ORIGINAL ARTICLE

The effect of organ preservation solutions on short-term outcomes after liver transplantation: a single-center retrospective study

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SUMMARY

The effect of preservation solutions on outcomes has been subject of many debates but the relative benefits of the various solutions remain unclear. We retrospectively compared short-term outcomes of 885 liver transplantations performed between 1/2000 and 12/2017 and preserved with either Histidine–Tryptophan–Ketoglutarate (HTK, $n = 190$), University of Wisconsin (UW, $n = 557$), or Institute George Lopez 1 preservation solution (IGL-1, $n = 139$). Inverse probability of treatment weighting (IPTW) was performed to account for baseline differences between groups and analyses were adjusted for confounders. In the IPTW analyses, peak AST within 7 days was 44% higher (95% CI 15–81%, $P < 0.001$) in HTK than in UW. Mean model of early allograft function (MEAF) score was 0.61 points (95% CI 0.12–1.10, $P = 0.01$) higher in HTK than in UW. Early allograft dysfunction (EAD) was more likely to occur with HTK compared to IGL-1 (IPTW OR = 2.87, 95% CI = 1.00–8.19, $P = 0.049$) and UW (IPTW OR = 1.75, 95% CI = 1.06–2.88, $P = 0.023$). The type of preservation solution had no impact on hospital stay, ICU stay, incidence of biliary strictures, or graft and recipient survival. HTK was the least effective on reducing graft injury and increased the probability of graft dysfunction after transplantation. UW and IGL-1 were equally effective in reducing graft injury and dysfunction.

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Key words
Histidine–Tryptophan–Ketoglutarate, IGL-1, liver transplantation, preservation solutions, University of Wisconsin

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Introduction

Liver transplantation (LTx) constitutes the ultimate treatment for end-stage liver disease [1]. However, the pool of suitable deceased organ donors is insufficient to satisfy the steadily increased demand for transplantation. To fill the gap between organ offer and transplant demand, criteria for organ donors acceptance have been liberated, including the so-called extended criteria donors (ECD), which presents characteristics and/or comorbidities previously considered unfit for organ transplantation (such as advanced age and hepatic steatosis, among others) [2,3]. Additionally, donations after circulatory death donors (DCD), in which the liver graft is exposed to a hit of warm ischemia before the cold storage phase, have been increasingly utilized in addition to the conventional donation after brain death donors (DBD). [2,4]. However, grafts procured from

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these higher-risk donors are more susceptible to ischemia–reperfusion injury (IRI), are at higher risk of graft failure or early complications, and may yield inferior outcomes in the long term [5,6].

IRI is unavoidable during LTx, and it is a key player in the pathogenesis of many early post-transplant complications, such as early allograft dysfunction (EAD) and biliary complications, and a determinant of long-term graft and patient survival [7]. Adequate preservation of liver grafts and prevention of severe IRI is therefore a cornerstone to the success of LTx [8]. The current standard for liver preservation is still static cold storage, during which the liver is maintained under hypothermic conditions after having been flushed in the donor with a cold preservation solution.

The most frequently used preservation solutions are University of Wisconsin (UW) and Histidine–Tryptophan–Ketoglutarate (HTK) [7,9,10]. Recently, Institute Georges Lopez 1 preservation solution (IGL-1) has been introduced in clinical practice [11]. Experimental studies have already been conducted [12,13], and clinical trials have shown promising results [14,15]. The relative benefits of each of these preservation solutions however remain incompletely characterized, especially regarding their effects on patient and graft survival and reduction of complications or dysfunction. The present study, through a retrospective single-center analysis, compared the effects on short-term outcomes of UW, HTK, or IGL-1 preservation solution.

Materials and methods

Population and study design

A clinical database was retrospectively reviewed to identify all adult recipients of a liver transplant performed at the University Hospitals of Leuven between January 1, 2000, and December 31, 2017. Re-transplantation and partial-graft LTx were not considered. Exclusion criteria included lack of information on the preservation solution utilized, storage with other or combinations of preservation solutions, and perioperative mortality. The identified LTx were divided in three groups according to the type of preservation solution that was used (UW, HTK, and IGL-1). Belgium operates with Eurotransplant [16], which is a nonprofit organization that promotes and manages cross-border sharing of organs for transplantation. Within the Eurotransplant region, considerable variation exists regarding the standard preservation solution utilized for organ storage and transport. Consequently, the choice of the preservation solution used to preserve imported grafts was beyond our control. HTK and UW were used over the entire study period, whereas IGL-1 was introduced from January 2015 onwards.

Donor-related variables considered were age, BMI, cause of death, donor type (DCD or DBD), donor peak aspartate transaminase (AST), donor risk index (DRI), donor hepatectomy time, and biliary duct flush during organ procurement. Recipient-related characteristics considered were age, gender, indication to transplantation, lab model for end-stage liver disease score (MELD), and balance of risk (BAR) score. Transplant-related data were cold ischemia time (CIT), implantation time, and total surgery time. Outcomes considered and compared between groups were peak AST within 7 days, model of early allograft function (MEAF) score, EAD, intensive care unit (ICU) stay, hospital stay, biliary strictures, and 1-year patient and graft survival.

CIT was defined as the time between the start of cold flush during the donor procedure and the graft being taken out of the ice box for implantation. Donor hepatectomy time was defined as the time from cold perfusion until the moment the graft left the donor body to be placed on melting ice. Implantation time was defined as the time between liver out of ice and reperfusion of both the portal vein and the hepatic artery. MEAF is a continuous score for graft dysfunction based on bilirubin, INR, and ALT within 3 days post-transplant [17]. EAD is a binary categorical score and was here defined according to Olthoff et al. [18].

Transplant procedure and postoperative care

All LTx were performed with classical replacement of the recipient vena cava using a veno-venous bypass, which also included a portal bypass in most of the cases. The vena porta was reconstructed in end-to-end fashion, while the hepatic artery anastomosis was preferentially performed on a carrel patch. Standard triple immunosuppression including calcineurin inhibitor, steroids, and antimetabolites was commenced shortly after transplantation.

The occurrence of biliary complications is routinely investigated in our center with a magnetic resonance cholangio-pancreatography (MRCP) one year after transplantation, whereas patients with clinical suspicion of biliary complications are screened with MRCP at earlier follow-up. Only clinically relevant biliary complications (provoking symptoms and/or sensible elevation of markers of cholestasis) and confirmed at radiology (in the presence of a patent hepatic artery) were considered as events in this analysis.

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Statistical analyses

Variables were compared between three groups based on preservation solution. Continuous variables are expressed as median (interquartile range, IQR), and differences between groups were tested using the nonparametric Kruskal–Wallis test. Nominal variables are expressed as numbers (%) and differences were assessed using a chi-squared test.

Linear models were used to compare the effect of preservation solutions on peak AST and MEAF, whereas logistic regression models were used for EAD and peak AST > 2000. The model for peak AST was fit on transformed values (log-transformed), and results were back-transformed to the original scale; therefore, differences were presented as ratios (and 95% confidence interval). Cox models were fit for biliary stricture, and 1-year patient and graft survival. Since graft loss without biliary stricture is a competing event for the occurrence of biliary strictures, cumulative incidence curves according to Nelson–Aalen estimates were shown. The time range for stricture was restricted to 12 months. Since in-ICU death and in-hospital death are competing risks for length of ICU and hospital stay, respectively, the same approach as for biliary strictures was used for these endpoints.

IGL-1 was introduced in our clinical practice in January 2015. Given the longevity of the study period and the evolution in donor profile over time, a significant difference in case mix between groups can be expected. In each of the models abovementioned, the risk of bias in this retrospective analysis was reduced as much as possible using an approach known as inverse probability of treatment weighting (IPTW). Each subject was weighted by its inverse probability of being in its specific group (propensity score), conditional to the following prespecified set of variables that are well-known to influence outcomes of interest: donor age, DRI, BMI, and cause of death; recipient age and gender; and cold ischemia time, donor hepatectomy time, and implantation time. LTx with missing information for the aforementioned variables ($n = 111$, 12.5%) were not considered for IPTW. The effects on outcomes of these potential confounders was preliminarily investigated in our population in univariable logistic regression models (no other multivariable analyses were performed; additional information in Supplementary Material, Supplementary Results, and Table S1). The aim of IPTW was to create a weighted sample in which the distribution of these variables was similar across groups. In the models, each individual is weighted by the inverse of its probability to belong to its group. Thus, the more typical a subject is for the group it belongs to, the lower its weight will be. The weights were normalized to the sample size in each group. For each of the confounding variables, the effect of the weighting was evaluated using unweighted and weighted chi-squared tests and linear models. Donor type was not included as a variable in the propensity model, since there were no DCD donors in the HTK group, and < 5% in the UW group. To further investigate the potential confounding effect of type of donor, a sensitivity analysis was also performed including only transplants of DBD grafts preserved with the preservation solutions considered. Additionally, the prespecified set of confounders utilized in the previous steps were added as covariates in each of the models to perform an IPTW adjusted analysis, also known as double robust approach.

Additional details on statistical methodology can be found in the Supplementary Materials. All analyses have been performed using SAS software, version 9.4 of the SAS System for Windows (SAS Institute Inc., Cary, NC).

Results

Between January 1, 2000, and December 31, 2017, 1154 full-size graft LTx were performed in the University Hospitals of Leuven. We excluded 269 patients because of lack of information about preservation solution used ($n = 110$), preservation with other or combinations of solutions ($n = 155$), and perioperative mortality ($n = 4$), leaving 885 patients for the analyses. One hundred ninety grafts were preserved with HTK (21.5%), 557 livers with UW (62.9%), and 139 with IGL-1 (15.7%). Fig. 1 provides a more detailed breakdown of the number of LTx performed per year, split by the preservation solution used.

The unweighted analysis showed significant differences between groups in donor and recipient characteristics such as donor age ($P = 0.015$), donor peak AST, hepatectomy time, cause of death, donor type, bile duct flush, DRI, CIT (all $P < 0.001$), recipient age ($P = 0.030$), Lab-MELD score ($P = 0.022$), BAR score ($P = 0.027$), indication for transplantation, implantation and surgery time, and era of transplantation (all $P < 0.001$) (Table 1; Table S2). Imbalances in baseline characteristics were all adequately corrected by IPTW (Table S3).

Hepatic injury

In the unweighted analysis, peak AST within 7 days after LTx in HTK was 46% higher (95% CI = 14–88%, $P = 0.001$) than in IGL-1 and 65% (95% CI = 34–103%,

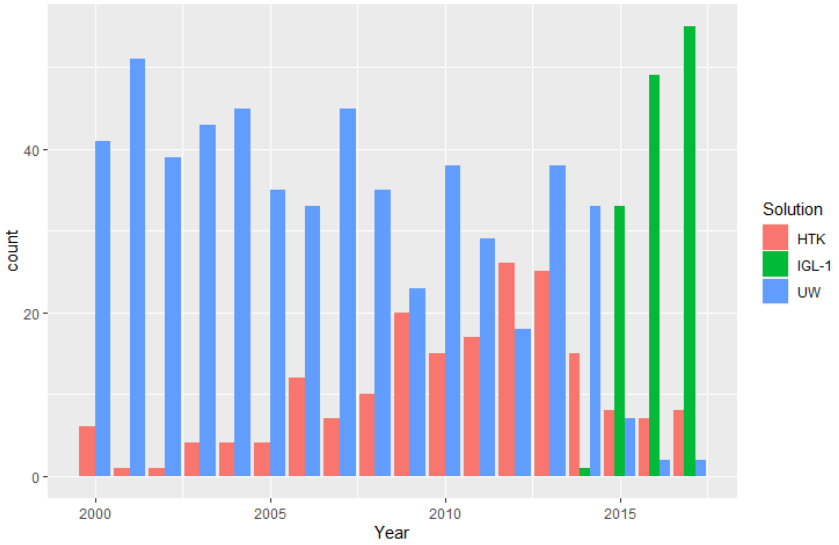

Figure 1 Barplot depicting the number of liver transplants performed per year, grouped by preservation solution.

$P < 0.001$) higher than that of UW (Table 2). The unweighted incidence of a post-transplant peak AST > 2000 was 28.4% in HTK, 12% in UW, and 12.5% in IGL-1 ($P < 0.001$). After weighting, peak AST within 7 days was 44% higher (95% CI = 15–81%; $P < 0.001$) in HTK than in UW but not different from that of IGL-1, whereas the incidence of post-transplant peak AST > 2000 was 24.7% in HTK, 11.7% in UW, and 10.4% in IGL-1 ($P = 0.001$; Table 2). A post-transplant peak AST > 2000 was more likely to occur with HTK than with UW (IPTW unadjusted OR = 2.46, 95% CI = 1.40–4.35; $P = 0.001$), but like that of IGL-1. No difference in odds was observed between UW and IGL-1. After adjusting (double robust approach), HTK remained associated with a post-transplant peak AST > 2000, with similar effect estimates (vs. UW, IPTW adjusted OR = 2.47, 95% CI = 1.36–4.47; $P = 0.001$; Table 3).

Graft function

The unweighted incidence of EAD was 41.7% in HTK, 24.6% in UW, and 17.1% in IGL-1 ($P < 0.001$). After weighting, these were 36.6%, 24.8%, and 16.8% ($P = 0.01$), respectively (Table 2). EAD was more likely to occur with HTK compared to either IGL-1 (IPTW unadjusted OR = 2.87, 95% CI = 1.00–8.19; $P = 0.049$) or UW (IPTW unadjusted OR = 1.75, 95% CI = 1.06–2.88; $P = 0.023$). UW and IGL-1 had similar effect on EAD (Table 3). The unweighted mean MEAF score was 0.90 points (95% CI = 0.47–1.34, $P < 0.001$) higher in HTK that in UW, and 0.61 points (95% CI = 0.04–1.17, $P = 0.031$) higher than IGL-1. After weighting, mean MEAF score was higher in HTK than in UW by 0.61 points (95% CI 0.12–1.10; $P = 0.01$) but did not differ from IGL-1 (Table 2). The weighted analysis adjusted for confounders confirmed that type of preservation solution had a significant impact on the probability to develop EAD ($P = 0.029$). Nevertheless, HTK did not show a significant odds ratio for EAD when compared to either UW (IPTW adjusted OR = 1.68, 95% CI = 1.00–2.84; $P = 0.053$) or IGL-1 (IPTW adjusted OR = 2.70, 95% CI = 0.85–8.59; $P = 0.110$) although the effect estimate was similar to that estimated by the IPTW unadjusted analysis (Table 3).

Biliary strictures

Within 1-year after transplantation, biliary strictures occurred in 48 (25.3%) patients in the HTK group, 110 (19.7%) in the UW group, and 24 (17.3%) in the IGL-1 group. The cumulative incidence curves for biliary strictures and its competing event (Nelson–Aalen estimates) are given in Fig. 2, suggesting no difference in the incidence of biliary strictures between preservation solutions ($P = 0.169$). There was no evidence of an impact of preservation solution on the risk of biliary strictures, neither in the IPTW unadjusted analysis nor in the IPTW adjusted model (Table 3).

Recipient and graft survival

There was no evidence that preservation solution influenced the duration of ICU or total hospitalization after LTx in the IPTW unadjusted analysis or after correction

Table 1. Overview of donor and recipient demographics according to the different preservation solutions used during liver transplantation.

	HTK (n = 190)	UW (n = 557)	IGL (n = 139)	P overall
Donor demographics				
Age (years)	51 (41–61)	53 (39–63)	56 (43–68)	0.015
BMI	24.2 (22.5–26.1)	24.2 (22.5–26.2)	24.5 (22.4–27.3)	0.568
Peak AST	49 (28.5–88.5)	41 (26–71)	70 (36–162)	<0.001
Hepatectomy time (min)	43 (33–56)	33 (24–44)	33 (25–42)	<0.001
Cause of death				<0.001
Trauma	98 (52.4)	242 (43.7)	58 (42.0)	
CVA	77 (41.2)	263 (47.5)	41 (29.7)	
Anoxia	10 (5.4)	32 (5.8)	26 (18.8)	
Other	2 (1.1)	17 (3.1)	13 (9.4)	
Donor type				<0.001
DBD	190 (100)	538 (96.6)	87 (63.0)	
DCD	0 (0.0)	19 (3.4)	51 (37.0)	
Biliary duct flush (yes)	85 (47.0)	477 (88.8)	129 (93.5)	<0.001
DRI	1.93 (1.59–2.24)	1.89 (1.54–2.25)	2.06 (1.65–2.63)	<0.001
CIT (h)	8.49 (7.25–9.63)	7.87 (6.33–9.68)	6.16 (4.60–7.82)	<0.001
Recipient demographics				
Age (years)	57.5 (47–65)	57 (49–64)	60 (52–66)	0.030
LabMELD	16.1 (10.6–27.9)	15.2 (10.5–22.1)	15.7 (10.9–21.1)	0.022
BAR	7 (3–10)	5 (3–10)	7 (4–12)	0.027
Gender (male)	114 (60.0)	338 (60.7)	88 (63.8)	0.760
Indication for transplantation				<0.001
ALF	29 (15.3)	34 (6.1)	2 (1.5)	
MED	8 (4.2)	19 (3.4)	6 (4.4)	
HCC	48 (25.4)	145 (26.0)	30 (21.9)	
Tumor	4 (2.1)	4 (0.72)	0 (0.0)	
PCLD	9 (4.7)	21 (3.8)	8 (5.8)	
Postethyl cirrhosis	24 (12.7)	119 (21.4)	39 (28.5)	
HCV cirrhosis	4 (2.1)	37 (6.6)	2 (1.5)	
HBV cirrhosis	3 (1.6)	16 (2.9)	2 (1.5)	
Cholestatic cirrhosis	19 (10.1)	67 (12.0)	22 (16.1)	
Other cirrhosis	10 (5.3)	13 (2.3)	3 (2.2)	
NASH cirrhosis	9 (4.8)	21 (3.8)	11 (8.0)	
Cryptogenic cirrhosis	4 (2.1)	22 (4.0)	7 (5.1)	
Thrombosis	9 (4.8)	28 (5.0)	3 (2.2)	
Others	9 (4.8)	11 (2.0)	2 (1.5)	
Surgery time (h)	6 (5–7.16)	5.5 (4.75–6.73)	6.88 (5.87–8.42)	<0.001
Implantation time (min)	79 (67–96)	80 (70–92)	62 (42–85)	<0.001
Era of transplantation (year)	2011 (2008–2013)	2006 (2003–2010)	2016 (2016–2017)	<0.001

Data are expressed as n (%) or median (IQR) when not differently indicated.

ALF, acute liver failure; AST, aspartate transaminase; BAR, balance of risk score; BMI, body mass index; CIT, cold ischemia time; DBD, donation after brain death donors; DCD, donation after circulatory death donors; DRI, donor risk index; CVA, cerebrovascular accident; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; LabMELD, lab model for end-stage liver disease score; MED, metabolic disease; PCLD, polycystic chronic liver disease; NASH, nonalcoholic steatohepatitis.

$P < 0.05$ was considered significant.

for confounders (Table 3). Nelson–Aalen estimates for the cumulative incidence of discharge from the ICU and hospital are given in Figures S1 and S2. Patient and graft survival curves are given in Fig. 3. Patient survival 1-year post-LTx was 87.9% (95% CI 83.3–92.0%) for HTK, 91.7% (95% CI 89.3–93.7%) for UW, and 92.8% (95% CI 87.2–95.9%) for IGL-1 (P = 0.17). Graft survival 1-year post-LTx was 87.9% (95% CI 83.3–92.0%) for HTK, 91.6% (95% CI 89.1–93.5%) for UW, and 90.6% (95% CI 84.7–94.3%) for IGL-1 (P = 0.28).

Table 2. Overview of transplantation outcomes. Results from both the unweighted and IPTW analysis are shown.

	HTK (n = 190)	UW (n = 557)	IGL (n = 139)	P overall
Peak AST within 7 days				
Unweighted	1061.03 (914.52; 1231.01)	644.29 (589.59; 704.06)	726.71 (626.61; 842.81)	<0.001
IPTW*	935.23 (791.81; 1104.63)	648.60 (593.72; 708.55)	761.29 (599.43; 966.86)	0.001
Peak AST within 7 days > 2000 (yes)				
Unweighted	48 (28.4%)	57 (12.0%)	16 (12.5%)	<0.001
IPTW*	41.7 (24.7%)	55.7 (11.7%)	13.4 (10.4%)	0.001
MEAF				
Unweighted	5.09 (4.78; 5.41)	4.19 (4.01; 4.37)	4.49 (4.14; 4.83)	<.0001
IPTW*	4.83 (4.46; 5.20)	4.22 (4.04;4.40)	4.73 (4.00; 5.47)	0.008
EAD (yes)				
Unweighted	70 (41.7)	116 (24.6)	22 (17.1)	<0.001
IPTW*	61.7 (36.6)	117.0 (24.8)	21.6 (16.8)	0.010

Data are expressed as n (%) or median (IQR) when not differently indicated.

AST, aspartate transaminase; EAD, early allograft dysfunction; MEAF, model of early allograft function.

$P < 0.05$ was considered significant.

*Adjusted after inverse probability of treatment weighting. Results are based on data from 774 (87.5%) patients with complete data (n = 169 for HTK, n = 474 for UW, and n = 129 for IGL-1).

Preservation solution was not associated with patient and graft survival at 1-year after transplantation (Table 3).

Sensitivity analysis

The sensitivity analysis was performed on 815 DBD livers transplanted during the study period. The results confirmed that preservation solutions influence graft injury (as measured by the post-transplant release of AST, or a peak of AST > 2000), but not the occurrence of biliary complications or 1-year patient and graft survival (Table S4). In contrast, in the sensitivity analysis preservation solution remained significantly associated with EAD after correcting for confounders in the IPTW adjusted analysis and HTK in particular retained its association with EAD (vs. IGL-1, IPTW adjusted OR: 4.02, 95% CI = 1.21–13.33; P = 0.018), similarly to what observed in the overall IPTW unadjusted analysis.

Discussion

The effect of preservation solutions on outcomes after LTx has been subject of many debates. Analyses from the United Network for Organ Sharing (UNOS) [19], European Liver Transplant Registry (ELTR) [20], and Eurotransplant [21] databases have shown conflicting results. Therefore, the relative benefits of various preservation solutions remain unclear.

This single-center study explored the effect of HTK, UW, and IGL-1 on short-term outcomes after liver transplantation, including > 800 LTx performed over a span of time of 18 years. As expected, donor demographics shifted over time toward profiles at higher risk. In particular, LTx in the IGL-1 group were performed more frequently with DCD donors, donors of older age and higher DRI. A double robust approach based on propensity score and additional adjustment for confounders was used to correct this imbalance as much as possible, whereas a sensitivity analysis was performed to further explore specific effects within DBD donors.

In all analyses, including the double robust approach, preservation solution was consistently found to be associated with the severity of graft injury (as measured by the post-transplant release of AST), with HTK being the less effective on protecting the liver graft. Indeed, not only was preserving the liver with HTK associated with a 44% (95% CI 15–81%) increase in peak AST compared to UW, but it was also associated with a 2-fold higher hazard of severe hepatic injury (peak AST > 2000) [22] compared to either UW or IGL-1. These findings are in line with previous studies associating HTK with a higher post-transplant transaminase peak when compared to UW [14,15]. In parallel, grafts preserved with HTK were more likely to develop EAD compared to either UW or IGL-1 and had a higher mean MEAF score compared to UW in our unadjusted analysis. Nevertheless, in the IPTW adjusted analysis

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Table 3. Comparison of short-term outcomes after transplantation of liver grafts preserved with HTK, UW, or IGL-1. Results are given for the unweighted, IPTW unadjusted, and IPTW adjusted (double robust) models

	Unweighted	*P*-value	IPTW unadjusted	*P*-value	IPTW adjusted*	*P*-value
Peak AST within 7 days, ratio between geometric means (95% CI)						
		<0.001		0.001		0.001
HTK vs. IGL-1	1.46 (1.14–1.88)		1.23 (0.87–1.74)		1.19 (0.86–1.66)	
HTK vs. UW	1.65 (1.34–2.03)		1.44 (1.15–1.81)		1.42 (1.13–1.77)	
IGL-1 vs. UW	1.13 (0.92–1.39)		1.17 (0.87–1.59)		1.19 (0.89–1.58)	
Peak AST within 7 days > 2000, OR (95% CI)						
		<0.001		0.001		0.001
HTK vs. IGL-1	2.78 (1.32–5.86)		2.81 (0.97–8.15)		2.88 (0.97–8.59)	
HTK vs. UW	2.90 (1.72–4.89)		2.46 (1.40–4.35)		2.47 (1.36–4.47)	
IGL-1 vs. UW	1.05 (0.51–2.13)		0.88 (0.32–2.43)		0.86 (0.30–2.45)	0.936
EAD, OR (95% CI)						
		<0.001		0.010		0.029
HTK vs. IGL-1	3.47 (1.79–6.74)		2.87 (1.00–8.19)		2.70 (0.85–8.59)	
HTK vs. UW	2.19 (1.40–3.41)		1.75 (1.06–2.88)		1.68 (1.00–2.84)	
IGL-1 vs. UW	0.63 (0.34–1.15)		0.61 (0.23–1.65)		0.62 (0.21–1.87)	
MEAF, difference in geometric means (95% CI)						
		<0.001		0.008		0.019
HTK vs. IGL-1	0.61 (0.04–1.17)		0.10 (−0.89–1.08)		0.03 (−0.95–1.01)	
HTK vs. UW	0.90 (0.47–1.34)		0.61 (0.12–1.10)		0.52 (0.06–0.98)	
IGL-1 vs. UW	0.30 (−0.17–0.76)		0.51 (−0.39–1.42)		0.49 (−0.43–1.41)	
ICU stay, HR (95% CI)†						
		0.012		0.081		0.018
HTK vs. IGL-1	1.00 (0.77–1.30)		1.09 (0.77–1.53)		1.19 (0.81–1.75)	
HTK vs. UW	0.80 (0.65–0.97)		0.88 (0.71–1.10)		0.90 (0.71–1.14)	
IGL-1 vs. UW	0.80 (0.64–0.99)		0.81 (0.61–1.09)		0.75 (0.54–1.04)	
Hospital stay, HR (95% CI)†						
		0.082		0.613		0.951
HTK vs. IGL-1	0.94 (0.72–1.22)		0.89 (0.59–1.33)		0.98 (0.61–1.58)	
HTK vs. UW	0.82 (0.67–1.02)		0.96 (0.77–1.19)		0.97 (0.76–1.23)	
IGL-1 vs. UW	0.88 (0.70–1.10)		1.08 (0.74–1.57)		0.99 (0.64–1.53)	
Biliary stricture, HR (95% CI)						
		0.095		0.190		0.369
HTK vs. IGL-1	1.67 (0.92–3.03)		1.49 (0.64–3.45)		1.40 (0.65–3.02)	
HTK vs. UW	1.38 (0.90–2.10)		1.33 (0.84–2.10)		1.22 (0.76–1.95)	
IGL-1 vs. UW	0.83 (0.48–1.42)		0.90 (0.41–1.97)		0.87 (0.43–1.74)	
1-year patient survival, HR (95% CI)						
		0.244		0.181		1.000
HTK vs. IGL-1	1.58 (0.64–3.95)		1.38 (0.41–4.67)		1.38 (0.41–4.67)	
HTK vs. UW	1.59 (0.83–3.07)		1.68 (0.78–3.60)		1.68 (0.78–3.60)	
IGL-1 vs. UW	1.00 (0.44–2.32)		1.22 (0.40–3.67)		1.22 (0.40–3.67)	
1-year graft survival, HR (95% CI)						
		0.286		0.284		1.000
HTK vs. IGL-1	1.22 (0.53–2.81)		1.27 (0.40–4.09)		1.27 (0.40–4.09)	
HTK vs. UW	1.55 (0.80–2.98)		1.55 (0.72–3.33)		1.55 (0.72–3.33)	
IGL-1 vs. UW	1.27 (0.60–2.70)		1.21 (0.43–3.47)		1.21 (0.43–3.47)	

Peak AST within 7 days was analyzed on log-transformed values.

AST, aspartate transaminase; EAD, early allograft dysfunction; HR, hazard ratio; ICU, intensive care unit; MEAF, model of early allograft function; OR, odds ratio.

$P < 0.05$ was considered significant.

*Adjusted for donor age, donor risk index (DRI), body mass index (BMI), cause of death; recipient age and gender; and cold ischemia time, donor hepatectomy time, and implantation time.

†For ICU and hospital stay, a HR > 1 refers to a higher probability to be discharged alive (hence, shorter length of stay).

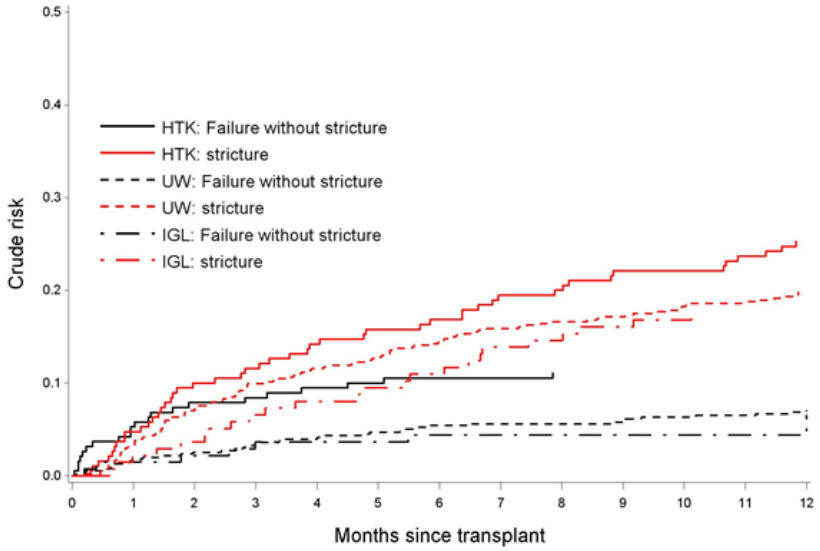


Figure 2 Cumulative incidence curves for biliary strictures and its competing event (graft failure without biliary stricture). Grays test for biliary strictures: $P = 0.169$.

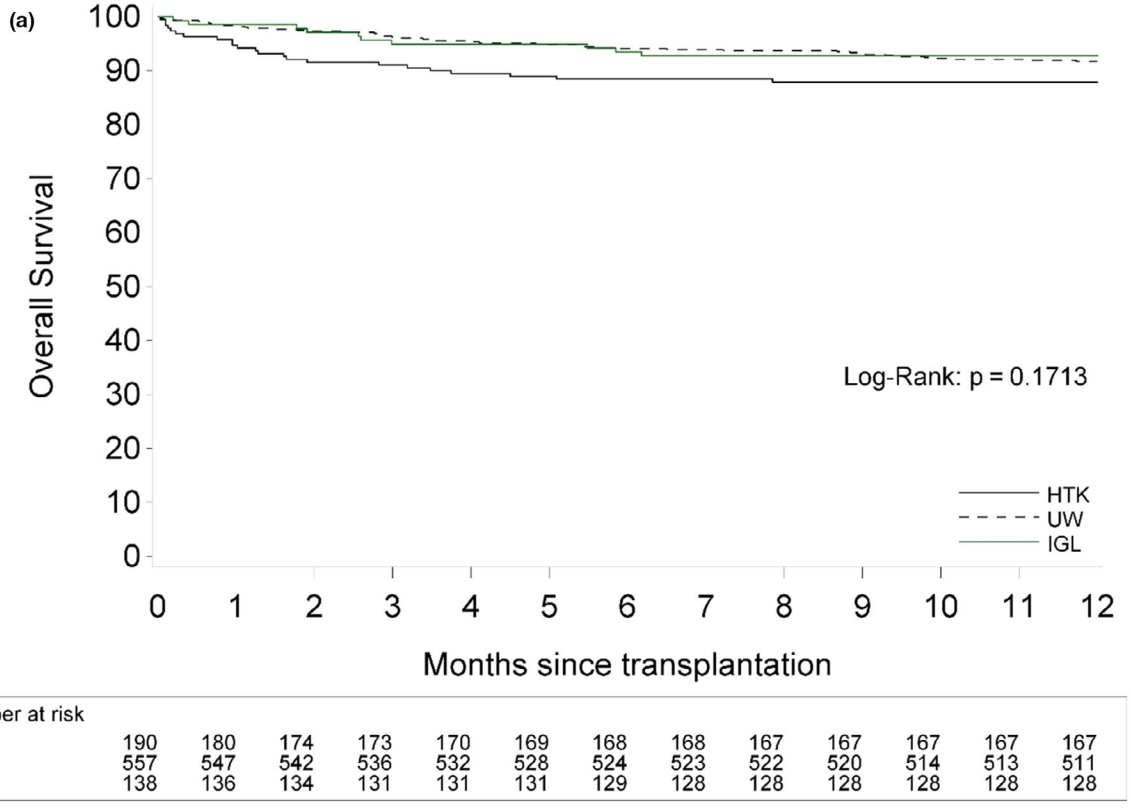
these differences, although having comparable magnitude, were not significant. As donor type was not included in the construction of the propensity scores, we cannot exclude that DCD donors still exerted a confounding effect on outcomes. Indeed, DCDs affected the probability to develop EAD (OR = 0.529, 95% CI 0.279–1.003, $P = 0.051$, Table S1). Additionally, in the sensitivity analysis, based on DBD LTx only, HTK was still associated with EAD in both the IPTW unadjusted analysis and the double robust approach. These findings suggest that with comparable baseline characteristics, a DBD graft preserved with HTK has higher probability to develop EAD, in line with previous large registry studies [19–21]

In both IPTW unadjusted and adjusted analysis, UW and IGL-1 seemed to be equally effective on protecting the liver graft from IRI and preventing EAD. However, as the weighted incidence of both post-transplant peak AST > 2000 and EAD was the lowest in the IGL-1 group, we cannot exclude that any possible advantage derived from the utilization of this preservation solution might have remained undetected in our analyses due to the relative small number of LTx performed with IGL-1.

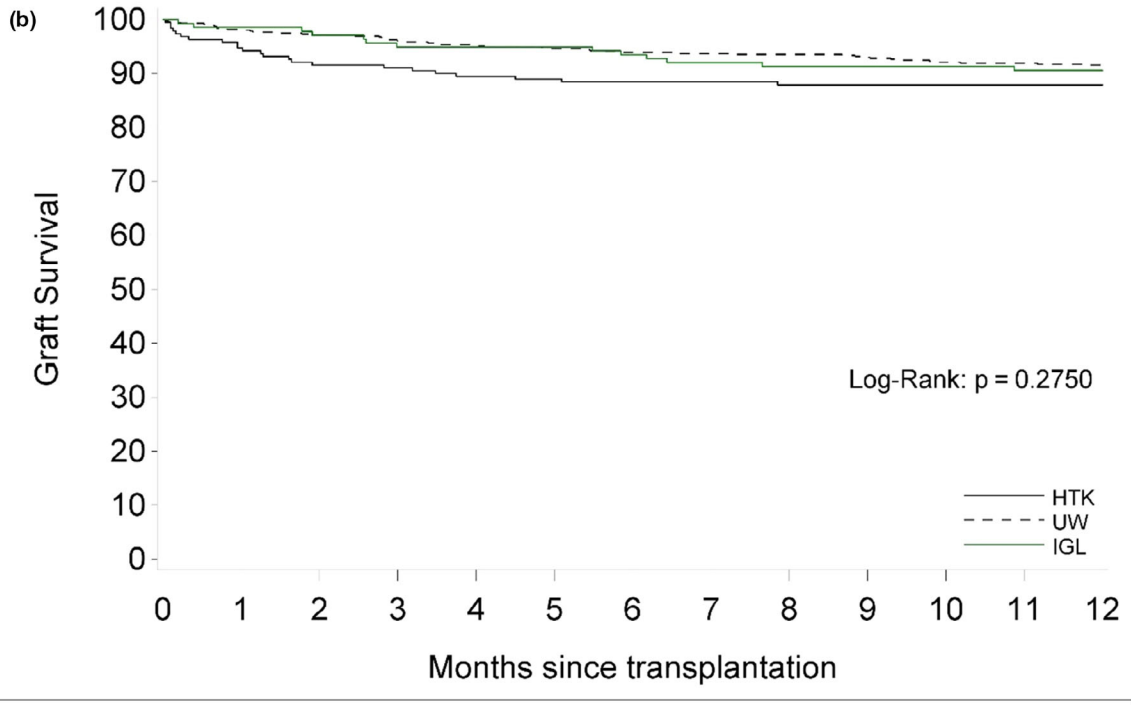
Intuitively, other factors, such as the duration of donor hepatectomy, cold ischemia, and implantation time, have likely influenced the severity of IRI and the probability to develop EAD. Nevertheless, the inverse probability of treatment weighting approach has accounted for baseline differences of these parameters sufficiently, allowing us to compare LTx with comparable duration of these surgical times (Table S3), thereby reducing their confounding effect. Furthermore, a recently performed retrospective analysis investigating the effect of donor hepatectomy and liver implantation time on the probability to develop EAD [23] showed that HTK is associated with the probability of this complication independently from the effect of all abovementioned surgical times, consistent with the findings reported herein.

Despite their association with post-transplant AST release and EAD, we did not observe a significant relationship between preservation solutions and the duration of both ICU and hospital stay, or graft and patient survival at 1-year after transplantation.

The major difference between the preservation solutions considered probably relate to viscosity. Preservation solutions characterized by high viscosity, such as UW, may in theory impair the flush out of livers with altered microcirculation, such as steatotic and DCD grafts [24]. In contrast, preservation solutions with low viscosity have been postulated to improve liver washout during procurement and to better protect from IRI and its complications, such as graft dysfunction and post-transplant cholangiopathy in particular [24,25]. HTK is characterized by low viscosity; nevertheless, its hypothesized beneficial effect on EAD was disproved in an earlier study of the UNOS database [26] and in a recent single-center study [27], and the observations from our analyses point toward the same direction. IGL-1 is also characterized by low viscosity, [28] and preliminary



Number at risk													
HTK	190	180	174	173	170	169	168	168	167	167	167	167	167
UW	557	547	542	536	532	528	524	523	522	520	514	513	511
IGL	138	136	134	131	131	131	129	128	128	128	128	128	128



Number at risk													
HTK	190	180	174	173	170	169	168	168	167	167	167	167	167
UW	557	546	541	535	531	527	523	522	521	519	513	512	510
IGL	138	136	134	131	131	131	129	127	126	126	126	125	125

Figure 3 Kaplan–Meier curves displaying the estimated 1-year survival probability for the three preservation solutions. (a) 1-year patient survival, (b) 1-year graft survival.

pre- and clinical studies suggested that IGL-1 may be particularly suited for the flush out of the microcirculation, thereby reducing the occurrence of post-transplant cholangiopathies [11,29–31]. In this transplant cohort, only a small number of biliary complications were observed and there was no association between the type of preservation solution and post-transplant cholangiopathy. Therefore, we cannot comment on previous findings associating IGL-1 with reduced incidence of biliary complications.

This study has its limitations. Graft steatosis influences the severity of IRI and the probability to develop EAD [32]. Nevertheless, the effect of this possible confounder could not be considered in our models because liver biopsies are not performed routinely in our center. The LTx included in this study have been performed in different eras, with significant changes in donor and recipient demographics. Although the weighting approach in part accounted for this era effect by leveling out the differences in baseline characteristics that reflect the temporal evolution of donors and candidate recipients of a liver transplantation, some potential confounders, such the expertise of the center and/or transplant surgeons, or other yet unidentified characteristics might have been inadequately accounted for, influencing outcomes. The weighted analyses were performed on subjects without missing values in the propensity model (12.5% of the patients had at least one variable missing). Therefore, it is assumed that subjects not included in the analysis were well represented by the ones that were included having the same confounder values, and an exploratory analysis comparing baseline characteristics of included and excluded subjects confirmed that this was the case (Table S5). Finally, as the aim of the study was to characterize the impact of different preservation solutions on short-term results after LTx, nine different outcomes were investigated by means of pairwise comparisons. No formal correction for multiple testing was applied. As such, these analyses should be considered exploratory and results interpreted accordingly. However, despite these inherent limitations, this study provides real-life results from a single center dealing with high-risk donors and contributes relevant additional information in particular on the effect on short-term outcomes of IGL-1, which became in recent years the standard preservation solution in several European countries and in Brazil [33].

In conclusion, in this study the preservation solution utilized to preserve the liver influenced the severity of ischemia–reperfusion injury and the occurrence of graft dysfunction, whereas no effect on other short-term outcomes was observed. Our findings suggest that HTK is the less effective on reducing graft injury and increases the probability of graft dysfunction after transplantation. Although UW and IGL-1 seemed to be equally effective, a possible advantage of the use of the latter cannot be entirely excluded and should be further investigated in larger studies.

Authorship

JVDE, JA, NG and JP: conceived the study. SF, JVDE and JA: collected, analyzed data, performed statistical analyses and drafted the manuscript. IJ, MSB, DM, NG and JP: gave important intellectual contribution and critically revised the manuscript.

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Conflicts of interest

The authors of this manuscript have conflicts of interest to declare. Professor J. Pirenne holds a KU Leuven chair of the IGL company. The other authors have no conflicts of interest to disclose. The IGL company had no role in the conduction of the study and reporting of the results of the clinical trial presented in this manuscript, in line with the editorial policy of Transplant International.

SUPPORTING INFORMATION

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

Supplementary Materials and Methods

Supplementary Results

Table S1 The effect of a prespecified set of confounder on the occurrence of peak AST>2000, EAD, and biliary stricture was explored in univariate analyses

Table S2 Donor and recipient demographics according to the different preservation solutions used during liver transplantation.

Table S3 Donor and recipient demographics before and after inverse probability of treatment weighting

Table S4 Results of a sensitivity analysis performed on donation after brain death liver transplantation only (n = 815), showing the effect of preservation solutions on the outcomes considered after weighting and after adjustment for confounders.

Table S5 Results from an explorative analysis comparing confounding characteristics of liver transplantations included and excluded from the inverse probability of treatment weighting.

Figure S1 Nelson-Aalen estimates for the cumulative incidence of discharge from hospital.

Figure S2 Nelson-Aalen estimates for the cumulative incidence of discharge from ICU.

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