Research Article
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A randomized-controlled trial of ischemia-free liver transplantation for end-stage liver disease

Zhiyong Guo[1,2,3,4,*,†], **Qiang Zhao**[1,2,3,†], **Zehua Jia**[1,2,3,†], **Changjun Huang**[1,2,3,†], Dongping Wang[1,2,3], Weiqiang Ju[1,2,3], Jian Zhang[5], Lu Yang[6], Shanzhou Huang[1,2,3], Maogen Chen[1,2,3], Xiaofeng Zhu[1,2,3], Anbin Hu[1,2,3], Yi Ma[1,2,3], Linwei Wu[1,2,3], Yinghua Chen[1,2,3], Ming Han[1,2,3], Yunhua Tang[1,2,3], Guodong Wang[1,2,3], Linhe Wang[1,2,3], Lifen Li[7], Wei Xiong[6], Zhiheng Zhang[1,2,3], Yuekun Shen[6], Zhaoxia Tang[7], Caihui Zhu[1,2,3], Xiaoxiang Chen[6], Xiaoguang Hu[7], Yiwen Guo[1,2,3], Honghui Chen[1,2,3], Yihao Ma[1,2,3], Tao Zhang[1,2,3], Shunwei Huang[7], Ping Zeng[1,2,3], Simei Lai[1,2,3], Tielong Wang[1,2,3], Zhitao Chen[1,2,3], Jinlong Gong[1,2,3], Jia Yu[1,2,3], Canhui Sun[8], Chang Li[8], Haiyi Tan[8], Yao Liu[1,2,3], Yuqi Dong[1,2,3], Chengjun Sun[1,2,3], Bing Liao[9], Jun Ren[10], Zhenhai Zhou[10], Schlegel Andrea[11], Nashan Björn[12], Changjie Cai[7], Fengqiu Gong[13], Jian Rong[14], Wenqi Huang[6], Xiangdong Guan[7], Pierre-Alain Clavien[15], Tullius G. Stefan[16], Jiefu Huang[1], Xiaoshun He[1,2,3,*]

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Background & Aims: Ischemia-reperfusion injury (IRI) has thus far been considered as an inevitable component of organ transplantation, compromising outcomes, and limiting organ availability. Ischemia-free organ transplantation is a novel approach designed to avoid IRI, with the potential to improve outcomes.
Methods: In this randomized-controlled clinical trial, recipients of livers from donors after brain death were randomly assigned to receive either an ischemia-free or a 'conventional' transplant. The primary endpoint was the incidence of early allograft dysfunction. Secondary endpoints included complications related to graft IRI.
Results: Out of 68 randomized patients, 65 underwent transplants and were included in the analysis. 32 patients received ischemia-free liver transplantation (IFLT), and 33 received conventional liver transplantation (CLT). Early allograft dysfunction occurred in two recipients (6%) randomized to IFLT and in eight (24%) randomized to CLT (difference −18%; 95% CI −35% to −1%; $p = 0.044$). Post-reperfusion syndrome occurred in three recipients (9%) randomized to IFLT and in 21 (64%) randomized to CLT (difference −54%; 95% CI −74% to −35%; $p < 0.001$). Non-anastomotic biliary strictures diagnosed with protocol magnetic resonance cholangiopancreatography at 12 months were observed in two recipients (8%) randomized to IFLT and in nine (36%) randomized to CLT (difference, −28%; 95% CI −50% to −7%; $p = 0.014$). The comprehensive complication index at 1 year after transplantation was 30.48 (95% CI 23.25–37.71) in the IFLT group *vs.* 42.14 (95% CI 35.01–49.26) in the CLT group (difference −11.66; 95% CI −21.81 to −1.51; $p = 0.025$).
Conclusions: Among patients with end-stage liver disease, IFLT significantly reduced complications related to IRI compared to a conventional approach.
Clinical trial registration: chictr.org. ChiCTR1900021158.

Introduction

Organ transplantation is the gold standard therapy for patients with end-stage organ failure.[1] Traditionally, donor organs are procured after cold flush with an organ preservation solution, stored on ice, and then urgently implanted to minimize ischemic times. The resulting ischemia-reperfusion injury (IRI) has therefore been considered an inevitable component of organ transplantation,[2] leading to a broad range of clinical complications in liver transplantation, including primary non-function, early allograft dysfunction (EAD), post-reperfusion syndrome, and non-anastomotic biliary strictures.[3] Recently, various machine perfusion technologies have been tested in clinical trials.[4–8] While conventional liver transplantation (CLT) subsequent to cold storage remains the standard of care, any machine perfusion technique is applied after a period of cold storage, with donor livers experiencing a period of ischemia before and after machine perfusion.

We have recently introduced an ischemia-free liver transplant (IFLT) technique during which the livers are procured, preserved, and implanted without interruption of normothermic, oxygenated blood supply.[9] Moreover, the concept of ischemia-free organ transplantation has also been piloted in kidney and heart transplantation,[10,11] demonstrating the broad

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* Corresponding authors. Address: Organ Transplant Center, The First Affiliated Hospital, Sun Yat-sen University, NO.58 Zhongshan Er Road, Guangzhou, 510080, China. Tel./fax: +86 20 87306082.
E-mail addresses: gdtrc@163.com (X. He), rockyucsf1981@126.com (Z. Guo).
† Zhiyong Guo, Qiang Zhao, Zehua Jia, and Changjun Huang contributed equally to this work as co-first authors
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applicability. Preliminary clinical results have also shown the safety and potential efficacy of IFLT.[12]

The IFLT-DBD (Ischemia-free Transplantation of Livers from Donors after Brain Death) trial was designed to assess whether the use of IFLT, compared to CLT using static cold storage, can reduce complications related to graft IRI in patients with end-stage liver disease.

Patients and methods

Study, settings and ethics

This trial was an investigator-initiated, single-center, open label, randomized-controlled trial conducted at The First Affiliated Hospital of Sun Yat-sen University. Patients were randomly assigned in a 1:1 ratio to receive an ischemia-free (IFLT group) or conventional (CLT group) liver transplant. The intraoperative and post-transplant care was performed according to standard protocols applied at our center. Detailed aspects of the study design are provided in the final trial protocol and the final statistical analysis plan with a summary of all changes in the supplementary files, and a prior protocol publication.[13] The Research Ethics Committee of our hospital approved the trial protocol, and all patients provided written informed consent.

Participants

Recipients

Adult patients (18–75 years) undergoing liver-only transplantation with a graft procured from donors after brain death in our hospital were eligible for inclusion. Patients were excluded if they underwent a partial or ABO-incompatible liver transplantation, if they had fulminant liver failure or a primary liver cancer beyond the UCSF (University of California at San Francisco) criteria,[14] or if they had a history of a previous organ transplantation.

Donors

Donors after brain death, over the age of 18 years, or over the age of 14 years with a body weight greater than 50 kg, were eligible for inclusion. Donors with a high risk of transmittable infections (human immunodeficiency virus infection and active tuberculosis), or donor malignancy transmission risk over 10% according to the Disease Transmission Advisory Committee category, were excluded.[15] All livers were procured at our hospital; written informed consents signed by donors' family members were obtained by coordinators from the Organ Procurement Organization of our institute. All donor livers were allocated by the China Organ Transplant Response System and none were from prisoners.

Randomization and blinding

Block randomization with varying block sizes of four or six was adopted. A subject randomization list was generated using a central randomization system by our statistician, with random allocation numbers automatically handled by the system to avoid bias. The trial did not impact the regular process of donor-recipient allocation. Randomization only took place when an eligible donor liver had been allocated to an eligible recipient. Otherwise, transplants proceeded but the recipients were not included in this trial.

Based on the nature of the surgical procedure, it was not possible to blind the surgical team to the group allocation. Involved radiologists assessing the magnetic resonance cholangiopancreatography were blinded to the transplant techniques used.

Surgical procedures

Fig. 1 provides a schematic depiction of the IFLT procedure. The Liver Assist device (Organ assist, The Netherlands) was primed with a leucocyte-depleted red blood cell-based perfusate (Table S1). After the donor liver was fully mobilized, the *in situ* normothermic machine perfusion circuit was established and perfusion began. The liver was then procured, moved to the organ reservoir of the Liver Assist, and underwent *ex situ* normothermic machine perfusion. Livers were considered transplantable if they met the VITTAL criteria.[16] After the recipient's diseased liver was removed, the donor liver was brought from the reservoir to the recipient peritoneal cavity. Liver implantation was conducted using a bicaval or piggyback technique. The anastomoses of the suprahepatic inferior vena cava, portal vein, and hepatic artery were performed under continuous *in situ* normothermic machine perfusion of the graft. Perfusion was discontinued after the donor liver had been revascularized, and all cannulas were removed. Then the biliary tract was reconstructed by end-to-end ductal anastomosis with a continuous suture. The techniques of CLT and additional details with videos are provided in our published reports and protocol.[9,12,13]

Outcomes

The primary endpoint was determined as the incidence of EAD within 7 days post-transplantation as defined by the Olthoff criteria: (i) peak aspartate aminotransferase or alanine aminotransferase >2,000 IU/L within the first 7 post-operative days, (ii) total bilirubin ≥10 mg/dl at day 7 post-transplantation, or (iii) international normalized ratio ≥1.6 at day 7 post-transplantation.[17]

Secondary endpoints included primary non-function;[18] post-reperfusion syndrome, defined as a >30% decline in mean arterial pressure compared to the baseline value before reperfusion that lasts for at least 1 min, occurring within 5 min of reperfusion of the donor liver;[19] biliary complications, including non-anastomotic strictures (assessed by magnetic resonance cholangiopancreatography [MRCP]), bile leakage, biliary anastomotic stenosis, and bile stones or sludge; lactate level at 1 h post-reperfusion based on arterial blood gas analysis; post-transplant liver function tests; patient and graft survival at 1, 6, and 12 months; duration of post-transplant intensive care unit and overall hospital stay.

Other endpoints included acute rejection, vascular complications at 1, 6, and 12 months; acute kidney injury within the first week; need for renal replacement therapy following transplantation; recipient infections within the first month; comprehensive complication index, adverse events, and severe adverse events at 1, 6, and 12 months; positive perfusate microbial culture rate; and organ discard rate.

Sample size calculation and statistical analysis

This study was a 1:1 parallel design, and the sample size calculation was based on our pilot study.[12] We assumed an incidence of EAD of 10% in the IFLT group and 40% in the CLT group. With a power of 80% (1-β) and significance level (α, two-sided) of 5%, we calculated that 32 patients were required for each arm. With the possibility that organs may be discarded, we increased the sample size by 5%. Ultimately, we planned to enroll a total of 68 patients (34 patients in each group).

The intention-to-treat population included recipients who were randomly assigned to either group and underwent liver transplantation. These patients were included for the efficacy analyses, regardless of compliance to treatment or attendance of follow-up visits, in addition to the safety analyses.

If data were missing, thus not enabling assessment of the primary endpoint, patients were assigned as having EAD. The primary endpoint was assessed with the between-group absolute risk differences and corresponding 95% CIs.

For the analysis of secondary outcomes, continuous data were analyzed using *t* test or Mann-Whitney *U* test; categorical data were analyzed using chi-square test or Fisher's exact test. In addition, we used the longitudinal mixed model with unstructured covariance structures for repeated measurements to analyze baseline changes at each time point. All of the model covariates were adjusted for age and sex. Treatment assignment, time and treatment × time interactions were included as fixed effects. The random intercept and slope model was applied. Random effects included intercept and time. Time-to-event outcomes were analyzed by the Kaplan-Meier method and significance of survival differences was determined with the log rank test. No multiple imputation was performed. All statistical analyses were based on two-sided tests; a *p* value less than 0.05 was considered to indicate statistical significance. Statistical analyses were performed using Stata version 14 (StataCorp).

Results

Patient characteristics

Between February 2019 and February 2021, a total of 192 patients were assessed for eligibility, 56 patients met the exclusion criteria; 35 patients did not receive a donor offer during the study period, 30 patients refused to join the trial, and three patients did not meet the inclusion criteria. Finally, 34 patients in each group were randomized. After randomization, three transplants had to be cancelled before any trial procedure had been started. One transplant was aborted in the CLT group because the patient was diagnosed with multiple extrahepatic malignancies in our mandatory chest CT scan immediately before operation implemented during the COVID-19 pandemic. Two transplants had to be cancelled in the IFLT group, one for a high fever immediately prior to surgery and one for withdrawal of informed consent. Ultimately, 32 patients were included in the IFLT group, and 33 patients were included in the CLT group for analysis of the primary endpoint (Fig. 2). Table 1 shows the clinical and baseline characteristics of both donors and recipients.

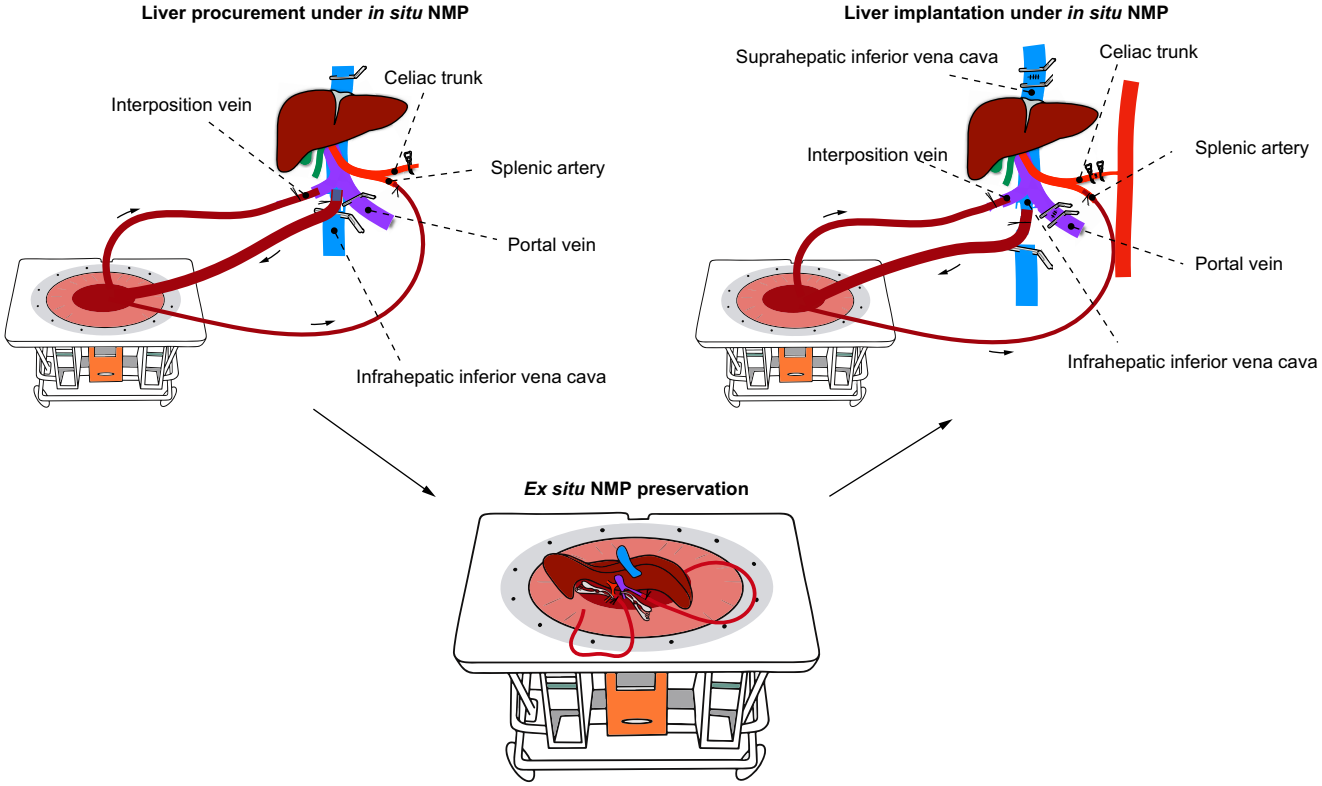

Fig. 1. Ischemia-free liver transplant procedure. During liver procurement, *ex situ* preservation and implantation, the graft is under continuous NMP using the Liver Assist device with perfusions of the portal vein via an interposition vein (right external iliac vein) and the hepatic artery via the splenic artery. NMP, normothermic machine perfusion.

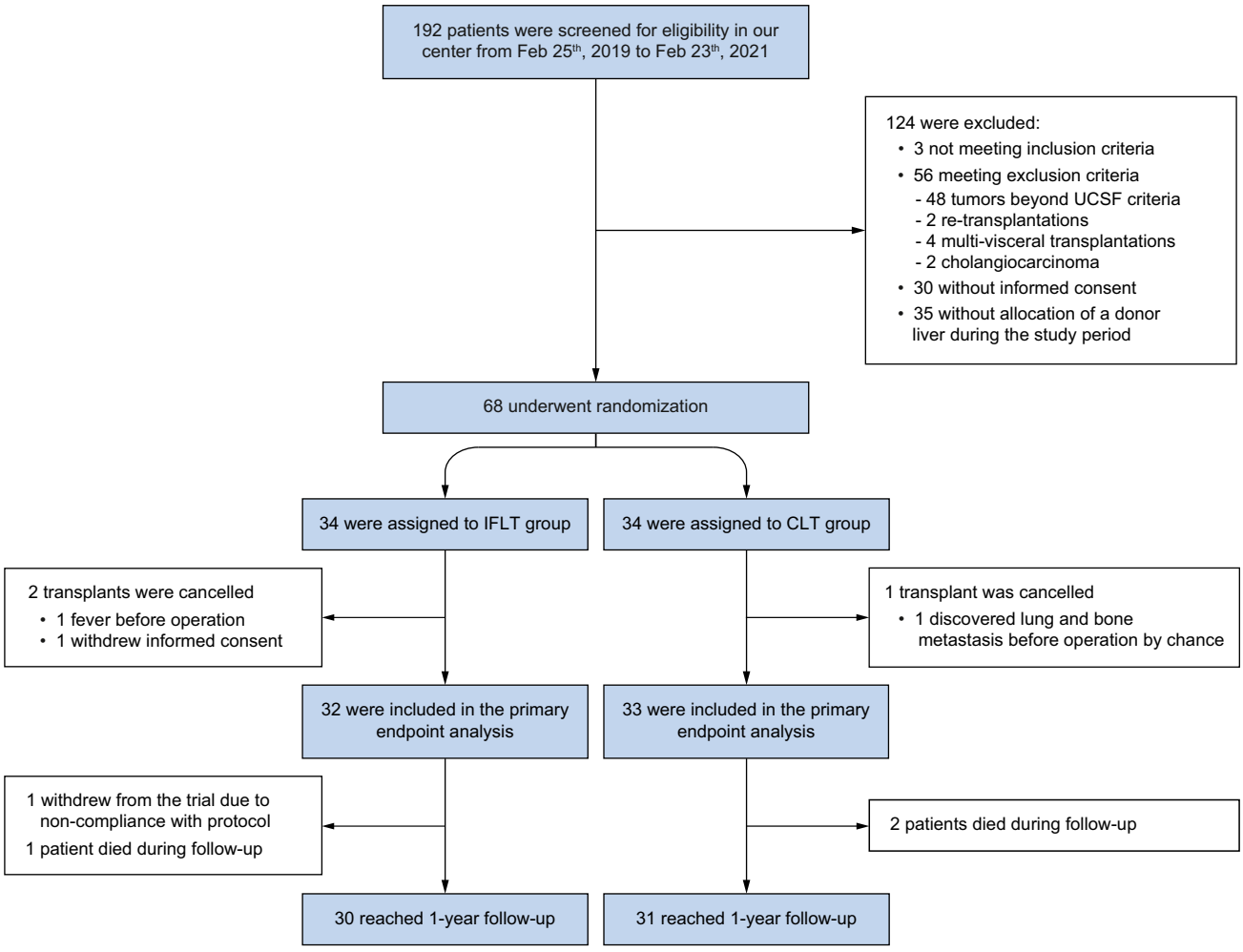

Fig. 2. Flow diagram of patient enrollment, randomization and follow-up. CLT, conventional liver transplantation; IFLT, ischemia-free liver transplantation; UCSF, University of California at San Francisco.

Assessment of graft viability in the IFLT group

Characteristics of the surgery and non-hepatic organ utilization in the two groups are summarized in Tables S2 and S3. In the IFLT group, all livers met not only the VITTAL criteria, but also the Groningen criteria for transplantation during *ex situ* normothermic machine perfusion (Figs. S1 and S2).[16,20] Briefly, they all had stable perfusion pressure and flow through both the portal vein and hepatic artery. The lactate levels fell below 2.5 mmol/L within 80 min of perfusion. All livers produced bile with a pH value higher than 7.8 and liver enzyme levels were stably low during perfusion. Interestingly, all grafts produced a rising perfusate glucose level and remained hyperglycemic despite the addition of insulin.

Primary outcome

The incidence of EAD was significantly lower in the IFLT group (6%, 2 out of 32 patients) compared to the CLT group (24%, 8 out of 33 patients), with an absolute risk difference of −18% (95% CI −35% to −1%; $p = 0.044$) (Table 2 and Table S4).

Consistent with those findings, peak alanine aminotransferase within the first week post-transplantation was reduced in the IFLT group compared to the CLT group (156 [IQR 103–263] IU/L *vs.* 439 [233–646] IU/L; $p < 0.001$). Likewise, peak aspartate aminotransferase within the first week post-transplantation was reduced in the IFLT recipients compared to controls (417 [235–715] IU/L *vs.* 1,010 [534–1,942] IU/L; $p < 0.001$). Bilirubin levels at post-operative day 7 were also lower in IFLT recipients (2.19 [1.33–3.11] mg/dl *vs.* 3.13 [1.85–6.63] mg/dl; $p = 0.03$). The results of liver function tests are shown in Fig. S3.

Secondary outcomes

Post-reperfusion syndrome occurred in 3 of 32 IFLT recipients (9%) compared to 21 of 33 CLT recipients (64%) (absolute risk difference −54%; 95% CI −74% to −35%; $p < 0.001$). Moreover, mean arterial pressure and body temperature were more stable during the early reperfusion stage in the IFLT group compared to the CLT group (Fig. S4). In addition, median lactate levels at 1 h after reperfusion were lower in the IFLT group (2.4 [2.00–3.05] mmol/L *vs.* 2.9 [2.30–4.10] mmol/L; $p = 0.031$). Median duration of intensive care unit stay was shorter in the IFLT group (36 [20–58] hours *vs.* 44 [36–83] hours; $p = 0.037$).

Non-anastomotic biliary strictures diagnosed with protocol MRCP at 12 months were observed in 2/26 (8%) patients receiving an IFLT compared to 9/25 (36%) patients in the

Table 1. Characteristics of donors and recipients.

	IFLT (n = 32)	CLT (n = 33)
Donor characteristics		
Age, median (IQR), yr	47 (39–55)	43 (33–49)
Male, No. (%)	22 (69)	21 (64)
Cause of death, No. (%)		
CVA	13 (41)	8 (24)
Hypoxia	2 (6)	4 (12)
Trauma	16 (50)	21 (64)
Other	1 (3)	0
Body mass index, mean (SD)[a]	22.9 (2.4)	22.6 (2.8)
Serum sodium, mean (SD), mmol/L	147.2 (11.2)	148.3 (11.3)
Macrovesicular steatosis, No. (%)		
None or mild (<30%)	30 (94)	30 (91)
Moderate (30%–60%)	2 (6%)	3 (9%)
Severe (>60%)	0	0
Extended criteria donor, No. (%)[b]	19 (59)	14 (42)
Donor risk index, mean (SD)[c]	1.4 (0.2)	1.4 (0.2)
Cold ischemia time, median (IQR), h[d]	n.a.	6.9 (6.6–7.3)
Machine perfusion time, median (IQR), h[e]	7.1 (6.7–7.6)	n.a.
Recipient characteristics		
Age, median (IQR), yr	53 (46–62)	54 (44–58)
Male, No. (%)	28 (88)	28 (85)
Body mass index, mean (SD)[a]	23.4 (3.0)	24.4 (3.3)
Indication for transplantation, No. (%)		
Hepatocellular carcinoma	13 (41)	18 (55)
Hepatitis B	15 (47)	12 (36)
Alcoholic	3 (9)	0
Miscellaneous[f]	1 (3)	3 (9)
Laboratory MELD score, median (IQR)[g]	15 (11–21)	16 (11–24)

CLT, conventional liver transplantation; CVA, cerebrovascular accident; IFLT, ischemia-free liver transplantation; MELD, model for end-stage liver disease; n.a., not applicable.
[a]The body mass index is the weight in kilograms divided by the square of the height in meters.
[b]The extended criteria donor would meet one of following criteria: Donor age >60-years-old; >25% macrovesicular steatosis; body mass index of donor >30; Before procurement, the latest serum sodium >165 mmol/L or aspartate aminotransferase >1,000 IU/L or alanine aminotransferase >1,000 IU/L or total bilirubin >3 mg/dl; intensive care unit therapy over 7 days.
[c]The donor risk index is a scoring system that was developed to quantitatively predict the risk of post-transplant graft failure in liver transplantation on the basis of donor risk factors.[14] The cold preservation time of the IFLT group is equal to zero.
[d]Cold ischemia time was defined as time between cold flush-out through aortic artery in the donor to reperfusion through portal vein in the recipient.
[e]Machine perfusion time was defined as time between machine perfusion through collateral portal vein in the donor to reperfusion through portal vein in the recipient.
[f]Miscellaneous indications for liver transplantation included one case of hepatitis C in the ischemia-free group, one case of Budd Chiari, one of biliary cholangitis and one of autoimmune hepatitis in the control group.
[g]The MELD score of recipients is an assessment method determining organ allocation priorities for liver transplantation in the United States. The laboratory MELD score is based on original laboratory variables ranging from 6 to 40 without MELD exception points.

control arm (absolute risk difference –28%; 95% CI –50% to –7%; $p = 0.014$; Table S5).[21,22] Table S6 summarizes the reasons why some patients missed scheduled MRCPs. In parallel, serum cholestasis markers (alkaline phosphatase and gamma-glutamyltransferase) were elevated in patients with non-anastomotic strictures (Table S7). Only one patient was symptomatic at 12 months after surgery in the control group, while no patient was symptomatic in the IFLT group. No patient with non-anastomotic strictures required an intervention in this trial.

Microbial cultures of perfusate were less likely to be positive in the IFLT group than in the CLT group (9% [3 out of 32] compared to 78% [25 out of 32], absolute risk difference –69%; 95% CI –86% to –51%; p <0.001). The microorganisms growing in the culture-positive perfusate were classified as pathogenic microorganisms and saprophytic flora.[24] The incidence of positive pathogenic microorganisms was 3% (1 out of 32) in the IFLT group and 44% (14 out of 32) in the CLT group (p <0.001).

The comprehensive complication index based on Clavien-Dindo Classification was significantly lower in the IFLT (30.48 [23.25–37.71]) than in the CLT (42.14 [35.01–49.26]) group at 1 year after transplantation (absolute risk difference –11.66; 95% CI –21.81 to –1.51; $p = 0.025$). Hospital stay, graft and patient survival at 1, 6, and 12 months and other post-operative complications were comparable between groups (Table 2; Fig. S5 and Table S8). There were three patient deaths during the trial. One patient in the IFLT group died of intracranial hemorrhage at 1 month post-operatively. In the CLT group, one patient died of primary graft non-function immediately after surgery, and one patient died of graft failure at post-operative month 5.

Adverse events

Overall, a total of eight serious adverse events were reported to the ethics committee in accordance with the trial protocol (Table S9). The distribution and severity of adverse events reported were comparable between groups (Table 3 and Table S10).

Discussion

The IFLT procedure is a novel technique designed to reduce IRI to an absolute minimum during organ procurement, preservation, and implantation.[9] Laboratory studies have shown that during IFLT, graft IRI is largely abrogated.[25] This first randomized-controlled trial demonstrated a significant reduction of EAD and other complications related to graft IRI in IFLT recipients compared to CLT recipients of livers from donors after brain death.

Although the role of EAD (Olthoff) as a study endpoint and surrogate for clinically relevant post-transplant complications is under debate, this parameter has been frequently used as primary endpoint in previous randomized-controlled trials with machine perfusion.[26] Herein, we show that IFLT can reduce the incidence of EAD. In support, peak liver enzyme levels were substantially reduced. In addition, bilirubin levels at 7 days were lower in the IFLT group. Moreover, lactate levels at 1 h after reperfusion were also lower in the IFLT recipients. Collectively, the use of IFLT was associated with improved early allograft function. We have shown before that liver transplant recipients with EAD have inferior 1-year patient and graft survival rates.[27] Nevertheless, as seen with other previously published randomized-controlled studies on liver machine perfusion,[6–8,28] the current trial was not powered to show a benefit of IFLT for graft/patient survival and it is therefore not surprising that the graft/patient survival rates were comparable in both groups.

Non-anastomotic biliary strictures represent fibrotic narrowing related to graft IRI.[6,29,30] This complication is the leading cause of re-transplantation and occurs in 2–12% of recipients of livers procured from brain dead donors.[31–34] In the current study, the incidence of non-anastomotic strictures based on protocol MRCP was reduced in the IFLT arm. Data from our previous study have shown that the integrity of microvilli in the common bile duct was better preserved in the IFLT *vs.* CLT group.[25] These results suggest that our IFLT procedure

Table 2. Primary and secondary endpoints.

	IFLT (n = 32)	CLT (n = 33)	Absolute risk difference (95% CI)[a]	*p* value
Primary endpoint				
Early allograft dysfunction, No. (%)	2 (6)	8 (24)	−18 (−35 to −1)	0.044
Secondary endpoints				
Peak ALT within 7 days, median (IQR), IU/L	156 (103–263)	439 (233–646)	NA	<0.001
Peak AST within 7 days median (IQR), IU/L	417 (235–715)	1,010 (534–1,942)	NA	<0.001
Tbil on POD 7, median (IQR), mg/dl	2.19 (1.33–3.11)	3.13 (1.85–6.63)	NA	0.028
INR on POD 7, median (IQR)	1.10 (1.04–1.19)	1.09 (1.02–1.18)	NA	0.610
Primary non-function, No. (%)	0	1 (3)	NA	NA[b]
Post-reperfusion syndrome, No. (%)[c]	3 (9)	21 (64)	−54 (−74 to −35)	<0.001
Lactate, median (IQR), mmol/L[d]	2.40 (2.00–3.05)	2.90 (2.30–4.10)	NA	0.031
Intensive care unit stay, median (IQR), h	36 (20–58)	44 (36–83)	NA	0.037
Anastomotic stenosis, No./total (%)				
POM 6	4/28(14)	2/29(7)	7 (−9 to 23)	0.364
POM 12	6/27(22)	2/25(8)	14 (−5 to 33)	0.156
Non-anastomotic stricture, No./total (%)[e]				
POM 6	2/28 (7)	8/29 (28)	−20 (−39 to −2)	0.043
Mild	1	4		
Moderate	1	4		
Severe	0	0		
POM 12	2/26 (8)	9/25 (36)	−28 (−50 to −7)	0.014
Mild	1	5		
Moderate	1	4		
Severe	0	0		
Comprehensive complication index[f]				
POM 1	24.81 (18.19 to 31.42)	35.35 (28.83 to 41.86)	−10.54 (−19.83 to −1.26)	0.027
POM 6	28.48 (21.01 to 35.96)	40.42 (33.06 to 47.77)	−11.93 (−22.42 to −1.44)	0.026
POM 12	30.48 (23.25 to 37.71)	42.14 (35.01 to 49.26)	−11.66 (−21.81 to −1.51)	0.025
Positive perfusate microbial culture, No. (%)	3 (9)	25 (78)	−69 (−86 to −51)	<0.001
Post-operative hospital stay, median (IQR), d	18 (16–24)	17 (14–24)	NA	0.479

ALT, alanine aminotransferase; AST, aspartate aminotransferase; INR, international normalized ratio; MELD, model for end-stage liver disease; NA, not assessed; POD, post-operative day; POM, post-operative month; Tbil, total bilirubin.

[a]Treatment effect is presented by mean difference or percentage difference with 95% CIs without adjustment for any confounding factors. Because of absence of events in one group or skewed continuous variables, some treatment differences were NA.

[b]Because of absence of events in one group, *p* value was NA.

[c]Post-reperfusion syndrome was defined as a >30% decline in mean arterial pressure compared to the baseline value before reperfusion that lasts for at least 1 min, occurring within 5 min of reperfusion of the donor liver.

[d]Lactate was measured by blood gas analysis before abdominal closure during recipient operation.

[e]Biliary complications were diagnosed by two experienced radiologists blindly according to protocol 6-month and 12-month MRCP images.[21] The classification of non-anastomotic stricture depends on severity, site, and number of lesions.[22]

[f]Comprehensive complication index is calculated to evaluate the severity of all post-operative complications.[23] Least square mean and 95% CIs derived from the mixed models were calculated and presented.

protects the bile duct. However, probably due to the use of low-risk donor livers, most of these strictures were asymptomatic, and none of them required an endoscopic or percutaneous intervention during our follow-up period of 1 year. It will be of interest to investigate whether IFLT can reduce symptomatic non-anastomotic biliary strictures following transplantation of high-risk donor livers in the future.

Table 3. Reported adverse events within 1 year after transplantation.

	IFLT (n = 32)[a]	CLT (n = 33)[a]
Total adverse events	272 (100%)	375 (100%)
Metabolism and nutrition disorders	73 (26.8%)	79 (21.1%)
Blood and lymphatic disorders	54 (21.0%)	70 (18.7%)
Gastrointestinal disorders	35 (12.9%)	41 (10.9%)
Respiratory, thoracic and mediastinal disorders	24 (8.8%)	36 (9.6%)
Hepatobiliary disorders	19 (7.0%)	31 (8.3%)
Cardiac and vascular disorders	16 (5.9%)	38 (10.1%)
Systemic disorders	16 (5.9%)	15 (4.0%)
Renal and urinary disorders	13 (4.8%)	22 (5.9%)
Infection	9 (3.3%)	15 (4.0%)
Skin and subcutaneous disorders	6 (2.2%)	9 (2.4%)
Nervous system disorders	5 (1.8%)	15 (4.0%)
Tumor recurrence	2 (0.7%)	1 (0.3%)
Musculoskeletal and connective tissue disorders	0	3 (0.8%)

[a]Data are n (%). The percentages are proportions of the total number of events rather than the total number of patients. No statistical test was available for these data because each patient could have more than one event.

Graft IRI does not only lead to local (graft) damage, but also contributes to remote injuries affecting the heart, lungs, kidneys, and intestine.[35] The post-reperfusion syndrome is reflective of cardiac complications immediately after graft reperfusion. This event occurs in 12–77% of liver transplant recipients,[36–38] with intraoperative cardiac arrest occurring in 1–3.7% recipients.[39,40] Our IFLT approach significantly reduced the incidence of post-reperfusion syndrome. Low body temperature, hepatic release of potassium and inflammatory cytokines are considered risk factors.[37] IFLT recipients did not experience low body temperature while CLT recipients did. Our previous analysis has also shown that the hepatic release of inflammatory cytokines including interleukin-1β, interleukin-6, and tumor necrosis factor-α was largely reduced in livers subjected to IFLT *vs.* CLT.[9,25] These results suggest that IFLT might protect against post-reperfusion syndrome by maintaining normal body temperature and inhibiting the release of inflammatory cytokines.

Early post-operative infections significantly contribute to morbidity and mortality among solid organ transplant

recipients.[41] The donated organ may be contaminated either by donor infections or as a consequence of the manipulation of the organ during procurement, preservation, and back-table preparation.[42–45] A multicenter study from Spain has shown that the prevalence of culture-positive preservation fluid was 62.5%.[46] The reported perfusate contamination rates were 45.6–77.8% in China.[47–49] A recent study has shown in rats that the efficiency of anti-infectious therapy is enhanced during *ex situ* hypothermic machine perfusion when compared to static cold storage.[50] In the current trial, the prevalence of culture-positive preservation fluid was substantially reduced in the IFLT *vs.* CLT group, suggesting a potential benefit of IFLT in protecting recipients from early post-operative infections.

Although both donors and recipients were considered of low risk, our approach reduced the comprehensive complication index within the first year post-transplantation. The index summarizes all post-operative complications, representing a highly clinically relevant endpoint.[23] Our data indicate that IFLT improves early transplant outcomes. Previous studies have shown that both normothermic machine perfusion and hypothermic oxygenated perfusion can efficiently support the transplantation of livers from extended criteria donors,[28,51] which are more prone to IRI than those from standard criteria donors. Normothermic machine perfusion is frequently used after a period of cold storage and grafts need to be rinsed prior to implantation.[7,8,51] Under these conditions, grafts undergo a "double hit" scenario of graft IRI. Comparable rates of non-anastomotic strictures have been seen with normothermic machine perfusion after cold storage compared to cold storage alone.[8,52] In contrast, graft IRI is largely prevented during IFLT because grafts are continuously perfused under normothermic, oxygenated conditions throughout transplantation.[25] Of note, using our IFLT approach, we have been able to successfully transplant livers with 90% macrovesicular steatosis, which are among the highest risk donor livers.[9] Multicenter, randomized trials are planned to compare the efficacy of the ischemia-free approach, normothermic machine perfusion preservation, and hypothermic oxygenated perfusion in transplantation of organs from extended criteria donors.

Our clinical trial has some limitations. Firstly, we calculated the sample size based on our previous clinical study.[12] Of note, the overall EAD incidence has declined in our center from 52.9% in 2018 to 20.9% in 2020, which explains the smaller than expected absolute risk difference of the primary endpoints between groups. Secondly, during our clinical study, the non-transportable Liver Assist was the only normothermic machine perfusion device available in China. Thus, IFLT could be conducted only when both donors and recipients were at the same institution. Transportable machine perfusion devices have recently become available and IFLT can now also be implemented when the donor and recipient are at different institutions. In addition, the technique of IFLT has been streamlined and simplified. This progress will promote wider application of the technique in the future.

In conclusion, among patients with end-stage liver disease, IFLT significantly reduced EAD and other IRI-related complications compared to CLT. Our novel approach of ischemia-free organ transplantation serves as a unique clinical model that will help to delineate the effects of IRI on transplant outcomes and organ utilization.

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]NHC key Laboratory of Assisted Circulation, Sun Yat-sen University, China; [5]State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, 510080, China; [6]Department of Anesthesiology, 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; [8]Department of Radiology, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, 510080, China; [9]Department of Pathology, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, 510080, China; [10]Department of Blood Transfusion, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, 510080, China; [11]General and Liver Transplant Surgery Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, 20100, Italy; [12]Organ Transplantation Center, The First Affiliated Hospital of the University of Science and Technology of China, Hefei, 230001, China; [13]Operating Room and Anesthesia Center, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, 510080, China; [14]Department of Cardiopulmonary Bypass, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, 510080, China; [15]Department of Surgery and Transplantation, Swiss HPB Center, University Hospital Zurich, Zurich, 8044, Switzerland; [16]Division of Transplant Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, 02115, MA, USA

Abbreviations

CLT, conventional liver transplant; EAD, early allograft dysfunction; IFLT, ischemia-free liver transplant; IRI, ischemia-reperfusion injury.

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Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Drs He and Guo had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors had access to the trial results, and reviewed and approved the final version of the manuscript for publication. X.H. served as the principal investigator for the study, contributed to study design, drafted the protocol, interpreted data and drafted the manuscript; Z.G. contributed to study design, drafted the protocol, interpreted data and drafted the manuscript; Q.Z., C.H. and Z.J. drafted the protocol, enrolled patients, analyzed and interpreted data, and drafted the manuscript; S.H. drafted the protocol, enrolled patients and collected data; D.W., W.J., M.C., X.Z., A.H., Yi Ma, L.W., Y.C., M.H., Y.T., G.W., T.W., Z.C., J.G. and J.Y. enrolled the patients and conducted the operations; L.W., Z.Zhang, C.Z., Y.G., H.C., Yihao Ma, Chengjun S. and T.Z. served as perfusionists and collected the perfusion data; L.Y., L.L., W.X., Y.S., Z.T., X.C., X.H., S.H., J.Ren, Z.Zhou, C.C., F.G., J.Rong, W.H. and X.G. contributed to the protocol drafting; P.Z., S.L., Y.L., Y.D., Canhui S., C.L., H.T. and B.L. collected the clinical data; J.Z. analyzed and interpreted the data; J.H., S.A., N.B., P.A.C. and T.G.S. participated in paper writing and editing.

Data availability statement

Data can be made available to researchers upon reasonable request.

Role of the funder/sponsor

The funders/sponsors had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

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Supplementary data

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References

Author names in bold designate shared co-first authorship.

[1] Morris PJ. Transplantation–a medical miracle of the 20th century. New Engl J Med 2004;351:2678–2680.
[2] Eltzschig HK, Eckle T. Ischemia and reperfusion–from mechanism to translation. Nat Med 2011;17:1391–1401.
[3] de Rougemont O, Dutkowski P, Clavien P-A. Biological modulation of liver ischemia-reperfusion injury. Curr Opin Organ Transplant 2010;15:183–189.
[4] de Meijer VE, Fujiyoshi M, Porte RJ. Ex situ machine perfusion strategies in liver transplantation. J Hepatol 2019;70:203–205.
[5] Guarrera JV, Henry SD, Samstein B, Odeh-Ramadan R, Kinkhabwala M, Goldstein MJ, et al. Hypothermic machine preservation in human liver transplantation: the first clinical series. Am J Transplant 2010;10:372–381.
[6] van Rijn R, Schurink IJ, de Vries Y, van den Berg AP, Cortes Cerisuelo M, Darwish Murad S, et al. Hypothermic machine perfusion in liver transplantation - a randomized trial. New Engl J Med 2021;384:1391–1401.
[7] Markmann JF, Abouljoud MS, Ghobrial RM, Bhati CS, Pelletier SJ, Lu AD, et al. Impact of portable normothermic blood-based machine perfusion on outcomes of liver transplant: the OCS liver PROTECT randomized clinical trial. JAMA Surg 2022;157:189–198.
[8] Nasralla D, Coussios CC, Mergental H, Akhtar MZ, Butler AJ, Ceresa CDL, et al. A randomized trial of normothermic preservation in liver transplantation. Nature 2018;557:50–56.
[9] **He X**, **Guo Z**, **Zhao Q**, Ju W, Wang D, Wu L, et al. The first case of ischemia-free organ transplantation in humans: a proof of concept. Am J Transplant 2018;18:737–744.
[10] **He X**, **Chen G**, **Zhu Z**, Zhang Z, Yuan X, Han M, et al. The first case of ischemia-free kidney transplantation in humans. Front In Med 2019;6:276.
[11] **Yin S**, **Rong J**, **Chen Y**, Cao L, Liu Y, Mo S, et al. Transplantation of a beating heart: a first in man. Lancet Reg Health West Pac 2022;23:100449.
[12] **Guo Z**, **Zhao Q**, **Huang S**, **Huang C**, Wang D, Yang L, et al. Ischaemia-free liver transplantation in humans: a first-in-human trial. Lancet Reg Health West Pac 2021;16:100260.
[13] **Huang C**, **Huang S**, **Tang Y**, Zhao Q, Wang D, Ju W, et al. Prospective, single-centre, randomised controlled trial to evaluate the efficacy and safety of ischaemia-free liver transplantation (IFLT) in the treatment of end-stage liver disease. BMJ open 2020;10:e035374.
[14] Yao FY, Ferrell L, Bass NM, Watson JJ, Bacchetti P, Venook A, et al. Liver transplantation for hepatocellular carcinoma: Expansion of the tumor size limits does not adversely impact survival. Hepatology 2001;33:1394–1403.
[15] Nalesnik MA, Woodle ES, Dimaio JM, Vasudev B, Teperman LW, Covington S, et al. Donor-transmitted malignancies in organ transplantation: assessment of clinical risk. Am J Transplant 2011;11:1140–1147.
[16] Laing RW, Mergental H, Yap C, Kirkham A, Whilku M, Barton D, 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:e017733.
[17] Olthoff KM, Kulik L, Samstein B, Kaminski M, Abecassis M, Emond J, et al. Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. Liver Transplant 2010;16:943–949.
[18] Johnson SR, Alexopoulos S, Curry M, Hanto DW. Primary nonfunction (PNF) in the MELD Era: an SRTR database analysis. Am J Transplant 2007;7:1003–1009.
[19] Aggarwal S, Kang Y, Freeman JA, Fortunato FL, Pinsky MR. Postreperfusion syndrome: cardiovascular collapse following hepatic reperfusion during liver transplantation. Transplant Proc 1987;19:54–55.
[20] **van Leeuwen OB**, **de Vries Y**, Fujiyoshi M, Nijsten MWN, Ubbink R, Pelgrim GJ, et al. Transplantation of high-risk donor livers after ex situ Resuscitation and assessment using combined Hypo- and normothermic machine perfusion: a prospective clinical trial. Ann Surg 2019;270:906–914.
[21] Valls C, Alba E, Cruz M, Figueras J, Andía E, Sanchez A, et al. Biliary complications after liver transplantation: diagnosis with MR cholangiopancreatography. AJR: Am J Roentgenol 2005;184:812–820.
[22] Buis CI, Verdonk RC, Van der Jagt EJ, van der Hilst CS, Slooff MJH, Haagsma EB, et al. Nonanastomotic biliary strictures after liver transplantation, part 1: radiological features and risk factors for early vs. late presentation. Liver Transplant 2007;13:708–718.
[23] Slankamenac K, Graf R, Barkun J, Puhan MA, Clavien P-A. The comprehensive complication index: a novel continuous scale to measure surgical morbidity. Ann Surg 2013;258:1–7.
[24] Oriol I, Sabé N, Tebé C, Veroux M, Boin IFSF, Carratalà J. Clinical impact of culture-positive preservation fluid on solid organ transplantation: a systematic review and meta-analysis. Transplant Rev (Orlando) 2018;32:85–91.
[25] **Guo Z**, **Xu J**, **Huang S**, **Yin M**, Zhao Q, Ju W, et al. Abrogation of graft ischemia-reperfusion injury in ischemia-free liver transplantation. Clin Translational Med 2022;12:e546.
[26] Martins PN, Rizzari MD, Ghinolfi D, Jochmans I, Attia M, Jalan R, et al. Design, analysis, and pitfalls of clinical trials using ex situ liver machine perfusion: the international liver transplantation Society consensus guidelines. Transplantation 2021;105:796–815.
[27] **Chen S**, **Wang T**, **Luo T**, He S, Huang C, Jia Z, et al. Prediction of graft survival post-liver transplantation by L-GrAFT risk score model, EASE score, MEAF scoring, and EAD. Front Surg 2021;8:753056.
[28] Czigany Z, Pratschke J, Froněk J, Guba M, Schöning W, Raptis DA, et al. Hypothermic oxygenated machine perfusion reduces early allograft injury and improves post-transplant outcomes in extended criteria donation liver transplantation from donation after brain death: results from a multicenter randomized controlled trial (HOPE ECD-DBD). Ann Surg 2021;274:705–712.
[29] de Vries Y, von Meijenfeldt FA, Porte RJ. Post-transplant cholangiopathy: classification, pathogenesis, and preventive strategies. Biochim Biophys Acta Mol Basis Dis 2018;1864:1507–1515.
[30] Gilbo N, Jochmans I, Sainz M, Pirenne J, Meurisse N, Monbaliu D. Reducing non-anastomotic biliary strictures in donation after circulatory death liver transplantation: cold ischemia time matters! Ann Surg 2017;266:e118–e119.
[31] Dubbeld J, Hoekstra H, Farid W, Ringers J, Porte RJ, Metselaar HJ, et al. Similar liver transplantation survival with selected cardiac death donors and brain death donors. Br J Surg 2010;97:744–753.
[32] Croome KP, McAlister V, Adams P, Marotta P, Wall W, Hernandez-Alejandro R. Endoscopic management of biliary complications following liver transplantation after donation from cardiac death donors. Can J Gastroenterol 2012;26:607–610.
[33] Abt P, Crawford M, Desai N, Markmann J, Olthoff K, Shaked A. Liver transplantation from controlled non-heart-beating donors: an increased incidence of biliary complications. Transplantation 2003;75:1659–1663.
[34] Meurisse N, Vanden Bussche S, Jochmans I, Francois J, Desschans B, Laleman W, et al. Outcomes of liver transplantations using donations after circulatory death: a single-center experience. Transplant Proc 2012;44:2868–2873.
[35] Nastos C, Kalimeris K, Papoutsidakis N, Tasoulis M-K, Lykoudis PM, Theodoraki K, et al. Global consequences of liver ischemia/reperfusion injury. Oxidative Med Cell longevity 2014;2014:906965.
[36] Ryu H-G, Jung C-W, Lee H-C, Cho Y-J. Epinephrine and phenylephrine pretreatments for preventing postreperfusion syndrome during adult liver transplantation. Liver Transplant 2012;18:1430–1439.
[37] Chui AK, Shi L, Tanaka K, Rao AR, Wang LS, Bookallil M, et al. Post-reperfusion syndrome in orthotopic liver transplantation. Transplant Proc 2000;32:2116–2117.
[38] Bukowicka B, Akar RA, Olszewska A, Smoter P, Krawczyk M. The occurrence of postreperfusion syndrome in orthotopic liver transplantation and its significance in terms of complications and short-term survival. Ann Transplant 2011;16:26–30.
[39] Aufhauser DD, Rose T, Levine M, Barnett R, Ochroch EA, Aukburg S, et al. Cardiac arrest associated with reperfusion of the liver during transplantation: incidence and proposal for a management algorithm. Clin Transplant 2013;27:185–192.

[40] Smith NK, Zerillo J, Kim SJ, Efune GE, Wang C, Pai S-L, et al. Intraoperative cardiac arrest during adult liver transplantation: incidence and risk factor analysis from 7 academic centers in the United States. Anesth analgesia 2021;132:130–139.
[41] Dorschner P, McElroy LM, Ison MG. Nosocomial infections within the first month of solid organ transplantation. Transpl Infect Dis 2014;16:171–187.
[42] Wakelin SJ, Casey J, Robertson A, Friend P, Jaques BC, Yorke H, et al. The incidence and importance of bacterial contaminants of cadaveric renal perfusion fluid. Transpl Int 2005;17:680–686.
[43] Grąt M, Ligocka J, Lewandowski Z, Barski K, Hołówko W, Skalski M, et al. Incidence, pattern and clinical relevance of microbial contamination of preservation fluid in liver transplantation. Ann Transpl 2012;17:20–28.
[44] Yansouni CP, Dendukuri N, Liu G, Fernandez M, Frenette C, Paraskevas S, et al. Positive cultures of organ preservation fluid predict postoperative infections in solid organ transplantation recipients. Infect Control Hosp Epidemiol 2012;33:672–680.
[45] Oriol I, Lladó L, Vila M, Baliellas C, Tubau F, Sabé N, et al. The Etiology, incidence, and impact of preservation fluid contamination during liver transplantation. PLoS One 2016;11:e0160701.
[46] Oriol I, Sabe N, Càmara J, Berbel D, Ballesteros MA, Escudero R, et al. The impact of culturing the organ preservation fluid on solid organ transplantation: a prospective multicenter cohort study. Open Forum Infect Dis 2019;6:ofz180.
[47] Yu X, Wang R, Peng W, Huang H, Liu G, Yang Q, et al. Incidence, distribution and clinical relevance of microbial contamination of preservation solution in deceased kidney transplant recipients: a retrospective cohort study from China. Clin Microbiol Infect 2019;25:595–600.
[48] Liu J, Xie Z, Li L, Lv H, An Y, Yi X, et al. Relationship between pathogens in organ preservation solution and infection of liver transplant recipients. Chin J Hepat Surg 2022;11:273–277.
[49] Chen Z, Zhang Y, Xue C, Tan J, Ma X, Jiang Q, et al. Positive rate, pathogen distribution and clinical significance of organ preservation solution culture. Chin J Inlect Control 2021;20:832–837.
[50] **Liang H**, **Zhang P**, Yu B, Liu Z, Pan L, He X, et al. Machine perfusion combined with antibiotics prevents donor-derived infections caused by multidrug-resistant bacteria. Am J Transplant 2022;22:1791–1803.
[51] **Mergental H**, **Laing RW**, Kirkham AJ, Perera MTPR, Boteon YL, Attard J, et al. Transplantation of discarded livers following viability testing with normothermic machine perfusion. Nat Commun 2020;11:2939.
[52] Gaurav R, Butler AJ, Kosmoliaptsis V, Mumford L, Fear C, Swift L, et al. Liver Transplantation Outcomes from Controlled Circulatory Death Donors: static cold storage *vs.* in situ normothermic regional perfusion *vs.* ex situ normothermic machine perfusion. Ann Surg 2022;275:1156–1164.

Journal of Hepatology, Volume *79*

Supplemental information

A randomized-controlled trial of ischemia-free liver transplantation for end-stage liver disease

Zhiyong Guo, Qiang Zhao, Zehua Jia, Changjun Huang, Dongping Wang, Weiqiang Ju, Jian Zhang, Lu Yang, Shanzhou Huang, Maogen Chen, Xiaofeng Zhu, Anbin Hu, Yi Ma, Linwei Wu, Yinghua Chen, Ming Han, Yunhua Tang, Guodong Wang, Linhe Wang, Lifen Li, Wei Xiong, Zhiheng Zhang, Yuekun Shen, Zhaoxia Tang, Caihui Zhu, Xiaoxiang Chen, Xiaoguang Hu, Yiwen Guo, Honghui Chen, Yihao Ma, Tao Zhang, Shunwei Huang, Ping Zeng, Simei Lai, Tielong Wang, Zhitao Chen, Jinlong Gong, Jia Yu, Canhui Sun, Chang Li, Haiyi Tan, Yao Liu, Yuqi Dong, Chengjun Sun, Bing Liao, Jun Ren, Zhenhai Zhou, Schlegel Andrea, Nashan Björn, Changjie Cai, Fengqiu Gong, Jian Rong, Wenqi Huang, Xiangdong Guan, Pierre-Alain Clavien, Tullius G. Stefan, Jiefu Huang, and Xiaoshun He

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Supplementary appendix. Immunosuppression Protocol

The immunosuppression therapy was divided into induction and maintenance phases as follows:

- Induction phase: Basiliximab (20mg) was administered intravenously during the operation and at post-operative day (POD) 4.
- Maintenance phase: The maintenance therapy began at POD 4. The mainstay of maintenance therapy are tacrolimus and mycophenolic acid. 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.

Fig. S1. Represented Photos of 32 Perfused Livers in the IFLT Group.

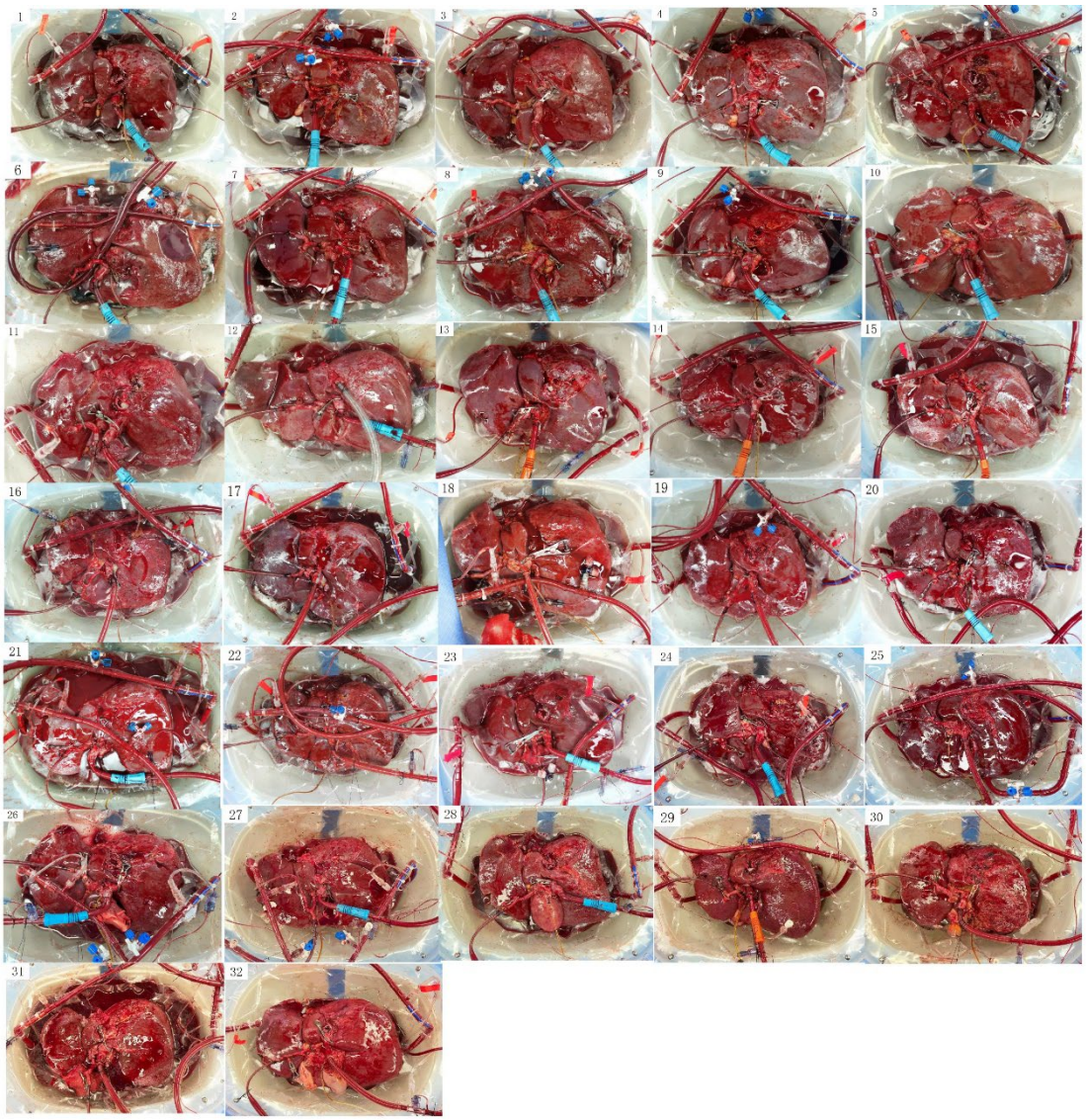
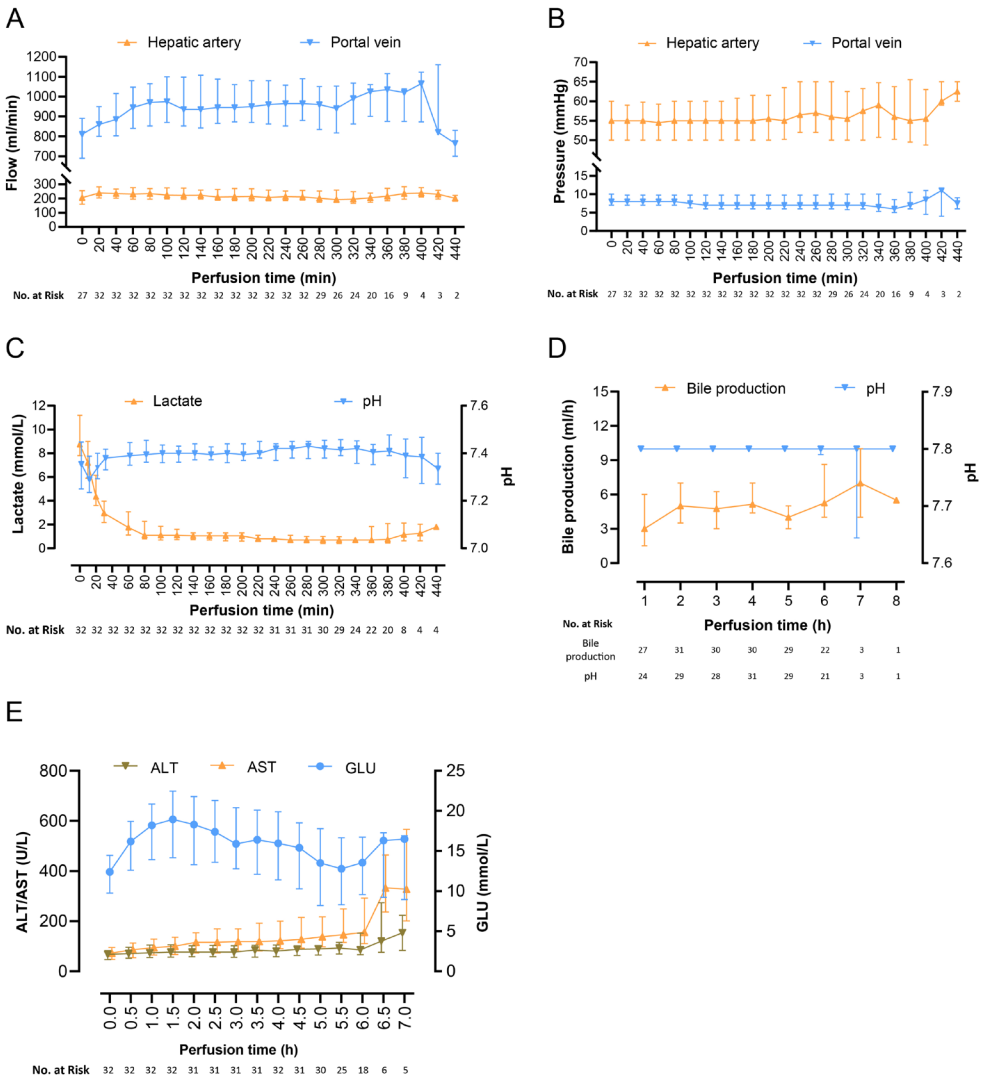
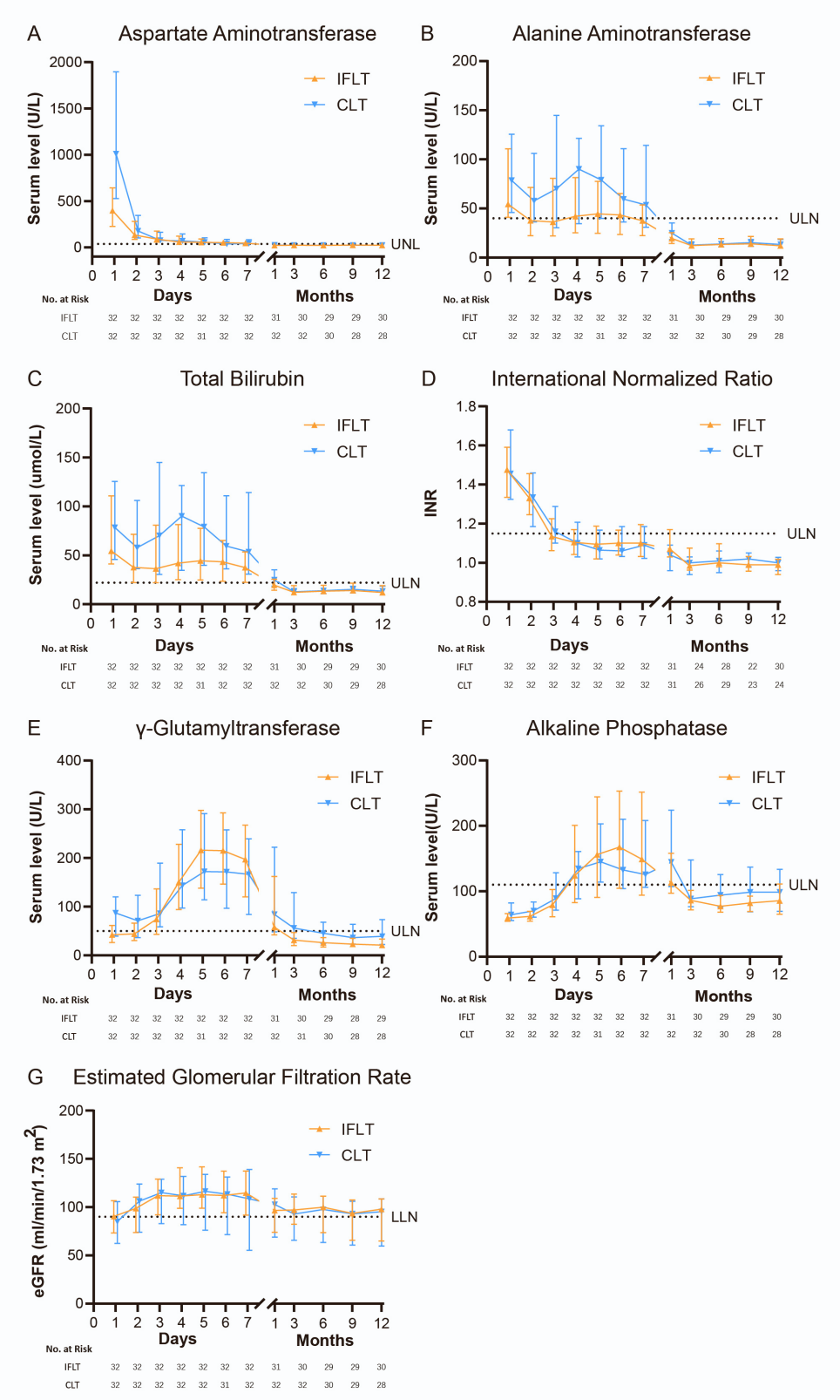


Fig. S2. Parameters of Normothermic Machine Perfusion in the IFLT Group.



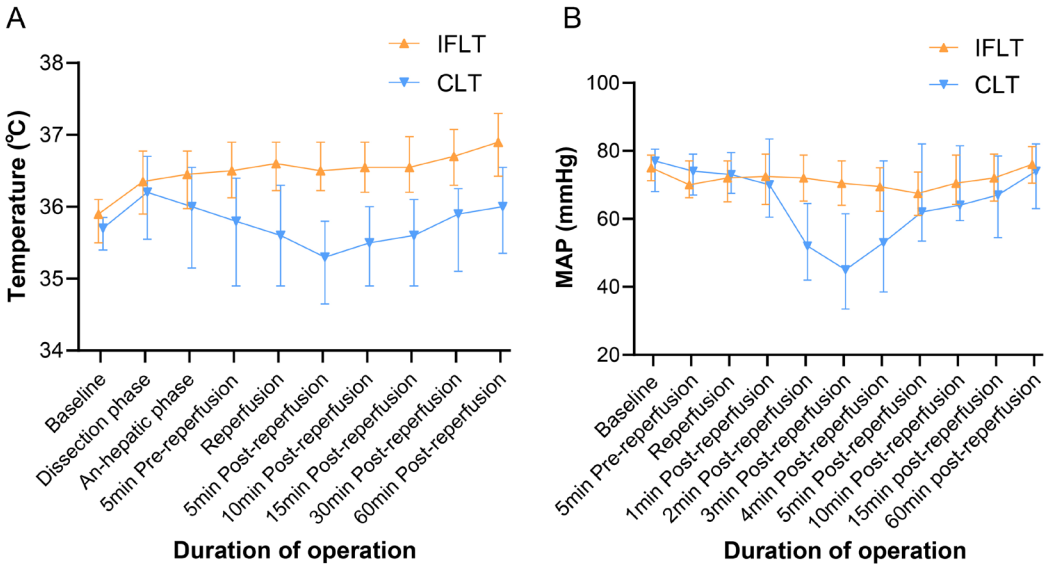
A, Flow of hepatic artery and portal vein during normothermic machine perfusion. **B**, Pressure of hepatic artery and portal vein during normothermic machine perfusion. **C**, Lactate and pH of perfusate by blood gas analysis during normothermic machine perfusion. **D**, Production and pH of bile during normothermic machine perfusion. **E**, Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and glucose (GLU) of perfusate during normothermic machine perfusion. **A-E**, All data are presented as median and interquartile range.

Fig. S3. Laboratory Analyses of Liver, Coagulation and Kidney Function after Transplantation.



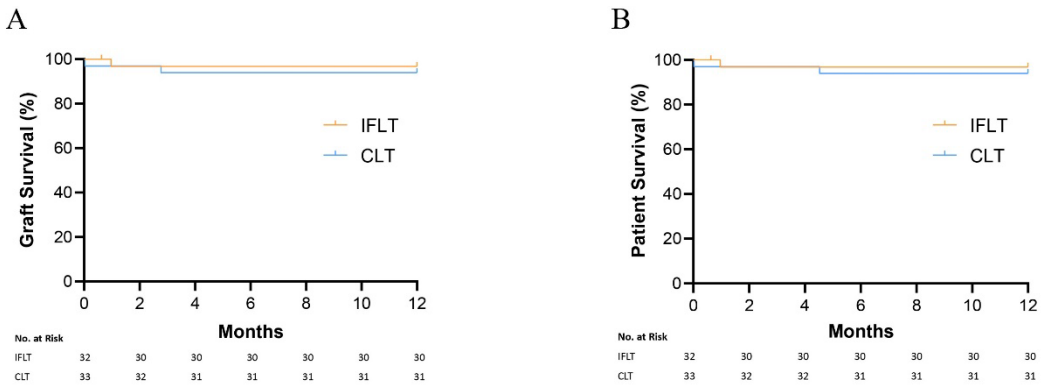
Data are presented as median and interquartile range. ULN, upper limit of normal. LLN, lower limit of normal.

Fig. S4. Body Temperature and Mean Arterial Pressure (MAP) of Recipients During IFLT and CLT.



Data are presented as median and interquartile range.

Fig. S5. Kaplan–Meier Curves of Graft/Patient Survival after Transplantation.



A, Kaplan-Meier curve of graft survival. *P* value = 0.589 (Log-rank test). B, Kaplan-Meier curve of patient survival. *P* value = 0.589 (Log-rank test).

Table S1. Standard Composition of the Perfusion Solution Used for Normothermic Machine Perfusion.

Components	**Quantity**
The washed red blood cells	10 U (2000 ml)
4% Succinylated gelatin injection	1200 ml
5% Sodium bicarbonate	100-150 ml
Heparin sodium injection (2mL:12500U)	37500 U
Calcium glubionate, intravenous solution 10ml:1g	40 ml
Magnesium sulfate injection (10ml:2.5g)	4 ml
Solu Medrol (Methylprednisolone sodium succinate for injection)	500 mg
Andamax (multi-trace elements injection)	10 ml
Metronidazole injection (100ml:0.5g)	100 ml
Imipenem and cilastatin sodium for injection	1g
Amino acid injection (250ml:12.5g)	250 ml

Specific quantity of each composition might be adjusted slightly according to available quantity of washed red blood cells before each machine perfusion

Table S2. Non-hepatic Organ Utilization in the Two Groups.

Transplanted donor organs	IFLT group (*n* = 32)	CLT group (*n* = 33)	*P* value
Left kidney	28 (87.5%)	30 (90.9%)	0.524
Right kidney	29 (90.63%)	29 (87.88%)	0.518
Heart	13 (40.63%)	16 (48.48%)	0.423
Lung	9(28.13%)	7 (21.21%)	0.721
Pancreas	0	1 (3.03%)	NA[a]
Corneal	23 (71.88%)	24 (72.73%)	0.934

Data are n (%). *P* values were calculated by chi-square tests. IFLT, ischemia-free liver transplantation; CLT, conventional liver transplantation.

[a] Because of absence of event in one group, *P* value was not assessed (NA).

Table S3. Summary of Liver Procedures.

Characteristics	IFLT (*n* = 32)	CLT (*n* = 33)	*P* value
Organ procurement duration, h	4.4 (4.0-5.2)	1.0 (0.8-1.1)	<0.001
Recipient operation duration, h	6.8 (6.2-7.7)	7.0 (6.0-8.5)	0.479
Implantation technique			0.265[a]
Bicaval	14 (44%)	19 (58%)	
Piggyback	18 (56%)	14 (42%)	
Anhepatic phase, min[b]	56 (46-65)	44 (40-48)	<0.001
Blood loss of recipient operation, ml	2015 (1635-2790)	2320 (1550-3000)	0.655
Blood transfusion of recipient operation			
Erythrocyte, ml	1300 (800-2100)	1900 (800-2200)	0.349
Plasma, ml	1550 (1200-1675)	1600 (1400-2150)	0.137
Platelet, ml	250 (0-250)	250 (0-250)	0.693

Data are n (%) or median (IQR). *P* values were calculated using Mann-Whitney test for continuous data.

[a] *P* value was calculated using chi-square test.

[b] Anhepatic phase was defined as time between portal vein occlusion to portal vein reopening in the recipient.

Table S4. Liver Functions Tests of Patients with Early Allograft Dysfunction (EAD).

Recipients	Peak AST (IU/L) within 7 days	Peak ALT (IU/L) within 7 days	INR on Day 7	Tbil on Day 7 (mg/dL)
IFLT-04	418	93	1.15	11.64
IFLT-31	734	257	1.69	19.43
CLT-06	4260	1161	1.11	15.44
CLT-08	2845	1330	0.88	2.20
CLT-09[a]	NA	NA	NA	NA
CLT-10	2372	346	0.92	0.99
CLT-24	4350	2183	1.10	1.94
CLT-27	1986	1312	1.77	10.14
CLT-28	5128	674	1.32	8.56
CLT-34	2665	618	1.21	3.65

ALT, alanine aminotransferase; AST, aspartate aminotransferase; INR, international normalized ratio; Tbil, total bilirubin.

[a]The patient was diagnosed with primary nonfunction (PNF) and passed away without any postoperative lab test results (NA). PNF was also classified as a form of EAD.[1,2]

Table S5. Image Characteristics of the Non-anastomotic Biliary Strictures with Protocol MRCP at POM6.

Group with Number	Characteristics	Classification
CLT-02	Stenosis in the right hepatic duct	Mild
CLT-05	Stenosis at the junction of the left and right hepatic duct	Mild
IFLT-06	Stenosis in the perihilar bile duct with dilatation in the left hepatic duct	Moderate
CLT-10	Stenosis in the right hepatic duct	Mild
CLT-15	Stenosis in the left hepatic duct	Mild
CLT-18	Stenosis at the junction of the left and right hepatic duct with dilatation in the right hepatic duct	Moderate
CLT-29	Stenosis in the right hepatic duct with proximal biliary dilatation	Moderate
IFLT-31	Stenosis in the right hepatic duct	Mild
CLT-32	Stenosis in the right hepatic duct with intrahepatic and extrahepatic biliary dilatation.	Moderate
CLT-34	Stenosis in the perihilar bile duct with intrahepatic biliary dilatation.	Moderate

Non-anastomotic biliary strictures were diagnosed by two experienced radiologists blindly according to protocol 6-month magnetic resonance cholangiopancreatography (MRCP) image.[3] The classification of non-anastomotic stricture depends on severity, site, and number of lesions.[4]

Table S6. Reasons for Missing Protocol Magnetic Resonance Cholangiopancreatography (MRCP) at POM 6 or POM 12

Reasons	IFLT	CLT	Total number[a]
Patient death prior to scheduled MRCP	2	4	6
Patient loss to follow-up	2	0	2
Covid-19 prevention and control policies	3	4	7
MRCP contradiction	0	2	2
Metastasized hepatocellular carcinoma	0	1	1
Follow-up wasn't at designated hospital	3	0	3
Patient rejected performing MRCP	0	1	1
Total	10	12	22

POM, postoperative month; Covid-19, coronavirus disease 2019.

[a] Each patient would have two times MRCP according to the protocol. The missing event number were displayed here.

Table S7. Comparison of Cholestatic Laboratory Tests in Patients with or without Non-anastomotic Biliary Strictures at POM 6 and POM 12.

Laboratory tests	**Radiological NAS**				***P* value**
	No		Yes		
POM 6	No. at risk/Missing	Median (IQR)	No. at risk/Missing	Median (IQR)	
Alkaline phosphatase (IU/L)	47/0	81 (70-98)	10/0	110 (80-166)	0.031
γ-Glutamyltransferase (IU/L)	47/0	31 (19-45)	10/0	64 (31-112)	0.039
Bilirubin (umol/L)[a]	47/0	14 (11-19)	10/0	14 (11-16)	0.975
POM 12					
Alkaline phosphatase (IU/L)	47/3	86 (67-109)	10/0	110 (96-138)	0.037
γ-Glutamyltransferase (IU/L)	47/3	24 (18-49)	10/0	57.5 (21-105)	0.093
Bilirubin (umol/L)[a]	47/3	12 (10-16)	10/0	17 (12-21)	0.072

P values were calculated by the Mann-Whitney test.

[a] To convert results for serum bilirubin from umol/L to mg/dL, divide results by 17.1.

Table S8. Additional End Points.

Outcome	**IFLT(*n* = 32)**	**CLT(*n* = 33)**	**Absolute difference (95% CI)**	***P* value**
Acute rejection				
POM 1	1 (3%)	2 (6%)	-0.03 (-0.13 to 0.07)	0.554
POM 6	2 (6%)	4 (13%)	-0.06 (-0.20 to 0.08)	0.391
POM 12	2 (6%)	4 (13%)	-0.06 (-0.20 to 0.08)	0.391
Vascular complications[a]	0	2 (6%)	NA	NA
Infection within POD 30	3 (9%)	6 (19%)	-0.09 (-0.26 to 0.08)	0.281
Acute kidney injury within POD 7[b]	5 (16%)	9 (28%)	-0.13 (-0.33 to 0.08)	0.227
Renal replacement therapy	0	2 (6%)	NA	NA

Data are n (%) or n/N (%), unless otherwise specified. Because of absence of events in one group, some treatment differences and p values were not assessed (NA). Treatment effect is presented by mean difference or percentage difference with 95% confidence interval (CI) without adjustment for any confounding factors.

[a] Vascular complications only included thrombosis, hemorrhage, embolism or stenosis of inferior vena cava, portal vein or hepatic artery.

[b] Acute kidney injury was diagnosed according to KDIGO criteria.[5]

Table S9. Serious Adverse Events.

Recipients	Events	Time from transplantation to first sign of events (days)	Duration of events (days)	Outcomes of events
IFLT-21	Upper gastrointestinal bleeding	30	7	Recovery
IFLT-22	Abdominal bleeding	6	< 1	Recovery
IFLT-34	Intracranial hemorrhage	28	1	Death
CLT-09	Primary graft non-function	< 1	< 1	Death
CLT-22	Incisional hernia	352	3	Recovery
CLT-27	Cerebral edema with acute kidney injury	2	8	Recovery
CLT-30	Liver failure	83	53	Death
CLT-34	Acute kidney injury	< 1	9	Recovery

Table S10. Grading of Adverse Events in Each Group

Clavien-Dindo classification[6]	IFLT (*n* = 32)	CLT (*n* = 33)	*P* value
Grade 1	171(32)	182(32)	0.99
Grade 2	77(27)	157(33)	0.19
Grade 3a	21(14)	30(19)	0.26
Grade 3b	2(2)	1(1)	0.98
Grade 4a	0	3(2)	NA[a]
Grade 4b	0	0	NA[a]
Grade 5	1(1)	2(2)	0.98

Data are presented as number of complications (number of patients with a complication). *P* values referred to comparison of numbers of patients and were calculated by chi-square tests.

[a] Because of absence of events in one group at least, *P* values were not assessed (NA).

References

1. Huang C, Huang S, Tang Y, et al. Prospective, single-centre, randomised controlled trial to evaluate the efficacy and safety of ischaemia-free liver transplantation (IFLT) in the treatment of end-stage liver disease. *BMJ Open.* 2020;10(5):e035374.
2. 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):943-949.
3. Valls C, Alba E, Cruz M, et al. Biliary complications after liver transplantation: diagnosis with MR cholangiopancreatography. *AJR Am J Roentgenol.* 2005;184(3):812-820.
4. Buis CI, Verdonk RC, Van der Jagt EJ, et al. Nonanastomotic biliary strictures after liver transplantation, part 1: Radiological features and risk factors for early vs. late presentation. *Liver Transpl.* 2007;13(5):708-718.
5. Khwaja A. KDIGO clinical practice guidelines for acute kidney injury. *Nephron Clin Pract.* 2012;120(4):c179-c184.
6. Dindo D, Demartines N, Clavien P-A. 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):205-213.