy of IFLT versus CLT in patients with end-stage liver disease.

Ethics and dissemination The protocol was reviewed and approved by the Ethics Committee of The First Affiliated Hospital of Sun Yat-sen University. The findings will be disseminated to the public through conference presentations and peer-reviewed scientific journals.

Trial registration number ChiCTR1900021158.

Keywords: Ischemia-free liver transplantation, normothermic machine perfusion, Ischemia-reperfusion injury, Donation after brain death, Early allograft dysfunction

INTRODUCTION

Background and rationale

Over the past several decades, liver disease has become one of the leading causes of death and illness worldwide.[1] It is considered that one-fifth of the population in China are suffered from some kind of liver disease, and the number of patients with end-stage liver disease remains high.[2] Liver transplantation has been acknowledged as the only effective treatment for end-stage liver diseases.[3] Although the short-term outcome of organ transplantation has made certain progress in recent years, the long-term outcome has not been significantly improved.[4] Meanwhile, organ shortage has become a worldwide issue, with a large number of patients dying on the waiting list for liver transplantation.[5 6] Hence, further improvement of transplant efficacy and expansion of donor organ pool have attracted great attentions in the field of organ transplantation.

During the process of conventional organ transplantation, organs are procured after a fast cold flush, preserved in 0-4°C solution, and implanted under hypothermic and hypoxia conditions.[7-9] The organs are suffering ischemic injuries during the whole transplant procedure, thus ischemia-reperfusion injury (IRI) is an inevitable event in all types of organ transplantation.[10 11] IRI not only brings about allograft dysfunction but also undermines the function of other organs, giving rise to a series of complications and even patient death.[12 13] The incidence of early graft dysfunction (EAD), primary nonfunction (PNF) and ischemic-type biliary lesions (ITBL) after liver transplantation are around 10-50%, 3-7% and 5-10%, respectively.[14-17] For decades, researchers have been making efforts to reduce the morbidity and mortality related to IRI. However, owing to the complex molecular mechanisms of IRI, interventions such as drugs, stem cells, and protective gases exert little effect.[18 19]

In the past decade, there have been tremendous achievements in the field of machine perfusion technology. It has been demonstrated that both normothermic machine perfusion (NMP) and hypothermic oxygenated machine perfusion (HOPE) can alleviate allograft IRI and improve transplant outcomes in animal experiments and clinical trials.[20-23] Particularly, conditions close to physiological status is provided to the grafts during *ex vivo* NMP. Therefore, not only organ repairing, but also graft viability assessment can be achieved during NMP. The technique has been successfully applied in lung, liver, kidney and heart transplantation.[20-26] However, under the current practice, ischemic injuries of organs first occur during procurement and preparation before the initiation of NMP, then once again occurs during implantation after NMP. Therefore, the organs might suffered "double hit" of IRI.

We therefore hypothesize that continuous oxygenated blood supply to the donor liver during the entire period of donor liver procurement, preservation and implantation could prevent IRI and significantly reduce the incidence of complications induced by IRI. We've established reliable ischemia-free liver transplantation (IFLT) technique in pigs, and reported the first case of IFLT in human.[27 28] During IFLT, the blood supply to the donor livers is continuously maintained throughout the whole process of procurement, preservation and implantation. Results of the first 14 cases of IFLT showed that EAD occurred in 1 case (7.1%), compared with 25 cases (53.2%) in 47 cases of conventional liver transplantation (CLT) with the standard static cold storage (SCS) at the same period. The peak alanine aminotransferase (AST) (369 U/L vs. 1502 U/L, P<0.001) and peak aspartate aminotransferase (ALT) (201 U/L vs. 689 U/L, P<0.001) within 7 days post-transplantation were significantly decreased in the IFLT versus CLT group. Histological studies showed that in sharp contrast to CLT, there was no augment in Suzuki score, hepatocyte apoptosis, inflammatory cytokines, chemokines and activation of inflammatory pathways after reperfusion in the IFLT group.

Although the pilot study suggests promising transplant outcomes of IFLT, the design of the study is non-randomized and patient selection bias cannot be ruled out. The purpose of this study was to further explore the efficacy and safety of IFLT in a prospective, randomized, controlled trial.

Objectives

Primary objective

To compare the incidence of IRI-related complications between IFLT and CLT recipients, as well as the allograft/recipient survival rate, to further validate the clinical efficacy and safety of IFLT.

Secondary objective

To compare the severity of allograft IRI between IFLT and CLT by laboratory analysis of peripheral blood and liver biopsy specimens.

METHODS

This protocol was designed in conformity with the Standard Protocol Items: Recommendations for Interventional Trials 2013 statement.[29]

Study setting

The study will be conducted at the The First Affiliated Hospital of Sun Yat-sen University.

Eligibility criteria

The following inclusion and exclusion criteria will be applied when recruiting donors and recipients. Only both donors and recipients meeting these criteria will be recruited into the study.

Donor inclusion criteria

1. Donation after brain death (DBD).

2. Over the age of 18 years, or over the age of 14 years with body weight >50 Kg.

3. The donor liver is allocated to the recipient of our own hospital.

Recipient inclusion criteria

1. Age of 18-75 years.

2. Patients with end-stage liver disease and active on the waiting list for liver transplantation.

3. Decided to receive liver grafts from deceased donors.

4. Able to give informed consent.

5. Able to comply with study protocol.

Donor exclusion criteria

1. Livers intended for split or reduced-size transplantation.

2. With high risk of transmitted infections (Human Immunodeficiency Virus infection and active tuberculosis).

3. The risk of donor malignancy transmission over 10% according to the Disease Transmission Advisory Committee (DTAC) categorizations.[30]

Recipient exclusion criteria

1. Waiting for multivisceral or combined organ transplantation.

2. ABO incompatible liver transplantation.

3. Primary liver cancer beyond University of California at San Francisco (UCSF) criteria.[31]

4. Fulminant liver failure.

5. Woman during pregnancy.

6. A history of organ transplantation.

7. With contraindications defined by American Association for the Study of Liver Diseases (AASLD) liver transplant practice guideline,[32] except model for end-stage liver disease (MELD) score < 15.

Interventions

Screening

All preoperative evaluation and eligibility scrutiny must be accomplished before randomization to ensure that the donor and recipient match all inclusion criteria. The investigator should create a screening log to record the details of all selected donors and recipients, to confirm their eligibility or ineligibility.

1. Donors: when a potential donor comes into sight, brain death judgement should be performed by two professional doctors. Medical history-taking and pre-operative evaluation should be conducted following the study protocol. Eligibility will be determined according to inclusion and exclusion criteria. The schedule for donor screening is summarized in Table 1.

2. Recipients: when an end-stage liver disease patient is enrolled in the waiting list, medical history-taking and preoperative evaluation should be conducted following the study protocol. Eligibility will be determined according to inclusion and exclusion criteria. The schedule for recipient screening is summarized in Table 2.

Donor and recipient matching

1. Donor livers are allocated to recipients by China Organ Transplant Response System (COTRS) according to blood type, patient's condition, waiting time and other routine allocation principles.

2. After an eligible donor liver is allocated to an eligible recipient in our center, the donor and recipient are assigned to Experimental group (IFLT group) or Control group (CLT group) based on randomization.

Surgical procedures (IFLT vs CLT)

IFLT

Donor livers from the IFLT group will undergo continuous NMP during procurement, preservation and implantation.[27]

1. Ischemia-free procurement of donor liver

Mobilization of the liver is conducted with a precision technique. A tube is placed in the common bile duct for bile drainage and the cystic duct is ligated. The celiac artery (CA), gastroduodenal artery (GDA), splenic artery (SA), inferior vena cava (IVC), and portal vein (PV) are well dissected. An 8Fr/12Fr arterial cannula is inserted into the GDA or SA without interruption of arterial supply for the liver from the CA. The arterial cannula is connected to the hepatic artery (HA) perfusion line of the Liver Assist (Organ Assist, Groningen, the Netherlands). A 3 cm-long right external iliac vein is harvested and end-to-side anastomosed to the portal

vein with partial blockage of the PV for constructinging an interposition vein. A straight 24Fr cannula is connected to the PV perfusion line of the Liver Assist and then inserted into the PV via the interposition vein. A 32-34Fr caval cannula is placed in the infrahepatic inferior vena cava (IHIVC) for outflow to the organ reservoir of the Liver Assist. The venous drainage of suprahepatic inferior vena cava (SHIVC) to the right atrium is blocked. Then the *in situ* circuit is established and NMP is started. The liver is harvested and transfered to the organ reservoir under continuous NMP. Immediately after the liver is removed from abdominal cavity, the kidneys are cold flushed via the cannula within the abdominal aorta and procured.

2. Ischemia-free preservation of donor liver

The liver is transferred to the perfusion device. The caval cannula is removed immediately when liver is moved to the organ reservoir. The liver graft is subjected to continuous *ex situ* NMP until allograft re-vascularization. The PV perfusion pressure is set at 6-10 mmHg with the targeted flow rate higher than 500 mL/min. The hepatic artery pressure is set at 50-60 mmHg with the targeted flow rate higher than 150 mL/min. During the NMP, the pressure and flow rate are monitored and adjusted within an appropriate range. Redundant tissues are removed from the liver and blood vessels. The SHIVC and IHIVC are examined for leaks by transiently blockage of the IVC. The bile tube is connected to a collection container. The amount of bile production is recorded and the biochemical parameters is monitored every 60 min. Perfusate samples are taken for blood gas analysis every 10-20 min and liver function tests every 30 min to monitor the biochemical parameters. The viability of the liver is assessed by blood gas analysis and liver function tests of the perfusate, as well as bile biochemical parameters as previously reported.[33] For the safety of patients, the viability of grafts during NMP is confirmed before we start the recipient surgical procedures.

3. Ischemia-free implantation of donor liver

The diseased liver is resected using a routine procedure. The donor IHIVC is re-cannulated and the SHIVC is blocked by a clamp. Then the donor liver is moved from the reservoir and placed in the recipient's abdominal cavity so that an *in situ* NMP circuit is re-established. The donor SHIVC is anastomosed to the recipient counterparts in a corresponding fashion using 3-0 Prolene based on bicaval or piggy-back technique. The donor PV and HA are anastomosed to the recipient counterparts in an end-to-end fashion using 5-0 and 7-0 Prolene, respectively. Because of the native and the artificial branches on the HA and PV, all these anastomoses are accomplished under continuous NMP of the allograft. After that, the clamps on the PV and HA are released, so that the native dual blood supply for the liver is re-established. At the same time, NMP is ceased after removal of the HA and PV cannula. Then the cannula within IHIVC is removed and around 200

mL perfusate within the liver is flushed out, followed by release of the clamp on the SHIVC. The anhepatic phase is over. The donor SA or GDA is ligated closed, and the interposition vein is sutured closed. The donor IHIVC is then anastomosed to the recipient IHIVC or ligated according to the bicaval or piggy-back technique used. The donor common bile duct is end-to-end anastomosed to the recipient common bile duct after withdrawal of the draining tube. After meticulous hemostasis and abdominal closure, the patient is sent to post-transplant intensive care unit (ICU).

4. Recording and assessment of NMP parameters

In the process of NMP, the stability and efficacy of perfusion is monitored, and the liver graft function is monitored by perfusate biochemical test and blood gas analysis. The perfusion parameters, regulatory measures, bile production and blood gas analysis results were recorded. NMP parameters are summarized in Table 3-7.

CLT

Following the standard *in situ* cold flushing procedure, the liver will be retrieved and placed in ice-cold University of Wisconsin Solution. Backtable preparation will be performed under standard procedure prior to implantation. After removal of the diseased liver, the donor liver is transferred to the abdominal cavity. Following anastomosis of IVC and PV, the vessels are re-opened to restore the blood supply of the allograft. Then the donor artery and bile duct are anastomosed successively. After meticulous hemostasis and abdominal closure, the patient is sent to ICU.

Intraoperative monitoring

Recipients' condition during operation and anesthesia will be recorded according to standard and norm in our center. Intraoperative monitoring will be conducted to compare the impacts of IFLT or CLT on the functions of donor liver and other organs such as heart, lung, kidney, intestine and brain.

Postoperative management.

Both groups are managed according to the patients' conditions and standard protocols of our center.

Follow-up

The patients will be followed up for 1 year. Postoperative visits will be performed on post-operative day (POD) 1-7, POD14 and each month post-transplantation. Biomedical values, complications, adverse events and medication administration records will be documented. Follow-up information are shown in Table 8-10.

Outcomes

Primary endpoint

The primary endpoint is the incidence of EAD within 7 days post-transplantation. The diagnosis of EAD is defined according to the presence of one or more of the following criteria.[34]

1. Peak aspartate amino transferase (AST) >2000 IU/L within the first 7 postoperative days.
2. Peak alanine aminotransferase (ALT) >2000 IU/L within the first 7 postoperative days.
3. Total bilirubin (Tbil) ⩾ 10 mg/dL on POD 7 (exclusion of biliary stricture).
4. International normalized ratio (INR) ⩾ 1.6 on POD 7.

Secondary endpoints

1. Post-transplant peak AST: in order to ensure consistency, serum AST will be measured 5-11 hours post-reperfusion on POD 1 and 6-8am on POD 2-7, and the peak level will be defined as the highest of these values (in IU/L).[35]

2. Post-transplant peak ALT: serum ALT will be measured and the peak level will be defined as AST.

3. Tbil on POD 7.

4. INR on POD 7.

5. AST, ALT, Tbil, INR, gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) at POD 1-7, POD 14, postoperative month (POM) 1, POM 6 and POM 12.

6. Lactate level at post-reperfusion 1h by arterial blood gas analysis.

7. Incidence of PNF: PNF is defined as unavoidable graft dysfunction requiring emergency re-transplantation or leading to death within first 10 days post-transplantation, in the absence of surgical or immunological factors.[19 36]

8. Post-reperfusion syndrome (PRS): PRS is defined as a decrease in mean arterial pressure ⩾30% in comparison with the baseline value, for at least 1 min, occurring during the first 5 min after reperfusion of the donor liver (without clamping of hepatic hilum).[37]

9. Biliary complications: including but not limited to bile leakage, anastomotic stenosis and ITBL. IBTL are nonanastomotic strictures and dilations involving only the biliary tree of the graft, in the absence of hepatic artery thrombosis.[38 39]

10. Patient survival status at POM 1, POM 6 and POM 12.

11. Graft survival status at POM 1, POM 6 and POM 12.

12. Length of post-transplant ICU care.

13. Length of post-transplant hospital stay.

Safety endpoints

1. Graft rejection at POM 1, POM 6 and POM 12, including clinically diagnosed rejection and pathologically confirmed rejection with Banff schema.[40]

2. Vascular complications at POM 1, POM 6 and POM 12, including thrombosis, hemorrhage, embolism and stenosis of IVC, PV and HA. Patients will undergo a color doppler ultrasound at each time point, and digital subtraction angiography (DSA) will be performed when necessary.

3. Acute kidney injury (AKI) within the first 7 postoperative days. AKI will be graded according to Kidney Disease: Improving Global Outcomes (KDIGO) staging system.[41]

4. Estimated glomerular filtration rate (eGFR) at POD 7, POD 14, POM 1, POM 6 and POM 12. eGFR will be judged according to chronic kidney disease epidemiology collaboration (CKD-EPI) creatinine equation.[42]

5. Need for renal replacement therapy following transplantation.

6. Recipient infection within POM 1. Infections will be defined on the basis of the standard criteria proposed by the Centers for Disease Control and Prevention.[43]

7. Cumulative complications at POM 1, POM 6 and POM 12. Complications will be graded according to the comprehensive complication index (CCI) based on Clavien-Dindo Classification.[44 45]

8. Adverse events (AE) and severe adverse events (SAE) will be assessed according to National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE) (version 5.0) criteria at POM 1, POM 6 and POM 12.

9. Positive perfusate microbial culture rate. At the end of SCS or NMP, a sample will be collected for microbiological culture (cold preservation solution or warm perfusate).

10. Organ discard rate.

Self-reported endpoints

Quality of life will be scored using an EQ-5D questionnaire obtained before transplantation and at POM 1, POM 6 and POM 12.

Exploratory endpoints

1. Molecular biological data of IRI and immune system are evaluated in serum, plasma, whole blood, liver specimen, bile duct tissue and perfusate at the above time points, during and after transplantation. The hepatic IRI is evaluated based on the Suzuki score,[46] bile duct IRI is evaluated based on Hansen BDI (bile duct injury)

score.[47 48]

2. Function tests of coagulation, heart, lung, kidney, intestine, brain and other organs at the above time points during and after transplantation.

3. The balance between medical expenditure and quality of life.

Sample size

This study is a 1:1 parallel design and the sample size calculation is based on our pilot study. It is estimated that EAD occurs 10% in the experimental (IFLT) group and 40% in the control (CLT) group. With a power of 80% (1-β) and significance level (α, two side) of 5%, we calculated that 32 patients needed to be enrolled in each arm. Considering the possibility of organ discard under special conditions, the sample size was increased by 5%. Ultimately, 34 patients in each arm, a total of 68 patients will be enrolled in the study.

Recruitment

Recruitment has been conducted from February 2019 until the target sample size is completed (patient recruitment is expected to be finalized in August 2020). The trial was designed as a prospective, randomized, controlled, single-center clinical trial in patients on the waiting list undergoing liver transplantation. Firstly, the donor and recipient eligibility will be assessed before transplantation. Informed consents will be obtained. All the donors have to be in our hospital and the donor livers will be allocated by COTRS. When a donor liver is allocated to an informed recipient of our own hospital, the recipient will be randomly assigned to IFLT or CLT group. The number of recipients in the two groups will be allocated to 1:1, and the grouping information is open labeled. Postoperative monitoring, treatment and nursing will be performed according to the same standards and procedures. Intraoperative parameters, liver graft function, post-transplant complications and patient/graft survival will be observed and recorded. After 12-month follow-up of the last enrolled recipient, the outcomes will be analyzed to evaluate the safety and efficacy of IFLT in human liver transplantation. The research brief flow chart is shown in Figure 1.

Randomization and blinding.

This study is a randomized controlled trial, and block randomization will be adopted for 1:1 random grouping. A subject randomization list will be generated using the proven central randomization system by the

statistician, and random allocation numbers will be automatically carried out by the system to avoid bias. When all the inclusion/exclusion criteria are fulfilled, the investigator will contact the central randomization system to get a random number, then the subject will be allocated to the experimental or control group based on the number. This is an open label study. Because of the nature of the surgical procedure, it is not possible to blind the surgical team for the group allocation. Outcome assessors will be blinded where possible. This includes the diagnostician interpreting the medical imaging examination as well as the histopathologists interpreting the biopsy specimens.

Data collection and management

Case Report Form (CRF) / electronic database

The investigators should input all subjects' original observation records timely, completely and correctly into the CRF. The data on the CRF will be transformed into an electronic database. The CRF and database will be reviewed by two independent inspectors for error checking and then the completed data will be handed over to the data manager. If there are questions about an CRF, the data manager will send Data Clarification Form (DCF) to the investigators and contact data inspectors to solve the doubtful points and return feedbacks. Data manager will confirm, modify and input data according to the feedbacks of investigators, and send DCF again if necessary.

Database Locking

After data review and confirmation, the data managers, main investigators, statistical analysts, sponsors and supervisors will jointly audit the data, and complete the final definition of the analysis population, then the data manager will lock the database. In general, locked databases or files should not be altered.

Data analysis

The final data will be submitted to the statistical analyst for statistical analysis.

Analysis plan

Analysis sets

1. Full analysis set: namely intention-to-treat (ITT), intention-to-treat analysis is a comparison of the treatment groups that includes all patients as originally allocated after randomization.

2. Per protocol set (PPS): per-protocol analysis is a comparison of treatment groups that includes only those

patients who completed the treatment originally allocated.

The primary endpoint and secondary endpoints of the study will be analyzed by ITT and PPS.

Statistics

1. The data analysis will be based on the principle of intention to treat (ITT), and all statistical analysis will be adopted two-sided test, and $P<0.05$ will be considered statistically significant. After the database is locked, the data analysis will be performed STATA 14·0 software (Stata Corp).

2. Demographic information and baseline characteristics will be analyzed using descriptive statistical analysis.

3. The primary endpoint will be analyzed using chi-square test and the absolute difference and 95% confidence interval will be calculated.

4. Analysis of secondary endpoint: the two-category variables will be analyzed using chi-square or logistic regression to report the odds ratio (OR value) after adjusting for confounding factors; for the continuous variables, t test will be utilized if the normal distribution is satisfied, otherwise the Mann-Whitney test will be utilized, or the mixed model with repeated measurement will be utilized to analyze the change of the individual from the baseline; the time data will be analyzed by Kaplan-Meier method and the log-rank test and Cox regression model will be also used.

5. Missing data: If EAD is missing, and it will be replaced by the worst value method and EAD will be considered. Secondary values will be replaced in two ways: (1) multiple imputation, missing values will be estimated by independent simulation variables according to the characteristics of the predicted values and the availability of the data. Linear regression will be utilized for continuous variables, logistic regression for binary categorical variables, ordered logistic regression for ordered multi-class variables, and disordered multi-class logistic regression for disordered multi-class variables. Twenty data sets will be created by the multiple imputation method, and the final result will be obtained by averaging the results of the twenty data sets using the Rubin rule, ensuring that the standard error of all regression coefficients takes into account the uncertainty of the simulation and the uncertainty of the estimate. (2) sensitivity analysis. Sensitivity analysis involves directly eliminating missing values，deeming treatment ineffectiveness as well as optimal and worst case analysis. Sensitivity analysis will be utilized to compare the consistency of the primary results analysis.

Monitoring and safety

AEs that occur during the period of the study should be addressed in accordance with well-established management criteria that will support the life and health of the study subjects. It is the responsibility of the investigators to collect and record all AEs occurring throughout the time of the study. All AEs will be documented on CRF. All SAEs should be reported to the superintendent and ethics committee by specialized SAE from. The causes and effects of SAE should be carefully assessed and the study should be suspension or termination when necessary. All SAEs will be followed up to resolution. Recording and reporting of AEs will continue until the last enrolled patient has accomplished 12 months of follow-up.

AEs and complications

AEs are defined as any unintended medical events that occur in patients participating in the trial. AE do not necessarily have a causal relationship with the trial. Complications are AEs that deviation from the ideal postoperative course, which is not inherent in the procedure and does not comprise a failure to cure.

The following scenarios are considered SAEs.

1. Death of the recipients.
2. Life-threatening complications.
3. Persistent or severe disability.
4. Significantly prolonged length of hospital stays.
5. Other severe events judged by the investigators.

AEs should be judged and graded according to the NCI-CTCAE 5.0 and documented in the CRF. Complications should be categorized by Clavein Dindo Classification and scored by CCI at each follow-up period.[44]

Withdrawal of trial

Withdrawal initiated by investigators

1. Severe violation of study protocol occurs due to donor liver, perfusion or recipient reasons in the process of procurement, preservation and implantation or even discarding the donor liver.
2. The subject suffers from certain disease that is not suitable for carrying on the study.
3. Safety and tolerance are disturbed by poor compliance.
4. Continued treatment will hurt the health of the subjects.

Withdrawal initiated by subjects

Subjects can decide to cease participation in this trial at any time for any reason. The reasons for their withdrawal should be acknowledged and documented.

Withdrawal procedure

1. If termination without liver transplant, the subject can be re-added to the waiting list.

2. If termination after liver transplant, the subject still can be receive standardized treatment, nursing and follow-up.

3. When a subject has an emergency that requires immediate termination of an ongoing liver transplant or subsequent therapy, the subject should be observed and evaluated accordingly while ensuring the safety.

The reasons and time points of any withdrawal should be clearly collected and documented, and the observation and evaluation should be carried out accordingly. When an AE occurs, it is necessary to track until it disappears. The CRF of any subject who received treatment but failed to complete the study should be retained and the last test results will be transferred to the final results. Treatment response, tolerance and AEs will be analyzed based on full data analysis.

Specimens collection

A written informed consent is required before all clinical specimens are collected. Complete and standard specimen enables the comparison between the experimental group and the control group under the same conditions, making the experimental data accurate and reliable. The sponsor will organize a clinical specimen collection team and establish a specimen bank. Body fluids, solid tissues and their derivatives (such as DNA, RNA, protein, etc.) will be collected and preserved for related research and experiments. It is responsibility of the investigators to participate in and supervise the process of specimen collection. All remaining samples are required to be destroyed within 15 years of the end of the clinical trial.

Before the operation, donor blood will be collected for extracting supernatant and periphera mononuclear blood cells. After organ retrieving, part of the spleen and iliac vessels will be preserved. In total, three excision biopsies will be harvested from the donor liver. The time points are: before retrieving, at the end of preservation, after graft re-vascularization. Two biopsies will be harvested from the donor common bile duct at the time points of immediately after procurement and before common bile duct anastomosis following graft re-vascularization. Perfusate samples will be collected repeatedly during machine perfusion. Bile (if produced) will be collected from a common bile duct tube. Recipient blood samples will be taken on pre-operation, POD 1, 3, 5, 7, 14 and every POM during follow-up period. Liver biopsy samples will be taken on POM 6 or rejection suspected.

DISCUSSION

Liver transplantation is an effective therapy for patients with end-stage liver disease. However, there are still many burdens that hamper the progress of liver transplantation. Donor liver IRI is an inevitable event in current transplant procedure that often compromises transplant outcomes and increases organ discard rate.[10-13] Tremendous achievements have been scored in the field of alleviating donor liver IRI.[19-21 23] Among these techniques, NMP has been successfully used in clinical practice in several transplant centers. David Nasralla et al reported that liver transplantation with the help of NMP is associated with a decrease of 50% graft injury and 50% organ discard rate, and an increase of 54% preservation time.[19] However, IRI still cannot be fully avoided due to the existence of hypothermia, ischemia and hypoxia in the surgical procedure. With surgical innovation, IFLT enables a complete elimination of hypothermia, ischemia and hypoxia during the whole transplant procedure.[27] Our pilot study has demonstrated its feasibility, safety and efficacy with diminished peak injury markers and lower incidence of EAD when compared to CLT.[49] Although the results of the pilot study are promising, the non-randomized design is a drawback. The purpose of this study is to further explore the efficacy and safety of IFLT in a randomized controlled trial.

A potential shortage of this study is the single source of DBD donors, with the exclusion of donation after cardiac death (DCD) donors because currently it is difficult to perform IFLT using DCD organs. However, it is possible to combine *in situ* regional normothermic perfuion (NRP) and IFLT in this group of donors. Donors with high risks of communicable infectious diseases or malignancy are excluded for the safety of participants. For recipients, patients with fulminant liver failure and primary hepatocellular carcinoma beyond UCSF criteria are not included in this trial for their high risk of complications and mortality. In addition, patients with a history of organ transplantation, multiple organ transplantation or combined organ transplantation and ABO incompatible liver transplantation are excluded, because different surgical or post-transplant management protocol is used in these patients.

Due to the increased organ shortage, more and more livers from extended criteria donors (ECD), such as elderly donors and donors with hepatosteatosis are used all over the world. When compared to organs the standard criteria donors (SCD), ECD organs are more vulnerable to IRI and are associated with high risks of morbidity and mortality.[5] Since IFLT is possibly able to prevent IRI, the ECD livers might be an appropriate indication for IFLT. Indeed, the first case of IFLT successfully resuscitated a donor liver with 85-90%

macrovesicular steatosis.[28] However, the donors are not limited to this group of donors in the current study, because the benefits of IFLT in either SCD or ECD livers have not been confirmed in a randomized study. In addition, IFLT is an extreme example of NMP. The advantage of NMP over static cold storage is still under debates, although a randomized study was recently reported.[19] Therefore, we did not design to compare IFLT with CLT using NMP as preservation method in the current study. Another randomized controlled study is under plan in our center to compare the safety and efficacy of these two methods in ECD livers.

The primary endpoint of this trial is the incidence of EAD after liver transplantation. EAD represents severe form of clinical IRI, serving as an important surrogate endpoint in liver transplantation.[50] The definition of EAD is largely based on serum AST/ALT in the recipient. The use of EAD (based on this definition) in the setting of NMP was recently criticized.[51] We agree that EAD is not a perfect endpoint. However, the first randomized controlled study used it as an important endpoint[19] and it is the most frequently used primary endpoint in the current registered trials concerning the use of machine perfusion techniques. The Food and Drug Administration (FDA) in the United States is insisting on EAD in the current NMP trials.[52] The Zurich group is using complication score (Clavien Dindo) in their current HOPE trial and the Groningen group is using ischemic cholangiopathy in DCD liver transplantation. Therefore, we included these two end-points as secondary and safety end-points in the current study.

Undoubtedly, there are limitations concerning the IFLT procedure. Firstly, the procedure is complicated and labor-intensive. Both experienced surgeons and perfusionists are required for a successful IFLT operation. Therefore, it is difficult to conduct a multicenter study at this moment. Further modification of the procedure is required. In addition, the NMP device used in our center is a non-transportable one. For this reason, the donors and recipients have to be from a same medical institution. In the future, simplified IFLT techniques with a portable NMP device is required to make the novel procedure road out to other centers.

In conclusion, this study is a single-center trial designed to assess the incidence of IRI-related complications between IFLT and CLT recipients, as well as the allograft/recipient survival, to further validate the efficacy and safety of IFLT. The results from this trial can provide important evidence for the potential benefits of IFLT.

Patient and public involvement

The patients and public are not involved in the design and conduct of the study.

Ethics and dissemination

This trial will be conducted in accordance with the principles of the 1964 Declaration of Helsinki and its later amendments. All organs utilized in this study are procured from brain dead volunteer donors. The diagnosis of brain death has to be made by two independent qualified neurologists. All donors are non-executed prisoners and no biological material is sourced from executed prisoners. Written informed contents have to be obtained from all the directive family members for each donor and all the organs have to be allocated through China Organ Transplantation Response System (COTRS). We have already provided evidence to the journal to verify the above statements.

The protocol was viewed and approved by the Ethics Committee of the First Affiliated Hospital, Sun Yat-sen University. The ethical approval number is [2019]037. All documents communicating with the ethics committee will be kept in the researcher's folder. If it is necessary to modify this protocol during clinical research, it should be reviewed by the hospital ethics committee and implemented after approval. Written informed consents should be obtained from each subject prior to organ allocation and randomization. The objectives and methods, the benefits, possible risks and solutions, specimen collection plan as well as corresponding compensation when hurts occur, should be clarified clearly for all subjects. All subjects have the rights to cease participation in this trial at any time for any reason. The informed consent for this study and any changes in the course of this study must be reviewed and approved by the ethics committee before applying.

With regard to dissemination, the results of this study will be published in an academic journal and presented at national and international conferences.

Author affiliations

[1]Organ Transplant Center, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, China

[2]Guangdong Provincial Key Laboratory of Organ Donation and Transplant Immunology, Guangzhou 510080, China

[3]Guangdong Provincial International Cooperation Base of Science and Technology (Organ Transplantation), Guangzhou 510080, China

[4]Department of Anesthesiology, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, China

[5]State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou 510080, China

[6]Department of Cardiopulmonary Bypass, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, China

[7]Surgical Intensive Care Unit, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, China

Acknowledgements We thank all volunteer donors for donating their organs for scientific research and clinical needs. We thank all members of the organ procurement organization, surgical intensive care unit, transplant anesthesia team and cardiopulmonary bypass team.

Contributors The initial study concept was derived from the initiating investigator study group (XSH, ZYG, QZ, WQJ, DPW, MGC, LWW, YM, ABH, XFZ). CJH, SZH and YHT drafted the manuscript. ZYG and JZ performed the sample size estimation. CJH, SZH, YHT, LY, ZHZ, ZBZ, LHW, CHZ, YXZ, CJS, WX, YKS, XXC, JR and CC participated in designing the study, preparing the revised protocol and are investigators at The First Affiliated Hospital, Sun Yat-sen University. All authors were involved in revising the manuscript and approved the final version.

Funding This work was supported by by grants as follows: the National Natural Science Foundation of China (81970564, 81471583 and 81570587), the Key Clinical Specialty Construction Project of National Health and Family Planning Commission of the People's Republic of China, the Guangdong Provincial Key Laboratory Construction Projection on Organ Donation and Transplant Immunology (2013A061401007 and 2017B030314018), Guangdong Provincial international Cooperation Base of Science and Technology (Organ Transplantation) (2015B050501002), Guangdong Provincial Natural Science Funds for Major Basic Science Culture Project (2015A030308010), Guangdong Provincial Natural Science Funds for Distinguished Young Scholars (2015A030306025), Special support program for training high level talents in Guangdong Province (2015TQ01R168), Pearl River Nova Program of Guangzhou (201506010014), and Science and Technology Program of Guangzhou (201704020150).

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval Approval was obtained from the Ethics Committee of The First Affiliated Hospital, Sun

Yat-sen University, China (1/2019).

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES:

1. Wang FS, Fan JG, Zhang Z, et al. The global burden of liver disease: the major impact of China. *Hepatology* 2014;**60**(6):2099-108.
2. Xiao J, Wang F, Wong NK, et al. Global liver disease burdens and research trends: Analysis from a Chinese perspective. *J Hepatol* 2019;**71**(1):212-21.
3. Zarrinpar A, Busuttil RW. Liver transplantation: past, present and future. *Nat Rev Gastroenterol Hepatol* 2013;**10**(7):434-40.
4. Saidi RF, Hejazii Kenari SK. Challenges of organ shortage for transplantation: solutions and opportunities. *Int J Organ Transplant Med* 2014;**5**(3):87-96.
5. Durand F, Renz JF, Alkofer B, et al. Report of the Paris consensus meeting on expanded criteria donors in liver transplantation. *Liver Transpl* 2008;**14**(12):1694-707.
6. Dubbeld J, Hoekstra H, Farid W, et al. Similar liver transplantation survival with selected cardiac death donors and brain death donors. *Br J Surg* 2010;**97**(5):744-53.
7. Starzl TE, Hakala TR, Shaw BW, Jr., et al. A flexible procedure for multiple cadaveric organ procurement. *Surg Gynecol Obstet* 1984;**158**(3):223-30.
8. Belzer FO, Southard JH. Principles of solid-organ preservation by cold storage. *Transplantation* 1988;**45**(4):673-6.
9. Maathuis MH, Leuvenink HG, Ploeg RJ. Perspectives in organ preservation. *Transplantation* 2007;**83**(10):1289-98.
10. Souidi N, Stolk M, Seifert M. Ischemia-reperfusion injury: beneficial effects of mesenchymal stromal cells. *Curr Opin Organ Transplant* 2013;**18**(1):34-43.
11. Solhjou Z, Uehara M, Bahmani B, et al. Novel Application of Localized Nanodelivery of Anti-Interleukin-6 Protects Organ Transplant From Ischemia-Reperfusion Injuries. *Am J Transplant* 2017;**17**(9):2326-37.
12. de Rougemont O, Dutkowski P, Clavien PA. Biological modulation of liver ischemia-reperfusion injury. *Curr Opin Organ Transplant* 2010;**15**(2):183-9.
13. Rauen U, de Groot H. New insights into the cellular and molecular mechanisms of cold storage injury. *J Investig Med* 2004;**52**(5):299-309.
14. Cursio R, Gugenheim J. Ischemia-Reperfusion Injury and Ischemic-Type Biliary Lesions following Liver Transplantation. *J Transplant* 2012;**2012**:164329.
15. Deschenes M. Early allograft dysfunction: causes, recognition, and management. *Liver Transpl* 2013;**19 Suppl 2**:S6-8.
16. Neves DB, Rusi MB, Diaz LG, et al. Primary graft dysfunction of the liver: definitions, diagnostic criteria and risk factors. *Einstein (Sao Paulo)* 2016;**14**(4):567-72.
17. Zhu Z, Tang Y, Huang S, et al. Donor liver apoptosis is associated with early allograft dysfunction and decreased short-term graft survival after liver transplantation. *Clin Transplant* 2018;**32**(12):e13438.
18. Eltzschig HK, Eckle T. Ischemia and reperfusion--from mechanism to translation. *Nat Med* 2011;**17**(11):1391-401.
19. Nasralla D, Coussios CC, Mergental H, et al. A randomized trial of normothermic preservation in liver transplantation. *Nature* 2018;**557**(7703):50-56.
20. Nasralla D, Ploeg R, Coussios C, et al. Outcomes from a Multinational Randomised Controlled Trial Comparing Normothermic Machine Perfusion with Static Cold Storage in Human Liver Transplantation. *American Journal of Transplantation* 2017;**17**:404-04.
21. Ceresa CDL, Nasralla D, Knight S, et al. Cold storage or normothermic perfusion for liver transplantation: probable application and indications. *Curr Opin Organ Transplant* 2017;**22**(3):300-05.
22. Moers C, Smits JM, Maathuis MH, et al. Machine perfusion or cold storage in deceased-donor kidney transplantation. *N Engl J Med* 2009;**360**(1):7-19.
23. Ravikumar R, Jassem W, Mergental H, et al. Liver Transplantation After Ex Vivo Normothermic Machine

Preservation: A Phase 1 (First-in-Man) Clinical Trial. *Am J Transplant* 2016;**16**(6):1779-87.

24. Cypel M, Yeung JC, Liu M, et al. Normothermic ex vivo lung perfusion in clinical lung transplantation. *N Engl J Med* 2011;**364**(15):1431-40.
25. Warnecke G, Moradiellos J, Tudorache I, et al. Normothermic perfusion of donor lungs for preservation and assessment with the Organ Care System Lung before bilateral transplantation: a pilot study of 12 patients. *Lancet* 2012;**380**(9856):1851-58.
26. Dhital KK, Iyer A, Connellan M, et al. Adult heart transplantation with distant procurement and ex-vivo preservation of donor hearts after circulatory death: a case series. *Lancet* 2015;**385**(9987):2585-91.
27. He X, Guo Z, Zhao Q, et al. The first case of ischemia-free organ transplantation in humans: A proof of concept. *Am J Transplant* 2018;**18**(3):737-44.
28. Zhao Q, Huang S, Wang D, et al. Does Ischemia Free Liver Procurement Under Normothermic Perfusion Benefit the Outcome of Liver Transplantation? *Ann Transplant* 2018;**23**:258-67.
29. Chan AW, Tetzlaff JM, Gotzsche PC, et al. SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. *BMJ* 2013;**346**:e7586.
30. Nalesnik MA, Woodle ES, Dimaio JM, et al. Donor-transmitted malignancies in organ transplantation: assessment of clinical risk. *Am J Transplant* 2011;**11**(6):1140-7.
31. Yao FY, Ferrell L, Bass NM, et al. Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. *Hepatology* 2001;**33**(6):1394-403.
32. Martin P, DiMartini A, Feng S, et al. Evaluation for liver transplantation in adults: 2013 practice guideline by the American Association for the Study of Liver Diseases and the American Society of Transplantation. *Hepatology* 2014;**59**(3):1144-65.
33. Laing RW, Mergental H, Yap C, et al. Viability testing and transplantation of marginal livers (VITTAL) using normothermic machine perfusion: study protocol for an open-label, non-randomised, prospective, single-arm trial. *BMJ Open* 2017;**7**(11):e017733.
34. Olthoff KM, Kulik L, Samstein B, et al. Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. *Liver Transpl* 2010;**16**(8):943-9.
35. Jochmans I, Monbaliu D, Pirenne J. The beginning of an end point: peak AST in liver transplantation. *J Hepatol* 2014;**61**(5):1186-7.
36. Broering DC, Topp S, Schaefer U, et al. Split liver transplantation and risk to the adult recipient: analysis using matched pairs. *J Am Coll Surg* 2002;**195**(5):648-57.
37. Siniscalchi A, Gamberini L, Bardi T, et al. Post-reperfusion syndrome during orthotopic liver transplantation, which definition best predicts postoperative graft failure and recipient mortality? *J Crit Care* 2017;**41**:156-60.
38. Novellas S, Caramella T, Fournol M, et al. MR cholangiopancreatography features of the biliary tree after liver transplantation. *AJR Am J Roentgenol* 2008;**191**(1):221-7.
39. Boraschi P, Donati F, Gigoni R, et al. Ischemic-type biliary lesions in liver transplant recipients: evaluation with magnetic resonance cholangiography. *Transplant Proc* 2004;**36**(9):2744-7.
40. Banff schema for grading liver allograft rejection: an international consensus document. *Hepatology* 1997;**25**(3):658-63.
41. Khwaja A. KDIGO clinical practice guidelines for acute kidney injury. *Nephron Clin Pract* 2012;**120**(4):c179-84.
42. Inker LA, Schmid CH, Tighiouart H, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med* 2012;**367**(1):20-9.
43. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. *Am J Infect Control* 2008;**36**(5):309-32.
44. Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a

cohort of 6336 patients and results of a survey. *Ann Surg* 2004;**240**(2):205-13.

45. Slankamenac K, Graf R, Barkun J, et al. The comprehensive complication index: a novel continuous scale to measure surgical morbidity. *Ann Surg* 2013;**258**(1):1-7.
46. Suzuki S, Toledo-Pereyra LH, Rodriguez FJ, et al. Neutrophil infiltration as an important factor in liver ischemia and reperfusion injury. Modulating effects of FK506 and cyclosporine. *Transplantation* 1993;**55**(6):1265-72.
47. op den Dries S, Westerkamp AC, Karimian N, et al. Injury to peribiliary glands and vascular plexus before liver transplantation predicts formation of non-anastomotic biliary strictures. *J Hepatol* 2014;**60**(6):1172-9.
48. Hansen T, Hollemann D, Pitton MB, et al. Histological examination and evaluation of donor bile ducts received during orthotopic liver transplantation--a morphological clue to ischemic-type biliary lesion? *Virchows Arch* 2012;**461**(1):41-8.
49. He X, Guo Z, Ju W, et al. Improved Transplant Outcomes in Ischemia-Free Liver Transplantation: A Report of the First 30 Cases. *American Journal of Transplantation* 2019;**19**:354-55.
50. Croome KP, Hernandezalejandro R, Chandok N. Early Allograft Dysfunction Is Associated With Excess Resource Utilization After Liver Transplantation. *Transplant Proc* 2013;**45**(1):259-64.
51. Dutkowski P, Guarrera JV, de Jonge J, et al. Evolving Trends in Machine Perfusion for Liver Transplantation. *Gastroenterology* 2019;**156**(6):1542-47.
52. Quintini C, Martins PN, Shah S, et al. Implementing an innovated preservation technology: The American Society of Transplant Surgeons' (ASTS) Standards Committee White Paper on Ex Situ Liver Machine Perfusion. *Am J Transplant* 2018;**18**(8):1865-74.

Figure legends

Figure 1 The brief flow chart of this study.

Abbreviations: AASLD, American Association for the Study of Liver Diseases; ALT, alanine aminotransferase; AST, aspartate transaminase; COTRS, China Organ Transplant Response System; DTAC, Disease Transmission Advisory Committee; EAD, early allograft dysfunction; HIV, Human Immunodeficiency Virus; ICU, intensive care unit; INR, international normalized ratio; MELD, model for end-stage liver disease; PNF, primary nonfunction; POD, postoperative day; POM, postoperative month; Tbil, total bilirubin; UCSF, University of California at San Francisco.

Table 1 The schedule for donor screening

Contents	Screening stage	Retrieval day
Time	-7day-0day	0day
Written informed consent	×	
Eligibility assessment	×	
Patient history	×	
Demographic data	×	
Vital signs	×	×
Physical examination	×	×
Standard routine blood tests	×	
Standard routine examinations	×	
Collection of blood specimens		×
Liver biopsy		×

Standard routine blood tests: blood type, blood/urine/stool routine test, coagulation function, communicable and infectious diseases, blood gas analysis, electrolytes, liver/renal/heart/ function tests.

Standard routine examinations: electrocardiogram, chest X ray, cardiac and abdominal color ultrasound, head/chest/ abdomen CT scan.

Table 2 The schedule for recipient screening

Contents	Screening stage	Transplant day
Time	-30day-0day	0day
Written informed consent	×	
Eligibility assessment	×	
Patient history	×	
Demographic data	×	
Vital signs	×	×
Physical examination	×	×
Performance status (ECOG)		×
Quality of life (EQ-5D)		×
Standard routine blood tests	×	
Standard routine examinations	×	
Collection of blood specimens		×
Liver biopsy		×
Others		

Standard routine blood tests: blood type, blood/urine/stool routine test, coagulation function, communicable and infectious diseases, electrolytes, liver/renal/heart/ function tests.

Standard routine examinations: electrocardiogram, chest X ray, lung function, cardiac and abdominal color ultrasound, abdomen CT scan.

Others: other parameters such as magnetic resonance, blood gas analysis, tumor markers and Hepatitis B virus DNA are performed according to clinical conditions

Abbreviations: ECOG, Eastern Cooperative Oncology Group; EQ-5D, EuroQol- 5 Dimension.

Table 3 Blood gas analysis during perfusion

Time (min)	10	20	30	40	60	80	100	120	140	160	180	200	...	420
pH														
PCO_2														
PO_2														
BE														
HCO_3^-														
sO_2														
Lac														
Na^+														
K^+														
Cl^-														
iCa														
GLU														
Hct														
Hb														

Abbreviations: pH, pondus hydrogenii; PCO_2, partial pressure of carbon dioxide; PO_2, partial pressure of oxygen; BE, base excess; HCO_3^-, bicarbonate ion; sO_2, oxygen saturation; Lac, lactate; Na^+,sodium ion; K^+ potassium ion; Cl^- chloride ion; GLU, glucose; iCa, Ionized Calcium; HCT, hematocrit; Hb, Haemoglobin.

Table 4 Regulation of perfusate

	Time (min)	0-20	20	20-40	40	40-60	60	60-80	80-100	...	420
Additive	Sterile water (ml)										
	Gelofusine (ml)										
	Alkaline solution (ml)										
	10% Calcium chloride (ml)										
	Heparin (U)										
	Vasoactive drugs										
	Gas										
	Others										

Table 5 Perfusion device parameters monitoring

Time	Hour	1h						2h						...	8h					
	Min	0	10	20	30	40	50	0	10	20	30	40	50		0	10	20	30	40	50
HA	Pressure																			
	Flow rate																			
	R																			
	T																			
PV	Pressure																			
	Flow rate																			
	R																			
	T																			

Abbreviations: HA, hepatic artery; PV, portal vein; R, resistance index; T, temperature

Table 6 Blood and perfusate biochemical monitoring

	Donor blood		Perfusate						
	Pre-procurement	Intraoperative	Post-modulation	0h	0.5h	1.0h	...	implantation	Post-reperfusion
K^+									
Na^+									
ALT									
AST									
ALP									
GGT									
LDH									
GLU									
Tbil									
Crea									
Osm									

Abbreviations: K^+, potassium ion; Na^+, sodium ion; ALT, alanine aminotransferase; AST, aspartate transaminase; ALP, alkaline phosphatase; GGT, glutamyl transpeptidase; LDH, lactate dehydrogenase; Tbil, total bilirubin; GLU, glucose; Crea, creatinine; Osm, osmotic pressure.

Table 7 Bile composition monitoring

	Procurement	1h	2h	3h	4h	5h	6h	7h	8h	Implantation	Reperfusion
Bile produce (ml/h)											
pH											
PCO_2											
PO_2											
BE											
HCO3-											
sO_2											
Lac											
Na^+											
K^+											
Cl^-											
iCa											
GLU											
Bile acid (umol/L)											
Cholesterol (umol/L)											
GGT (U/L)											
Tbil (umol/L)											
LDH (U/L)											

Abbreviations: pH, pondus hydrogenii; PCO_2, partial pressure of carbon dioxide; PO_2, partial pressure of oxygen; BE, base excess; HCO_3^-, bicarbonate ion; sO_2, oxygen saturation; Lac, lactate; Na^+,sodium ion; K^+ potassium ion; Cl^- chloride ion; GLU, glucose; GGT, glutamyl transpeptidase; Tbil, total bilirubin ; LDH, lactate dehydrogenase.

Table 8 Follow-up period 1(POD1-7)

	POD 1[$]	POD 2	POD 3	POD 4	POD 5	POD 6	POD 7
Hb (g/L)							
WBC (10^9/L)							
PLT (10^9/L)							
NEUT%							
CRP							
ALT (U/L)							
AST (U/L)							
Tbil (umol/L)							
LDH (U/L)							
Crea (mmol/L)							
ALB (g/L)							
PA (mg/L)							
ALP (U/L)							
GGT (U/L)							
PCT							
CK							
CK-MB							
Myoglobin							
High-sensitivity troponin							
Endotoxin							
Serum amylase							
Serum lipase							
Ca^{2+}							
Serum cystatin							
Urea							
Complement C1q							
P^{2+}							
Retinol binding protein							
BNP							
Ammonia (mg/L)							
PT (S)							
INR							
Fbg (g/L)							
D-Dimer (mg/L)							
PH*							
BE*							
Lac (mmol/L)*							
PO2 (mmHg)*							
PCO2 (mmHg)*							
HCO3- (mmol/L)*							
Collection of blood specimens	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y

Blood culture #	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y
US #	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y
Chest X-ray #	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y
Complications	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y
Adverse events	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y
Medication Records	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y
Recipient survival status	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y
Graft survival status	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y

$ Blood examination 5-11 hours after reperfusion.

* If the recipient is still in ICU or needs arterial blood gas analysis.

& Blood culture: When the patient's body temperature is above 38℃, the blood culture should be carried out according to the patient's condition.

Every other day within 1 week after operation.

Complications, adverse events and medication records are recorded in detail in the case report form.

Abbreviations: Hb, Haemoglobin; WBC, white blood cell; PLT, platelets; NEUT%, neutrophilic granulocyte percentage; CRP, C-reactive protein; ALT, alanine aminotransferase; AST, aspartate transaminase; Tbil, total bilirubin; LDH, lactate dehydrogenase; Crea, creatinine; ALB, albumin; PA, prealbumin; ALP, alkaline phosphatase; GGT, glutamyl transpeptidase; PCT, procalcitonin; CK, creatine kinase; CK-MB, creatine kinase isoenzyme; P^{2+}, phosphonium ion; BNP, brain natriuretic peptide; PT, prothrombin time; INR, international Normalized Ratio; Fbg, fibrinogen; pH, pondus hydrogenii; BE, base excess; Lac, lactate; PO_2, partial pressure of oxygen; PCO_2, partial pressure of carbon dioxide; HCO_3^-, bicarbonate ion; US, ultrasound

Table 9 Follow-up period 2 (POD14 and discharge day)

	POD14	Discharge Day
Hb (g/L)		
WBC (10^9/L)		
PLT (10^9/L)		
NUET%		
CRP*		
PCT*		
PT (S)		
INR		
Fbg (g/L)		
D-Dimer (mg/L)*		
ALT (U/L)		
AST (U/L)		
Tbil (umol/L)		
LDH (U/L)		
Crea (mmol/L)		
ALB (g/L)		
PA (mg/L)		
ALP (U/L)		
GGT (U/L)		
Ammonia (mg/L) *		
Collection of blood specimens		
US *		
Performance status (ECOG)		
Quality of life (EQ-5D)		
Complications	$\square_0$N $\square_1$Y	$\square_0$N $\square_1$Y
Adverse events	$\square_0$N $\square_1$Y	$\square_0$N $\square_1$Y
Medication Records	$\square_0$N $\square_1$Y	$\square_0$N $\square_1$Y
Recipient survival status	$\square_1$N $\square_1$Y	$\square_0$N $\square_1$Y
Graft survival status	$\square_0$N $\square_1$Y	$\square_0$N $\square_1$Y

* If necessary.

Complications, adverse events and medication records are recorded in detail in the case report form.

Hb, Haemoglobin; WBC, white blood cell; PLT, platelets; NEUT%, neutrophilic granulocyte percentage; CRP, C-reactive protein; PCT, procalcitonin; PT, prothrombin time; INR, international Normalized Ratio; Fbg, fibrinogen;ALT, alanine aminotransferase; AST, aspartate transaminase; Tbil, total bilirubin; LDH, lactate dehydrogenase; Crea, creatinine; ALB, albumin; PA, prealbumin; ALP, alkaline phosphatase; GGT, glutamyl transpeptidase; US, ultrasound; ECOG, Eastern Cooperative Oncology Group; EQ-5D, EuroQol- 5 Dimension.

Table 10 Follow-up period (Within one year after discharge)

	POM1	POM2	POM3	POM4	POM4	POM5	…	POM12
Hb (g/L)								
WBC (10^9/L)								
PLT (10^9/L)								
NUET%								
CRP*								
PCT*								
PT (S)								
INR								
Fbg (g/L)								
D-Dimer (mg/L)*								
ALT (U/L)								
AST (U/L)								
Tbil (umol/L)								
LDH (U/L)								
Crea (mmol/L)								
ALB (g/L)								
PA (mg/L)								
ALP (U/L)								
GGT (U/L)								
Ammonia (mg/L)*								
Collection of blood specimens								
MRCP#								
US*								
Performance status (ECOG)								
Quality of life (EQ-5D)								
Complications	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y
Adverse events	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y
Medication Records	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y
Recipient survival status	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y
Graft survival status	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y

* If necessary.

all patients undergo an MRCP at 6 months and 12 months after transplantation

Complications, adverse events and medication records are recorded in detail in the case report form.

Hb, Haemoglobin; WBC, white blood cell; PLT, platelets; NEUT%, neutrophilic granulocyte percentage; CRP, C-reactive protein; PCT, procalcitonin; PT, prothrombin time; INR, international Normalized Ratio; Fbg, fibrinogen;ALT, alanine aminotransferase; AST, aspartate transaminase; Tbil, total bilirubin; LDH, lactate

dehydrogenase; Crea, creatinine; ALB, albumin; PA, prealbumin; ALP, alkaline phosphatase; GGT, glutamyl transpeptidase; US, ultrasound; MRCP, magnetic resonance cholangiopancreatography; ECOG, Eastern Cooperative Oncology Group; EQ-5D, EuroQol- 5 Dimension.

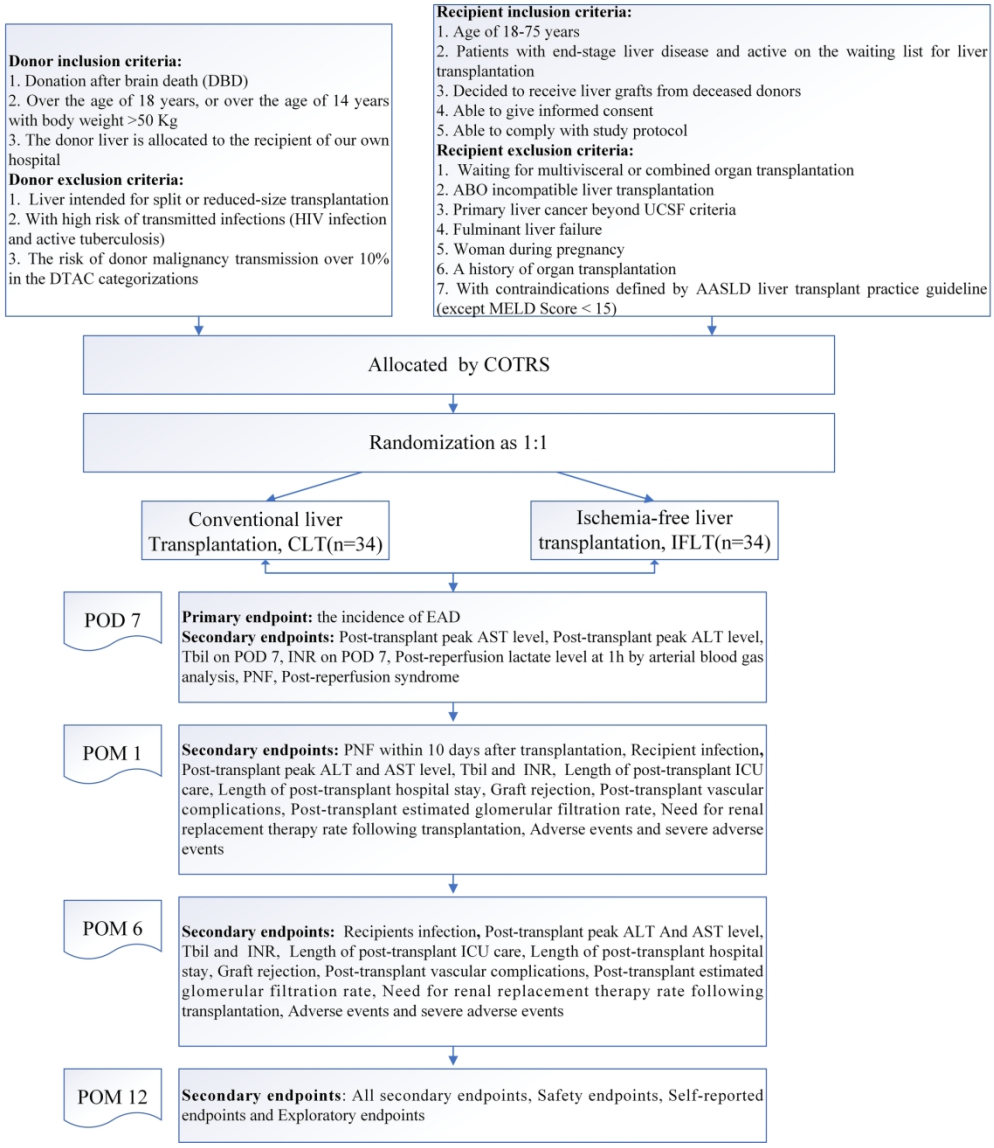


Figure 1 The brief flow chart of this study.

Abbreviations: AASLD, American Association for the Study of Liver Diseases; ALT, alanine aminotransferase; AST, aspartate transaminase; COTRS, China Organ Transplant Response System; DTAC, Disease Transmission Advisory Committee; EAD, early allograft dysfunction; HIV, Human Immunodeficiency Virus; ICU, intensive care unit; INR, international normalized ratio; MELD, model for end-stage liver disease; PNF, primary nonfunction; POD, postoperative day; POM, postoperative month; Tbil, total bilirubin; UCSF, University of California at San Francisco

180x205mm (300 x 300 DPI)

BMJ Open

Study protocol for a prospective, single-centre, randomized, controlled trial to evaluate the efficacy and safety of ischaemia-free liver transplantation (IFLT) in the treatment of end-stage liver disease

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-035374.R1
Article Type:	Protocol
Date Submitted by the Author:	17-Feb-2020
Complete List of Authors:	Huang, Changjun; The First Affiliated Hospital, Sun Yat-sen University, Organ Transplant Center; The First Affiliated Hospital, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Organ Donation and Transplant Immunology Huang, Shanzhou; The First Affiliated Hospital, Sun Yat-sen University; The First Affiliated Hospital, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Organ Donation and Transplant Immunology Tang, Yunhua; The First Affiliated Hospital, Sun Yat-sen University, Organ Transplant Center; The First Affiliated Hospital, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Organ Donation and Transplant Immunology Zhao, Qiang; The First Affiliated Hospital, Sun Yat-sen University, Organ Transplant Center; The First Affiliated Hospital, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Organ Donation and Transplant Immunology Wang, Dongping; The First Affiliated Hospital of Sun Yat-sen University, Organ Transplant Center; The First Affiliated Hospital, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Organ Donation and Transplant Immunology Ju, Weiqiang; The First Affiliated Hospital, Sun Yat-sen University, Organ Transplant Center; The First Affiliated Hospital, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Organ Donation and Transplant Immunology Yang, Lu; The First Affiliated Hospital, Sun Yat-sen University, Department of Anesthesiology Zhang, Jian; Sun Yat-Sen University Zhongshan Ophthalmic Center, State Key Laboratory of Ophthalmology Wu, Linwei; The First Affiliated Hospital, Sun Yat-sen University, Organ Transplant Center; The First Affiliated Hospital, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Organ Donation and Transplant Immunology Chen, Maogen; The First Affiliated Hospital, Sun Yat-sen University, Organ Transplant Center; The First Affiliated Hospital, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Organ Donation and Transplant Immunology Zhang, Zhiheng; The First Affiliated Hospital, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Organ Donation and Transplant Immunology; The First Affiliated Hospital, Sun Yat-sen University, Organ

	Transplant Center Zhu, Zebin; The First Affiliated Hospital, Sun Yat-sen University, Organ Transplant Center; The First Affiliated Hospital, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Organ Donation and Transplant Immunology Wang, Linhe; The First Affiliated Hospital, Sun Yat-sen University, Organ Transplant Center; The First Affiliated Hospital, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Organ Donation and Transplant Immunology Zhu, Caihui; The First Affiliated Hospital, Sun Yat-sen University, Organ Transplant Center; The First Affiliated Hospital, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Organ Donation and Transplant Immunology Zhang, Yixi; The First Affiliated Hospital, Sun Yat-sen University, Organ Transplant Center; The First Affiliated Hospital, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Organ Donation and Transplant Immunology Sun, Chengjun; The First Affiliated Hospital, Sun Yat-sen University, Organ Transplant Center; The First Affiliated Hospital, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Organ Donation and Transplant Immunology Xiong, Wei; The First Affiliated Hospital, Sun Yat-sen University, Department of Anesthesiology Shen, Yuekun; The First Affiliated Hospital, Sun Yat-sen University, Department of Anesthesiology, Chen, Xiaoxiang; The First Affiliated Hospital, Sun Yat-sen University, Department of Anesthesiology, Ma, Yi; The First Affiliated Hospital, Sun Yat-sen University, Organ Transplant Center ; The First Affiliated Hospital, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Organ Donation and Transplant Immunology Hu, Anbin; The First Affiliated Hospital, Sun Yat-sen University, Organ Transplant Center; The First Affiliated Hospital, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Organ Donation and Transplant Immunology Zhu, Xiaofeng; The First Affiliated Hospital, Sun Yat-sen University, Organ Transplant Center; The First Affiliated Hospital, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Organ Donation and Transplant Immunology Rong, Jian; The First Affiliated Hospital, Sun Yat-sen University, Department of Cardiopulmonary Bypass Cai, Changjie; The First Affiliated Hospital, Sun Yat-sen University, Surgical Intensive Care Unit Guo, Zhiyong; The First Affiliated Hospital, Sun Yat-sen University, Organ Transplant Center; The First Affiliated Hospital, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Organ Donation and Transplant Immunology He, Xiaoshun; The First Affiliated Hospital, Sun Yat-sen University, Organ Transplant Center; The First Affiliated Hospital, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Organ Donation and Transplant Immunology
Primary Subject Heading:	Surgery
Secondary Subject Heading:	Gastroenterology and hepatology
Keywords:	TRANSPLANT SURGERY, Hepatobiliary surgery < SURGERY, Hepatology < INTERNAL MEDICINE

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Study protocol for a prospective, single-centre, randomized, controlled trial to evaluate the efficacy and safety of ischaemia-free liver transplantation (IFLT) in the treatment of end-stage liver disease

Changjun Huang[1,2,3,†], Shanzhou Huang[1,2,3,†], Yunhua Tang[1,2,3,†], Qiang Zhao[1,2,3], Dongping Wang[1,2,3], Weiqiang Ju[1,2,3], Lu Yang[4], Jian Zhang[5], Linwei Wu[1,2,3], Maogen Chen[1,2,3], Zhiheng Zhang[1,2,3], Zebin Zhu[1,2,3], Linhe Wang[1,2,3], Caihui Zhu[1,2,3], Yixi Zhang[1,2,3], Chengjun Sun[1,2,3], Wei Xiong[4], Yuekun Shen[4], Xiaoxiang Chen[4], Yi Ma[1,2,3], Anbin Hu[1,2,3], Xiaofeng Zhu[1,2,3], Jian Rong[6], Changjie Cai[7], Zhiyong Guo[1,2,3,*], Xiaoshun He[1,2,3,*]

[1]Organ Transplant Center, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, China

[2]Guangdong Provincial Key Laboratory of Organ Donation and Transplant Immunology, Guangzhou 510080, China

[3]Guangdong Provincial International Cooperation Base of Science and Technology (Organ Transplantation), Guangzhou 510080, China

[4]Department of Anesthesiology, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, China

[5]State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou 510080, China

[6]Department of Cardiopulmonary Bypass, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, China

[7]Surgical Intensive Care Unit, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, China

*Corresponding authors: Zhiyong Guo, and Xiaoshun He, Organ Transplant Center, The First Affiliated Hospital, Sun Yat-sen University, NO.58 Zhongshan Er Road, Guangzhou 510080, China (Tel: 86-20-87306082; Fax: 86-20-87306082; E-mail: rockyucsf1981@126.com and gdtrc@163.com)

[†]The first three authors contributed equally to this work and should be considered as co-first authors.

Strengths and limitations of this study

This study is the first to compare the efficacy and safety of ischaemia-free liver transplantation (IFLT) and conventional liver transplantation (CLT) in the treatment of end-stage liver disease in a randomized controlled clinical trial.

The study will answer the question: "Can IFLT reduce ischaemia-reperfusion injury (IRI)-related unfavourable impacts and achieve better transplant outcomes?"

The inclusion of a series of well-designed endpoints and multiple research parameters will enable an in-depth analysis of the effects of IFLT on human liver transplantation.

The randomization design will allow us to achieve a homogeneous distribution of patients between IFLT and CLT.

The open-label design is considered a limitation of this trial, and due to the nature of the surgical procedure, it is not possible to blind the surgical team to the group allocation.

ABSTRACT

Introduction During conventional liver transplantation (CLT), ischaemia-reperfusion injury (IRI) is inevitable and is associated with complications such as early allograft dysfunction (EAD), primary non-function, and ischaemic-type biliary lesions. We have established a novel procedure called ischaemia-free liver transplantation (IFLT). The results from a pilot study suggest that IFLT might prevent IRI and yield better transplant outcomes than CLT. The purpose of this study was to further assess the efficacy and safety of IFLT versus CLT in patients with end-stage liver disease.

Methods and analysis This is an investigator-initiated, open-label, phase III, prospective, single-centre randomized, controlled trial on the effects of IFLT in patients with end-stage liver disease. Adult patients (18-75 years) eligible for liver transplantation will be screened for participation in this trial and will be randomized between the IFLT group (n=34) and the CLT group (n=34). In the IFLT group, the donor liver will be procured, preserved and implanted with continuous normothermic machine perfusion (NMP). In the CLT group, the donor liver will be procured after a fast cold flush, preserved in 0-4°C solution, and implanted under hypothermic and hypoxic conditions. Patients in both groups will be managed according to the standard protocol of our centre. The primary endpoint is the incidence of EAD after liver transplantation. Intraoperative

and postoperative parameters of donor livers and recipients will be observed and recorded, and postoperative liver graft function, complications, and recipient and graft survival will be evaluated. After a 12-month follow-up of the last enrolled recipient, the outcomes will be analysed to evaluate the safety and efficacy of IFLT versus CLT in patients with end-stage liver disease.

Ethics and dissemination The protocol was reviewed and approved by the Ethics Committee of The First Affiliated Hospital of Sun Yat-sen University. The findings will be disseminated to the public through conference presentations and peer-reviewed scientific journals.

Trial registration number ChiCTR1900021158.

Keywords: Ischaemia-free liver transplantation, normothermic machine perfusion, Ischaemia-reperfusion injury, Donation after brain death, Early allograft dysfunction

INTRODUCTION

Background and rationale

Over the past several decades, liver disease has become one of the leading causes of death and illness worldwide.[1] One-fifth of the population in China suffers from some kind of liver disease, and the number of patients with end-stage liver disease remains high.[2] Liver transplantation has been acknowledged as the only effective treatment for end-stage liver diseases.[3] Although the short-term outcome of organ transplantation has made certain progress in recent years, the long-term outcome has not been significantly improved.[4] Meanwhile, organ shortage has become a worldwide issue, with a large number of patients dying on the waiting list for liver transplantation.[5 6] Hence, further improvement of transplant efficacy and expansion of the donor organ pool have attracted great attention in the field of organ transplantation.

During the process of conventional organ transplantation, organs are procured after a fast cold flush, preserved in 0-4°C solution, and implanted under hypothermic and hypoxic conditions.[7-9] The organs can suffer ischaemic injuries during the whole transplant procedure, so ischaemia-reperfusion injury (IRI) is an inevitable event in all types of organ transplantation.[10 11] IRI not only brings about allograft dysfunction but also undermines the function of other organs, giving rise to a series of complications and even patient death.[12 13] The incidences of early graft dysfunction (EAD), primary nonfunction (PNF) and ischaemic-type biliary lesions (ITBL) after liver transplantation are around 10-50%, 3-7% and 5-10%, respectively.[14-17] For decades, researchers have been making efforts to reduce the morbidity and mortality related to IRI. However, owing to the complex molecular mechanisms of IRI, interventions such as drugs, stem cells, and protective gases exert little effect.[18 19]

In the past decade, there have been tremendous achievements in the field of machine perfusion technology. It has been demonstrated that both normothermic machine perfusion (NMP) and hypothermic oxygenated machine perfusion (HOPE) can alleviate allograft IRI and improve transplant outcomes in animal experiments and clinical trials.[20-23] Particularly, conditions close to physiological status are provided to the grafts during *ex vivo* NMP. Therefore, not only organ repair but also graft viability assessment can be achieved during NMP. The technique has been successfully applied in lung, liver, kidney and heart transplantation.[20-26] However, under the current practice, ischaemic injuries of organs first occur during procurement and preparation, before the initiation of NMP, and then once again during implantation after NMP. Therefore, the organs might suffer a “double hit” of IRI.

We therefore hypothesize that continuous oxygenated blood supply to the donor liver during the entire period of donor liver procurement, preservation and implantation could prevent IRI and significantly reduce the incidence of complications induced by IRI. We established a reliable ischaemia-free liver transplantation (IFLT) technique in pigs and reported the first case of IFLT in humans.[27 28] During IFLT, the blood supply to the donor livers is continuously maintained throughout the whole process of procurement, preservation and implantation. The results of the first 14 cases of IFLT showed that EAD occurred in 1 case (7.1%), compared with in 25 (53.2%) out of 47 cases of conventional liver transplantation (CLT) with standard static cold storage (SCS) in the same period. The peak alanine aminotransferase (AST) (369 U/L vs. 1502 U/L, $P<0.001$) and peak aspartate aminotransferase (ALT) (201 U/L vs. 689 U/L, $P<0.001$) within 7 days post-transplantation were significantly decreased in the IFLT versus the CLT group. Histological studies showed that in sharp contrast to CLT, there was no augmentation in the Suzuki score, hepatocyte apoptosis, inflammatory cytokines, chemokines or activation of inflammatory pathways after reperfusion in the IFLT group.

Although the pilot study suggested promising transplant outcomes of IFLT, the design of the study was non-randomized, and patient selection bias could not be ruled out. The purpose of this study was to further explore the efficacy and safety of IFLT in a prospective, randomized, controlled trial.

Objectives

Primary objective

To compare the incidence of IRI-related complications between IFLT and CLT recipients, as well as the allograft/recipient survival rate, to further validate the clinical efficacy and safety of IFLT.

Secondary objective

To compare the severity of allograft IRI between IFLT and CLT by laboratory analysis of peripheral blood and liver biopsy specimens.

METHODS

This protocol was designed to conform with the Standard Protocol Items: Recommendations for Interventional Trials 2013 statement.[29]

Study setting

The study will be conducted at The First Affiliated Hospital of Sun Yat-sen University.

Eligibility criteria

The following inclusion and exclusion criteria will be applied when recruiting donors and recipients. Only donors and recipients meeting these criteria will be recruited into the study.

Donor inclusion criteria

1. Donation after brain death (DBD).

2. Over the age of 18 years, or over the age of 14 years with body weight >50 kg.

3. The donor liver is allocated to a recipient at our own hospital.

Recipient inclusion criteria

1. Age 18-75 years.

2. End-stage liver disease and active on the waiting list for liver transplantation.

3. Agreed to receive liver grafts from deceased donors.

4. Able to give informed consent.

5. Able to comply with the study protocol.

Donor exclusion criteria

1. Livers intended for split or reduced-size transplantation.

2. High risk of transmitted infections (human immunodeficiency virus infection and active tuberculosis).

3. Risk of donor malignancy transmission over 10% according to the Disease Transmission Advisory Committee (DTAC) categorizations.[30]

Recipient exclusion criteria

1. Waiting for multivisceral or combined organ transplantation.

2. ABO-incompatible liver transplantation.

3. Primary liver cancer beyond the University of California at San Francisco (UCSF) criteria.[31]

4. Fulminant liver failure.

5. Current pregnancy.

6. A history of organ transplantation.

7. Contraindications defined by the American Association for the Study of Liver Diseases (AASLD) liver transplant practice guidelines,[32] except model for end-stage liver disease (MELD) score < 15.

Interventions

Screening

All preoperative evaluation and eligibility scrutiny must be accomplished before randomization to ensure that the donor and recipient match all inclusion criteria. The investigator will create a screening log to record the details of all selected donors and recipients to confirm their eligibility or ineligibility.

1. Donors: When a potential donor comes into sight, brain death should be confirmed by two doctors. Medical history-taking and preoperative evaluation should be conducted following the study protocol. Eligibility will be determined according to inclusion and exclusion criteria. The schedule for donor screening is summarized in Table 1.

2. Recipients: When an end-stage liver disease patient is enrolled on the waiting list, medical history-taking and preoperative evaluation should be conducted following the study protocol. Eligibility will be determined according to inclusion and exclusion criteria. The schedule for recipient screening is summarized in Table 2.

Donor and recipient matching

1. Donor livers are allocated to recipients by the China Organ Transplant Response System (COTRS) according to blood type, patient condition, waiting time and other routine allocation principles.

2. After an eligible donor liver is allocated to an eligible recipient in our centre, the donor and recipient are assigned to the experimental group (IFLT group) or control group (CLT group) based on randomization.

Surgical procedures (IFLT vs CLT)

IFLT

Donor livers from the IFLT group will undergo continuous NMP during procurement, preservation and implantation.[27]

1. Ischaemia-free procurement of donor liver

Mobilization of the liver is conducted with a precise technique. A tube is placed in the common bile duct for bile drainage, and the cystic duct is ligated. The celiac artery (CA), gastroduodenal artery (GDA), splenic artery (SA), inferior vena cava (IVC), and portal vein (PV) are well dissected. An 8-Fr/12-Fr arterial cannula is inserted into the GDA or SA without interruption of arterial supply for the liver from the CA. The arterial cannula is connected to the hepatic artery (HA) perfusion line of the Liver Assist (Organ Assist, Groningen, the Netherlands). A 3-cm-long right external iliac vein is harvested and end-to-side anastomosed to the portal

vein with partial blockage of the PV for constructing an interposition vein. A straight 24-Fr cannula is connected to the PV perfusion line of the Liver Assist and then inserted into the PV via the interposition vein. A 32-34-Fr caval cannula is placed in the infrahepatic inferior vena cava (IHIVC) for outflow to the organ reservoir of the Liver Assist. The venous drainage of the suprahepatic inferior vena cava (SHIVC) to the right atrium is blocked. Then the *in situ* circuit is established, and NMP is started. The liver is harvested and transferred to the organ reservoir under continuous NMP. Immediately after the liver is removed from the abdominal cavity, the kidneys are cold-flushed via the cannula within the abdominal aorta and procured.

2. Ischaemia-free preservation of donor liver

The liver is transferred to the perfusion device. The caval cannula is removed immediately when the liver is moved to the organ reservoir. The liver graft is subjected to continuous *ex situ* NMP until allograft re-vascularization. The PV perfusion pressure is set at 6-10 mmHg with a targeted flow rate higher than 500 mL/min. The hepatic artery pressure is set at 50-60 mmHg with a targeted flow rate higher than 150 mL/min. During the NMP, the pressure and flow rate are monitored and adjusted to within an appropriate range. Redundant tissues are removed from the liver and blood vessels. The SHIVC and IHIVC are examined for leaks by transient blockage of the IVC. The bile tube is connected to a collection container. The amount of bile production is recorded, and the biochemical parameters is monitored every 60 min. Perfusate samples are taken for blood gas analysis every 10-20 min and liver function tests every 30 min to monitor the biochemical parameters. The viability of the liver is assessed by blood gas analysis and liver function tests of the perfusate, as well as bile biochemical parameters, as previously reported.[33] For the safety of patients, the viability of grafts during NMP is confirmed before we start the recipient surgical procedures.

3. Ischaemia-free implantation of donor liver

The diseased liver is resected using a routine procedure. The donor IHIVC is re-cannulated, and the SHIVC is blocked by a clamp. Then the donor liver is moved from the reservoir and placed in the recipient's abdominal cavity so that an *in situ* NMP circuit is re-established. The donor SHIVC is anastomosed to the recipient counterparts in a corresponding fashion using 3-0 Prolene based on the bicaval or piggy-back technique. The donor PV and HA are anastomosed to the recipient counterparts in an end-to-end fashion using 5-0 and 7-0 Prolene, respectively. Because of the native and artificial branches on the HA and PV, all these anastomoses are accomplished under continuous NMP of the allograft. After that, the clamps on the PV and HA are released so that the native dual blood supply for the liver is re-established. At the same time, NMP ceases after removal of the HA and PV cannula. Then the cannula within IHIVC is removed, and around 200 mL perfusate

within the liver is flushed out, followed by release of the clamp on the SHIVC. The anhepatic phase is over. The donor SA or GDA is ligated closed, and the interposition vein is sutured closed. The donor IHIVC is then anastomosed to the recipient IHIVC or ligated according to the bicaval or piggy-back technique used. The donor common bile duct is end-to-end anastomosed to the recipient common bile duct after withdrawal of the draining tube. After meticulous haemostasis and abdominal closure, the patient is sent to the post-transplant intensive care unit (ICU).

4. Recording and assessment of NMP parameters

In the process of NMP, the stability and efficacy of perfusion are monitored, and liver graft function is monitored by perfusate biochemical tests and blood gas analysis. The perfusion parameters, regulatory measures, bile production and blood gas analysis results are recorded. NMP parameters are summarized in Table 3-7.

CLT

Following the standard *in situ* cold flushing procedure, the liver will be retrieved and placed in ice-cold University of Wisconsin solution. Back-table preparation will be performed under standard procedures prior to implantation. After removal of the diseased liver, the donor liver is transferred to the abdominal cavity. Following anastomosis of the IVC and PV, the vessels are re-opened to restore the blood supply of the allograft. Then the donor artery and bile duct are anastomosed successively. After meticulous haemostasis and abdominal closure, the patient is sent to the ICU.

Intraoperative monitoring

The recipient's condition during operation and anaesthesia will be recorded according to the standards and norms of our centre. Intraoperative monitoring will be conducted to compare the impacts of IFLT or CLT on the functions of the donor liver and other organs, such as the heart, lung, kidney, intestine and brain.

Postoperative management.

Both groups are managed according to the patients' conditions and standard protocols of our centre.

Follow-up

The patients will be followed up for 1 year. Postoperative visits will be performed on postoperative day (POD) 1-7, POD14 and each month post-transplantation. Biomedical values, complications, adverse events and medication administration records will be documented. Follow-up information is shown in Tables 8-10.

Outcomes

Primary endpoint

The primary endpoint is the incidence of EAD within 7 days post-transplantation. The diagnosis of EAD is defined according to the presence of one or more of the following criteria.[34]

1. Peak aspartate amino transferase (AST) >2000 IU/L within the first 7 postoperative days.
2. Peak alanine aminotransferase (ALT) >2000 IU/L within the first 7 postoperative days.
3. Total bilirubin (Tbil) ≥ 10 mg/dL on POD 7 (exclusion of biliary stricture).
4. International normalized ratio (INR) ≥ 1.6 on POD 7.

Secondary endpoints

1. Post-transplant peak AST: To ensure consistency, serum AST will be measured 5-11 hours post-reperfusion on POD 1 and 6-8 am on POD 2-7, and the peak level will be defined as the highest of these values (in IU/L).[35]

2. Post-transplant peak ALT: serum ALT will be measured, and the peak level will be defined as AST.

3. Tbil on POD 7.

4. INR on POD 7.

5. AST, ALT, Tbil, INR, gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) at POD 1-7, POD 14, postoperative month (POM) 1, POM 6 and POM 12.

6. Lactate level at 1 hour post-reperfusion by arterial blood gas analysis.

7. Incidence of PNF: PNF is defined as unavoidable graft dysfunction requiring emergency re-transplantation or leading to death within the first 10 days post-transplantation, in the absence of surgical or immunological factors.[19 36]

8. Post-reperfusion syndrome (PRS): PRS is defined as a decrease in mean arterial pressure ≥30% in comparison with the baseline value, for at least 1 min, occurring during the first 5 min after reperfusion of the donor liver (without clamping of hepatic hilum).[37]

9. Biliary complications include but are not limited to bile leakage, anastomotic stenosis and ITBL. IBTLs are nonanastomotic strictures and dilations involving only the biliary tree of the graft in the absence of hepatic artery thrombosis.[38 39]

10. Patient survival status at POM 1, POM 6 and POM 12.

11. Graft survival status at POM 1, POM 6 and POM 12.

12. Length of post-transplant ICU care.

13. Length of post-transplant hospital stay.

Safety endpoints

1. Graft rejection at POM 1, POM 6 and POM 12, including clinically diagnosed rejection and pathologically confirmed rejection with the Banff schema.[40]

2. Vascular complications at POM 1, POM 6 and POM 12, including thrombosis, haemorrhage, embolism and stenosis of IVC, PV and HA. Patients will undergo a colour Doppler ultrasound at each time point, and digital subtraction angiography (DSA) will be performed when necessary.

3. Acute kidney injury (AKI) within the first 7 postoperative days. AKI will be graded according to the Kidney Disease: Improving Global Outcomes (KDIGO) staging system.[41]

4. Estimated glomerular filtration rate (eGFR) at POD 7, POD 14, POM 1, POM 6 and POM 12. eGFR will be judged according to the chronic kidney disease epidemiology collaboration (CKD-EPI) creatinine equation.[42]

5. Need for renal replacement therapy following transplantation.

6. Recipient infection within POM 1. Infections will be defined on the basis of the standard criteria proposed by the Centers for Disease Control and Prevention.[43]

7. Cumulative complications at POM 1, POM 6 and POM 12. Complications will be graded according to the comprehensive complication index (CCI) based on Clavien-Dindo Classification.[44 45]

8. Adverse events (AE) and severe adverse events (SAE) will be assessed according to National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE) (version 5.0) criteria at POM 1, POM 6 and POM 12.

9. Positive perfusate microbial culture rate. At the end of SCS or NMP, a sample will be collected for microbiological culture (cold preservation solution or warm perfusate).

10. Organ discard rate.

Self-reported endpoints

Quality of life will be scored using the EQ-5D questionnaire obtained before transplantation and at POM 1, POM 6 and POM 12.

Exploratory endpoints

1. Molecular biological data of IRI and the immune system will be evaluated in serum, plasma, whole blood, liver specimens, bile duct tissue and perfusate at the above time points during and after transplantation. The hepatic IRI will be evaluated based on the Suzuki score.[46] Bile duct IRI will be evaluated based on the Hansen

bile duct injury (BDI) score.[47 48]

2. Functional tests of coagulation, heart, lung, kidney, intestine, brain and other organs at the above time points during and after transplantation.

3. The balance between medical expenditure and quality of life.

Sample size

This study has a 1:1 parallel design, and the sample size calculation is based on our pilot study. It is estimated that EAD will occur in 10% of the experimental (IFLT) group and 40% of the control (CLT) group. With a power of 80% (1-β) and significance level (α, two-sided) of 5%, we calculated that 32 patients need to be enrolled in each arm. Considering the possibility of organ discard under special conditions, the sample size was increased by 5%. Ultimately, 34 patients in each arm, for a total of 68 patients, will be enrolled in the study.

Recruitment

Recruitment began in February 2019 and will go until the target sample size is reached (expected: August 2020). The trial was designed as a prospective, randomized, controlled, single-centre clinical trial in patients on the waiting list undergoing liver transplantation. First, donor and recipient eligibility will be assessed before transplantation. Informed consent will be obtained. All the donors have to be in our hospital, and the donor livers will be allocated by COTRS. When a donor liver is allocated to an informed recipient of our own hospital, the recipient will be randomly assigned to the IFLT or CLT group. The number of recipients in the two groups will be allocated 1:1, and the grouping information will be open label. Postoperative monitoring, treatment and nursing will be performed according to the same standards and procedures. Intraoperative parameters, liver graft function, post-transplant complications and patient/graft survival will be observed and recorded. After a 12-month follow-up of the last enrolled recipient, the outcomes will be analysed to evaluate the safety and efficacy of IFLT in human liver transplantation. The research overview flow chart is shown in Figure 1.

Randomization and blinding

This study is a randomized controlled trial, and block randomization will be adopted for 1:1 random grouping.

A subject randomization list will be generated using a proven central randomization system by the statistician, and random allocation numbers will be automatically handled by the system to avoid bias. When all the inclusion/exclusion criteria are fulfilled, the investigator will contact the central randomization system to get a random number, and then the subject will be allocated to the experimental or control group based on the number. This is an open-label study. Because of the nature of the surgical procedure, it is not possible to blind the surgical team to the group allocation. Outcome assessors will be blinded where possible. This includes the diagnostician interpreting the medical imaging examination as well as the histopathologists interpreting the biopsy specimens.

Data collection and management

Case report form (CRF)/electronic database

The investigators should input all subjects' original observation records timely, completely and correctly into the CRF. The data on the CRF will be transformed into an electronic database. The CRF and database will be reviewed by two independent inspectors for error checking, and then the completed data will be handed over to the data manager. If there are questions about a CRF, the data manager will send the Data Clarification Form (DCF) to the investigators and contact data inspectors to solve the doubtful points and return feedback. The data manager will confirm, modify and input data according to the feedback of investigators and send the DCF again if necessary.

Database locking

After data review and confirmation, the data managers, main investigators, statistical analysts, sponsors and supervisors will jointly audit the data and complete the final definition of the analysis population. Then the data manager will lock the database. In general, locked databases or files should not be altered.

Data analysis

The final data will be submitted to the statistical analyst for statistical analysis.

Analysis plan

Analysis sets

1. Full analysis set: intention-to-treat (ITT). Intention-to-treat analysis is a comparison of the treatment groups that includes all patients as originally allocated after randomization.

2. Per-protocol set (PPS): Per-protocol analysis is a comparison of treatment groups that includes only those patients who completed the treatment originally allocated.

The primary endpoint and secondary endpoints of the study will be analysed by ITT and PPS.

Statistics

1. The data analysis will be based on the principle of intention-to-treat (ITT), and all statistical analyses will adopt a two-sided test; $P<0.05$ will be considered statistically significant. After the database is locked, the data analysis will be performed in STATA 14.0 software (Stata Corp).

2. Demographic information and baseline characteristics will be analysed using descriptive statistical analysis.

3. The primary endpoint will be analysed using the chi-square test, and the absolute difference and 95% confidence interval will be calculated.

4. Analysis of secondary endpoint: The two-category variables will be analysed using the chi-square test or logistic regression to report the odds ratio (OR value) after adjusting for confounding factors. For the continuous variables, Student's t test will be utilized if the normal distribution is satisfied; otherwise, the Mann-Whitney test will be utilized, or the mixed model with repeated measurement will be utilized to analyse the change of the individual from the baseline. The time data will be analysed by the Kaplan-Meier method, and the log-rank test and a Cox regression model will also be used.

5. Missing data: If EAD is missing, it will be replaced by the worst-value method, and EAD will be considered. Secondary values will be replaced in two ways: (1) multiple imputation and missing values will be estimated by independent simulation variables according to the characteristics of the predicted values and the availability of the data. Linear regression will be utilized for continuous variables, logistic regression for binary categorical variables, ordered logistic regression for ordered multi-class variables, and disordered multi-class logistic regression for disordered multi-class variables. Twenty data sets will be created by the multiple imputation method, and the final result will be obtained by averaging the results of the twenty data sets using the Rubin rule, ensuring that the standard error of all regression coefficients takes into account the uncertainty of the simulation and the uncertainty of the estimate. (2) Sensitivity analysis. Sensitivity analysis involves directly eliminating missing values, deeming treatment ineffectiveness as well as optimal and worst-case analysis. Sensitivity analysis will be utilized to compare the consistency of the primary results.

Monitoring and safety

AEs that occur during the study should be addressed in accordance with well-established management criteria that will support the life and health of the study subjects. It is the responsibility of the investigators to collect and record all AEs occurring throughout the study. All AEs will be documented on the CRF. All SAEs should be reported to the superintendent and the ethics committee in a specialized SAE from. The causes and effects of SAE will be carefully assessed, and the study will be suspended or terminated if necessary. All SAEs will be followed up to resolution. Recording and reporting of AEs will continue until the last enrolled patient has accomplished 12 months of follow-up.

AEs and complications

AEs are defined as any unintended medical events that occur in patients participating in the trial. An AE does not necessarily have to have a causal relationship with the trial. Complications are AEs that deviate from the ideal postoperative course, are not inherent in the procedure, and do not comprise a failure to cure.

The following scenarios are considered SAEs.

1. Death of the recipient.
2. Life-threatening complications.
3. Persistent or severe disability.
4. Significantly prolonged hospital stay.
5. Other severe events as judged by the investigators.

AEs should be judged and graded according to NCI-CTCAE 5.0 and documented in the CRF. Complications should be categorized by Clavien-Dindo classification and scored against the CCI at each follow-up period.[44]

Withdrawal of trial

Withdrawal initiated by investigators

The investigators will initiate withdrawal in any of the following circumstances:

1. Severe violation of the study protocol occurs due to donor liver, perfusion or recipient reasons in the process of procurement, preservation, implantation or even discarding of the donor liver.
2. The subject suffers from certain diseases that are not suitable for participating in the study.
3. Safety or tolerance is disturbed by poor compliance.
4. Continued treatment will hurt the health of the subjects.

Withdrawal initiated by subjects

Subjects can decide to cease participation in this trial at any time for any reason. The reasons for their withdrawal should be acknowledged and documented.

Withdrawal procedure

1. If the subject withdraws without liver transplant, the subject can be re-added to the waiting list.
2. If the subject withdraws after liver transplant, the subject still can be receive standardized treatment, nursing and follow-up.
3. When a subject has an emergency that requires immediate termination of an ongoing liver transplant or subsequent therapy, the subject should be observed and evaluated accordingly while ensuring safety.

The reasons and time points of any withdrawal should be clearly collected and documented, and the observation and evaluation should be carried out accordingly. When an AE occurs, it must be tracked until it disappears. The CRF of any subject who received treatment but failed to complete the study should be retained, and the last test results will be transferred as the final results. Treatment response, tolerance and AEs will be analysed based on full data analysis.

Specimen collection

Written informed consent is required before all clinical specimens are collected. Complete and standard specimens enable the comparison between the experimental group and the control group under the same conditions, making the experimental data accurate and reliable. The sponsor will organize a clinical specimen collection team and establish a specimen bank. Body fluids, solid tissues and their derivatives (such as DNA, RNA, protein, etc.) will be collected and preserved for related research and experiments. It is the responsibility of the investigators to participate in and supervise the process of specimen collection. All remaining samples must be destroyed within 15 years of the end of the clinical trial.

Before the operation, donor blood will be collected for extracting supernatant and peripheral mononuclear blood cells. After organ retrieval, part of the spleen and iliac vessels will be preserved. In total, three excision biopsies will be harvested from the donor liver: before retrieval, at the end of preservation, and after graft re-vascularization. Two biopsies will be harvested from the donor common bile duct: immediately after procurement and before common bile duct anastomosis following graft re-vascularization. Perfusate samples will be collected repeatedly during machine perfusion. Bile (if produced) will be collected from a

common bile duct tube. Recipient blood samples will be taken preoperatively; on PODs 1, 3, 5, 7, and 14; and in every POM during the follow-up period. Liver biopsy samples will be taken at POM 6 or when rejection is suspected.

DISCUSSION

Liver transplantation is an effective therapy for patients with end-stage liver disease. However, there are still many burdens that hamper the progress of liver transplantation. Donor liver IRI is an inevitable event in the current transplant procedure that often compromises transplant outcomes and increases the organ discard rate.[10-13] Tremendous achievements have been made in the field of alleviating donor liver IRI.[19-21 23] Among these techniques, NMP has been successfully used in clinical practice in several transplant centres. David Nasralla et al reported that liver transplantation with the help of NMP is associated with a decrease of 50% in graft injury and 50% in the organ discard rate and an increase of 54% in preservation time.[19] However, IRI still cannot be fully avoided due to the existence of hypothermia, ischaemia and hypoxia in the surgical procedure. With surgical innovation, IFLT has enabled complete elimination of hypothermia, ischaemia and hypoxia during the whole transplant procedure.[27] Our pilot study demonstrated its feasibility, safety and efficacy, with diminished peak injury markers and lower incidence of EAD compared to CLT.[49] Although the results of the pilot study were promising, the non-randomized design was a drawback. The purpose of this study is to further explore the efficacy and safety of IFLT in a randomized controlled trial.

A potential shortcoming of this study is the single source of DBD donors and the exclusion of donation after cardiac death (DCD) because it is difficult to perform IFLT using DCD organs. However, it is possible to combine *in situ* regional normothermic perfusion (NRP) and IFLT in such donors. Donors with high risks of communicable infectious diseases or malignancy are excluded for the safety of participants. For recipients, patients with fulminant liver failure and primary hepatocellular carcinoma beyond the UCSF criteria are not included in this trial due to their high risk of complications and mortality. In addition, patients with a history of organ transplantation, multiple-organ transplantation or combined organ transplantation and ABO-incompatible liver transplantation are excluded because different surgical or post-transplant management protocols are used in these patients.

Due to the increased organ shortage, an increasing number of livers from extended-criteria donors (ECDs), such as elderly donors and donors with hepatosteatosis, are used worldwide. When compared to

organs of standard-criteria donors (SCD), ECD organs are more vulnerable to IRI and are associated with high risks of morbidity and mortality.[5] Since IFLT might be able to prevent IRI, ECD livers might be an appropriate indication for IFLT. Indeed, the first case of IFLT successfully resuscitated a donor liver with 85-90% macrovesicular steatosis.[28] However, the donors are not limited to ECD donors in the current study, because the benefits of IFLT in either SCD or ECD livers have not been confirmed in a randomized study. In addition, IFLT is an extreme example of NMP. The advantage of NMP over static cold storage is still under debate, although a randomized study was recently reported.[19] Therefore, we did not design this study to compare IFLT with CLT using NMP as a preservation method in the current study. Another randomized controlled study is being planned in our centre to compare the safety and efficacy of these two methods in ECD livers.

The primary endpoint of this trial is the incidence of EAD after liver transplantation. EAD represents a severe form of clinical IRI, serving as an important surrogate endpoint in liver transplantation.[50] The definition of EAD is largely based on serum AST/ALT in the recipient. The use of EAD (based on this definition) in the setting of NMP was recently criticized.[51] We agree that EAD is not a perfect endpoint. However, the first randomized controlled study used it as an important endpoint[19], and it is the most frequently used primary endpoint in the current registered trials concerning the use of machine perfusion techniques. The Food and Drug Administration (FDA) of the United States insists on EAD in the current NMP trials.[52] The Zurich group is using the complication score (Clavien-Dindo) in their current HOPE trial, and the Groningen group is using ischaemic cholangiopathy in DCD liver transplantation. Therefore, we included these two endpoints as secondary and safety endpoints in the current study.

Undoubtedly, there are limitations concerning the IFLT procedure. First, the procedure is complicated and labour-intensive. Both experienced surgeons and perfusionists are required for a successful IFLT operation. Therefore, it is difficult to conduct a multicentre study at this moment. Further modification of the procedure is required. In addition, the NMP device used in our centre is non-transportable. For this reason, the donors and recipients have to be from the same medical institution. In the future, simplified IFLT techniques with a portable NMP device are required to yield a novel multicentre procedure.

In conclusion, this study is a single-centre trial designed to assess the incidence of IRI-related complications between IFLT and CLT recipients, as well as allograft/recipient survival, to further validate the efficacy and safety of IFLT. The results from this trial can provide important evidence for the potential benefits of IFLT.

Patient and public involvement

The patients and public were not involved in the design and conduct of the study.

Ethics and dissemination

This trial will be conducted in accordance with the principles of the 1964 Declaration of Helsinki and its later amendments. All organs utilized in this study will be procured from brain-dead volunteer donors. The diagnosis of brain death has to be made by two independent, qualified neurologists. Donors were not prisoners, and no biological material was sourced from prisoners. Written informed content has to be obtained from all the directive family members for each donor, and all the organs have to be allocated through the China Organ Transplantation Response System (COTRS). We have already provided evidence to the journal to verify that the above criteria will be met.

The protocol was viewed and approved by the Ethics Committee of the First Affiliated Hospital, Sun Yat-sen University. The ethical approval number is [2019]037. All documents communicating with the ethics committee will be kept in the researcher's folder. If it is necessary to modify this protocol during clinical research, it will be reviewed by the hospital ethics committee and implemented after approval. Written informed consent will be obtained from each subject prior to organ allocation and randomization. The objectives and methods, benefits, possible risks and solutions, specimen collection plan and corresponding compensation when damage occurs will be clarified clearly for all subjects. All subjects have the right to cease participation in this trial at any time for any reason. Informed consent for this study and any changes in the course of this study must be reviewed and approved by the ethics committee before applying.

With regard to dissemination, the results of this study will be published in an academic journal and presented at national and international conferences.

Author affiliations

[1]Organ Transplant Center, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, China

[2]Guangdong Provincial Key Laboratory of Organ Donation and Transplant Immunology, Guangzhou 510080, China

[3]Guangdong Provincial International Cooperation Base of Science and Technology (Organ Transplantation), Guangzhou 510080, China

[4]Department of Anesthesiology, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080,

China

[5]State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou 510080, China

[6]Department of Cardiopulmonary Bypass, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, China

[7]Surgical Intensive Care Unit, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, China

Acknowledgements We thank all volunteer donors for donating their organs for scientific research and clinical needs. We thank all members of the organ procurement organization, surgical intensive care unit, transplant anaesthesia team and cardiopulmonary bypass team.

Contributors The initial study concept was derived from the initiating investigator study group (XSH, ZYG, QZ, WQJ, DPW, MGC, LWW, YM, ABH, XFZ). CJH, SZH and YHT drafted the manuscript. ZYG and JZ performed the sample size estimation. CJH, SZH, YHT, LY, ZHZ, ZBZ, LHW, CHZ, YXZ, CJS, WX, YKS, XXC, JR and CC participated in designing the study, preparing the revised protocol and are investigators at The First Affiliated Hospital, Sun Yat-sen University. All authors were involved in revising the manuscript and approved the final version.

Funding This work was supported by by grants as follows: the National Natural Science Foundation of China (81970564, 81471583 and 81570587), the Key Clinical Specialty Construction Project of National Health and Family Planning Commission of the People's Republic of China, the Guangdong Provincial Key Laboratory Construction Projection on Organ Donation and Transplant Immunology (2013A061401007 and 2017B030314018), Guangdong Provincial international Cooperation Base of Science and Technology (Organ Transplantation) (2015B050501002), Guangdong Provincial Natural Science Funds for Major Basic Science Culture Project (2015A030308010), Guangdong Provincial Natural Science Funds for Distinguished Young Scholars (2015A030306025), Special support program for training high level talents in Guangdong Province (2015TQ01R168), Pearl River Nova Program of Guangzhou (201506010014), and Science and Technology Program of Guangzhou (201704020150).

Competing interests None.

Patient consent for publication Not required.

Ethics approval Approval was obtained from the Ethics Committee of The First Affiliated Hospital, Sun Yat-sen University, China (1/2019).

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES:

1. Wang FS, Fan JG, Zhang Z, et al. The global burden of liver disease: the major impact of China. *Hepatology* 2014;**60**(6):2099-108.
2. Xiao J, Wang F, Wong NK, et al. Global liver disease burdens and research trends: Analysis from a Chinese perspective. *J Hepatol* 2019;**71**(1):212-21.
3. Zarrinpar A, Busuttil RW. Liver transplantation: past, present and future. *Nat Rev Gastroenterol Hepatol* 2013;**10**(7):434-40.
4. Saidi RF, Hejazii Kenari SK. Challenges of organ shortage for transplantation: solutions and opportunities. *Int J Organ Transplant Med* 2014;**5**(3):87-96.
5. Durand F, Renz JF, Alkofer B, et al. Report of the Paris consensus meeting on expanded criteria donors in liver transplantation. *Liver Transpl* 2008;**14**(12):1694-707.
6. Dubbeld J, Hoekstra H, Farid W, et al. Similar liver transplantation survival with selected cardiac death donors and brain death donors. *Br J Surg* 2010;**97**(5):744-53.
7. Starzl TE, Hakala TR, Shaw BW, Jr., et al. A flexible procedure for multiple cadaveric organ procurement. *Surg Gynecol Obstet* 1984;**158**(3):223-30.
8. Belzer FO, Southard JH. Principles of solid-organ preservation by cold storage. *Transplantation* 1988;**45**(4):673-6.
9. Maathuis MH, Leuvenink HG, Ploeg RJ. Perspectives in organ preservation. *Transplantation* 2007;**83**(10):1289-98.
10. Souidi N, Stolk M, Seifert M. Ischemia-reperfusion injury: beneficial effects of mesenchymal stromal cells. *Curr Opin Organ Transplant* 2013;**18**(1):34-43.
11. Solhjou Z, Uehara M, Bahmani B, et al. Novel Application of Localized Nanodelivery of Anti-Interleukin-6 Protects Organ Transplant From Ischemia-Reperfusion Injuries. *Am J Transplant* 2017;**17**(9):2326-37.
12. de Rougemont O, Dutkowski P, Clavien PA. Biological modulation of liver ischemia-reperfusion injury. *Curr Opin Organ Transplant* 2010;**15**(2):183-9.
13. Rauen U, de Groot H. New insights into the cellular and molecular mechanisms of cold storage injury. *J Investig Med* 2004;**52**(5):299-309.
14. Cursio R, Gugenheim J. Ischemia-Reperfusion Injury and Ischemic-Type Biliary Lesions following Liver Transplantation. *J Transplant* 2012;**2012**:164329.
15. Deschenes M. Early allograft dysfunction: causes, recognition, and management. *Liver Transpl* 2013;**19 Suppl 2**:S6-8.
16. Neves DB, Rusi MB, Diaz LG, et al. Primary graft dysfunction of the liver: definitions, diagnostic criteria and risk factors. *Einstein (Sao Paulo)* 2016;**14**(4):567-72.
17. Zhu Z, Tang Y, Huang S, et al. Donor liver apoptosis is associated with early allograft dysfunction and decreased short-term graft survival after liver transplantation. *Clin Transplant* 2018;**32**(12):e13438.
18. Eltzschig HK, Eckle T. Ischemia and reperfusion--from mechanism to translation. *Nat Med* 2011;**17**(11):1391-401.
19. Nasralla D, Coussios CC, Mergental H, et al. A randomized trial of normothermic preservation in liver transplantation. *Nature* 2018;**557**(7703):50-56.
20. Nasralla D, Ploeg R, Coussios C, et al. Outcomes from a Multinational Randomised Controlled Trial Comparing Normothermic Machine Perfusion with Static Cold Storage in Human Liver Transplantation. *American Journal of Transplantation* 2017;**17**:404-04.
21. Ceresa CDL, Nasralla D, Knight S, et al. Cold storage or normothermic perfusion for liver transplantation: probable application and indications. *Curr Opin Organ Transplant* 2017;**22**(3):300-05.
22. Moers C, Smits JM, Maathuis MH, et al. Machine perfusion or cold storage in deceased-donor kidney transplantation. *N Engl J Med* 2009;**360**(1):7-19.
23. Ravikumar R, Jassem W, Mergental H, et al. Liver Transplantation After Ex Vivo Normothermic Machine

Preservation: A Phase 1 (First-in-Man) Clinical Trial. *Am J Transplant* 2016;**16**(6):1779-87.

24. Cypel M, Yeung JC, Liu M, et al. Normothermic ex vivo lung perfusion in clinical lung transplantation. *N Engl J Med* 2011;**364**(15):1431-40.
25. Warnecke G, Moradiellos J, Tudorache I, et al. Normothermic perfusion of donor lungs for preservation and assessment with the Organ Care System Lung before bilateral transplantation: a pilot study of 12 patients. *Lancet* 2012;**380**(9856):1851-58.
26. Dhital KK, Iyer A, Connellan M, et al. Adult heart transplantation with distant procurement and ex-vivo preservation of donor hearts after circulatory death: a case series. *Lancet* 2015;**385**(9987):2585-91.
27. He X, Guo Z, Zhao Q, et al. The first case of ischemia-free organ transplantation in humans: A proof of concept. *Am J Transplant* 2018;**18**(3):737-44.
28. Zhao Q, Huang S, Wang D, et al. Does Ischemia Free Liver Procurement Under Normothermic Perfusion Benefit the Outcome of Liver Transplantation? *Ann Transplant* 2018;**23**:258-67.
29. Chan AW, Tetzlaff JM, Gotzsche PC, et al. SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. *BMJ* 2013;**346**:e7586.
30. Nalesnik MA, Woodle ES, Dimaio JM, et al. Donor-transmitted malignancies in organ transplantation: assessment of clinical risk. *Am J Transplant* 2011;**11**(6):1140-7.
31. Yao FY, Ferrell L, Bass NM, et al. Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. *Hepatology* 2001;**33**(6):1394-403.
32. Martin P, DiMartini A, Feng S, et al. Evaluation for liver transplantation in adults: 2013 practice guideline by the American Association for the Study of Liver Diseases and the American Society of Transplantation. *Hepatology* 2014;**59**(3):1144-65.
33. Laing RW, Mergental H, Yap C, et al. Viability testing and transplantation of marginal livers (VITTAL) using normothermic machine perfusion: study protocol for an open-label, non-randomised, prospective, single-arm trial. *BMJ Open* 2017;**7**(11):e017733.
34. Olthoff KM, Kulik L, Samstein B, et al. Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. *Liver Transpl* 2010;**16**(8):943-9.
35. Jochmans I, Monbaliu D, Pirenne J. The beginning of an end point: peak AST in liver transplantation. *J Hepatol* 2014;**61**(5):1186-7.
36. Broering DC, Topp S, Schaefer U, et al. Split liver transplantation and risk to the adult recipient: analysis using matched pairs. *J Am Coll Surg* 2002;**195**(5):648-57.
37. Siniscalchi A, Gamberini L, Bardi T, et al. Post-reperfusion syndrome during orthotopic liver transplantation, which definition best predicts postoperative graft failure and recipient mortality? *J Crit Care* 2017;**41**:156-60.
38. Novellas S, Caramella T, Fournol M, et al. MR cholangiopancreatography features of the biliary tree after liver transplantation. *AJR Am J Roentgenol* 2008;**191**(1):221-7.
39. Boraschi P, Donati F, Gigoni R, et al. Ischemic-type biliary lesions in liver transplant recipients: evaluation with magnetic resonance cholangiography. *Transplant Proc* 2004;**36**(9):2744-7.
40. Banff schema for grading liver allograft rejection: an international consensus document. *Hepatology* 1997;**25**(3):658-63.
41. Khwaja A. KDIGO clinical practice guidelines for acute kidney injury. *Nephron Clin Pract* 2012;**120**(4):c179-84.
42. Inker LA, Schmid CH, Tighiouart H, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med* 2012;**367**(1):20-9.
43. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. *Am J Infect Control* 2008;**36**(5):309-32.
44. Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a

cohort of 6336 patients and results of a survey. *Ann Surg* 2004;**240**(2):205-13.

45. Slankamenac K, Graf R, Barkun J, et al. The comprehensive complication index: a novel continuous scale to measure surgical morbidity. *Ann Surg* 2013;**258**(1):1-7.
46. Suzuki S, Toledo-Pereyra LH, Rodriguez FJ, et al. Neutrophil infiltration as an important factor in liver ischemia and reperfusion injury. Modulating effects of FK506 and cyclosporine. *Transplantation* 1993;**55**(6):1265-72.
47. op den Dries S, Westerkamp AC, Karimian N, et al. Injury to peribiliary glands and vascular plexus before liver transplantation predicts formation of non-anastomotic biliary strictures. *J Hepatol* 2014;**60**(6):1172-9.
48. Hansen T, Hollemann D, Pitton MB, et al. Histological examination and evaluation of donor bile ducts received during orthotopic liver transplantation--a morphological clue to ischemic-type biliary lesion? *Virchows Arch* 2012;**461**(1):41-8.
49. He X, Guo Z, Ju W, et al. Improved Transplant Outcomes in Ischemia-Free Liver Transplantation: A Report of the First 30 Cases. *American Journal of Transplantation* 2019;**19**:354-55.
50. Croome KP, Hernandezalejandro R, Chandok N. Early Allograft Dysfunction Is Associated With Excess Resource Utilization After Liver Transplantation. *Transplant Proc* 2013;**45**(1):259-64.
51. Dutkowski P, Guarrera JV, de Jonge J, et al. Evolving Trends in Machine Perfusion for Liver Transplantation. *Gastroenterology* 2019;**156**(6):1542-47.
52. Quintini C, Martins PN, Shah S, et al. Implementing an innovated preservation technology: The American Society of Transplant Surgeons' (ASTS) Standards Committee White Paper on Ex Situ Liver Machine Perfusion. *Am J Transplant* 2018;**18**(8):1865-74.

Figure legends

Figure 1 Brief flow chart of this study.

Abbreviations: AASLD, American Association for the Study of Liver Diseases; ALT, alanine aminotransferase; AST, aspartate transaminase; COTRS, China Organ Transplant Response System; DTAC, Disease Transmission Advisory Committee; EAD, early allograft dysfunction; HIV, human immunodeficiency virus; ICU, intensive care unit; INR, international normalized ratio; MELD, model for end-stage liver disease; PNF, primary nonfunction; POD, postoperative day; POM, postoperative month; Tbil, total bilirubin; UCSF, University of California at San Francisco.

Table 1 The schedule for donor screening

Contents	Screening stage	Retrieval day
Time	-7day-0day	0day
Written informed consent	×	
Eligibility assessment	×	
Patient history	×	
Demographic data	×	
Vital signs	×	×
Physical examination	×	×
Standard routine blood tests	×	
Standard routine examinations	×	
Collection of blood specimens		×
Liver biopsy		×

Standard routine blood tests: blood type, blood/urine/stool routine test, coagulation function, communicable and infectious diseases, blood gas analysis, electrolytes, liver/renal/heart/function tests.

Standard routine examinations: electrocardiogram, chest X-ray, cardiac and abdominal colour ultrasound, and head/chest/abdomen CT scan.

Table 2 The schedule for recipient screening

Contents	Screening stage	Transplant day
Time	-30 days-day 0	Day 0
Written informed consent	×	
Eligibility assessment	×	
Patient history	×	
Demographic data	×	
Vital signs	×	×
Physical examination	×	×
Performance status (ECOG)		×
Quality of life (EQ-5D)		×
Standard routine blood tests	×	
Standard routine examinations	×	
Collection of blood specimens		×
Liver biopsy		×
Others		

Standard routine blood tests: blood type, blood/urine/stool routine test, coagulation function, communicable and infectious diseases, electrolytes, liver/renal/heart/function tests.

Standard routine examinations: electrocardiogram, chest X-ray, lung function, cardiac and abdominal colour ultrasound, and abdomen CT scan.

Other parameters, such as magnetic resonance, blood gases, tumour markers and hepatitis B virus DNA, are measured according to clinical conditions.

Abbreviations: ECOG, Eastern Cooperative Oncology Group; EQ-5D, EuroQol-5 Dimension.

Table 3 Blood gas analysis during perfusion

Time (min)	10	20	30	40	60	80	100	120	140	160	180	200	...	420
pH														
PCO_2														
PO_2														
BE														
HCO_3^-														
sO_2														
Lac														
Na^+														
K^+														
Cl^-														
iCa														
GLU														
Hct														
Hb														

Abbreviations: pH, pondus hydrogenii; PCO_2, partial pressure of carbon dioxide; PO_2, partial pressure of oxygen; BE, base excess; HCO_3^-, bicarbonate ion; sO_2, oxygen saturation; Lac, lactate; Na^+, sodium ion; K^+, potassium ion; Cl^- chloride ion; GLU, glucose; iCa, ionized calcium; HCT, haematocrit; Hb, haemoglobin.

Table 4 Regulation of perfusate

	Time (min)	0-20	20	20-40	40	40-60	60	60-80	80-100	...	420
Additive	Sterile water (ml)										
	Gelofusine (ml)										
	Alkaline solution (ml)										
	10% Calcium chloride (ml)										
	Heparin (U)										
	Vasoactive drugs										
	Gas										
	Others										

Table 5 Perfusion device parameter monitoring

	Hour	1						2						...	8					
Time	Min	0	10	20	30	40	50	0	10	20	30	40	50		0	10	20	30	40	50
HA	Pressure																			
	Flow rate																			
	R																			
	T																			
PV	Pressure																			
	Flow rate																			
	R																			
	T																			

Abbreviations: HA, hepatic artery; PV, portal vein; R, resistance index; T, temperature

Table 6 Blood and perfusate biochemical monitoring

	Donor blood		Perfusate						
	Pre-procurement	Intraoperative	Post-modulation	0h	0.5h	1.0h	...	implantation	Post-reperfusion
K^+									
Na^+									
ALT									
AST									
ALP									
GGT									
LDH									
GLU									
Tbil									
Crea									
Osm									

Abbreviations: K^+, potassium ion; Na^+, sodium ion; ALT, alanine aminotransferase; AST, aspartate transaminase; ALP, alkaline phosphatase; GGT, glutamyl transpeptidase; LDH, lactate dehydrogenase; Tbil, total bilirubin; GLU, glucose; Crea, creatinine; Osm, osmotic pressure.

Table 7 Bile composition monitoring

	Procurement	1h	2h	3h	4h	5h	6h	7h	8h	Implantation	Reperfusion
Bile produce (ml/h)											
pH											
PCO_2											
PO_2											
BE											
HCO3-											
sO_2											
Lac											
Na^+											
K^+											
Cl^-											
iCa											
GLU											
Bile acid (μmol/L)											
Cholesterol (μmol/L)											
GGT (U/L)											
Tbil (μmol/L)											
LDH (U/L)											

Abbreviations: pH, pondus hydrogenii; PCO_2, partial pressure of carbon dioxide; PO_2, partial pressure of oxygen; BE, base excess; HCO_3^-, bicarbonate ion; sO_2, oxygen saturation; Lac, lactate; Na^+, sodium ion; K^+ potassium ion; Cl^- chloride ion; GLU, glucose; GGT, glutamyl transpeptidase; Tbil, total bilirubin; LDH, lactate dehydrogenase.

Table 8 Follow-up period 1 (PODs 1-7)

	POD 1[$]	POD 2	POD 3	POD 4	POD 5	POD 6	POD 7
Hb (g/L)							
WBC (10^9/L)							
PLT (10^9/L)							
NEUT%							
CRP							
ALT (U/L)							
AST (U/L)							
Tbil (μmol/L)							
LDH (U/L)							
Crea (mmol/L)							
ALB (g/L)							
PA (mg/L)							
ALP (U/L)							
GGT (U/L)							
PCT							
CK							
CK-MB							
Myoglobin							
High-sensitivity troponin							
Endotoxin							
Serum amylase							
Serum lipase							
Ca^{2+}							
Serum cystatin							
Urea							
Complement C1q							
P^{2+}							
Retinol binding protein							
BNP							
Ammonia (mg/L)							
PT (S)							
INR							
Fbg (g/L)							
D-Dimer (mg/L)							
PH*							
BE*							
Lac (mmol/L)*							
PO2 (mmHg)*							
PCO2 (mmHg)*							
HCO3- (mmol/L)*							
Collection of blood specimens	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y

Blood culture #	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y
US #	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y
Chest X-ray #	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y
Complications	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y
Adverse events	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y
Medication Records	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y
Recipient survival status	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y
Graft survival status	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y	□0N□1Y

$ Blood examination 5-11 hours after reperfusion.

* If the recipient is still in the ICU or needs arterial blood gas analysis.

& Blood culture: When the patient's body temperature is above 38°C, blood culture should be carried out according to the patient's condition.

Every other day within 1 week after operation.

Complications, adverse events and medication records are recorded in detail in the case report form.

Abbreviations: Hb, haemoglobin; WBC, white blood cells; PLT, platelets; NEUT%, neutrophilic granulocyte percentage; CRP, C-reactive protein; ALT, alanine aminotransferase; AST, aspartate transaminase; Tbil, total bilirubin; LDH, lactate dehydrogenase; Crea, creatinine; ALB, albumin; PA, prealbumin; ALP, alkaline phosphatase; GGT, glutamyl transpeptidase; PCT, procalcitonin; CK, creatine kinase; CK-MB, creatine kinase isoenzyme; P^{2+}, phosphonium ion; BNP, brain natriuretic peptide; PT, prothrombin time; INR, international normalized ratio; Fbg, fibrinogen; pH, pondus hydrogenii; BE, base excess; Lac, lactate; PO_2, partial pressure of oxygen; PCO_2, partial pressure of carbon dioxide; HCO_3^-, bicarbonate ion; US, ultrasound

Table 9 Follow-up period 2 (POD 14 and discharge day)

	POD 14	Discharge Day
Hb (g/L)		
WBC (10^9/L)		
PLT (10^9/L)		
NEUT%		
CRP*		
PCT*		
PT (S)		
INR		
Fbg (g/L)		
D-Dimer (mg/L)*		
ALT (U/L)		
AST (U/L)		
Tbil (μmol/L)		
LDH (U/L)		
Crea (mmol/L)		
ALB (g/L)		
PA (mg/L)		
ALP (U/L)		
GGT (U/L)		
Ammonia (mg/L) *		
Collection of blood specimens		
US *		
Performance status (ECOG)		
Quality of life (EQ-5D)		
Complications	$\square_0$N $\square_1$Y	$\square_0$N $\square_1$Y
Adverse events	$\square_0$N $\square_1$Y	$\square_0$N $\square_1$Y
Medication Records	$\square_0$N $\square_1$Y	$\square_0$N $\square_1$Y
Recipient survival status	$\square_1$N $\square_1$Y	$\square_0$N $\square_1$Y
Graft survival status	$\square_0$N $\square_1$Y	$\square_0$N $\square_1$Y

* If necessary.

Complications, adverse events and medication records are recorded in detail in the case report form.

Hb, haemoglobin; WBC, white blood cells; PLT, platelets; NEUT%, neutrophilic granulocyte percentage; CRP, C-reactive protein; PCT, procalcitonin; PT, prothrombin time; INR, international normalized ratio; Fbg, fibrinogen; ALT, alanine aminotransferase; AST, aspartate transaminase; Tbil, total bilirubin; LDH, lactate dehydrogenase; Crea, creatinine; ALB, albumin; PA, prealbumin; ALP, alkaline phosphatase; GGT, glutamyl transpeptidase; US, ultrasound; ECOG, Eastern Cooperative Oncology Group; EQ-5D, EuroQol-5 Dimension.

Table 10 Follow-up period (within one year after discharge)

	POM 1	POM 2	POM 3	POM 4	POM 5	POM 6	…	POM 12
Hb (g/L)								
WBC (10^9/L)								
PLT (10^9/L)								
NEUT%								
CRP*								
PCT*								
PT (S)								
INR								
Fbg (g/L)								
D-Dimer (mg/L)*								
ALT (U/L)								
AST (U/L)								
Tbil (μmol/L)								
LDH (U/L)								
Crea (mmol/L)								
ALB (g/L)								
PA (mg/L)								
ALP (U/L)								
GGT (U/L)								
Ammonia (mg/L)*								
Collection of blood specimens								
MRCP#								
US*								
Performance status (ECOG)								
Quality of life (EQ-5D)								
Complications	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y
Adverse events	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y
Medication Records	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y
Recipient survival status	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y
Graft survival status	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y	□$_0$N□$_1$Y

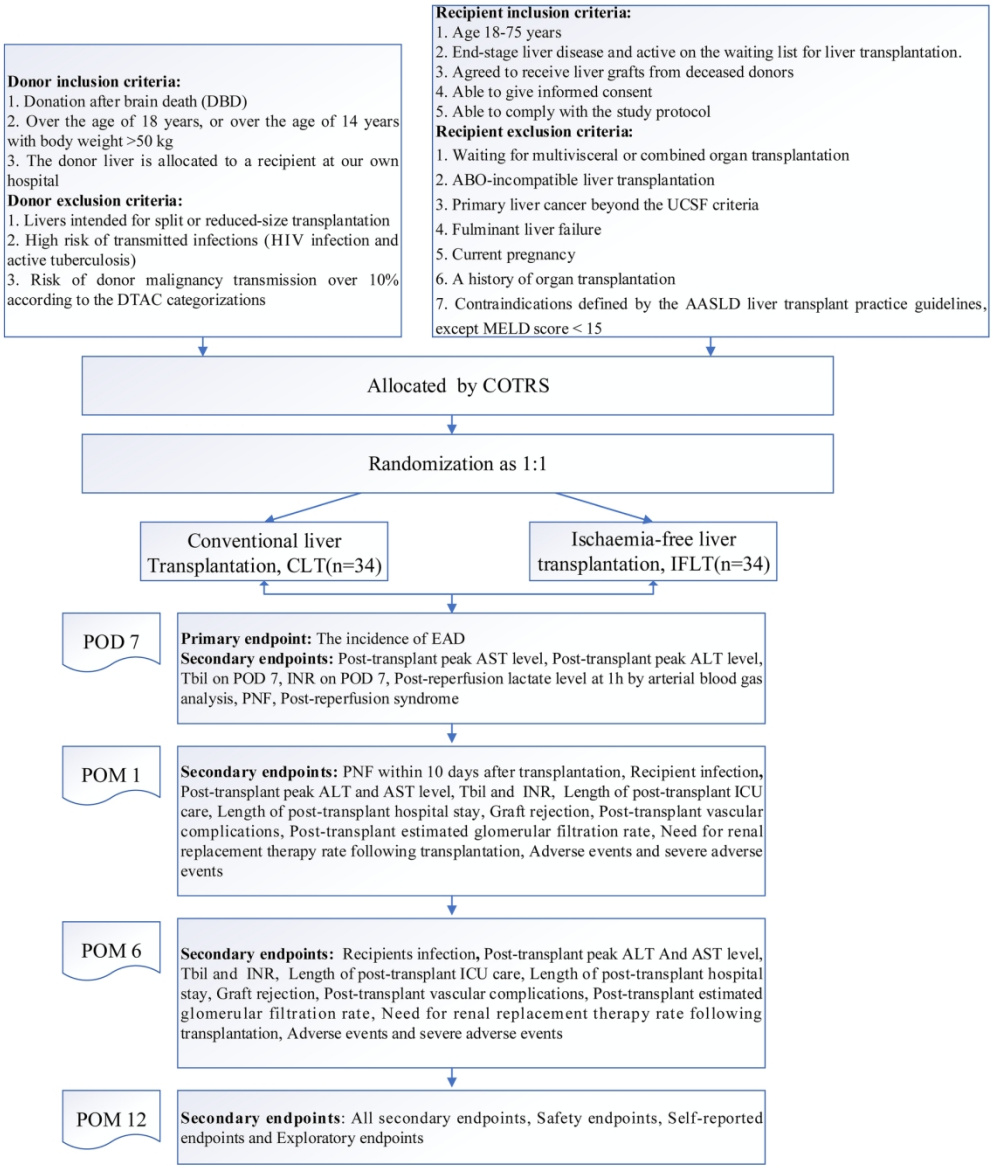
* If necessary.

all patients undergo MRCP at 6 months and 12 months after transplantation

Complications, adverse events and medication records are recorded in detail in the case report form.

Hb, haemoglobin; WBC, white blood cells; PLT, platelets; NEUT%, neutrophilic granulocyte percentage; CRP, C-reactive protein; PCT, procalcitonin; PT, prothrombin time; INR, international normalized ratio; Fbg, fibrinogen; ALT, alanine aminotransferase; AST, aspartate transaminase; Tbil, total bilirubin; LDH, lactate dehydrogenase;

Crea, creatinine; ALB, albumin; PA, prealbumin; ALP, alkaline phosphatase; GGT, glutamyl transpeptidase; US, ultrasound; MRCP, magnetic resonance cholangiopancreatography; ECOG, Eastern Cooperative Oncology Group; EQ-5D, EuroQol-5 Dimension.



Abbreviations: AASLD, American Association for the Study of Liver Diseases; ALT, alanine aminotransferase; AST, aspartate transaminase; COTRS, China Organ Transplant Response System; DTAC, Disease Transmission Advisory Committee; EAD, early allograft dysfunction; HIV, human immunodeficiency virus; ICU, intensive care unit; INR, international normalized ratio; MELD, model for end-stage liver disease; PNF, primary nonfunction; POD, postoperative day; POM, postoperative month; Tbil, total bilirubin; UCSF, University of California at San Francisco.

160x189mm (300 x 300 DPI)

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	ItemNo	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	1,3
	2b	All items from the World Health Organization Trial Registration Data Set	Yes (available on register ChiCTR1900021158)
Protocol version	3	Date and version identifier	n/a
Funding	4	Sources and types of financial, material, and other support	20
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	19,20
	5b	Name and contact information for the trial sponsor	1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	20
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	n/a

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-5
	6b	Explanation for choice of comparators	4-5
Objectives	7	Specific objectives or hypotheses	
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7-9
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	8
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	n/a
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	n/a

Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9-12
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	12
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	12
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	12
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	12-13
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	12-13
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	12-13

Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	12-13
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	12-13
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	13, 25-35
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	13
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	13
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13-14
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	14
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	14
Methods: Monitoring			

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	14-15
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	n/a
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	15-16
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	14-15
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	19
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	19
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	19
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	7,25-26
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	20

Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	13
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	n/a
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	19
	31b	Authorship eligibility guidelines and any intended use of professional writers	19
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n/a
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	n/a All participants have signed the written informed consent. If requested, we can provide.
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	16

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.