The Effect of Histidine-tryptophan-ketoglutarate Solution and University of Wisconsin Solution: An Analysis of the Eurotransplant Registry

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Background. Both University of Wisconsin (UW) and histidine-tryptophan-ketoglutarate (HTK) solutions are currently used in the Eurotransplant region for preservation of liver allografts. Previous studies on their effect have led to a lot of discussion. This study aims to compare the effect of HTK and UW on graft survival. **Methods.** First liver transplantations in recipients 18 years or older from January 1, 2007, until December 31, 2016, were included. Graft survival was compared for livers preserved with HTK and UW at 30 days, 1, 3, and 5 years. Multivariable analysis of risk factors was performed and outcome was adjusted for important confounders. **Results.** Of all 10628 first liver transplantations, 8176 (77%) and 2452 (23%) were performed with livers preserved with HTK and UW, respectively. Kaplan-Meier curves showed significant differences in graft survival between HTK and UW at 30 days (89% vs 93%, *P*=<0.001), 1 year (75% vs 82%, *P*=<0.001), 3 years (67% vs 72%, *P*<0.001), and at 5 years (60% vs 67%, *P*<0.001). No significant differences in outcome were observed in separate analyses of Germany or non-German countries. In multivariable analysis, UW was associated with a decreased risk of graft loss at 30 days (HR 0.772, *P*=0.002) and at 1 year (0.847 (0.757-0.947). When adjusted for risk factors, no differences in long term outcome could be detected. **Conclusions.** Because the use of preservation fluids is clustered geographically, differences in outcome by preservation fluids are strongly affected by regional differences in donor and recipient characteristics. When adjusted for risk factors, no differences in graft survival exist between transplantations performed with livers preserved with either HTK or UW.

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Ischemic injury sustained during organ preservation influences posttransplantation outcomes in an important way. Throughout the process of organ preservation, preservation fluids are used. In the donor, the liver is perfused with cold preservation fluid after cross-clamping of the aorta. It is then packed in a sterile bag filled with this same fluid in a box with ice after hepatecomy.[1] In the transplant hospital, the organ is perfused before transplantation using the same preservation fluid. Almost all livers within Eurotransplant (ET) are preserved by this "cold storage." Other preservation techniques, such as machine perfusion, are currently only performed in an experimental way.

Several preservation fluids are used within the ET region although most countries use either University of Wisconsin (UW) solution or histidine-tryptophan-ketoglutarate (HTK) solution.[2] The choice of preservation fluid is thought to be important for outcome and a difference in effect on outcome has often been studied. First, studies on the topic could not detect significant differences in short- and long-term patient and graft survivals[2-7] (Table 1). This might have been a result

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TABLE 1.

Studies on the use and effect of perfusion fluids in deceased donor liver transplantation

Author	Year	Journal	Short description	Perfusion fluid	No. patients	Graft survivals						Best
						30 d	3 mo	6 mo	1 y	3 y	5 y	
Adam et al[8]	2015	AJT	Retrospective study on the ELTR database	HTK	8696				77%	69%	64%	UW
				UW	24562				83%	75%	69%	
				CE	7756				82%	73%	68%	
				IGL	1855				82%	75%	68%	
Kaltenborn et al[9]	2014	BMC gastroenterology	Double-center, retrospective study	HTK	1838	No effect in 3 month graft survival, HTK beneficial on long term graft survival in univariate but not in multivariable analysis						HTK
				UW	1314							
Stewart et al[10]	2009	AJT	Retrospective study on the UNOS database	HTK	4755	HTK vs UW, OR 1.2 (1.04-1.39, $P < 0.012$) on early graft loss (<30 days) in multivariable analysis						UW
				UW	12673							
Rayya et al[7]	2008	Transplant procedure	Single-center, retrospective study	HTK	69	90%			71%	71%		UW
				UW	68	90%			78%	75%		
Mangus et al[6]	2008	Liver transplantation	Single-center, retrospective study in ECD livers	HTK	204		89%		84%			HTK
				UW	231		88%		83%			
Meine et al[3]	2006	Transplant procedure	Single-center, randomized, prospective study	HTK	37	No significant differences in 2 years graft survival (death censored)						N/A
				UW	65							
Avolio et al[5]	2006	Transplant procedure	Single-center study	HTK	14			86%				
				UW	21			81%				
Mangus et al[4]	2006	Liver transplantation	Single-center, retrospective study	HTK	174	92%		86%	81%			UW
				UW	204	92%		86%	82%			
Erhard et al[2]	1994	Transplant Int.	Prospective, randomized study	HTK	30		87%			77%		HTK
				UW	30		80%			74%		

of the frequent single-center design and low numbers of included transplantations. A larger study by Stewart et al[10] showed HTK to be associated with a higher risk of early graft loss (<30 days) as compared with UW in the United Network for Organ Sharing (UNOS) database. It contributed to a gradual change to UW although some centers prefer HTK for the lower viscosity and lower costs.

More recent studies of Kaltenborn et al[9] and Adam et al[8] presented conflicting results on the issue. Kaltenborn showed only minimal differences between HTK and UW, whereas Adam et al found HTK to be associated with a significant increased risk of long-term graft loss (at least up to 5 years) as compared with UW in the European Liver Transplant Registry (ELTR).[8] Several remarks and concerns with the design of the study and its conclusions were placed by Nashan et al.[11] Most important concerns were with including living donation, insufficient risk adjustment and the overrepresentation of German livers in the HTK group. Germany uses HTK exclusively and it has a model for end-stage liver disease (MELD)-based allocation combined with one of the lowest donor rates of Europe.[12] The difference in long-term outcome that was attributed to HTK in this study might rather reflect inferior outcomes in general in Germany. In response, Adam et al published an analysis without living donors and German centers and more recently, an analysis based on propensity score matching.[8,13] This analysis matched patients on ABO compatibility; recipient ischemic time of 6 hours or longer; sex; study period (2003-2007 vs 2008-2012); recipient age, 60 years or older, donor age, 55 years or older; whole liver; urgency of transplantation; hepatocellular carcinoma; recipient HIV status; and centers performing more than 10 liver transplantations from living donors. Although an association between HTK and graft loss could be seen, we believe that interregional differences in donor, transplant, and recipient characteristics were insufficiently taken into account.

This study aims to evaluate the effect of HTK and UW on short- and long-term outcomes after liver transplantation in the Eurotransplant region, with adequate adjustment for (regional) differences in donor, transplant, and recipient factors.

PATIENTS AND METHODS

Data Selection

All first transplantations from deceased donor livers performed in adult recipients (≥18 years) from January 1, 2007, until December 31, 2016, were included. Transplantations with livers from donors after circulatory death (DCD) (n = 771), split allografts (n = 380) and allografts from donors outside of Eurotransplant were excluded. When information on the used preservation fluids was missing (n = 160) or when preserved with other preservation fluids than HTK or UW fluid (Celsior n = 18, Eurocollins = 1, IGL-1 n = 79 and other n = 216) transplantations were also excluded as well as transplantations performed in patients with a high-urgency status (n = 888), with a combination other than liver/kidney and transplantations performed in Göttingen.[14] Transplantations were categorized in either HTK or UW according to the preservation fluid that was used during procurement and subsequent transport.

Follow-up data were obtained from the Eurotransplant Network Information System and ET Liver Registry up to September 2017. All data were anonymized for transplant center and patient related data with exception of country. The study protocol was approved by the Eurotransplant Liver Intestine Advisory Committee, and no ethical statement was required according to European guidelines and Dutch law.

Data Analysis

Laboratory values were converted to standardized units and in case of missing values <2%, median values were used; gamma-glutamyl-transpeptidase (GGT) 38 U/L (1.8%) and recipient body mass index (BMI) 25.8 (0%). The Eurotransplant-Donor Risk Index (ET-DRI)[15] was calculated for all transplanted livers and the simplified recipient risk index (sRRI)[16] was calculated for all recipients based on most recent laboratory MELD score before transplantation. With the ET-DRI and sRRI the donor-recipient model (DRM) was calculated for all transplantations.[17] Serum creatinin value was set at 4 mg/d therapy according to ET guidelines for patients receiving renal replacement, MELD score was rounded to the nearest whole value (range, 6-40). Donor hepatitis C antibody (HCVAb), donor hepatitis B core antibody (HBcAb), recipient HCVAb, dialysis of the recipient before transplantation and a history of diabetes in the donor were considered negative if not tested or missing. Rescue allocation is a center-oriented allocation after patient-oriented allocation and is started for short allocation time or medical reasons. Clinical characteristics were summarized by median and 25% and 75% interquartile ranges (IQR) and number and percentage (N/%) for respectively continuous and categorical variables. Numerical and categorical factors between groups were compared using Kruskall-Wallis and χ^2 tests.

Outcome Measures

Primary outcomes used in the analyses were 30 days, 1-, 3-, and 5-year non–death-censored graft survival. Secondary outcomes were 30 days, 1-, 3-, and 5-year patient survival (PS). Graft survival was defined as the period between date of transplantation and date of retransplantation or patient death. Patient survival was defined as the period between date of transplantation and date of patient death. Outcome was analyzed by Kaplan Meier analysis and log-rank tests when stratified by preservation fluid category (HTK, UW). Results were also stratified for transplantation region and preservation fluid (Germany+HTK, Germany+UW and non-Germany+HTK, non-Germany+UW).

Risk Factors

To identify risk factors associated with graft survival, multivariable analysis was performed in a Cox regression analysis (backward selection) for all transplantations and included factors described to be associated with graft survival.[15,17-19] These factors included donor age, cause of death, sex, BMI, latest GGT, HBcAb, HCVAb, history of diabetes, Recipient age, sex, BMI, laboratory MELD score at transplantation, etiology of primary liver disease, liver/kidney combination, dialysis before transplantation, total ischemic time, rescue allocation, allocation region (local, regional, extraregional), and year of transplantation (continuous). Graft survival was then adjusted for all risk factors associated with 5-year graft survival in Germany, non-German countries and all transplantations. A potential effect of preservation fluids in HCC patients or in livers with longer cold ischemic times was described in literature.[8] This potential relation was analyzed with Kaplan-Meier analysis and in a Cox-regression analysis when adjusted for risk factors.

For all analyses a Wald *P* value less than 0.05 was considered significant. Survival analyses were performed using Kaplan-Meier survival models and multivariable analyses were performed using Cox regression models. All analyses were performed with SPSS (version 24.0).

RESULTS

Within the study period, 10628 first liver transplantations were included. Median donor age of all transplantations was 55 years old (IQR, 45-67) and median donor BMI 26 (IQR, 24-28). Cerebrovascular accident was the most frequent cause of death (62%) followed by trauma (20%). Near half of donors was allocated extraregionally (46%) and median ET-DRI was 1.84. Most recipients were male (70%) and had a median age 56 years old and median BMI of 25. Transplanted recipients had a median laboratory MELD score of 16 and a median match MELD score of 24. Alcoholic disease was most frequent primary diagnosis (27%) followed by malignant disease (25%) and other cirrhosis (14%). The majority of transplantations was performed in Germany (62%) followed by Belgium (12%) and Austria (10%). Median sRRI was 1.86 and median DRM was 2.77.

Preservation Fluid Category

Of all transplantations, 8176 (77%) and 2452 (23%) were performed with livers preserved with HTK and UW, respectively. The relative use of UW decreased from 36% in 2007 to 18% in 2016, whereas the use of HTK increased from 64% to 82% (Figure 1). Within donor countries strong preference for either HTK or UW during procurement was seen. HTK is preferred in Hungary (100%), Germany (98%), Slovenia (97%) and Austria (84%), whereas UW is preferred in The Netherlands (98%), Croatia (83%), Belgium (73%) and, with very small numbers, Luxembourg (100%).

Median donor age and BMI were significantly higher in the HTK group as compared with the UW group (56 years vs 55 years, $P < 0.001$) and (26 years vs 25 years, $P < 0.001$), respectively. Cause of death of the donor was significantly

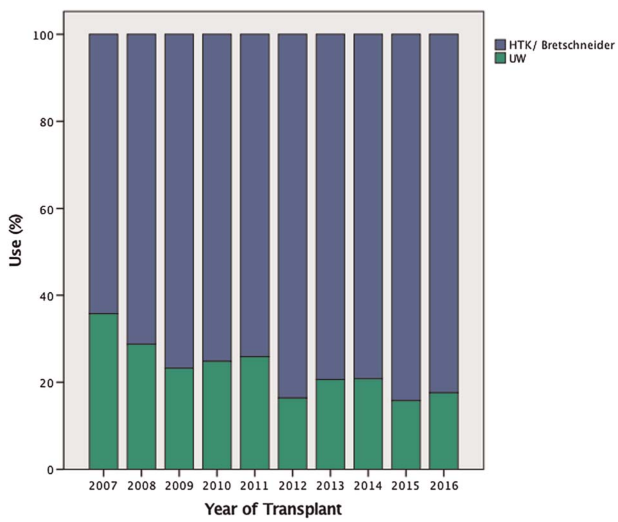

FIGURE 1. The use of HTK and UW in the Eurotransplant region.

TABLE 2.

Donor and recipient characteristics per perfusion fluid, n = 10826

	HTK Bretschneider (n = 8176)	UW (n = 2452)	HTK vs UW
	(n (%)/ Median (25%/ 75% percentile)	(n (%)/ Median (25%/ 75% percentile)	*P*
Donor factors			
Donor age, y	56 (45-67)	55 (43-65)	<0.001
Height, cm	174 (165-180)	174 (167-180)	0.097
Weight, kg	80 (70-90)	76 (68-85)	<0.001
BMI	26 (24-28)	25 (23-28)	<0.001
Last GGT, U/L	43 (22-99)	31 (17-62)	<0.001
Sex (male)	4445 (54)	1366 (56)	0.241
Cause of death			<0.001
Anoxia	1020 (13)	82 (3)	
Circulational	113 (1)	158 (6)	
CNS tumor	44 (1)	19 (1)	
CVA/stroke	5129 (63)	1484 (61)	
Trauma	1426 (17)	648 (26)	
Other	443 (5)	61 (3)	
Diabetes, y	816 (10)	173 (7)	<0.001
Transplant factor			
Total ischemic time, h	8.6 (6.3-11.0)	7.3 (5.0-9.6)	<0.001
Allocation region			<0.001
Local	1980 (24)	1004 (41)	
Regional	1902 (23)	892 (36)	
Extraregional	4294 (53)	556 (23)	
Rescue (yes)	2613 (32)	389 (16)	<0.001
Country			<0.001
Germany	6147 (75)	463 (19)	
Hungary	221 (3)	11 (0)	
The Netherlands	124 (2)	465 (19)	
Belgium	476 (6)	752 (31)	
Croatia	196 (2)	593 (24)	
Slovenia	149 (2)	9 (0)	
Austria	863 (11)	159 (7)	
ET-DRI	1.90 (1.59 -2.24)	1.66 (1.40-1.92)	<0.001
Recipient factor			
Age, y	56 (49-62)	57 (49-62)	0.093
Height, cm	174 (168-180)	173 (167-180)	0.003
Weight, kg	80 (69-90)	78 (68-90)	0.019
BMI	26 (23-29)	26 (23-29)	0.390
Laboratory MELD	16 (11-27)	16 (11-23)	0.001
Match MELD	25 (16-31)	22 (17-27)	<0.001
Exceptional MELD (yes)	2753 (34)	790 (32)	0.181
Sex (male)	5759 (70)	1696 (69)	0.228
Dialysis pretransplant	1002 (12)	157 (6)	<0.001
Primary diagnosis			<0.001
Metabolic	264 (3)	91 (4)	
Acute	158 (7)	28 (1)	
Cholestatic	906 (10)	267 (11)	
Alcoholic	2112 (24)	716 (29)	
Malignant	2060 (24)	628 (26)	
HBV	316 (4)	94 (4)	
HCV	867(10)	211 (9)	
Other cirrhosis	1146 (13)	295 (12)	
Other	347 (5)	122 (5)	

TABLE 2. (Continued)

	HTK Bretschneider (n = 8176)	UW (n = 2452)	HTK vs UW
LabMELD category			<0.001
<15	3515 (43)	1040 (42)	
15-25	2446 (30)	930 (38)	
25-35	1136 (14)	329 (13)	
35+	1079 (13)	153 (6)	
sRRI	1.87 (1.58-2.23)	1.86 (1.58-2.17)	<0.001
DRM	2.85 (2.31-3.51)	2.56 (2.09-3.08)	<0.001

different between both groups ($P < 0.001$); less trauma (17% vs 26%) and more often anoxia (13% vs 3%) were registered as cause death in the HTK group. Total ischemic times were longer in the HTK group in comparison to the UW group (8.6 hours vs 7.3 hours) and HTK livers were more often accepted in rescue allocation (32% vs 16%, $P < 0.001$). The median ET-DRI was significantly higher in the HTK group (1.90 vs 1.66, $P < 0.001$).

Recipient age and BMI were not different in both the UW and HTK group with a median of 56 years old ($P = 0.093$) and BMI of 26 ($P = 0.390$), respectively. Although both groups had a similar median laboratory MELD score, the distribution was not equal ($P < 0.001$). As compared with the UW group, the HTK group has a higher proportion of transplanted MELD 25-35 (14% vs 13%) and MELD 35+ recipients (13% vs 6%). Also, the match MELD did vary between HTK and UW (25 vs 22, $P < 0.001$). Median sRRI showed only minor differences, whereas the DRM was significantly higher in the HTK group 2.85 versus 2.56 ($P < 0.001$), data shown in Table 2.

Outcome

For all transplantations, graft survival at 30 days, 1, 3 and 5 years was 90%, 77%, 68%, and 62%, respectively. Graft survival was significantly better in the UW group as compared with HTK at 30 days (93% vs 89%, $P = <0.001$), 1 year (82% vs 75%, $P = <0.001$), 3 years (72% vs 67%, $P < 0.001$), and at 5 years (67% vs 60%, $P < 0.001$), as shown in Figure 2A. Similar differences were found in PS; transplantations with UW-preserved livers showed better PS as compared with HTK at 30 days (95% vs 93%, $P = <0.001$), 1 year (86% vs 79%, $P = <0.001$), 3 years (78% vs 71%, $P < 0.001$), and at 5 years (72% vs 65%, $P = <0.001$), as shown in Figure 2B.

Within Germany, 6174 transplantations were performed with HTK and 463 with UW. In non-German countries, 2029 and 1989 transplantations were performed with HTK- and UW-preserved livers, respectively. Outcome stratified for transplantation region (Germany/non-Germany) and preservation fluid (HTK/UW) showed significantly lower overall graft survival in Germany. Within both regions, a trend for a slightly higher graft survival on short term was seen for UW-preserved livers as compared with HTK livers. On long-term, HTK livers showed a trend toward better graft survival. This was observed in Germany at 30 days (HTK 87% vs UW 88%), 1 year (HTK, 72% vs UW, 73%), 3 years (HTK 64% vs UW 64%), and at 5 years (HTK, 57% vs UW, 56%). In non-Germany this was also observed at 30 days (HTK 93% vs 94%), 1 year (HTK, 83% vs

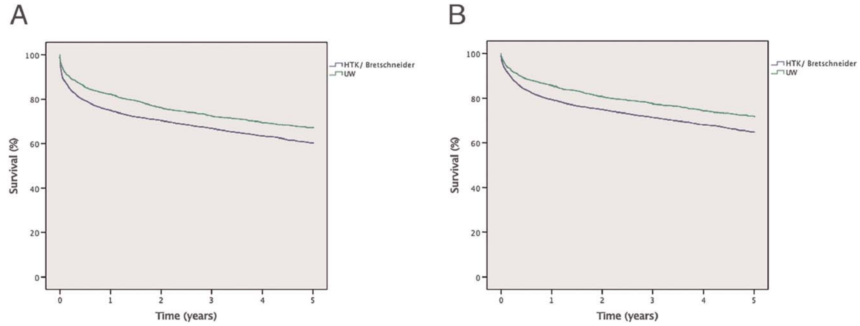

FIGURE 2. Kaplan-Meier survival analysis by preservation fluid (n = 10628). A, Graft survival. B, Patient survival.

84%),3 years (HTK, 76% vs UW, 74%) and at 5 years (70% vs 70%) (data shown in Figure 3). Differences in outcome within both regions were not statistically significant at any time point.

Risk Factors

In multivariable analysis, donor age, total ischemic time, donor last GGT, a history of diabetes in the donor, allocation region, rescue, recipient age, sex, etiology of liver disease, dialysis before transplantation, laboratory MELD score and year of transplantation were associated with 5-year graft survival. An association between outcome and preservation fluids could only be detected on short-term. UW was associated with a decreased risk of graft loss at 30 days (hazard ratio [HR], 0.762; 95% confidence interval [CI], 0.643-0.902; $P = 0.002$) and at 1 year (HR, 0.835; 95% CI, 0.746-0.0.934; $P = 0.002$), data are shown in Table 3. When adjusted for all risk factors associated with 5-years graft survival, no difference could be detected between both preservation fluids in transplantations performed in Germany ($P = 0.572$) (Figure 4A) or non-Germany ($P = 0.522$) (Figure 4B). In all transplantations, also no difference in long-term outcome could be shown (data are shown in Figure 4C).

Risk Groups

Of all transplantations, 3527 (33%) of patients had a registered HCC. Patients with HCC had lower graft survival when transplanted with a liver preserved with HTK (n = 2747) as compared with livers preserved with UW (n = 780) at 30 days (90% vs 93%, $P = 0.013$) and at 1 year (77% vs 81%, $P = 0.006$). When adjusted for other risk factors, a potential effect of HTK or UW in HCC patients was not observed at 30 days ($P = 0.557$) or at 1 year ($P = 0.424$).

When transplantations were stratified according to the ELTR total ischemic times categories, 3 groups were identified; livers transplanted with <=6 hours (n = 2700), 6-12 hours (n = 6231) and > =12 hours (n = 1697) of cold ischemic time. Only in transplantations performed with livers with 6 to 12 hours of cold ischemic time a statistically significant difference between HTK and UW could be observed (60% vs 69%, $P < 0.001$) (data are shown in **Figure S1a-c, SDC,** http://links.lww.com/TP/B622). When adjusted for other risk factors, or when analyzed per region (Germany vs non-Germany) this potential negative impact of HTK in livers with longer cold ischemic times was not observed (data are shown in **Figure S2-3a, b, c, SDC,** http://links.lww.com/TP/B622).

DISCUSSION

This study shows that HTK is used in the majority of organ transplantations within Eurotransplant. The use of HTK is increasing, in contrast to UW. Overall graft survival is lower for livers preserved with HTK, but these results are strongly affected by regional differences in donor, recipient and transplant characteristics. When adjusted for these risk factors, no difference between HTK and UW could be observed.

The issue of preservation fluids remains an important point of discussion in liver transplantation. Although evidence is still considered nonconclusive, different preservation fluids are currently used. This study shows that although UW is internationally considered the golden standard, the relative use of UW within ET is decreasing, whereas the use of HTK is increasing. To compare the effect of both preservation fluids, we have tried to ensure a homogenous study population. We have excluded all pediatric recipients, those receiving living related livers, livers from DCD donors, split livers and transplantations in high-urgent patients. Even with these strict inclusion criteria, this study includes a sufficiently high number of transplantations to detect minor differences in outcome and to perform an adequate multivariable analysis. The unfavorable characteristics of the group of livers preserved with HTK are likely to have contributed to the inferior graft survival and PS. We have therefore separated our analysis per region, and have adjusted outcome for risk factors to interpret the differences in graft survival and PS. The high completeness for important data like total ischemic times and MELD

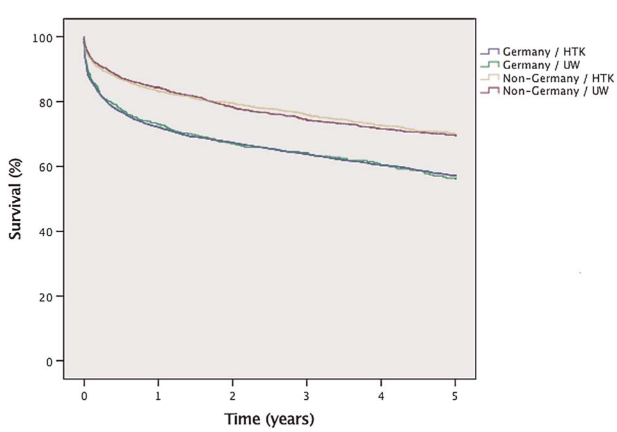

FIGURE 3. Kaplan Meier survival analysis of graft survival by preservation fluid and transplant region (Germany vs non-Germany), (n = 10628).

TABLE 3.

Multivariable analysis of factors associated with graft survival

	30 d		1 y		3 y		5 y	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Donor factor								
Preservation fluid: (HTK) UW	0.762 (0.643-0.902)	0.002	0.835 (0.746-0.934)	0.002	[a]		[a]	
Age, y	*		1.007 (1.004-1.009)	<0.001	1.009 (1.006-1.011)	<0.001	1.009 (1.006-1.011)	<0.001
Total ischemic time, h	1.031 (1.015-1.047)	<0.001	1.026 (1.015-1.037)	<0.001	1.017 (1.007-1.026)	0.001	1.016 (1.007-1.025)	0.001
Last GGT	1.001 (1.001-1.002)	<0.001	1.001 (1.000-1.001)	0.006	1.000 (1.000-1.001)	0.020	1.000 (1.000-1.001)	0.016
BMI	1.013 (1.000-1.027)	0.050	[a]		[a]		[a]	
Diabetes: (no) yes	1.299 (1.076-1.570)	0.007	1.214 (1.065-1.385)	0.004	1.231 (1.097-1.382)	<0.001	1.207 (1.080-1.348)	0.001
Allocation (local)	[a]			0.039		0.003		0.001
Regional	[a]		1.077 (0.954-1.215)	0.230	1.078 (0.972-1.196)	0.154	1.074 (0.974-1.185)	0.151
Extraregional	[a]		1.158 (1.033-1.297)	0.012	1.182 (1.072-1.303)	0.001	1.190 (1.085-1.305)	<0.001
Rescue: (no) yes	1.345 (1.159-1.560)	<0.001	1.212 (1.091-1.346)	<0.001	1.218 (1.113 -1332)	<0.001	1.219 (1.121-1.326)	<0.001
Recipient factor								
Age	[a]		1.011 (1.006-1.015)	<0.001	1.012 (1.007-1.016)	<0.001	1.011 (1.008-1.015)	<0.001
Sex: (female) male	[a]		1.143 (1.040-1.256)	0.005	1.177 (1.083-1.280)	<0.001	1.183 (1.092-1.280)	<0.001
BMI	1.016 (1.003-1.029)	0.017	[a]		[a]		[a]	
Etiology (metabolic)		0.002		0.005		<0.001		<0.001
Acute	1.897 (1.206-2.984)	0.006	1.389 (0.987-1.954)	0.059	1.372 (1.008-1.866)	0.044	1.398 (1.035-1.889)	0.029
Cholestatic	1.103 (0.751-1.622)	0.616	1.135 (0.871-1.480)	0.348	1.057 (0.836-1.336)	0.646	1.102 (0.877-1.383)	0.404
Alcoholic	0.918 (0.642-.1.313)	0.641	0.990 (0.773-1.267)	0.935	0.926 (0.745-1.152)	0.491	0.990 (0.802-1.223)	0.928
Malignant	1.016 (0.704-1.466)	0.932	1.074 (0.832-1.385)	0.585	1.116 (0.894-1.394)	0.332	1.195 (0.964-1.481)	0.105
HBV	1.023 (0.653-1.602)	0.921	0.872 (0.634-1.201)	0.402	0.887 (0.672-1.171)	0.399	0.913 (0.698-1.194)	0.505
HCV	1.119 (0.764-1.640)	0.563	1.271 (0.978-1.652)	0.073	1.408 (1.120-1.769)	0.003	1.476 (1.183-1.843)	0.001
Other cirrhosis	0.943 (0.648-1.372)	0.758	1.010 (0.780-1.308)	0.940	1.002 (0.798-1.258)	0.986	1.052 (0.843-1.312)	0.655
Other/unknown	1.283 (0.816-2.016)	0.280	0.986 (0.706 -1.378)	0.936	0.786 (0.581-1.062)	0.117	0.823 (0.616-1.098)	0.186
SLK (yes)	0.578 (0.371-0.901)	0.016	0.748 (0.567-0.986)	0.039	[a]		[a]	
Dialysis pretransplant (no) yes	1.417 (1.153-1.742)	0.001	1.489 (1.296-1.709)	<0.001	1.231 (1.097-1.382)	<0.001	1.402 (1.246-1.578)	<0.001
LabMELD (<15)		<0.001		<0.001		<0.001		<0.001
≥ 15 and < 25	1.044 (0.889-1.226)	0.598	1.083 (0.970-1.209)	0.158	1.041 (0.947-1.143)	0.405	1.042 (0.954-1.138)	0.363
≥ 25 and < 35	1.356 (1.100-1.671)	0.004	1.580 (1.374-1.817)	<0.001	1.434 (1.268-1.623)	<0.001	1.347 (1.196-1.516)	<0.001
≥ 35	1.776 (1.403-2.248)	<0.001	1.976 (1.683-2.320)	<0.001	1.799 (1.560-2.075)	<0.001	1.705 (1.487-1.956)	<0.001
Year of transplantation (2007)	0.975 (0.954-0.998)	0.030	0.979 (0.964-0.995)	0.009	0.984 (0.970-0.997)	0.984	0.985 (0.972-0.999)	0.033

[a]No statistical significance and not in the equation.
The following factors were not statistically significantly associated with outcome at the measured time points: donor sex, cause of death, HBcAb, HCVAb, recipient sex, HCVAb.

score add to the reliability of our findings. Although performed with care, risk adjustment may still not be sufficient as is inherent to the retrospective design. We considered graft survival as primary outcome and did not have information on biliary complications or early bile production. This is a potential limitation, because some studies found suggestions for more posttransplantation bile production and less biliary complications in livers that were preserved with HTK.[20] However, biliary complications will likely also affect graft-survival in the long run.

The presented results of inferior *unadjusted* graft survival between HTK and UW are in line with the previously published study by the ELTR.[8] The ELTR study attributed this inferior long-term outcome to the use of HTK. Interesting, because the risk of HTK on graft loss was one of the lowest of all risk factors and only just statistically significant (risk ratio 1.1, *P* = 0.02) in over 34 500 transplantations.[8] Based on our findings, differences in long-term outcome in particular, are more likely to reflect differences in donor, recipient and transplant risks than an effect of the preservation fluid itself. When these differences are adequately taken into account no statistically significant difference could be detected between HTK and UW. This finding is in accordance to other studies that could not show any significant differences between HTK and UW.[2-7] Although this could be a result of an inadequate power due to small numbers, also Kaltenborn et al[9] neither have shown a difference in risk between both fluids despite a sizeable data set (summary in Table 1). A slightly better *short term* graft survival in livers preserved with UW, as reported by Stewart et al,[10] may be present according to the risk adjusted survival in non-German countries (Figure 3B).

Some studies have also described a more pronounced effect of preservation fluids in several subgroups. This would affect livers from DCD donors,[10] livers with total ischemic times longer than 12 hours,[8] patients with a HCC[8] and split liver allografts.[8] A potential difference in DCD donors and split procedures could not be analyzed because these were excluded in this study. Differences in the other mentioned subgroups (categorical total ischemic time groups, HCC recipients) were not confirmed in this study or did not persist when adjusted for other risk factors.

To correctly interpret differences in outcome between several preservation fluids, the hypothesized causative pathway is important. The mechanism through which HTK would

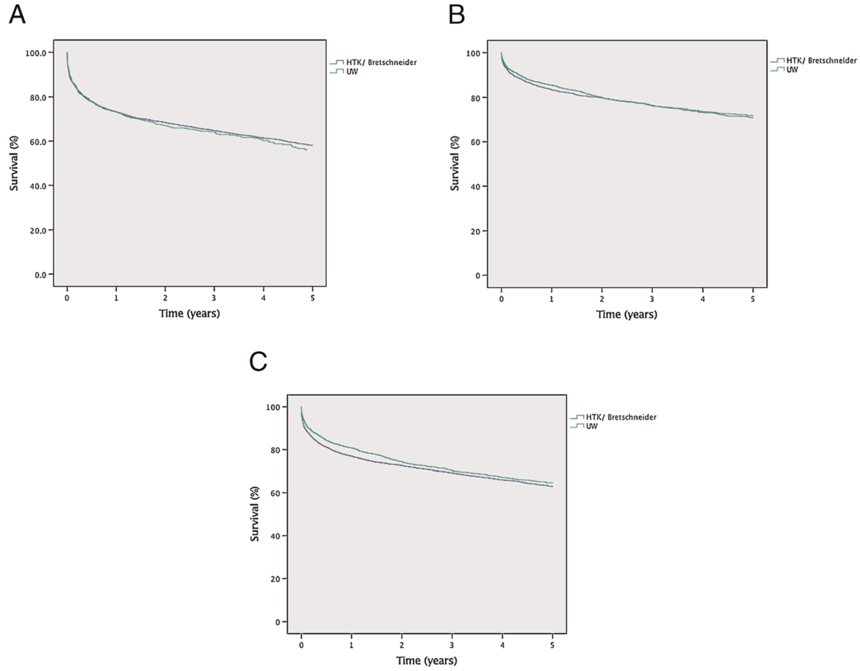

FIGURE 4. Risk adjusted graft survival. A, Germany adjusted for all separate risk factors. B, Non-Germany adjusted for all separate risk factors. C, All transplantations adjusted for all separate risk factors.

be inferior is, however, currently still unclear. It could be related to differences in composition and viscosity[2] which might lead to different effects in liver cell volume, efficiency of washout or to the presence of antioxidant agents.[21,22] These effects would, in theory, especially affect short term graft survival.

The differences in donor, transplant and patient characteristics between HTK and UW are primarily a result of the national choice of preservation fluids. Germany, for example, used HTK in 97% of all procurements and in 93% of their transplantations (the difference is because of international exchange within Eurotransplant). When compared with all HTK transplantations in Eurotransplant, 75% of all HTK-preserved livers are transplanted in Germany. A country that has been struggling with one of the lowest DBD donor rates in Europe[12] and has implemented a MELD based allocation system. Both are likely to impact posttransplantation outcome in a negative way (Figure 4). Because of the low donation rates, limits for liver allografts have been stretched and liver grafts are in general of lower quality; higher donor age, lab values and BMI. Also, because of the shortage of grafts, the waiting list expands and recipients will only be able to receive an offer when their MELD-score raises.[23]

For this reason, outcome was stratified for Germany versus all other countries. It is therefore interesting, that transplantations with HTK livers showed a trend for similar or better graft survival as compared with UW in both regions although this difference was not statistically significant. This statistical phenomenon where findings in subgroups are apparently contradictory to overall results is called a Simpson's paradox. It can exist when different sample sizes are compared of groups with different outcome. In this case, because of discrepancies in the use of preservation fluids between countries with different posttransplantation outcome. The latter affects outcome of UW livers in Germany: Germany almost exclusively uses HTK so livers perfused with UW are likely to originate from other ET countries. This is the case for livers that were not accepted for transplantation in the donor country.

The significant differences in outcome within Eurotransplant are also observed when results from ET are compared with the United States. The presented 1-year graft survival rates in non-German countries of about 83% are significantly lower than the approximately 90% 1-year graft survival for first liver transplantations in the United States in 2016.[24] We believe that a difference in liver quality between ET and the US attributes to this difference in outcome. This difference in donor quality was shown by Blok et al in 2012[25] and is evident for donor age; about 66% of all livers used for a transplant in the United States in 2016 were from donors younger than 50 years old[24] as compared with 36% in ET (median age, 55 years).[23] This might be a result of regulation on center outcome as is done in the United States or by an assumed higher shortage of organs in ET. Regardless of the reason(s), the difference in donor quality shows that centers in ET have expanded their criteria for acceptable donors to increase the number of patients that can be transplanted and to decrease waiting list mortality. This strategy, however, comes at the cost of slightly inferior posttransplantation outcome.

In deciding what preservation fluid to use, the experience of surgeon and center should be the most important consideration. Our results indicate that no significant difference exists between both preservation fluids. Other aspects, like the lower viscosity, which is often appreciated by clinicians and the lower costs associated with the use of HTK might then also be taken into account.

CONCLUSIONS

The use of preservation fluids differs significantly per country within the Eurotransplant region. Histidine-tryptophan-ketoglutarate is being used in the majority of liver transplantations and its use is increasing, in contrast to the use of UW. This retrospective database analysis shows that differences in outcome by preservation fluids are caused by regional differences in donor, recipient and transplant characteristics. These differences, rather than the used preservation fluid, cause the difference in outcome. When adjusted for these risk factors, no differences in graft survival exist between transplantations performed with livers that are preserved with either HTK or UW.

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