2017-06-12

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Donor Diabetes and Prolonged Cold Ischemia Time Synergistically Increase the Risk of Graft Failure After Liver Transplantation

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Background. Both prolonged cold ischemia time (CIT) and donor history of diabetes mellitus (DM) are associated with reduced graft survival after liver transplantation. However, it is unknown whether the adverse effect of prolonged CIT on posttransplant graft survival is more pronounced after transplant with DM versus non-DM donor grafts. **Methods.** The study sample included 58 226 liver transplant recipients (2002-2015) from the Scientific Registry of Transplant Recipients. Multivariable Cox survival regression with interaction analysis was used to quantify the extent to which history of donor DM (n = 6478) potentiates the adverse effect of prolonged (≥8 hours) CIT (n = 18 287) on graft survival. **Results.** Donor DM and CIT 8 hours or longer were each associated with increased risk of graft failure (GF) (adjusted hazard ratio [aHR], 1.19; 95% confidence interval [CI], 1.06-1.35 and aHR, 1.42; 95% CI, 1.32-1.53, respectively) compared with transplanted grafts without either risk factor. However, the combination of DM and CIT 8 hours or longer was associated with a higher risk of GF than either factor alone (aHR, 1.79; 95% CI, 1.55-2.06) and had a synergy index of 1.30. The interaction was significant on a multiplicative scale in the later postoperative period, days 31 to 365 (P = 0.047). **Conclusions.** These results suggest that liver grafts from DM donors are more susceptible to the adverse effects of prolonged CIT than livers from non-DM donors. We need to be cognizant that they are more susceptible to ischemic injury, and this may be considered during the allocation process.

(*Transplantation Direct* 2017;3: e173; doi: 10.1097/TXD.0000000000000692. Published online 12 June, 2017.)

Liver transplantation (LT) provides an effective treatment for patients with end-stage liver disease. Due to persistent organ shortages, approximately 2000 patients die while on the liver waiting list every year.[1] To overcome this gap, there has been an increase in the use of organs from deceased donors that have multiple comorbidities and other factors that are associated with increased risk of graft failure (GF).

The prevalence of diabetes mellitus (DM) among US adults in 2011 was 23.7 million and this number is expected to increase to 29.6 million in 2030.[2] Only few studies regarding the effect of donor DM on the risk of GF have been carried out.[3,4] It has been suggested that DM in donor livers may be associated with adverse outcomes posttransplant.[3] DM often results in systemic vascular damage (diabetic microangiopathy)[5] and is a risk factor for chronic hepatic injury due to nonalcoholic fatty liver disease and subsequent progression to more advanced liver diseases.[6]

Prolonged cold ischemia time (CIT) is a well-established risk factor for GF. Ischemic injury damages the liver graft at the cellular level and may lead to primary nonfunction, delayed graft function, and ischemic cholangiopathy.[7] Ischemia-reperfusion injury (IRI) is associated with the release of reactive

Received 3 March 2017. Revision requested 12 April 2017.

Accepted 29 April 2017.

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Funding of this study was supported by a research grant of the Maag, Lever, Darm Stichting (MLDS), the Netherlands.

The authors declare no conflicts of interest.

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I.M.A. participated in research design, drafting the work, and data analysis. No conflict of interest to declare. N.H.D. participated in research design, drafting the work, and data analysis. R.J.P. participated in research design, critical revising of the work, and data analysis. A.B. participated in research design, critical revising of the work, and data analysis. P.N.M. participated in research design, critical revising of the work, and data analysis.

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ISSN: 2373-8731

DOI: 10.1097/TXD.0000000000000692

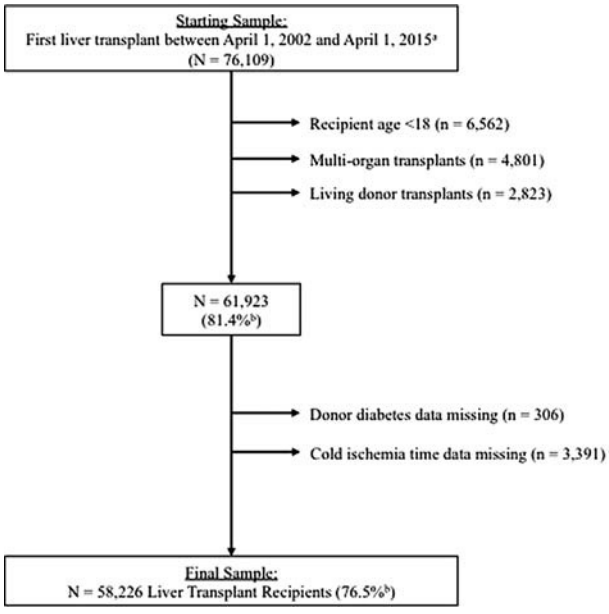

FIGURE 1. Study Inclusion/exclusion criteria flowchart. Scientific Registry of Transplant Recipients. [a] Retransplants were not included in analyses. [b] Percent = $N_{remaining}$ / $N_{starting\ sample}$. [c] Extreme values (>18 hours) change to missing.

oxygen species and proinflammatory mediators, which causes damage of the hepatic sinusoidal epithelium and severe hepatic microcirculatory impairment.[8]

We hypothesized that prolonged cold ischemia aggravates preexisting microvascular changes that are seen in diabetic donors grafts, leading to inferior graft survival. We explored this question using over a decade of comprehensive US Transplant Registry data and quantified the extent to which the effects of donor DM on risk of GF 1 year post-LT are modified by prolonged CIT.

MATERIALS AND METHODS

Study Design

This retrospective cohort study includes subjects that underwent first LT between April 1, 2002, and April 1, 2015, using Scientific Registry of Transplant Recipients (SRTR) data (Figure 1). Exclusion criteria included: recipient age, younger than 18 years (n = 6562), multiorgan (n = 4801) or living donor (n = 2823) transplants, and patients with missing or extreme values (>18 hours for CIT) on key predictor variables, donor DM (n = 306) and CIT (n = 3391). Retransplants were also excluded from analysis.

The primary study endpoint was 1-year GF, defined as time to all-cause GF or retransplant. 1-month and 3-year GF were included as secondary outcomes to investigate potential early, and long-term consequences of IRI. Separate survival models were fit for 1-year and 3-year results with appropriate censoring as each person either died or was lost to follow up. The primary exposure variables were prolonged CIT (≥8 hours) and history of donor DM (DM+) versus no DM (DM−). CIT was evaluated as a potential effect measure modifier of donor DM on graft survival rate. CIT threshold was dichotomized into CIT 8 hours or longer versus CIT less than 8 hours based on previous publications.[3] Sensitivity analyses were done for CIT of 6, 8, and 10 hours as well as using up to 5 categories of CIT. The results were not presented in the final analyses, because they did not meaningfully change the results.

Potential confounders included both donor and recipient factors and were identified based on established clinical evidence and literature review.[3,4,9] Recipient characteristics were collected perioperatively pretransplant and included age, sex, race/ethnicity, body mass index (BMI), history of diabetes, last laboratory Model for End-Stage Liver Disease (MELD) score, United Network for Organ Sharing (UNOS) Status 1, primary liver diagnosis, hepatitis C status, Child-Pugh score (and individual components albumin, bilirubin, encephalopathy, and ascites), medical condition (intensive care unit, hospitalized, or home) and whether patients were receiving ventilatory support. Donor characteristics included Donor Risk Index and its components (age, race/ethnicity,

TABLE 1.

Recipient pretransplant characteristics by donor DM status

Characteristics[a]	Donor diabetes (n = 6478)	No donor diabetes (n = 51 748)	*P*
Age, y	55.1 ± 9.6	53.9 ± 10.1	<0.001
Women	29.6	32.7	<0.001
Race/ethnicity			
White	72.2	72.2	
Hispanic	12.8	12.8	
African American	9.5	9.2	
Other	5.5	5.8	0.78
BMI[b]			
< 18.5	1.6	1.8	
18.5 to < 25	25.9	27.0	
25 to < 30	33.5	34.3	
≥ 30	36.0	33.5	0.001
Diabetes	24.7	22.7	<0.001
Primary liver diagnosis			
Noncholestatic	63.6	64.2	
Cholestatic	7.4	7.8	
Malignancy	20.3	17.9	
Acute hepatic necrosis	4.0	4.9	
Other	4.7	5.2	
Hepatitis C	39.3	42.1	<0.001
UNOS status 1	2.1	3.0	<0.001
MELD (last laboratory)			
< 15	32.9	30.1	
15-29	48.6	47.6	
30-34	7.9	8.7	
35-40	10.6	13.6	<0.001
Albumin	3.0 ± 0.7	3.0 ± 0.7	0.65
Medical condition			
Home	74.5	71.0	
Hospitalized	15.9	17.2	
ICU	9.7	11.8	<0.001
Ventilatory support	3.8	5.1	<0.001

SRTR 2002-2015 (N = 58 226).

[a] Mean ± standard deviation or column percentage (variables with missing data may not add up to 100%).

[b] <18.5: underweight; 18.5-25: normal; 25-30: overweight; ≥30: obese.

ICU, intensive care unit.

TABLE 2.

Donor characteristics by donor DM status

Characteristics[a]	Donor diabetes (n = 6478)	No donor diabetes (n = 51 748)	P
Donor Risk Index[b]	1.88 ± 0.48	1.57 ± 0.45	<0.001
Age, y	54.0 ± 12.8	40.4 ± 16.9	<0.001
Women	44.7	40.0	<0.001
Race/ethnicity			
White	56.7	68.7	
African American	25.6	16.2	
Other	17.8	15.2	<0.001
BMI[c]			
< 18.5	1.6	3.3	
18.5- < 25	22.2	39.1	
25 to < 30	31.2	33.6	
≥ 30	44.6	23.6	<0.001
Height, cm	170.1 ± 10.4	171.8 ± 10.6	<0.001
Hypertension	79.6	29.3	<0.001
Cause of death			
Head trauma	12.0	38.6	
Anoxia	28.2	19.7	
Stroke	57.5	39.1	
Other	2.3	2.6	<0.001
Donation after cardiac death	9.7	11.4	<0.001
Split/partial liver	0.2	1.5	<0.001
Allocation type			
Local	68.3	72.8	
Regional	23.5	22.5	
National	8.3	4.8	<0.001

SRTR 2002-2015 (N = 58 226).

[a] Mean ± standard deviation or column percentage (variables with missing data may not add up to 100%).

[b] Donor risk index includes age, race/ethnicity, cause of death, donation after cardiac death, partial/split liver, height, allocation type, CIT (Feng et al).

[c] <18.5: underweight; 18.5- < 25: normal; 25- < 30: overweight; ≥30: obese.

cause of death, donation after cardiac death, partial/split liver, height, allocation type), sex, BMI, and history of hypertension.

Statistical Analyses

Variables were assessed for missingness and extreme values; variables with more than 5% missing values were not included in tables or regression analyses. A full list of variables in the SRTR database can be found on the associated website.[10] Complete-case analyses were conducted, whereas only subjects with complete data on all variables included in the statistical equation were included in the models. We evaluated the relationships between potential confounders and the primary exposure variable using *t* tests for continuous variables and χ^2 tests for categorical variables. Results are expressed as mean ± SD unless otherwise indicated.

Graft survival rates were estimated and tested using the Kaplan-Meier method and log-rank tests, respectively. Cox proportional hazards survival regression was used to examine the relationships between the primary predictors of interest and graft survival. Multivariable models were built for each predictor (donor DM, CIT) separately and then in combination using a forward (manual) approach, sequentially adding conceptually meaningful groups of variables while assessing model fit. Only variables that were statistically significant were retained in the final model. Model goodness-of-fit and proportional hazards assumptions were assessed graphically and confirmed with the Grønnesby and Borgan test using martingale residuals.[11]

Effect measure modification was assessed by evaluating departures from additivity using dummy variables for each possible combination of DM and CIT (short CIT, DM- (referent group) / short CIT, DM+ / prolonged CIT, DM- / prolonged CIT, DM+) and multiplicative effects were assessed using a product term (interaction) for DM x CIT.

Results are expressed as hazard ratios (HR) (95% confidence intervals [CIs]); *P* value of 0.05 or less was considered significant. All analyses were conducted using Stata software version 13 (StataCorp LP).

RESULTS

Sample Characteristics

After excluding 3697 patients with missing data on key variables (only considering variables with <5% missingness) the final sample used in complete cases analyses included 58 226 subjects. The average age was 54.1 (±10.0) years and 1/3 (32.4%) were women. About one-quarter (72.2%) of the sample was White, compared to 12.8% Hispanic and 9.3% African American. The average MELD score was 20.9 ± 9.7. Eleven percent (n = 6478) of donors had a history of DM and 88.9% (n = 51 748) did not.

Table 1 shows recipient pretransplant characteristics by donor DM status. Recipient characteristics were comparable among those that received a diabetic versus nondiabetic donor graft.

Table 2 shows donor characteristics by donor DM status. DM donors were older and more likely to be African American or Hispanic. A greater proportion of DM donors was obese and/or suffered from hypertension. DM donors more often died from anoxia or stroke than trauma.

GF

The median time at risk was 38 months and 11.7% (6797 subjects) experienced GF at any point during the study period. Graft survival at 30 days and 1 year posttransplant was 97.5% (1456 events) and 93.0% (3755 events), respectively.

Table 3 shows HRs for GF within 1 year posttransplant for DM and CIT. The GF rate was higher for diabetic donor

TABLE 3.

HRs (95% CIs) for 1-year GF by donor DM status and CIT

Primary determinants	Rate[a] n (%)	Unadjusted model, Crude HR (95% CI)	Combined model, adjusted[b] HR (95% CI)
Prolonged CIT	1503 (8.9)	1.48 (1.38-1.58)	1.43 (1.33-1.53)
Not prolonged	2252 (6.2)	1.00	1.00
Donor diabetes	571 (9.7)	1.46 (1.33-1.59)	1.22 (1.11-1.34)
No diabetes	3184 (6.7)	1.00	1.00

SRTR 2002-2015 (N = 58 226).

[a] KM Life table-calculated GF rate, $P < 0.001$ for difference in survival between groups.

[b] Adjusted for: recipient age, race/ethnicity, BMI, primary liver diagnosis, hepatitis C, UNOS status 1, albumin, ascites, medical condition (ICU/hospitalized/home), ventilatory support; donor age, race/ethnicity, height, cause of death, donation after cardiac death, split/partial liver.

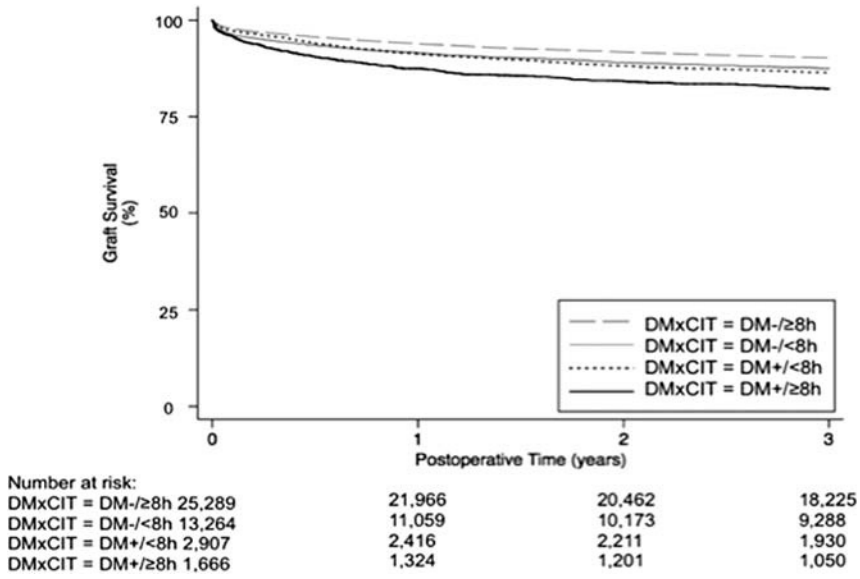

FIGURE 2. Kaplan-Meier curves: Liver graft survival up to 3 years posttransplant, stratified by donor DM status and short (<8 hours) versus long (≥8 hours) CIT. SRTR 2002-2010 (N = 33 980).

grafts and for prolonged CIT. Adjustment did not meaningfully alter (>10% change in estimate) the HR for risk associated with CIT. The only risk factor that meaningfully altered the HR for DM (and remained statistically significant) in the final models was donor age.

Figure 2 illustrates the (unadjusted) Kaplan-Meier curves by DM and CIT strata. GF rate was highest in the immediate postoperative period (days 0-30) for all groups. Graft survival was lowest for patients that received a diabetic donor graft with prolonged CIT for up to 3 years posttransplant and survival functions were significantly different across strata on log-rank test ($P < 0.001$).

Table 4 shows the HRs for patients with either or both risk factors relative to neither. On adjusted analyses, the probability of GF within 1 year of transplant was highest for recipients with a combination of donor DM and prolonged CIT compared to for recipients of grafts with short CIT from donors without any history of DM (adjusted HR, 1.79; 95% CI, 1.55-2.06). The unadjusted HRs for subjects with either factor alone versus neither factor were comparable, but the HR for donor DM (relative to subjects with neither factor) declined by about 16% after adjustment.

After multivariable adjustment, 10% of the risk for grafts with both prolonged CIT and donor DM history was attributable to interactive effect between CIT and DM, suggestive of effect measure modification, with a synergy index of 1.30. In other words, there are likely grafts that would fail in the presence of both factors that would not otherwise fail without the added injury from prolonged CIT in a given range of time. Thus, although the product term (multiplicative interaction)

TABLE 4.

HRs (95% CI) for 1-year GF stratified by donor DM status and CIT relative to neither risk factor

		1-y GF	
	Rate[a]	Crude	Adjusted[b]
	n (%)	HR (95% CI)	
Prolonged CIT and DM+	298 (12.6)	2.23 (1.95-2.55)	1.79 (1.55-2.06)
Prolonged CIT, no DM	2401 (8.4)	1.46 (1.36-1.57)	1.42 (1.32-1.53)
DM+, short CIT	905 (8.4)	1.41 (1.26-1.59)	1.19 (1.06-1.35)
DM–, short CIT	6262 (5.9)	1.00 (referent)	1.00 (referent)

[a] KM Life table-calculated GF rate, $P < 0.001$ for difference in survival between groups.

[b] Adjusted for: recipient age, race/ethnicity, BMI, primary liver diagnosis, hepatitis C, UNOS status 1, albumin, ascites, medical condition (ICU/hospitalized/home), ventilatory support; donor age, race/ethnicity, height, cause of death, donation after cardiac death, split/partial liver.

TABLE 5.

HRs (95% CIs) for GF for recipients of diabetic donor grafts and the effect of prolonged CIT

	GF[a]			
	HR (95% CI)			
	Days 0-30		Days 31-365	
	Unadjusted	Adjusted[b]	Unadjusted	Adjusted[c]
Donor diabetes[d]	1.34 (1.10-1.64)	1.18 (0.96-1.45)	1.46 (1.26-1.69)	1.19 (1.02-1.38)
Diabetic donor graft exposed to prolonged CIT[e]	2.14 (1.71-2.68)	1.77 (1.40-2.25)	2.29 (1.94-2.71)	1.84 (1.55-2.19)
P[f]	0.35	0.30	0.048	0.047

SRTR 2002-2015 (N = 58 226).

[a] GF: time from transplant to GF for any reason.

[b] Adjusted for recipient age, race/ethnicity, primary liver diagnosis, UNOS Status 1, medical condition (ICU/hospitalized/home), ventilatory support; donor age, race/ethnicity, cause of death, donation after cardiac death, split liver, allocation type, and donor height.

[c] Adjusted for: recipient age, race/ethnicity, BMI, primary liver diagnosis, hepatitis C, UNOS status 1, albumin, ascites, medical condition (ICU/hospitalized/home), ventilatory support; donor age, race/ethnicity, height, cause of death, donation after cardiac death, split/partial liver.

[d] HR for recipients of grafts from donors with a history of DM versus grafts from donors with no history of DM and CIT <8 hours.

[e] HR for recipients of grafts from donors with a history of DM and prolonged CIT (>8 hours) versus no history of DM and CIT <8 hours.

[f] Significance for interaction between donor DM (yes/no) and CIT (yes/no) variables; significant P values in bold emphasis.

for CIT and DM was not significant for overall 1-year GF, we further subdivided postoperative periods into 0 to 30 days and 31 to 365 days and found that there was a significant synergistic interaction between DM and CIT in the latter postoperative period (Table 5).

DISCUSSION

We used the SRTR database to analyze the extent to which donor DM modifies the effect of prolonged CIT on the risk of GF after LT. Our results show that the combination of prolonged CIT in a diabetic liver graft has a synergistic effect on the risk of GF.

Our results are supported by prior literature on GF risk associated with donor DM and further advance the field by providing evidence on the interaction between DM and CIT. A recent study describing 26 645 liver transplant recipients demonstrated that recipients of diabetic donor grafts have an increased risk of mortality after LT (HR, 1.11; 95% CI, 1.02-1.19).[3] In this study, 34.8% of recipients of grafts from DM donors experience GF compared with 27.8% of recipients that received a non-DM donor graft ($P < 0.001$). Another study evaluated 27 033 transplant cases and showed that donor diabetes was a strong independent risk factor for GF (HR, 1.20; $P = 0.006$) in hepatitis C virus positive transplant recipients.[4] Segev et al showed no effect modification by donor DM on the effects of prolonged CIT. Our results did not show a significant interaction between CIT and donor DM on overall 1-year GF either. The synergistic interaction between DM and CIT was significant in the latter postoperative period only.[9]

Type 2 DM is associated with hepatic steatosis, a form of nonalcoholic fatty liver disease, which can progress to nonalcoholic steatohepatitis.[12,13] Hepatic fat accumulation can result in liver inflammation through the release of various cytokines and ultimately cause liver fibrosis.[5,14,15] Several studies have shown that steatotic livers are more likely to experience IRI, leading to worse clinical outcomes after LT.[16,17]

Furthermore, the microvascular changes in diabetic donor grafts, like damage to the sinusoidal lining cells and disruption of the microvasculature, impair the hepatic microcirculation. Prolonged CIT can aggravate these microvascular changes and consequently increase susceptibility to IRI.[18-21] Surprisingly, the impact of donor DM status on graft survival was more pronounced 30 days after LT. This seems to be counterintuitive, but can be explained by the fact that donor DM status can increase the incidence of acute rejection episodes and ischemic cholangiopathy. To support this hypothesis, a recent study on 88 primary LTs demonstrated a significant association between donor DM and ischemic-type biliary lesions with an HR of 9.5 ($P = 0.009$), suggesting that DM promotes chronic changes in the biliary vessels, thereby increasing susceptibility to IRI.[22]

There were several limitations to our study. First, it should be noted that the statistical significance was only marginal. Second, this is a retrospective analysis and it is impossible to eliminate allocation biases. As is common in analyses of large administrative databases, missing data and reliability of the entered data must be thoroughly evaluated and appropriately handled in analyses, and in our study, we did not use any variables missing more than 5% of values. In addition, we were also unable to evaluate the direct effect of steatosis in the donor graft, because biopsy results are not captured in this database. To address this limitation, we evaluated BMI as a surrogate of graft steatosis but found it was not associated with GF after multivariable adjustment and was not retained in any of our final models. Lastly, we were not able to assess the impact of the duration of DM, since the SRTR does not have a variable that quantifies duration of DM. Future suggestions would be to investigate whether increasing GF rates occur with increasing CIT and duration of DM in a study with a smaller sample size.

Over the last decade more marginal donor organs are being accepted to increase the shrinking donor organ pool. This study demonstrates for the first time that liver grafts from a DM donor are more susceptible to prolonged CIT compared to nondiabetic donor grafts. The risk of GF within 1 year after LT is significantly higher in liver grafts from DM donors with CIT 8 hours or longer compared with diabetic liver grafts with CIT less than 8 hours. Although the outcomes of diabetic liver grafts are acceptable, we need to be cognizant that they are more susceptible to ischemic injury in transit and IRI intraoperatively.

Expediting allocation of diabetic donor grafts will help to reduce CIT and therefore decrease graft injury. In conclusion, this study confirmed that donor DM status is an independent risk factor and contributes synergistically with prolonged CIT to reduce graft survival.

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