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Short recipient warm ischemia time improves outcomes in deceased donor liver transplantation

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ORIGINAL ARTICLE

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SUMMARY

While adverse effects of prolonged recipient warm ischemia time (rWIT) in liver transplantation (LT) have been well investigated, few studies have focused on possible positive prognostic effects of short rWIT. We aim to investigate if shortening rWIT can further improve outcomes in donation after brain death liver transplant (DBD-LT). Primary DBD-LT between 2000 and 2019 were retrospectively reviewed. Patients were divided according to rWIT (≤30, 31–40, 41–50, and >50 min). The requirement of intraoperative transfusion, early allograft dysfunction (EAD), and graft survival were compared between the rWIT groups. A total of 1,256 patients of DBD-LTs were eligible. rWIT was ≤30min in 203 patients (15.7%), 31–40min in 465 patients (37.3%), 41–50min in 353 patients (28.1%), and >50min in 240 patients (19.1%). There were significant increasing trends of transfusion requirement ($P < 0.001$) and increased estimated blood loss (EBL, $P < 0.001$), and higher lactate level ($P < 0.001$) with prolongation of rWIT. Multivariable logistic regression demonstrated the lowest risk of EAD in the WIT ≤30min group. After risk adjustment, patients with rWIT ≤30 min showed a significantly lower risk of graft loss at 1 and 5-years, compared to other groups. The positive prognostic impact of rWIT ≤30min was more prominent when cold ischemia time exceeded 6 h. In conclusion, shorter rWIT in DBD-LT provided significantly better post-transplant outcomes.

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Key words
cold ischemia time, complications, early allograft dysfunction, implantation, survival

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Introduction

In solid organ transplantation, ischemia-reperfusion injury of the graft is inevitable. Several studies have investigated the effects of ischemia damage on graft function and outcomes in liver transplantation (LT) and sought to identify the medical and surgical strategies to mitigate the adverse impact of such damage [1]. In LT, graft ischemia times comprise cold ischemia time (CIT) and warm ischemia time (WIT). Cold ischemia time is defined as the time between donor cross-clamping (and flushing), and the removal of the liver from cold preservation solution immediately before implantation. In donation after brain death donor (DBD) LTs, the only WIT is recipient WIT (rWIT), which is defined as the time between the removal of the liver from cold preservation solution to portal reperfusion. On the other hand, in donation after circulatory death donor (DCD) LTs, WIT occurs both in donor and recipient surgeries. WIT in DCD donor surgery is

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typically defined as the time between donor extubation and cross-clamping [2]. A variety of strategies, such as normothermic regional perfusion of the liver graft for DCD donors, have been proposed and utilized to alleviate the negative effects of donor WIT. However, rWIT, essentially equivalent to "liver graft implantation time", might be considered "fixed-time". In other words, it would not allow for much improvement given that modern LT surgical techniques are already generally well sophisticated.

Warm ischemia of the liver graft is more deleterious to hepatocytes and high energy demands begin at 20^{O}C of the liver core temperature [3]. It has been well reported that prolonged rWIT is associated with poor LT outcomes [4–6]. Rana et al. reported that rWIT of 60 min or longer, compared to less than 60 min, was significantly associated with worse 3–months patient survival [7]. Typically, rWIT ranges between 30–50 min, which is usually considered as an "acceptable length" of rWIT [6–9]. However, it has not been well studied whether keeping rWIT shorter than the acceptable time, more specifically within 30 min, could lead to further improvement in LT outcomes.

In this study, we focused on the prognostic impact of rWIT in DBD-LT and hypothesized that further improvement of outcomes can be achieved by shortening rWIT liver graft implantation time in DBD-LT. This study aimed to assess the prognostic impact of rWIT on DBD-LT outcomes by primarily focusing on possible positive prognostic effects of short rWIT.

Methods

Study population

Our institution's prospective maintained transplant surgery database was retrospectively queried to identify patients who underwent LT between January 2000 and September 2019. Patients undergoing primary LT from DBD donors were included. Exclusion criteria included transplantation from DCD donors, patients undergoing retransplantation, simultaneous multi-organ transplantation, and living donor LT. Our Institutional Review Board approved the investigation.

Surgical management

Donor livers were flushed in situ with histidine-tryptophan-ketoglutarate (HTK) or University of Wisconsin (UW) solution, and the portal flush was added on the back table. Note that the preferred preservation solution at our center and transplant region is HTK; however, we do occasionally receive livers imported from regions that utilize a UW flush. The hepatic venous outflow reconstruction selected during the LT depended on intraoperative factors and surgeon preference, and was either piggyback or bicaval technique. Portal reperfusion was performed prior to arterial reperfusion in all cases. CIT was defined as the time between donor cross-clamping and the removal of the liver from cold preservation solution immediately before implantation. rWIT was defined as the time between removing the liver from cold preservation solution to portal reperfusion (namely, the time required to complete caval and portal venous anastomosis). This was followed by the completion of the hepatic arterial anastomosis.

Post-transplant management

All patients were transferred postoperatively to the surgical intensive care unit, followed by transfer to the Transplant Surgery unit, and regular clinical and laboratory follow-up occurred. Postoperative maintenance immunosuppression with tacrolimus, mycophenolate mofetil, and steroids was utilized.

Analysis of post-transplant outcomes

The patients were categorized according to rWIT: ≤ 30, 31–40, 41–50, and >50 min. Primary outcomes evaluated were graft and patient survival, which were compared among the rWIT subcategories. The hazards of graft loss were adjusted for the following variables. Recipient variables included age, gender, ethnicity, body mass index (BMI), diabetes, Karnofsky Score, etiology of liver disease, model for end-stage liver disease (MELD) score at transplant, cold ischemia time (CIT), dialysis requirement, presence of ascites and hepatic encephalopathy, hepatic vein reconstruction techniques (piggyback vs. bicaval), and the era of transplantation (2000–2004, 2005–2010, 2011–2014, 2015–2019). Donor variables included age, gender, and BMI. The effects of rWIT on graft survival were further evaluated after stratification by CIT (≤6 and >6 hours).

The secondary outcomes assessed were intraoperative transfusion requirement and post-transplant early allograft dysfunction (EAD)[10]. Our institutional database includes EAD data for all LTs performed after January 1, 2012. The hazards of EAD were adjusted for the following recipient and donor characteristics: age, gender, ethnicity, body mass index (BMI), diabetes, Karnofsky

Score, dialysis requirement, presence of ascites and hepatic encephalopathy, era of transplantation, and donor risk index (DRI)[11].

To investigate the direct and indirect effects of rWIT on the occurrence of EAD and graft loss, mediation analysis were conducted. The exposure variable (rWIT) can have a direct effect on outcomes such as EAD and graft loss. Intraoperative blood loss may be a risk factor for EAD and/or graft loss as well. The association between rWIT and endpoints (EAD, graft loss) might be mediated by intraoperative blood loss. The direct and indirect effects of rWIT on the endpoints were assessed using causal mediation analysis. The total amount of packed red blood cell transfusion and autologous transfusion during LT surgery was used as a substitute for intraoperative blood loss.

Statistical analysis

Descriptive data for continuous variables were reported as mean (±standard deviation) or median with interquartile range and compared using the student's *t*-test and Kruskal–Wallis test. Categorical variables were reported as numbers and percentages and were compared using chi-square. Trends of values according to rWIT were assessed using the Jonckheere-Terpstra test. Graft and patient survival were analyzed using the Kaplan-Meier method, and the subgroups compared using log-rank tests. Trends of survival according to rWIT were checked using the log-rank trend test. Cox proportional hazard regression models were constructed to identify predictors for post- LT graft loss. Similarly, predictors of EAD were evaluated using a logistic regression model. Regression with a backward model selection was considered to determine risk factors for graft loss and the occurrence of EAD. Mediation analysis was performed using PROCESS v3.4.1 created by Preacher and Hayes. A *P*-value <0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 25 (IBM Corp., Armonk, NY).

Results

During the study period, 1,815 LTs were performed, of which 1,290 patients met the inclusion criteria. rWIT data were available for 1,261 patients. This included 203 patients (15.7%) with a rWIT ≤ 30 min, 465 patients (36%) with a rWIT 31–40 min, 353 patients (27.4%) with a rWIT 41–50 min, and 240 patients (18.6%) with a rWIT >50 min. Mean rWIT was 48.6 min in the years 2000–2004, 44.2 min in 2005–2010, 38.0 min in 2011–2014, and 38.2 min in 2015–2019. The mean rWIT was 45.4 min in LTs performed before 2011 and 37.9 min in LTs performed after January 1, 2011 ($P < 0.001$). Baseline recipient, donor, and intraoperative characteristics are shown in Table 1. There were significant differences in the choice of hepatic vein implantation technique. While the inferior vena cava (IVC)-sparing technique was used in 96.6% in the group of rWIT ≤ 30 min, this was used in 62.1% in the group of rWIT >50 min ($P < 0.001$). The likelihood of achieving rWIT ≤ 30 min was 8 folds higher in the IVC-sparing technique (piggyback) compared to the bicaval technique (odds ratio 8.22, 95%CI: 3.77–17.51, $P < 0.001$).

Intraoperative factors and rWIT

Table 2 demonstrates comparisons of median intraoperative transfusion requirements, blood loss, INR (international normalized ratio), and lactate levels at the end of LT surgeries between the rWIT groups. There was a significant difference in all types of transfusion requirements, EBL, and median lactate level, according to rWIT. The trend of transfusion requirement, EBL, and lactate levels in an association with length of rWIT was checked by the Jonckheere-Terpstra test, which demonstrated that there were significant increasing trends of transfusion of packed red blood cells (PRBC) requirement (PRBC, $P < 0.001$; autologous transfusion, $P < 0.001$; cryoprecipitate, $P < 0.001$; FFP (fresh frozen plasma), $P < 0.001$; platelet, $P < 0.001$), EBL ($P < 0.001$), and lactate level ($P < 0.001$) with prolongation of rWIT.

EAD and rWIT

EAD data was available for transplants performed since 2012 ($n = 431$). Overall, EAD occurred in 106 patients (24.6%). This included 20/111 (18.0%) of patients with rWIT ≤ 30 min, 32/170 (18.8%) of those with rWIT 31–40 min, 31/104 (29.8%) of those with rWIT 41–50 min, and 23/46 (50.0%) of those with rWIT > 50 min ($P < 0.001$). Multivariable logistic regression demonstrated a significantly higher risk of EAD in the groups of WIT 41–50 min ($P = 0.029$, HR 2.2 [1.1–4.5]), and WIT >50 min ($P < 0.001$, odds ratio 6.3 [2.7–14.8]) to EAD, compared to WIT ≤ 30 min (Fig. S1).

Graft loss and mortality

Log-rank tests showed a statistically significant 5-year graft and patient survival benefit when comparing rWIT

Table 1. Baseline recipient and donor characteristics according to recipient warm ischemia time.

rWIT	≤30 min (n = 203)	31–40 min (n = 465)	41–50 min (n = 353)	>50 min (n = 240)	P value
Recipient factors					
Age (year), mean ± SD	55.6 ± 10.4	54.8 ± 9.2	55.2 ± 9.4	53.2 ± 9.7	0.04
Age group (%)					
≤50	49 (24.1)	101 (21.7)	72 (20.4)	76 (31.7)	
51–60	71 (35.0)	212 (45.6)	163 (46.2)	105 (43.8)	
61–70	74 (36.5)	146 (31.4)	110 (31.2)	54 (22.5)	
>70	9 (4.4)	6 (1.3)	8 (2.3)	5 (2.1)	
Gender (male) (%)	119 (58.6)	305 (65.6)	234 (66.3)	168 (70.0)	0.09
Ethnicity (black), number (%)	26 (12.8)	75 (16.1)	51 (14.4)	49 (20.4)	0.13
BMI, mean ± SD	28.8 ± 5.2	29.2 ± 6.0	29.5 ± 5.8	29.2 ± 5.8	0.58
Laboratory MELD (MELD-Na) score at transplant, mean ± SD	21.1 ± 9.8	21.0 ± 9.5	20.1 ± 8.6	19.6 ± 8.6	0.20
Comorbidities (%)					
DM	51 (25.1)	104 (22.4)	76 (21.6)	64 (26.8)	0.43
Hyperlipidemia	29 (15.6)	53 (12.4)	29 (9.0)	19 (8.8)	0.07
HTN	69 (37.1)	171 (40.1)	129 (40.1)	81 (37.5)	0.83
CVA	5 (2.7)	13 (3.3)	6 (2.0)	9 (4.8)	0.37
CAD	21 (11.4)	32 (8.0)	24 (8.2)	18 (9.6)	0.55
Primary liver disease (%)					
Hepatitis C	77 (38.1)	203 (43.7)	156 (44.2)	101 (42.1)	0.52
Alcohol	60 (29.7)	155 (33.3)	103 (29.2)	63 (26.2)	0.25
NASH	27 (13.4)	44 (9.5)	32 (9.1)	21 (8.8)	0.33
AIH	10 (5.0)	11 (2.4)	8 (2.3)	7 (2.9)	0.26
PSC	11 (5.4)	26 (5.6)	21 (5.9)	20 (8.3)	0.49
PBC	5 (2.5)	18 (3.9)	14 (4.0)	6 (2.5)	0.62
Acute liver failure	4 (2.0)	10 (2.2)	7 (2.0)	11 (4.6)	0.17
HCC (%)	59 (29.2)	129 (27.7)	106 (30.0)	56 (23.3)	0.33
Karnofsky score (%) 10%–30%	31 (15.7)	67 (14.8)	35 (10.3)	21 (9.3)	0.056
Grade 3 or 4 hepatic encephalopathy (%)	25 (12.8)	60 (13.5)	33 (10.1)	16 (8.1)	0.17
Dialysis (%)	15 (7.4)	25 (5.4)	17 (4.8)	9 (3.8)	0.38
Moderate/severe ascites (%)	57 (29.2)	135 (30.3)	82 (25.0)	51 (25.8)	0.35
Donor and surgical factors					
Age (year), mean ± SD	44.5 ± 16.9	43.1 ± 16.8	45.4 ± 16.2	43.1 ± 16.5	0.17
Gender (male), number (%)	102 (50.2)	254 (54.6)	203 (57.5)	150 (62.5)	0.057
Ethnicity (Black) number (%)	44 (21.7)	83 (17.8)	71 (20.1)	46 (19.2)	0.68
BMI, mean ± SD	28.2 ± 7.0	27.9 ± 6.7	28.1 ± 6.4	28.6 ± 7.0	0.62
CIT (hours), mean ± SD	5.4 ± 1.4	5.6 ± 1.4	5.9 ± 1.8	6.16 ± 1.7	<0.001
CIT > 6 hours (%)	51 (25.1)	154 (33.2)	147 (42.1)	116 (48.3)	<0.001
IVC-sparing hepatic vein reconstruction* (%)	196 (96.6)	408 (87.7)	263 (74.5)	149 (62.1)	<0.001

AIH, autoimmune hepatitis; ALF, acute liver failure; BMI, body mass index; CAD, coronary artery disease; CIT, cold ischemia time; CVA, cerebrovascular accident; DM, diabetes mellites; HCC, hepatocellular carcinoma; HTN, hypertension; MELD, Model for End-Stage Liver Disease; NASH, nonalcoholic steatohepatitis; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis.

*Piggyback or cavocavostomy technique.

≤ 30 min to all other rWIT subgroups (Fig. 1). rWIT ≤ 30 min provided significant 1-year graft survival compared to rWIT 41–50 min and rWIT >50 min, and significant 1-year patient survival compared to rWIT > 50 min. Log-rank trend tests showed that decreasing trends of 1 and 5-year graft survival rates with increasing rWIT were significant ($P < 0.001$ and 0.003, respectively).

After risk adjustment, patients with rWIT ≤ 30 min maintained a significantly lower risk of graft loss at 1

Table 2. Comparisons of intraoperative factors according to recipient warm ischemia time.

rWIT	≤30 min (n = 203)	31–40 min (n = 465)	41–50 min (n = 353)	>50 min (n = 240)	P value*	P value for trend†
EBL (mL), median (IQR)	1500 [1000, 2550]	1900 [1000, 3000]	2000 [1200, 4000]	2675 [1500, 5525]	<0.001	<0.001
Transfusion						
PRBC (unit), median (IQR)	3.0 [1.0, 6.0]	3.0 [1.0, 6.0]	3.00 [1.0, 6.0]	4.00 [2.0, 8.0]	<0.001	<0.001
Autologous (unit), median (IQR)	2.0 [1.0, 3.0]	2.0 [1.0, 4.0]	3.0 [1.0, 5.0]	3.0 [2.0, 7.0]	<0.001	<0.001
Cryoprecipitate (unit), median (IQR)	1.00 [0, 3.0]	0 [0, 5.0]	0.5 [0.0, 10.0]	3.0 [0.0, 10.0]	<0.001	<0.001
FFP (unit), median (IQR)	5.0 [2.0, 9.0]	6.0 [3.0, 10.0]	6.0 [4.0, 11.0]	8.0 [4.0, 13.0]	<0.001	<0.001
Platelet (unit), median (IQR)	0 [0, 2.0]	0 [0, 6.0]	1.0 [0, 6.0]	5.0 [0, 10.0]	<0.001	<0.001
INR at end of surgery, median (IQR)	2.10 [1.83, 2.58]	2.08 [1.77, 2.44]	2.20 [2.00, 2.51]	2.06 [1.74, 2.41]	0.26	0.46
Lactate at end of surgery, median (IQR)	2.40 [1.80, 3.88]	3.60 [2.00, 5.80]	4.25 [2.62, 6.57]	5.00 [2.73, 7.58]	<0.001	<0.001

EBL, estimated blood loss; FFP, fresh frozen plasma; PRBC, packed red blood cells.

*Kruskal–Wallis test.

†Jonckheere-Terpstra test.

and 5- years. The risk of 1-year graft loss was significantly lower in the groups of rWIT ≤30min, compared to 31–40min (HR 0.45, 95%CI: 0.23–0.90, P = 0.02), 41–50min (HR 0.43, 95%CI: 0.21–0.88, P = 0.02), and >50min (HR 0.27, 95%CI: 0.13–0.55, P < 0.001) (Fig. 2a). Similar findings were observed when assessing the risk of 5-year graft loss (Fig. 2b). There was no significant difference when comparing the risk between the three groups (31–40 min vs. 41–50 min vs. >50 min). Table 3 presents the final multivariable Cox regression model with a backward selection for 1 and 5-year graft loss. Independent risk factors for 1-year graft loss included recipient BMI >30 (ref. BMI 25–30), Grade 3 or 4 encephalopathy, Karnofsky score 10–30%, MELD score, acute liver failure as primary liver disease, cold ischemia time, and female donor, along with rWIT. Independent risk factors for 5-year graft loss included Grade 3 or 4 encephalopathy, acute liver failure as primary liver disease, and the presence of hepatocellular carcinoma, and older donor age, along with rWIT.

Prognostic impact of rWIT in association with CIT

Cold ischemia time was an independent risk factor for graft loss. A threshold to stratify the risk was a CIT of 6 hours (Fig. S2). Because CIT and rWIT might have synergistic effects on outcomes, the impact of rWIT was assessed by stratifying the cohort of patients into CIT: ≤6 hours and >6 hours. The protective effect of rWIT ≤30 min from graft loss was more pronounced in the subgroup of patients with a CIT of greater than 6 hours (Fig. S3). In the longer CIT group, the shortest rWIT group showed a lower risk of graft loss at 1 and 5-years, compared with rWIT 31–40 min, 41–50 min, and >50 min. In the shorter CIT group, the difference of risk was significant between the shortest and longest rWIT groups (≤30min vs. >50min), but the risk was similar between the shortest group and the groups of 31–40min or 41–50min (Fig. 3).

Of note, we evaluated the positive effect of short rWIT according to different CIT cut-offs. There were only 82 and 21 patients who had CIT of 8–10 hours and 10 hours or longer, respectively. When comparing 1-year graft survival rates in the group with CIT of 8 hours or longer according to rWIT, 1-year graft survival rates were 90% (n = 27), 82.1% (n = 39), 77.3% (n = 27), and 74.1% (n = 10) in those with rWIT of 30 min or shorter, 31–40 min, 41–50 min, and >50 min, respectively (P = 0.778). While the difference did not reach statistical significance, likely due to the

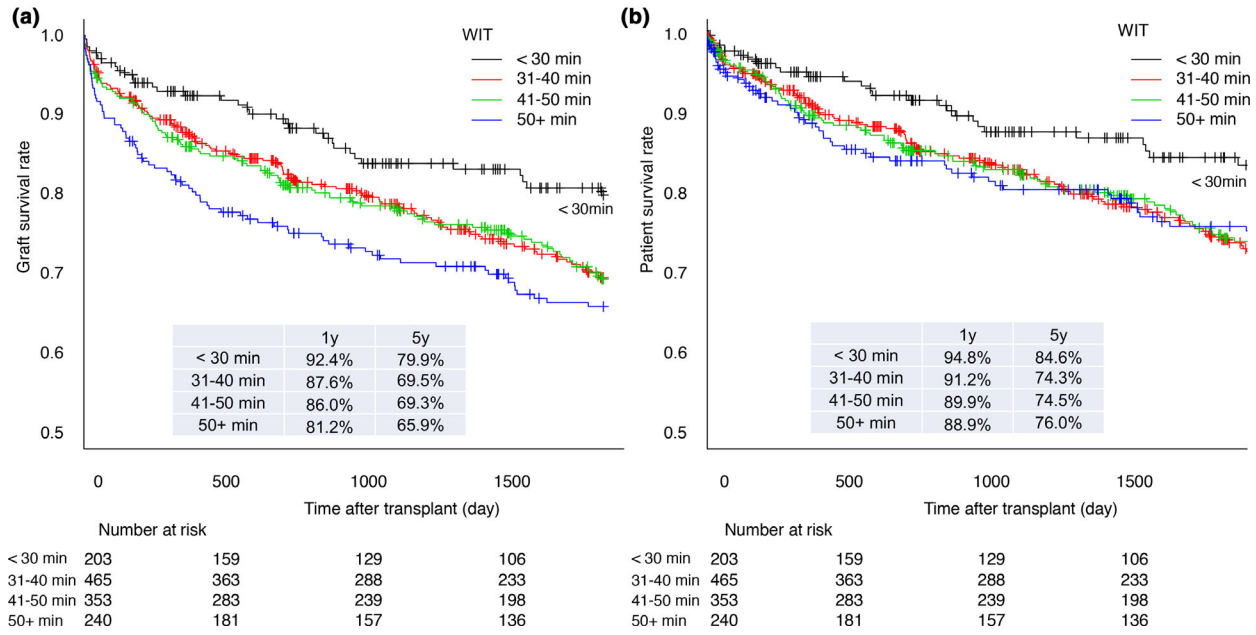


Figure 1 Graft and patient survival according to recipient warm ischemia time.

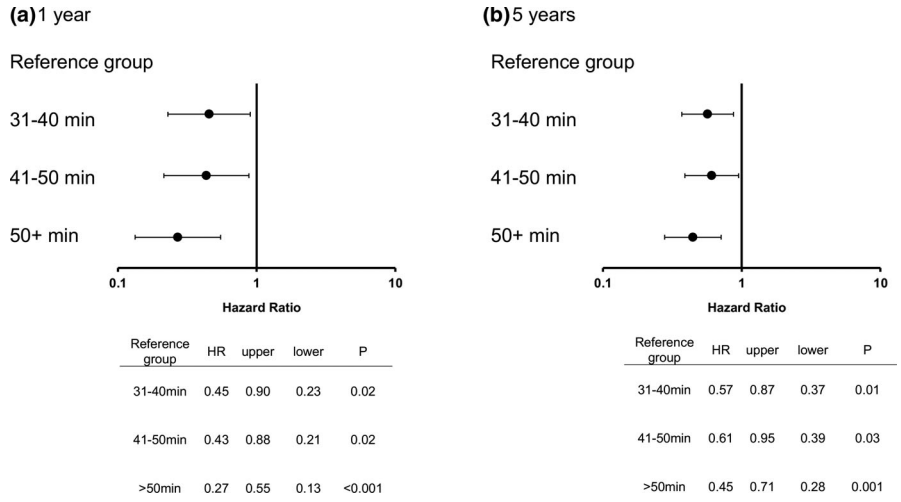


Figure 2 Adjusted hazards of 1 and 5-year graft loss in the group of recipient warm ischemia time of 30 min or shorter.

small number of patients, we still observed a trend towards superior outcomes in the shorter rWIT group.

Mediation analysis

Mediation analysis demonstrated a significant direct positive effect of shorter rWIT on graft outcome, compared to rWIT >50 min (Effect –0.517 [95% CI: −1.032, −0.001], P = 0.050). It also showed significant indirect effects of shorter rWIT on graft outcome via intraoperative blood loss (≤30 min: Effect –0.121 [95% CI: –0.234, −0.035]), 31–40 min: Effect –0.096 [95% CI: –0.198, −0.025], 41–50 min: Effect –0.074 [95% CI: –0.163, −0.015]) (ref. rWIT >50 min) (Fig. 4a). Shorter rWIT had a significant direct effect on occurrence of EAD when comparing rWIT > 50 min to both rWIT ≤30 min (Effect –1.328 [95% CI: –2.124, −0.531], $P = 0.001$) and 31–40 min (Effect –1.346 [95% CI: –2.073, −0.619], $P < 0.001$), while no significant indirect effect via intraoperative blood loss was demonstrated (Fig. 4b).

Discussion

This study evaluated the prognostic impact of rWIT in LT, which demonstrated that shortening rWIT within

Table 3. Final model of multivariable Cox regression analysis with backward selection for risk factors for 1 and 5-year graft loss.

	1-year graft loss				5-year graft loss			
Variable	*P* value	HR	95%CI Lower	95%CI Upper	*P* value	HR	95%CI Lower	95%CI Upper
rWIT (ref. ≤ 30min)								
31–40 min	0.02	2.20	1.11	4.36	0.01	1.76	1.15	2.70
41–50 min	0.02	2.30	1.14	4.67	0.03	1.64	1.05	2.57
50+ min	<0.001	3.70	1.82	7.53	0.001	2.25	1.41	3.59
CIT, per min increase	0.02	1.002	1.000	1.004	0.057	1.001	1.000	1.002
Recipient BMI (ref. 20–24.9)								
25–30	0.46	1.22	0.72	2.05	–	–	–	–
>30	0.04	1.67	1.02	2.72	–	–	–	–
<20	0.53	0.63	0.15	2.68	–	–	–	–
Grade 3 or 4 encephalopathy	0.02	1.93	1.12	3.33	0.001	1.81	1.26	2.60
Karnofsky 10–30% (ref. 40–100%)	0.04	1.77	1.02	3.09	–	–	–	–
MELD score at transplant	0.03	0.97	0.95	0.997	–	–	–	–
Primary liver disease								
Acute liver failure	0.003	3.23	1.48	7.05	0.050	1.93	1.001	3.70
Hepatocellular carcinoma	–	–	–	–	0.005	1.47	1.13	1.92
Donor age	–	–	–	–	<0.001	1.01	1.01	1.02
Donor gender female	0.02	1.53	1.08	2.16	–	–	–	–

Variables for the final model were selected using a backward elimination method. Risks were adjusted for recipient factors (primary liver disease [ALD, hepatitis C, NASH, PSC, PBC, AIH, acute liver failure], presence of hepatocellular carcinoma, age, gender, race, BMI, diabetes, grade 3 or 4 encephalopathy, moderate to severe ascites, dialysis requirement, Karnofsky score, MELD score, CIT, recipient WIT, surgical technique for hepatic vein reconstruction [piggyback/cavocavostomy vs bicaval techniques] and transplant year), and donor factors (donor age, race, gender, BMI).

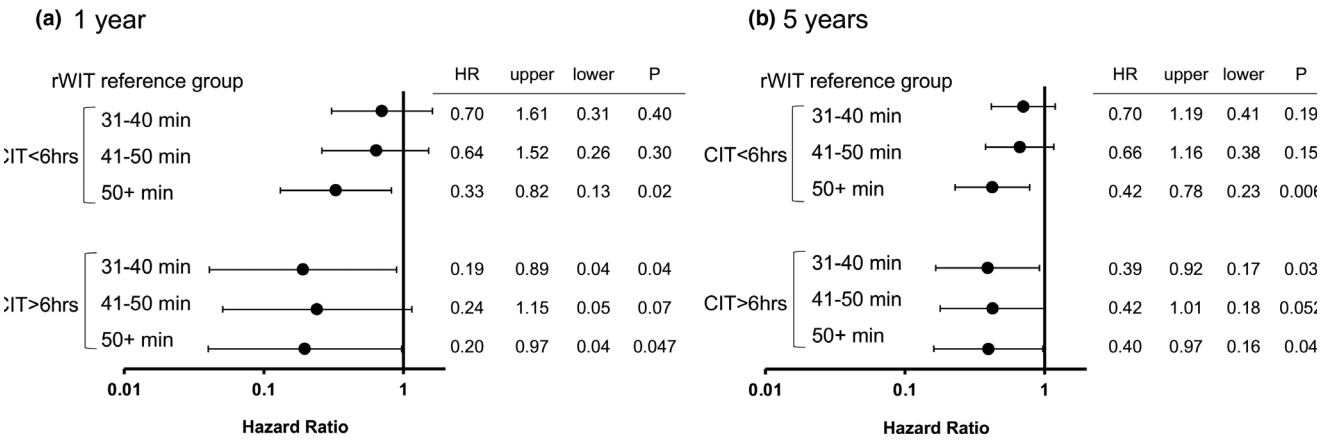


Figure 3 Adjusted hazards of graft loss in the group of recipient warm ischemia time of 30 min or shorter according to cold ischemia.

30 min could improve post-LT outcomes. Notably, the positive prognostic effects of short rWIT (≤30 min) were more prominent when CIT exceeded 6 hours, suggesting that liver graft damage due to prolonged CIT could be offset by a short rWIT. While the requirement of intraoperative transfusion also decreased by shortening rWIT, there would be a concern that the prognostic effects of short rWIT were the result of a smaller amount of intraoperative blood transfusion, but not necessarily directly causative of poor outcomes. In fact, a larger amount of blood transfusion was significantly associated with poor outcomes. It is unclear if prolonged rWIT caused more blood loss and transfusion, which led to poorer outcomes or vice versa. To address

(a)

B: Effect of WIT on blood loss

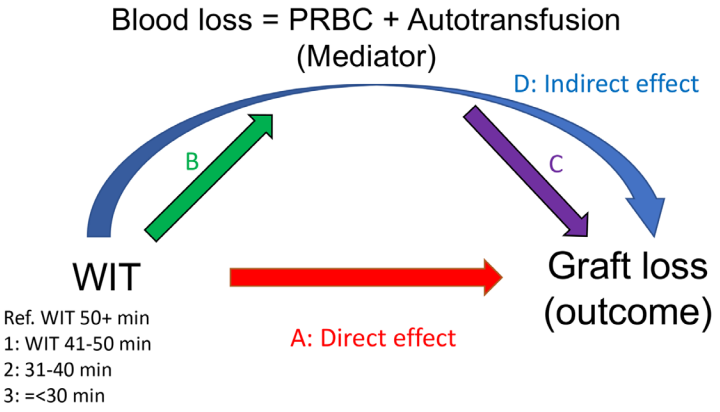
	coeff	p	LLCI	ULCI
1	-5.030	.000	-7.401	-2.659
2	-6.494	.000	-8.731	-4.256
3	-8.194	.000	-10.791	-5.597

C: Effect of blood loss on Graft loss

Coeff	p	LLCI	ULCI
0.015	0.005	0.005	0.025

D: Indirect effect of WIT on Graft loss

	Effect	Boot SE	Boot LLCI	Boot ULCI
1	**-.074**	**.038**	**-.163**	**-.015**
2	**-.096**	**.045**	**-.198**	**-.025**
3	**-.121**	**.051**	**-.234**	**-.035**



A: Direct effect of WIT on Graft loss

	Effect	p	LLCI	ULCI
1	-.164	.460	-.598	.270
2	-.232	.273	-.647	.183
3	**-.517**	**.050**	**-1.032**	**-.001**

(b)

B: Effect of WIT on blood loss

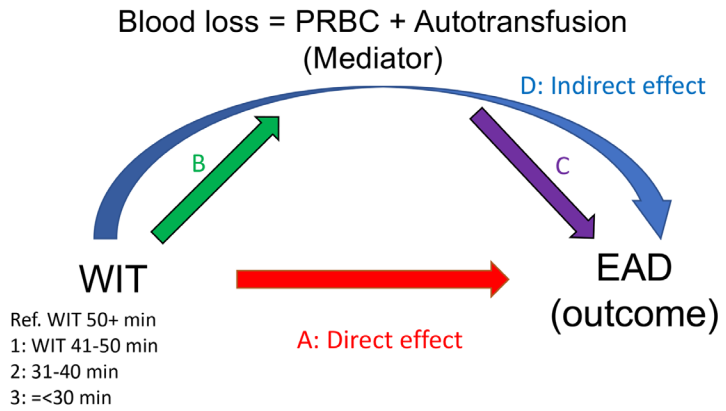
	coeff	p	LLCI	ULCI
1	-13.618	.000	-18.637	-8.600
2	-13.727	.000	-18.439	-9.016
3	-16.961	.000	-21.931	-11.991

C: Effect of blood loss on Graft loss

Coeff	p	LLCI	ULCI
.0079	0.277	-.0064	.0222

D: Indirect effect of WIT on Graft loss

	Effect	Boot SE	Boot LLCI	Boot ULCI
1	-.108	.127	-.421	.080
2	-.109	.130	-.434	.079
3	-.134	.151	-.495	.103



A: Direct effect of WIT on Graft loss

	Effect	p	LLCI	ULCI
1	-.694	.068	-1.439	.052
2	**-1.346**	**.000**	**-2.073**	**-.619**
3	**-1.328**	**.001**	**-2.124**	**-.531**

Figure 4 Mediation Analysis evaluating the direct and indirect effects of recipient warm ischemia time on occurrence of a) graft loss and b) early allograft dysfunction.

this concern, we conducted mediation analysis to assess the direct and indirect effects of rWIT on outcomes associated with intraoperative blood transfusion. The mediation analysis showed that rWIT had a significant direct effect and an indirect effect via intraoperative blood loss on graft outcomes. These results confirmed that rWIT had a direct impact on the risk of graft loss after LT, as well as an association with intraoperative blood loss, which indirectly increased the risk of graft loss. While the adverse effects of prolonged rWIT have been well recognized, the novelty of this study is focusing on the positive prognostic impact of shorter rWIT, compared to "acceptable rWIT of 30–50 min" and "prolonged rWIT (>50 min)". The results of this study emphasize the potential of LT outcome improvement by shortening rWIT within 30 min and reaffirm the adverse impact of prolonged rWIT.

Short rWIT might enhance liver graft function immediately after reperfusion and quickly reverse intraoperative coagulopathy, which could lower intraoperative blood loss and transfusion requirement. As hypothesized, there were significant trends of increasing requirement of blood transfusion as rWIT was prolonged. However, we acknowledge that reasons for blood loss and transfusion requirements during LT are multifactorial. Possible causes include severe preoperative coagulopathy, difficult hepatectomy, marginal donor liver graft, and/or severe fibrinolysis. DCD-LT, retransplant, or multi-organ transplant were excluded to homogenize the study cohort and alleviate the concerns about technical difficulties, and donor quality. Of note, we were unable to identify the exact period of blood loss and transfusion requirement during each LT surgery, especially before or after reperfusion of the liver graft. Therefore, it is difficult to draw a firm conclusion on the association between short rWIT and a lower amount of intraoperative blood transfusion. However, reports from other groups have indicated the association between ischemia-reperfusion injury of the liver graft and intraoperative blood loss and transfusion, which our results corroborate [12, 13]. Hence, the roles of shorter rWIT in decreasing intraoperative blood loss and transfusion should be emphasized.

Similarly, the occurrence of EAD after LT would be multifactorial [14]. Many other studies have suggested possible risk factors for EAD [15, 16]. Bastos-Neves et al. reported that prolonged rWIT (40 min or longer) was one of the risk factors [17]. In this study, recipient and donor characteristics were adjusted to assess the risk of rWIT for EAD. The risk adjustment model showed that the risk of EAD was, compared to WIT of 30 min or shorter, significantly higher in the groups of rWIT of 41–50 min and >50min, and similar to the groups of 31–40 min. EAD in LT is a well-known prognostic factor [18, 19]. Shortening rWIT may lead to a decreased risk of EAD, which can improve short and long-term graft and patient outcomes.

Another important finding of this study is the possible role of short rWIT in offsetting the risk of prolonged CIT. Cold ischemia time is also a well-known prognostic factor in LT. In the liver donor risk index formula, 8 hours was suggested as a cut-off associated with worse outcomes [11]. In our series, CIT remained as an independent risk factor for 1-year graft loss, and CIT of 6 hours stratified the risk of liver graft loss (Fig. S2). Because CIT and rWIT might have confounding and/or synergistic effects on outcomes, a subgroup analysis was conducted by grouping patients according to CIT to determine the prognostic effects of rWIT in association with CIT. This subgroup analysis showed that positive prognostic effects of short rWIT were more evident in the prolonged CIT group. These findings provide critical insight into donor and operative management. Transplant teams attempt to shorten CIT by optimizing the timing and logistics of donor and recipient surgeries and liver graft transportation from a donor hospital. However, there may be occasions when the CIT is prolonged due to unexpected intraoperative findings or other reasons. It is worth acknowledging that shortening rWIT could offset the adverse impact of prolonged CIT.

Reducing ischemia time, both CIT and rWIT is crucial to maintain liver graft quality and improve LT outcomes [20]. rWIT, reflective of the "time for liver graft implantation", may be affected by surgical implantation techniques such as the piggyback and bicaval techniques. In fact, the piggyback technique was significantly associated with a short rWIT, compared to the bicaval technique. Transplant centers may have their own preference, and, in fact, our center preferentially selected the piggyback technique, which was used in over 80% of LTs in this series. It should be emphasized that the findings of this study do not necessarily recommend the piggyback technique over the bicaval technique, because the technical challenges and difficulties might bias the selection of the hepatic vein reconstruction technique during LT. In our series, the piggyback group had significantly better graft survival compared to the bicaval group on univariable analysis, but this did not remain as an independent factor by adjusting risk for other recipient and donor factors, including rWIT (Table 3). We analyzed the impact of rWIT in the piggyback and bicaval groups separately.

The effects of rWIT were significant in the piggyback group, but not in the bicaval group (Data not shown). However, this discrepancy might be due to the small number of patients in the bicaval group. The pros and cons of these techniques are beyond the scope of this study. Instead, we emphasize the importance of avoiding unnecessary extra time during the implantation. Sophistication and standardization of implantation techniques are essential. Also, keys for successful implantation include efficient teamwork with the operating room staff and anesthesiologists. Preoperative briefing with the operating room staff is crucial to let team members better understand the sequence of procedures and prepare for necessary instruments, and devices required during the surgery, especially during liver graft implantation. During the implantation or immediately before the reperfusion of the liver graft, patients may develop hemodynamic instability and surgeons might need to wait until this is optimized. Proactive optimization of the hemodynamic state by experienced liver anesthesiologists would avoid this holding time. There are occasional technical difficulties in the implantation because of lack of abdominal space, recipient body habitus (deep abdominal cavity), larger liver graft size relative to recipient body size, portal vein thrombosis, and vessel tissue quality. Interestingly, we did not observe significant differences in rWIT according to the presence of portal vein thrombosis, donor BMI, recipient BMI, donor-recipient gender match, or presence of ascites. The piggyback technique was the only factor associated with short rWIT, compared to the bicaval technique. Optimal strategies to shorten rWIT may include good preparation and exposure of the implantation field, and preoperative assessment of recipient and liver graft size match, allowing consistent implantation procedures without significant deviation. It may be beneficial for each transplant center to re-explore the surgical techniques and strategies of their procedures to consistently achieve short rWIT or at least strive to avoid prolonged rWIT. Changing and improving small details could lead to a significant time difference. The message of this investigation is not that a surgeon must hasten implantation time at all costs, which could itself affect outcomes in a negative fashion. Rather, this study emphasizes the impact of a short rWIT on graft and recipient outcomes and encourages the surgeon and transplant center to modify implantation techniques, when possible and safe, to optimize these outcomes.

This study is retrospective and performed at a single center, limiting the generalizability of these findings in other transplant centers. While one of the strengths of this study is the large number of cases (over 1200 LTs from our 20-year experience), the historical bias would be another limitation. To alleviate this issue, the transplant year (era) was also included in the risk adjustment. The prognostic effects of rWIT (liver implantation time) and LT outcomes have been studied elsewhere. However, the novelty of this study, which focused on the protective impact of short rWIT, should be emphasized. Also, it would be valuable and meaningful for transplant teams to acknowledge the potential to mitigate the adverse impact of prolonged CIT by shortening rWIT. It is difficult to prove the relationship between short rWIT, intraoperative blood loss, and transfusion requirements. The presence of two different venous reconstructive techniques further adds to this complexity. That said, our multivariate regression model did adjust for venous reconstruction technique when analyzing graft and patient survival. We further performed Mediation Analysis to demonstrate a direct relationship (irrespective of blood loss) between rWIT and graft survival/EAD. In addition, although the context of acute liver failure can differ from that of other indications for liver transplantation, we decided to include this pathology in the study, as it does constitute part of the spectrum of disease states that are indications for liver transplantation within our inclusion criteria. Acute liver failure was the indication for transplant in only 32 patients (2.5%). We did adjust for the etiology of liver disease in our multivariate analysis of graft and patient survival, so we believe that the effect of this variable on our reported outcomes is minimal. Lastly, to homogenize the cohort, this study did not include living donor LT, DCD-LT, retransplant, or combined organ transplants. Consequently, we are unable to comment on the possible effects of rWIT in these cases. However, there would be no reason for not seeing a similar positive impact of short rWIT in these cases.

In conclusion, this study revealed that keeping rWIT within 30 min might decrease the risk of EAD and improve short and long-term outcomes after LT. Protective effects of short rWIT were more prominent when CIT exceeded 6 hours, which suggested that shorter rWIT might offset the negative impact of prolonged CIT. Ischemic damage to the liver graft is one of the biggest hurdles for successful LT, and many medical and surgical strategies have been proposed to counteract its effect. Because LT surgeries have been well standardized, we might think that there would not remain much room for improvement in the liver graft implantation techniques to shorten rWIT. However, it would be beneficial to revisit the basics of LT surgeries and explore possibilities to shorten rWIT.

Authorship

Al-Kurd: study concept, data acquisition, manuscript writing, data analysis. Kitajima: study concept, manuscript writing, data analysis. Shamma: data acquisition, interpretation data, critical revision. Ivanics: interpretation data, critical revision. Collins: study concept, interpretation data, critical revision. Rizzari: study concept, interpretation data, critical revision. Yoshida: study concept, interpretation data, critical revision. Abouljoud: study concept, interpretation data, critical revision. Nagai: study concept, manuscript writing, data analysis, interpretation data.

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Conflict of interest

Nothing to disclose. No writing assistance.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Adjusted odds ratio of early allograft dysfunction according to recipient warm ischemia times (ref. 30 min or shorter).

Figure S2. One-year graft survival according to cold ischemia time.

Figure S3. Graft survival according to cold ischemia and recipient warm ischemia times.

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