Research paper

# Ischaemia-free liver transplantation in humans: a first-in-human trial

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## ARTICLE INFO

## ABSTRACT

Background Ischaemia-reperfusion injury is considered an inevitable component of organ transplantation, compromising organ quality and outcomes. Although several treatments have been proposed, none has avoided graft ischaemia and its detrimental consequences.

Methods Ischaemia-free liver transplantation (IFLT) comprises surgical techniques enabling continuous oxygenated blood supply to the liver of brain-dead donor during procurement, preservation, and implantation using normothermic machine perfusion technology. In this non-randomised study, 38 donor livers were transplanted using IFLT and compared to 130 conventional liver transplants (CLT).

Findings Two recipients (5•3%) in the IFLT group experienced early allograft dysfunction, compared to 50•0% in patients receiving conventional transplants (absolute risk difference, 44•8%; 95% confidence interval, 33•6-55•9%). Recipients of IFLT had significantly reduced median (IQR) peak aspartate aminotransferase levels within the first week compared to CLT recipients (365, 238-697 vs 1445, 791-3244 U/L, p<0•001); likewise, median total bilirubin levels on day 7 were significantly lower (2•34, 1•39-4•09

mg/dL) in the IFLT group than in the CLT group (5•10, 1•90-11•65 mg/dL) (p<0•001). Moreover, IFLT recipients had a shorter median intensive care unit stay (1•48, 0•75-2•00 vs 1•81, 1•00-4•58 days, p=0•006). Both one-month recipient (97•4% vs 90•8%, p=0•302) and graft survival (97.4% vs 90•0%, p=0•195) were better for IFLT than CLT, albeit differences were not statistically significant. Subgroup analysis showed that the extended criteria donor livers transplanted using the IFLT technique yielded faster post-transplant recovery than did the standard criteria donor livers transplanted using the conventional approach.

Interpretation IFLT provides a novel approach that may improve outcomes, and allow the successful utilisation of extended criteria livers.

Panel: Research in context

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Research in context

**Evidence before the study**

We searched PubMed using the terms 'machine perfusion', '*ex vivo* perfusion', and 'transplantation' for relevant articles in any language published until September 20, 2020. We searched for clinical studies on organ transplantation using machine perfusion as a preservation method. Previous studies have documented the safety and efficacy of normothermic machine perfusion for liver, heart, lung and kidney transplantation. Previous studies have also shown an advantage of machine perfusion under hypo- and normothermic conditions compared to static cold storage. Ischaemia-free organ transplantation is an entirely new approach. Our group has shown the feasibility of this approach in a previous case report. Ischaemia-free liver transplantation (IFLT) has not been tested before systematically in a clinical trial.

**Added value of the study**

This is the first in-human clinical trial of ischaemia-free organ transplantation, confirming that this approach is feasible and safe and improves outcomes while dramatically reducing the consequences of ischaemia-reperfusion injury. In addition, extended criteria donor livers after IFLT had a faster recovery when compared to standard criteria donor livers after conventional liver transplantation.

**Implications of all the available evidence**

An insufficient supply of organs currently limits the success of transplantation. IFLT can improve early graft function and has the potential to reduce IRI. Therefore, IFLT represents a novel approach allowing the successful utilisation of marginal organs with an improved outcome.

## 1. Introduction

Ischaemia-reperfusion injury (IRI) is an inevitable component of transplantation. Sequelae of IRI include early allograft dysfunction (EAD), primary nonfunction (PNF), and augmentation of alloimmune responses with more frequent rejections in liver transplantation [1]. An optimised utilisation of extended criteria donor (ECD) organs has the clinical potential to close the gap between demand and supply in organ transplantation [2, 3]. However, ECD organs are frequently not used or discarded as they are more vulnerable to IRI, associated with a higher risks of morbidity and mortality when compared to standard criteria donor (SCD) organs [4].

Great efforts have been made to reduce IRI over the years. Those efforts include approaches of ischaemic preconditioning, the use of therapeutic gases, pharmacological interventions, stem cell and gene therapy [5]. However, success has been limited. As an alternative to standard static cold storage (SCS), *ex-situ* normother-

mic machine perfusion (NMP) can provide oxygenated blood supply to the organ. The advantages of NMP have been tested either in a post-SCS approach (end-ischaemic NMP) or as a continuous treatment once organs are procured (preservation NMP) [6]. Although NMP provides benefits in the liver [7], heart [8, 9], lung [10, 11], and kidney transplants [12], they are not able to avoid the consequences of IRI. The sequelae of IRI have also been shown to initiate potent innate immune responses leading to augmented alloimmune responses with more frequent rejections [13]. From an academic perspective it will therefore be of great value to study the link of injury and alloimmunity in a clinical model avoiding IRI.

To avoid graft ischaemia entirely, we have established a novel procedure called ischaemia-free liver transplantation (IFLT), during which liver grafts are procured, preserved, and implanted under continuous NMP [14]. In the current study, we assessed the efficacy and safety of IFLT versus conventional liver transplantation (CLT) in patients with end-stage liver disease.

## 2. Methods

### 2.1. Study setting and participants

The design of this study is a single-centre, prospective, non-randomised, controlled trial. All donation after brain death (DBD) donors, older than 12 years, were eligible for inclusion. All donors were from the voluntary citizen-based organ donation system, and the organs were allocated through the China Organ Transplant Response System (COTRS) based on emergency of the disease and length of waiting time [15]. All adult recipients (>18 years) with end-stage liver disease waiting for a first whole liver transplant were eligible; excluded were patients undergoing combined organ transplantation, multi-visceral transplantation, split liver transplantation, and those receiving ABO-blood group incompatible transplants. As the NMP device used in this study was not transportable, only organs from donors located in The First Affiliated Hospital of Sun Yat-sen University were included.

The group allocation was non-randomised, although participants in both groups had to fullfil the above criteria. Eligible patients were approached for consent to receive IFLT when both NMP device disposables and perfusionists were available. Patients and their family were informed (1) the incidence of common complications during and post-liver transplantation by using the conventional procedure, (2) the IFLT technique was an entirely new developed one, with the potential benefits of reduced IRI and complications, as well as the potential risks of additional warm ischaemic injury of the grafts due to technical issues. IFLT proceeded in consented patients who agreed to receive IFLT. The patients underwent

CLT when the NMP device disposable or perfusionist was unavailable or when they refused to receive IFLT. These patients were informed the expected incidence of common complications during and post-liver transplantation.

We tried to include all the DBD livers which were from our hospital and allocated to recipients in our hospital during the study period. The acceptability of a graft depends on the recipient's conditions, donor risk factors, assessment of the livers by the procurement surgeon and biopsy findings if necessary. In the control group, the decision was made by the senior surgeon team in our centre when it was a difficult one. We had pre-defined acceptability criteria based on the NMP parameters in the IFLT group. Since it took time to prepare the matched washed red blood cells and perfusate for the perfusion when the IFLT was planned to be conducted, a liver graft was included in the study before its final acceptance for organ transplantation.

Standardised post-transplant care was provided in both groups, including fluid management, antibiotic and anti-hepatitis B virus (HBV) prophylaxis, immunosuppression, and surveillance ultrasonography. In our centre, induction therapy was performed by administrating a dose of 20 mg anti-IL-2 receptor antibody intraoperatively and on post-transplant day 4. Tacrolimus and mycophenolate mofetil (MMF) were on post-transplant day 4. The initial dose of tacrolimus was 0.04 mg/kg/d, and the target trough level was 8-10 ng/ml within the first three months, and 6-8 ng/ml thereafter. A dose of 500-750 mg MMF was given twice a day.

The study protocol (ChiCTR-OPN-17012090) was approved by the Ethical Committee of The First Affiliated Hospital, Sun Yat-sen University.

### 2.2. IFLT procedure

Fig. 1 and Video 1 detail the technical aspects of IFLT.

Before procurement, the NMP device (Liver Assist, Groningen, Netherlands) was primed with leucocyte-depleted red blood cells (approximately 1.2 litres) in addition to 1.2 litres of succinylated gelatin supplemented with heparin, magnesium sulphate, calcium chloride, and amino acids (appendix p 2). Two rotary pumps provide a pulsatile flow to the hepatic artery and a continuous flow to the portal vein. Once the liver was fully mobilized in the donor, a 12 Fr cannula was inserted into the splenic or gastroduodenal artery without interruption of the arterial supply to the liver from the celiac artery. A 32 Fr cannula was placed in the infrahepatic inferior vena cava and connected to the organ reservoir of the NMP device. A 24 Fr cannula was inserted into the portal vein via an interposition vein graft (the donor right external iliac vein) and connected to the portal vein line of the device. The arterial cannula was then connected to the arterial line of the device. Once the *in-situ* NMP circuit was established and perfusion began, the liver was procured and moved to the organ reservoir of the Liver Assist.

On the Liver Assist device, the liver underwent *ex-situ* NMP. NMP is used to protect the grafts from IRI and assess graft viability in IFLT. Livers were considered suitable for transplantation if they met all of the following criteria during *ex-situ* NMP: (i) the livers produced bile, (ii) the lactate level decreased to < 2•0 mmol/L within 90 min, (iii) the perfusate pH value was greater than 7•30, (iv) the arterial flow was greater than 150 ml/min, and the portal venous flow was greater than 500 ml/min, and (v) the graft had a homogeneous appearance with soft consistency of the parenchyma. The livers remained on the device for 2-9 h depending on the progress of the recipient procedure.

After the hepatectomy of the diseased liver was completed, the donor liver was moved from the reservoir to the recipient peritoneal cavity. Liver implantation was performed using a bicaval (caval replacement) or piggy-back (caval preservation) technique. Notably, based on the continuous *in-situ* NMP of the liver via the splenic artery and the interposition vein on the portal vein, the anastomoses of the suprahepatic inferior vena cava (to the counterpart in the bicaval technique, or to the common orifice of the left and middle hepatic vein in the piggy-back technique), portal vein and hepatic artery were conducted with continuous blood supply to the graft. Once the liver had been re-vascularised, NMP was discontinued, and all cannulas were removed. The anastomosis of the infrahepatic inferior vena cava were done in the bicaval technique.

### 2.3. CLT procedure

Brain dead donors underwent a standard *in-situ* cold flushing procedure with University of Wisconsin (UW) solution. The liver was retrieved, placed in 0-4°C UW solution and stored on ice. A standard back-table preparation was performed before implantation. After removing the recipient's liver, the donor liver was transferred to the abdominal cavity with a standard bicaval or piggy-back liver transplantation. Once the anastomoses of the inferior vena cava and portal vein were completed, the blood supply to the allograft resumed. Subsequently, the hepatic artery and bile ducts were anastomosed.

In both IFLT and CLT groups, 37500U Heparin Sodium Injection was used before the donor livers were harvested. During recipient operation, fresh plasma, cryoprecipitate and fibrinogen were used according to the results of thromboelastogram (TEG).

### 2.4. Subgroup dividing

Grafts used for IFLT or CLT were assigned as either SCD or ECD. ECD livers were defined if at least one of the following criteria was met: (1) donor age >60 years; (2) hypernatremia (serum Na+ >165 mmol/L); (3) >30% macrovesicular steatosis by biopsy; (4) donor serum aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >1,000 IU/L or total bilirubin (Tbil) >3 mg/dL at the time of organ offer; (5) or cold ischaemia time (CIT) ≥12 hours.

### 2.5. Outcome measures and observation period

Patients were followed for one year post-transplantation. The primary end-point was the incidence of EAD. EAD assignment was based on elevated AST or ALT level peak in >2000 U/L within the first week, international normalised ratio (INR)≥1•6 or Tbil≥10 mg/dL on day 7 post-transplantation [16].

The secondary endpoints were primary non-function (PNF), biliary complications, vascular complications, clinical acute rejection, need for renal replacement therapy (RRT) within 30 days, length of stay in the intensive care unit (ICU), total length of hospital stay, as well as one-month and one-year graft and patient survival. PNF was defined as graft failure immediately after transplantation requiring urgent re-transplantation or leading to patient death [7]. Biliary complications, including anastomotic stricture, non-anastomotic biliary stricture (NAS), biliary leak, and biliary stone, were diagnosed by magnetic resonance cholangiopancreatography, endoscopic retrograde cholangiopancreatography, or percutaneous transhepatic cholangiography [17]. Notably, NAS were defined by criteria established by Carlijn et al.: any stricture, dilatation, or irregularity of the intra- or extrahepatic bile ducts of the liver graft, after exclusion of isolated strictures at the bile duct anastomosis and hepatic artery thrombosis (HAT) [18]. Vascular complications, including HAT and portal vein thrombosis, were diagnosed by ultrasonic examination, and finally confirmed by visceral angiography [19]. Moreover, clinical acute rejection, need for renal replacement therapy (RRT) within 30 days, length of stay in the intensive care unit (ICU), total length of hospital stay, in addition graft and patient survival by one and 12 months were recorded.
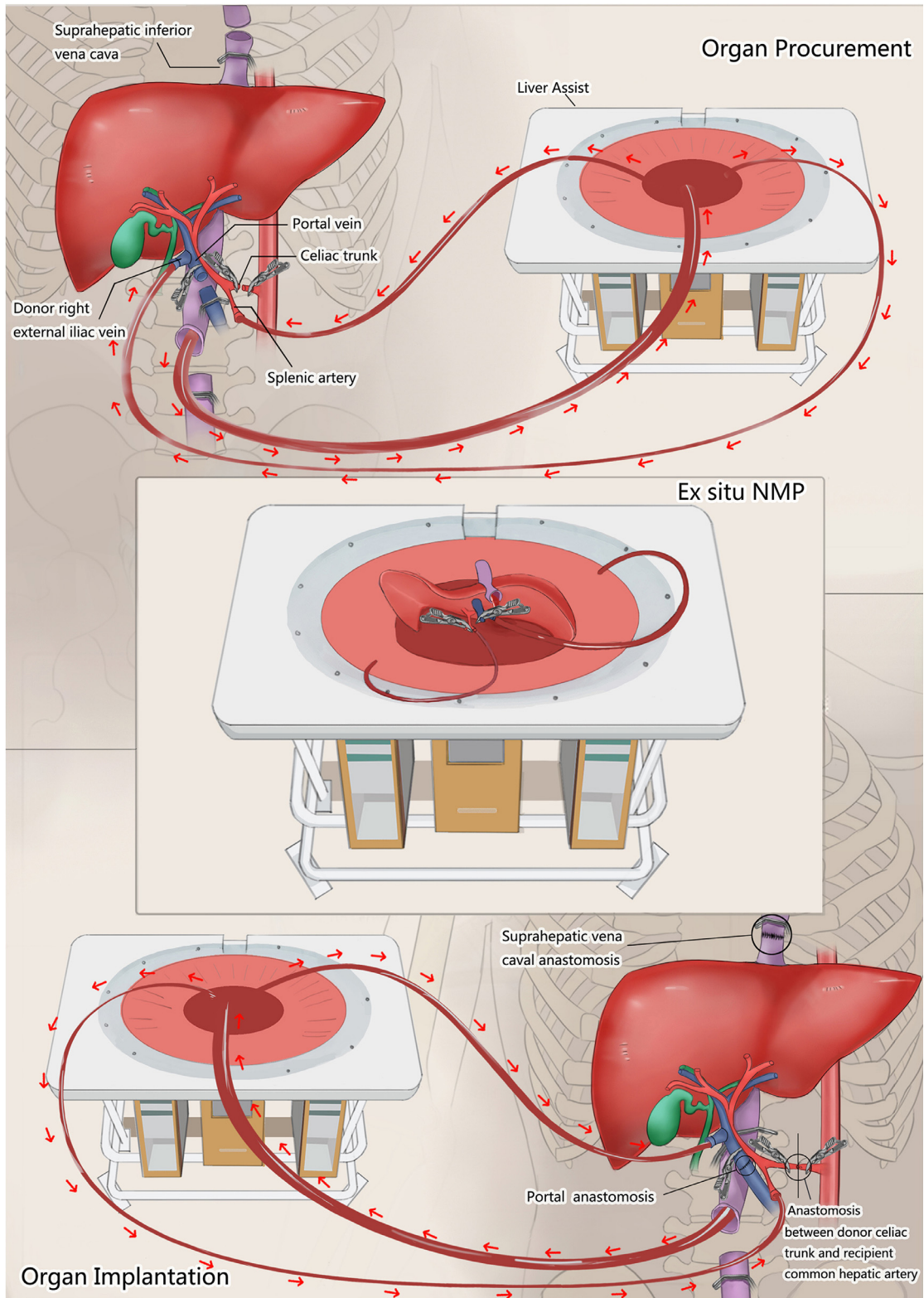
**Fig. 1. Ischaemia-free Liver Transplant Procedure.** Liver procurement, *ex situ* preservation, and implantation under normothermic machine perfusion (NMP) using the Liver Assist device with cannulation of the donor infrahepatic vena cava, interposition vein (right external iliac vein) on the portal vein, and splenic artery.

## 2.6. Statistical analysis

In the original study protocol, the peak AST level was selected as the primary end-point and 15 participants were planned to be included in the IFLT group. However, we considered EAD as a more comprehensive outcome measure than the peak AST level alone for assessing the safety and feasibility of IFLT. Therefore, the primary end-point and sample size were amended. The sample size of 36 patients per trial group was estimated to provide the trial with 80% power to detect a 30 percentage-point difference between the IFLT group and the control group, at a two-sided alpha level of 0.05. Based on the incidence of EAD in our centre, it was expected that 45% of the patients in the control group and 15% of the patients in the IFLT group would have an EAD. We, therefore, aimed to include at least 38 patients in each group. The inclusion of participants in the IFLT group was much slower than in the CLT group. We therefore included all the patients who met the inclusion criteria and underwent CLT during the study period to reduce selection bias. The increased sample size in the control group meant that the study was more powered to detect an overall absolute difference of 30 percentage points in the incidence of EAD.

In addition to outcome measures, donor and recipient characteristics were expressed as the median (inter quartile range, IQR) or the mean $\pm$ standard deviation (SD) for continuous parameters and in percentages for nominal parameters. Continuous parameters were compared with two-tailed Student's t-tests or two-tailed Mann-Whitney nonparametric tests. Fisher's exact test was used to compare categorical parameters. For categorical outcomes, absolute risk differences between groups were calculated using exact unconditional methods based on the Farrington-Manning score statistic, expressed as percentage points with 95% confidence intervals (CIs) [20]. Linear graphs of perfusion parameters, blood gas analysis, and bile examination were presented as the median and range. To explore the efficacy of IFLT versus CLT in using ECD livers, subgroup analyses were performed for ECD and SCD. A p value $<0.05$ was considered to be statistically significant. Data analysis was performed using Statistical Product and Service Solutions (SPSS) 22.0 (IBM, New York, USA).

The original and final versions of the protocol, as well as the summary of the changes and their rationales can be found in Appendix 2.

## 2.7. Role of the funding source

Funding did not influence study design, data collection, data analysis, data interpretation, or writing of the manuscript. The corresponding authors had full access to all data at any time and take final responsibility for the submission.

## 3. Results

### 3.1. Surgery

From January 1st, 2017 to March 12th, 2019, 412 donor livers were allocated to our centre for transplantation. A total of 40 donor livers underwent the IFLT protocol. One liver in the IFLT group had to be assigned to the control group as the hepatic artery pump of the device stopped running (due to a defect of the disposable) immediately after the donor liver was moved from the donor peritoneal cavity to the organ reservoir. Moreover, one liver was discarded because of slow lactate clearance and macroscopic fibrosis secondary to HBV infection. A total of 168 donor livers met both donor and recipient inclusion criteria and were successfully transplanted. Thirty-eight patients underwent IFLT and 130 patients underwent CLT (Fig. 2). Donor and recipient characteristics are summarised in Table 1. All donor sex, age, body mass in-

dex (BMI), causes of death, donor types, and donor risk index (DRI) were comparable between the two groups. There were 12 (31•6%) and 29 (22•3%) ECD organs in the IFLT and CLT groups, respectively (appendix p 3). Moreover, recipient age, sex, model for end-stage liver disease (MELD) score [21], or primary diagnosis of liver diseases were comparable. Organ utilisation rates of the kidneys, hearts, lungs and pancreas were not different for donors proceeding with IFLT or CLT (appendix p 4), and outcomes for renal transplants were comparable between the two groups (appendix pp 5, 12).

The organ procurement time was longer in the IFLT versus CLT group (204, 190-220 min vs 45, 40-59 min, p<0•001) (Table 1) because efforts were made to fully dissect the donor liver in the IFLT group, and hemodynamics were stable in both groups. The anhepatic phase of recipient operations were comparable between the two groups (p=0•949), although the median (IQR) duration of the recipient operations was shorter in the IFLT group than in the CLT group (385, 340-445 min vs 445, 380-512 min, p<0•001). There was no cold ischaemia time (CIT) in the IFLT group, and the median CIT in the CLT group was 369 (329-450) min. The median NMP duration time in the IFLT group was 240 (160-360) min. The bicaval implantation technique was more frequently used in the IFLT recipients, and the piggy-back implantation technique was more frequently used in the CLT recipients (Table 1). During transplantation, IFLT recipients had a higher mean ($\pm$ SD) body temperature during the an-hepatic phase (35•69 $\pm$ 0•70°C vs 34•66 $\pm$ 3•14°C, p=0•006) and one hour after graft revascularisation (36•30 $\pm$ 0•72°C vs 35•33 $\pm$ 0•96°C, p<0.001) (appendix p 13). There was no significant difference in intraoperative mean arterial pressure, use of norepinephrine and dopamine, blood loss, use of red blood cells and fresh frozen plasma, as well as post-transplant INR, prothrombin time (PT), and fibrinogen (Fbg) levels (all p values > 0.05) (Table 1, and appendix pp 6-7, 14).

Supplemental figure 4 shows the NMP parameters and biochemical analysis of the perfusate in the 38 IFLT cases (appendix p 15). The pressure and flow of both portal vein and hepatic artery were stable throughout the entire IFLT procedure. The partial pressures of oxygen (pO$_2$) and carbon dioxide (pCO$_2$) were between 150-250 mmHg and 35-45 mmHg, respectively. Biochemical analysis of the perfusate showed that the pH values were within normal ranges (7•35-7•45). The lactate level declined rapidly from 6•63 $\pm$ 1•37 mmol/L at the initiation of NMP to less than 2•0 mmol/L. All perfused livers continued producing bile during the entire procedure.

### 3.2. Post-transplant Recovery

Table 2 summarises the primary and secondary endpoints. Only two (5•3%) patients developed EAD in the IFLT group compared to 65 (50•0%) in the CLT group (p<0•001); the majority (41/65, 63•1%) of EAD cases in the CLT group met the Tbil criteria (appendix pp 8-9). No patient suffered PNF in the IFLT group, while four cases of PNF occurred in the CLT group. The median (IQR) peak AST levels within the first week were significantly lower in the IFLT group (365, 238-697 U/L) compared to the CLT group (1445, 791-3244 U/L) (p<0•001). Likewise, the peak ALT levels were much lower in the IFLT group (155, 106-306 U/L compared to 694, 368-1176 U/L for CLT livers, p<0•001). In addition, the Tbil levels by day 7 were significantly lower in the IFLT versus CLT group (2•34, 1•39-4•09 mg/dL vs 5•10, 1•90-11•65 mg/dL, p<0•001). Moreover, IFLT recipients demonstrated lower cumulative levels of liver injury markers, including AST, ALT, Tbil and lactate dehydrogenase (LDH) in the early phase post-transplantation (appendix p 16). Finally, IFLT recipients showed significantly lower gamma-glutamyl transpeptidase (GGT) and alkaline phosphatase (ALP) levels by one year post-transplantation (GGT: 49•1 $\pm$ 37•6 U/L vs 103•6 $\pm$ 134•0 U/L,
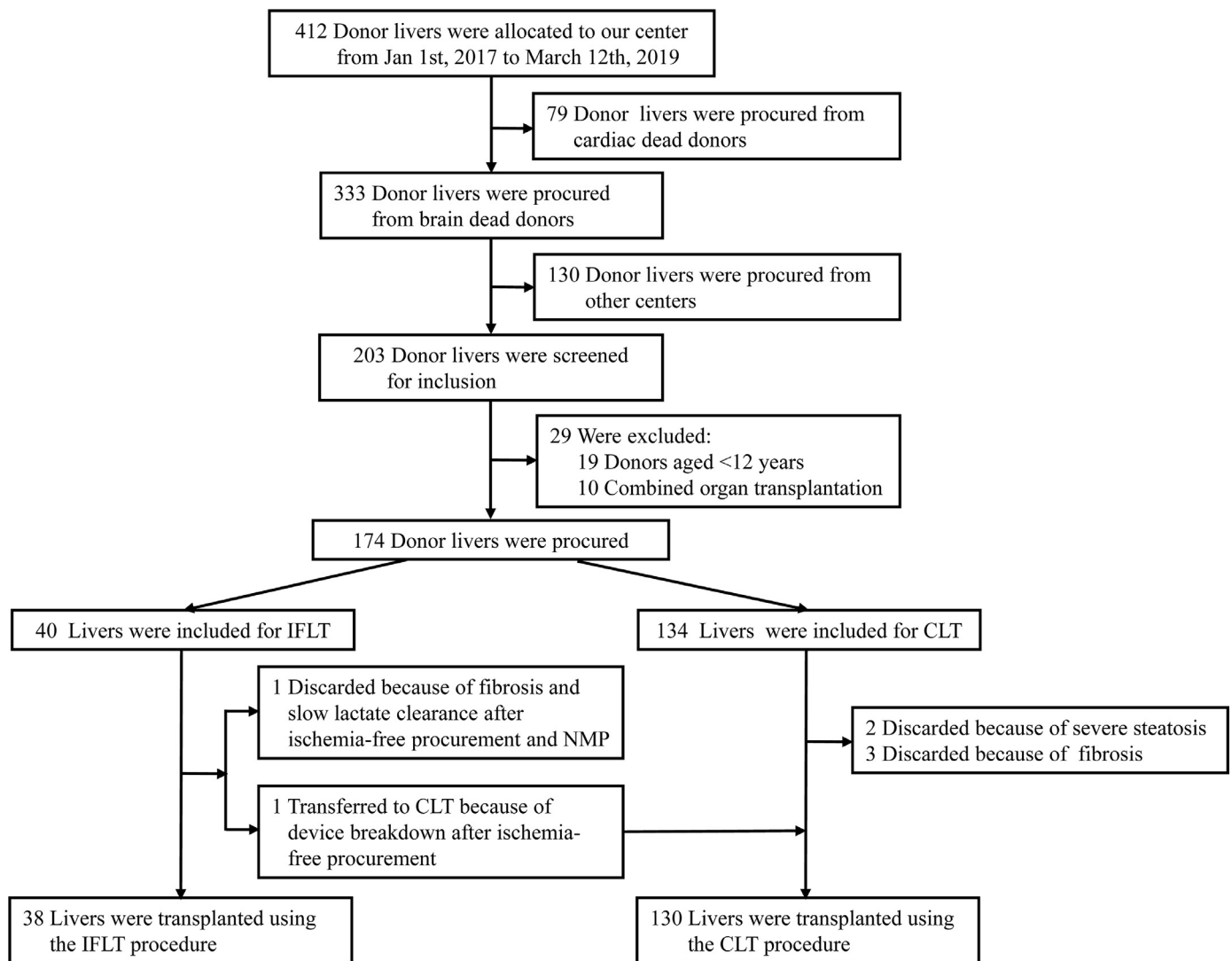
**Fig. 2. Screening, Selection, and Follow-Up.** A total of 412 donor livers were screened from January 1st, 2017, to March 12th, 2019, 79 livers from donations after cardiac death (DCD) and 130 livers from other centres were excluded; 203 brain dead donors were considered for inclusion into the study. 19 livers from paediatric donors and 10 livers used for combined organ transplantation were excluded. Of the remaining 174 livers, 40 livers were assigned to the ischaemia-free liver transplantation (IFLT) group, and 134 livers were included for conventional liver transplantation (CLT). Out of the 40 livers in the IFLT group, one liver was discarded because of fibrosis and slow lactate clearance after ischaemia-free procurement and *ex situ* normothermic machine perfusion (NMP); one liver had to be re-assigned to the CLT group because of a technical problem with the perfusion disposable. Of the 134 livers for CLT, two were discarded because of severe steatosis and three were discarded because of fibrosis. Eventually, 38 livers were transplanted using the IFLT procedure and 130 livers were transplanted using the CLT procedure.

p=0•048; ALP: 83•7 ± 22•9 U/L vs 131•2 ± 133•4 U/L, p=0•044) (appendix p 16).

IFLT recipients had a shorter median (IQR) post-transplant ICU stay (1•48, 0•75-2•00 days vs 1•81, 1•00-4•58 days, p=0•006). Although not significant, only 2/38 (5•3%) patients in the IFLT group needed renal replacement treatment compared to 22/130 (16•9%) recipients in the CLT group (p=0•111). There were comparable biliary complications between the IFLT group (10•5% with no NAS) and the CLT group (18•5% with five NAS) (p=0•326). Clinical allograft rejection occurred in two patients (5•3%) in the IFLT group by one year compared to nine patients (6•9%) in the CLT group (p=1•000). Vascular complications were comparable between the two groups (p=0•686).

Recipients in the IFLT group showed improved one-month patient (97•4% vs 90•8%, p=0•302) and graft (97•4% vs 90•0%, p=0•195) survival rates. Ten patients (7.7%) died of PNF or HAT in the CLT group, while none died of these two complications in the IFLT group (appendix p 10). Improved outcomes were also observed by one year for patient (92•1% vs. 82•3%, p=0•142) and graft survival (89.5% vs. 81•5%, p=0•326) in the IFLT group. However, these differences were not statistically significant.

### 3.3. Outcomes for ECD livers

To further delineate the potential advantages of IFLT in using ECD livers, we performed a subgroup analysis. SCD and ECD livers in the IFLT recipients demonstrated comparable post-transplant peak AST/ALT and Tbil levels within the first week (appendix pp 11, 17). Moreover, ECD livers in the IFLT group showed improved transaminases compared to SCD in the CLT group (peak AST: 385, 283-853 U/L vs 1325, 729-2725 U/L, p<0•001; peak ALT: 259, 108-356 U/L vs 658, 360-1067 U/L, p<0•001) (appendix pp 11, 17). EAD occurred in only one recipient (8•3%) of the IFLT_ECD subgroup compared to 48•3% (p=0•030) and 50•5% (p=0•006) in the CLT_ECD and CLT_SCD subgroups, respectively (appendix pp 11, 17).

**Table 1**
Baseline demographic and clinical characteristics of donors and recipients. *

| Characteristics | IFLT (n=38) | CLT (n=130) | p value† |
|---|---|---|---|
| **Donor** | | | |
| Age (years) | 36·0 ± 14·2 | 37· 2 ± 12·2 | 0·597 |
| Sex | | | 0.249 |
| Male | 31 (81·6%) | 94 (72·3%) | |
| Female | 7 (18·4%) | 36 (27·7%) | |
| BMI (kg/m$^2$) | 22·3 ± 2·2 | 22·5 ± 2·5 | 0·643 |
| Cause of death | | | 0·123 |
| Head trauma | 21 (55·3%) | 55 (42·3%) | |
| Anoxia | 3 (7·9%) | 9 (6·9%) | |
| Cerebrovascular accident | 11 (28·9%) | 62 (47·7%) | |
| Other‡ | 3 (7·9%) | 4 (3·1%) | |
| Type | | | 0·284 |
| Extended criteria donor | 12 (31·6%) | 29 (22·3%) | |
| Standard criteria donor | 26 (68·4%) | 101 (77·7%) | |
| Donor risk index | 1·344 ± 0·235 | 1·361 ± 0·209 | 0·656 |
| **Recipient** | | | |
| Age (years) | 50·8 ± 11·3 | 50·2 ± 9·6 | 0·741 |
| Sex | | | 0·523 |
| Male | 34 (89·5%) | 120 (92·3%) | |
| Female | 4 (10·5%) | 10 (7·7%) | |
| MELD score | 24·0 ± 3·7 | 24·0 ± 4·1 | 0·931 |
| HBV infection | | | 1·000 |
| (+) | 32 (84·2%) | 110 (84·6%) | |
| (−) | 6 (15·8%) | 20 (15·4%) | |
| Primary diagnosis | | | 0·214 |
| Hepatocellular carcinoma | 18 (47·4%) | 69 (53·1%) | |
| Hepatitis B cirrhosis | 18 (47·4%) | 44 (33·8%) | |
| Other§ | 2 (5·3%) | 17 (13·1%) | |
| **Operation** | | | |
| Liver retrieval time (min) | 204 (190-220) | 45 (40-59) | <0·001 |
| Anhepatic phase (min) | 49 (44-55) | 51 (39-62) | 0·949 |
| Recipient operation time (min) | 385 (340-445) | 445 (380-512) | <0·001 |
| Cold ischaemia time (min) | 0 | 369 (329-450) | <0·001 |
| NMP duration time (min) | 240 (160-360) | NA | NA |
| Implantation method | | | 0·016 |
| Bicaval | 26 (68·42%) | 58 (44·62%) | |
| Piggy-back | 12 (31·58%) | 72 (55·38%) | |
| Blood loss (ml) | 2000 (1000-2500) | 2000 (1000-3000) | 0·601 |
| Intraoperative use of RBCs (ml) | 800 (500-1400) | 1125 (600-1840) | 0·157 |
| Intraoperative use of FFP (ml) | 1600 (1000-2200) | 1550 (1000-2200) | 0·927 |

* Data are presented as n (%), mean ± standard deviation, or median (inter quartile range, IQR). IFLT, ischaemia-free liver transplantation; CLT, conventional liver transplantation; BMI, body mass index; ECD, extended criteria donor; SCD, standard criteria donor; MELD, model for end-stage liver disease; HBV, hepatitis B virus; NMP, normothermic machine perfusion; NA, not applicable; RBC, red blood cell; FFP, fresh frozen plasma.
† P values apply to comparisons of IFLT vs. CLT groups calculated with Fisher's exact test for discrete variables, and with a 2-tailed Student's T-test or Mann-Whitney test for continuous variables.
‡ Other cause of death: bacterial encephalitis, viral encephalitis, organophosphorus poisoning.
§ Other primary disease: primary sclerosing cholangitis, primary biliary cirrhosis, alcoholic cirrhosis, hepatitis C cirrhosis, Budd-Chiari syndrome, cholangiocarcinoma or liver cirrhosis of an unknown origin.

## 4. Discussion

During conventional organ transplantation, oxygenated blood supply is completely interrupted during procurement, preservation, and implantation. The restoration of oxygenated blood supply (graft re-vascularisation) subsequent to ischaemia exacerbates the initial cellular damage, a process that has been well characterized as IRI, albeit the pathophysiological complexity of this event remains only poorly understood [1]. Many therapeutic interventions have been proposed and tested [5]. While some have shown an amelioration of the detrimental sequelae of IRI, there has yet not been a systematic approach of testing the absence of IRI. Our IFLT approach provides thus an entirely novel approach with unique clinical and research opportunities. Indeed, we have been able to show in a large clinical series that IFLT is not only feasible and safe, but also leading to a significant improvement in outcomes.

Various types of machine perfusion technologies have been used in clinical practice, including hypothermic machine perfusion (HMP), hypothermic oxygenated perfusion (HOPE), NMP, subnormothermic machine perfusion (SNP), and controlled oxygenated

rewarming (COR) [6, 7, 22-27]. These novel preservation methods are potentially able to assess graft viability and improve transplant outcomes. Nevertheless, those approaches may, at best, reduce some of the detrimental consequences of IRI. However, grafts remain to suffer from ischaemia and subsequent IRI. We have introduced a novel surgical approach by creating a tri-branch structure of the portal vein, celiac artery, and retrohepatic inferior vena cava, enabling a continuous blood supply at body temperature switching between *in vivo* blood perfusion, and NMP during organ procurement and implantation, thus avoiding IRI.

Although the DBD donors were young with normal BMI in our country, the incidence of EAD is 36·4-54·8% during 2015-2017 according to the Chinese national database, which is much higher compared to rates reported by centres in the Western world [7, 28]. The deceased organ donation system has been established since 2015 in our country. The potential donors often suffered hypotension, hypoxia, anaemia, hypoalbuminemia, hypernatremia and infections before organ donation, because the family members would sign informed consents of refusal of active resuscitation and symptomatic therapies when patient death was irriversible, and

**Table 2**
Outcomes in the IFLT and CLT groups. *

| Outcomes | IFLT (N=38) | CLT (N=130) | Absolute Risk Difference (95% CI) ‡ | p value† |
|---|---|---|---|---|
| EAD | 2 (5•3%) | 65 (50•0%) | 44•8 (33•6, 55•9) | <0•001 |
| Peak AST (U/L) within 7 days | 365 (238-697) | 1445 (791-3244) | •• | <0•001 |
| Peak ALT (U/L) within 7 days | 155 (106-306) | 694 (368-1176) | •• | <0•001 |
| Tbil (mg/dL) on POD 7 | 2•34 (1•39-4•09) | 5•10 (1•90-11•65) | •• | <0•001 |
| INR on POD 7 | 1•12 (1•07-1•22) | 1•10 (1•04-1•17) | •• | 0•385 |
| PNF | 0 | 4 (3•1%) | •• | 0•575 |
| ICU stay (days) | 1•48 (0•75-2•00) | 1•81 (1•00-4•58) | •• | 0•006 |
| Post-transplant hospital stay (days) | 19•5 (15-33) | 21•5 (16-29) | •• | 0•795 |
| Biliary complications | 4 (10•5%) | 24 (18•5%) | 8•0 (-3•9, 19•8) | 0•326 |
| Non-anastomotic stricture | 0 | 5 (3•8%) | •• | |
| Anastomotic stricture | 3 (7•9%) | 16 (12•3%) | •• | |
| Biliary leak | 1 (2•6%) | 2 (1•5%) | •• | |
| Biliary stone | 0 | 1 (0•8%) | •• | |
| Acute rejection | 2 (5•3%) | 9 (6•9%) | 1•7 (-6•7, 10•0) | 1•000 |
| Vascular complications | 1 (2•6%) | 8 (6•2%) | 3•5 (-3•0, 10•1) | 0•686 |
| Need for RRT within 30 days | 2 (5•3%) | 22 (16•9%) | 11•7 (2•1, 21•2) | 0•111 |
| One-month patient survival | 37 (97•4%) | 118 (90•8%) | -6.6 (-13.7, 0.5) | 0•302 |
| One-month graft survival | 37 (97•4%) | 117 (90•0%) | -7.4 (-14.6, -0.1) | 0•195 |
| One-year patient survival | 35 (92•1%) | 107 (82•3%) | -9•8 (-20•6, 1•0) | 0•142 |
| One-year graft survival | 34 (89•5%) | 106 (81•5%) | -7•9 (-19•8, 3•9) | 0•326 |

* Data are presented as n (%) or as median (inter quartile range, IQR). AST, aspartate aminotransferase; ALT, alanine aminotransferase; CLT, conventional liver transplantation; CI, confidence interval; EAD, early allograft dysfunction; ICU, intensive care unit; IFLT, ischaemia-free liver transplantation; INR, international normalised ratio; NAS, non-anastomotic biliary stricture; PNF, primary nonfunction; POD, post-operation day; RRT, renal replacement therapy; Tbil, total bilirubin.

† P values were calculated with Fisher's exact test for discrete variables and with a 2-tailed Mann-Whitney test for continuous variables.

‡ Absolute risk differences were expressed as percentages with 95% CI and calculated using exact unconditional methods based on the Farrington-Manning score statistic.

the ICU phycisians lacked of experience and resource for treating the potential donors. These conditions might explain the high EAD rate in our country. In the current study, a substantially lower EAD rate was documented in the IFLT versus CLT groups. EAD in one patient was defined due to elevated AST level because of intrahepatic hematoma. Another one was defined due to elevated Tbil level because of biliary stricture in the IFLT group. These two cases of EAD do not necessarily represent a state of organ dysfunction.

Recently, the definition of EAD has been challenged as an endpoint in studies related to NMP as liver enzyme might be "washed out" during perfusion [29]. However, the AST/ALT concentrations in the perfusate were consistently low. In addition, more than half of EAD cases were defined by the Tbil criteria in this study. These results suggest that the "washed out" effect cannot explain the difference in the incidence of EAD between the two groups. It has also been reported that EAD cannot predict graft survival [30]. Nevertheless, the patient and graft survival rates of recipients with EAD in our study were significantly compromised than those without EAD in the CLT group (appendix p 18). Moreover, the incidence of peak AST >5000 U/L, which is a predictor of inferior graft survival [31], was significantly lower in the IFLT group than in the CLT group (0 vs. 13•1%, p<0•001). No patient died of PNF in the IFLT group, while four patients died of PNF in the CLT group. Therefore, IFLT can largely avoid the detrimental consequence of IRI.

ECD livers are those from older donors, livers with >30% macrovesicular steatosis, and livers with long CIT or hypernatremia. The ECD livers account for about 12% of all transplanted livers during 2002-2016 in the United States [32]. Their susceptibility to IRI remains the major limitation of the maximun utilisation of these organs. In 2016, approximately 1600 (more than 20%) of all liver allografts from deceased donors were discarded [33]. For instance, most centres around the world would not accept a liver with a macrovesicular hepatosteatosis grade

>60% [34]. We have previously demonstrated the potential of our clinical IFLT approach by utilising a donor liver with 85-90% macrovesicular hepatosteatosis [14]. In the current study, by using IFLT, the ECD livers yielded comparable graft function as the SCD livers. Moreover, the ECD livers in the IFLT group recovered even faster with a much lower incidence of EAD than the SCD livers in the CLT group. During IFLT, IRI is largely avoided, and the graft viability is easily assessed. Therefore, IFLT might represent a promising approach for utilising ECD livers.

We consider the practice of IFLT can be generalised to other centers. Firstly, our group has developed a multiple organ procurement protocol when IFLT is conducted. No extrahepatic organ lost during the IFLT procurement process. The recovery rates of non-extrahepatic grafts and renal transplant outcomes were comparable between the CLT and IFLT groups. However, the whole multi-organ procurement team should be informed of the prolonged procurement time when IFLT is performed. Besides, The shorter time for the recipient operation in the IFLT group may have been related to a reduced time for back-table preparation and post-revascularization haemostasis of donor livers. Secondly, although the majority of the participants in this study were male with HBV infection and normal BMI, the IFLT procedure can be performed regardless of the gender, primary diagnosis and BMI of patients. Thirdly, additional cost associated with IFLT was about 5000 Euro per case. We consider it is cost-effective, because IFLT can avoid PNF, reduce incidence of EAD and length of ICU stay, particularly when a high-risk donor liver is used. Finally, the technical difficulties can be overcome. One liver intended to undergo IFLT was transferred to the control group due to a defect of the perfusion disposable. Others have previously reported on a discarded liver after NMP due to a technical failure with portal cannulation [35]. Therefore, a back-up preservation method should be available at any time, when using IFLT. Critical for the success is also a well-

trained perfusionist and surgeon team. It is relevant to avoid redundant portal vein and twisted perfusion lines. A parachuting anastomosis technique for suprahepatic inferior vena cava should be used if the abdominal cavity is small during implantation.

There are limitations in the current study. Firstly, a stationary machine perfusion device was used in this study, and the livers were all locally procured in both groups. A simplified technique with a portable device is under development in our centre to enable distant procurement. Secondly, there might be patient selection bias because of the non-randomized design, although no significant difference in either donor or recipient characteristics has been found, and the surgery and health provider team had the same expertise for the treatment of patients in both groups. We are currently working on a randomised controlled trial (ChiCTR1900021158) in our centre to confirm the above findings. Finally, although IFLT represents a unique approach, the potential benefits of IFLT over liver transplantation using preservation NMP, particularly in ECD livers, will need to be defined.

In conclusion, IFLT can provide an efficient approach which can potentially improve transplant outcomes, and increase the availability of organs for transplantation. Importantly, it has been shown that NMP is safe and feasible in the heart [8, 9], lung [10, 11], and kidney preservation [12]. Therefore, it is possible that the concept of ischaemia-free organ transplantation can also be adopted to organs other than the liver. Indeed, the techniques of ischaemia-free kidney transplantation have been established by our centre [36]. This novel approach may change current practice in organ transplantation.

## Contributors

XH, ZG, WJ, QZ, SH, and CH contributed to the trial design and data interpretation. CH, DW, LY, MC, LWu, ZZha, ZZhu, LWa, CZ, YZ, YT, CS, WX, YS, XC, JY, TW, YM, AH, YC, XZ, JR, CC, FG, XW, and WH contributed to data collection. WJ, QZ, ZG, SH, JZ, and CH analysed and interpreted the data. ZG, SH, and CH drafted the manuscript, which was critically reviewed and revised by XH, WJ, QZ, XL, DSCK, SGT, and JH. All authors reviewed and approved the final manuscript.

## Declaration of Competing Interest

The authors declare no conflict of interest.

## Data Sharing

Original data statistical analysis plan, informed consent form, and ethics committee approval are available and can be shared upon request. All available participant data will be de-identified. To access data, a request should be submitted to the corresponding author with a scientific proposal including objectives. The steering committee of this study will discuss and evaluate all requests and decide whether data sharing is appropriate. Data will only be shared after a data sharing agreement is fully executed.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.lanwpc.2021.100260.

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