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Hepatic ischemia reperfusion injury is associated with acute kidney injury following donation after brain death liver transplantation

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Summary

Donation after cardiac death liver transplant recipients have an increased frequency of acute kidney injury (AKI). This suggests that hepatic ischemia-reperfusion injury may play a critical role in the pathogenesis of AKI after liver transplantation. The aim of this single-center study was to determine if hepatic ischemia-reperfusion injury, estimated by peak peri-operative serum amino-transferase (AST), is associated with AKI following donation after brain death (DBD) liver transplantation. A total of 296 patients received 298 DBD liver transplants from January 2007 to June 2011. The incidence of AKI was 35.9%. AKI was a risk factor for chronic kidney disease ($P = 0.037$) and mortality ($P = 0.002$). On univariate analysis, peak AST correlated with peak creatinine ($P < 0.001$) and peak change in creatinine from baseline ($P < 0.001$). Peak AST was higher in AKI patients ($P < 0.001$). The incidence of AKI in patients with a peak AST of <1500, 1500–2999 and ≥3000 U/l was 26.1%, 39.8% and 71.2%, respectively ($P < 0.001$). On multiple logistic regression analysis, peak AST was independently associated with the development of AKI ($P < 0.001$). In conclusion, hepatic ischemia-reperfusion injury demonstrates a strong relationship with peri-operative AKI in DBD liver transplant recipients.

Introduction

Acute kidney injury (AKI) is a major cause for morbidity and mortality after liver transplantation [1–4]. In addition to the prolonged recovery period and greater financial cost, AKI is increasingly recognized as an independent risk factor for short-term mortality in the intensive care setting [1,2,4,5]. Moreover, AKI can cause permanent structural damage, with progressive tubulo-interstitial fibrosis and long-term implications for renal function [3,6–8]. Liver transplant patients with postoperative acute renal failure are twice as likely to develop chronic kidney disease [3].

The etiology of AKI after liver transplantation is multi-factorial. Most studies focus on recipient factors and immunosuppression [1]. However, we have recently shown that donation after cardiac death (DCD) liver transplantation is associated with an increased frequency of AKI [4]. Furthermore, in DCD recipients peak peri-operative serum aspartate amino-transferase (AST), a surrogate marker of hepatic ischemia-reperfusion injury is the only variable related to renal dysfunction [4]. Hepatic ischemia-reperfusion injury is associated with a systemic inflammatory response, which is the common pathway for the multiple organ dysfunction of sepsis and other inflammatory disorders [9–12]. Therefore, it follows that hepatic ischemia-reperfusion injury, by driving a systemic inflammatory response, may play a critical and potentially modifiable role in the pathogenesis of AKI after DCD liver transplantation [13,14].

Despite the growing utilization of DCD organs, the majority of liver grafts continue to be sourced from

donation after brain death (DBD) [15,16]. Whether hepatic ischemia-reperfusion injury contributes to AKI after DBD liver transplantation remains unclear. If the findings in DCD recipients are echoed, it follows that the increasing acceptance of marginal DBD grafts may have negative consequences for postliver transplant renal function.

The aim of this study was to determine if hepatic ischemia-reperfusion injury, estimated by peak peri-operative serum AST, is associated with AKI following DBD liver transplantation.

Methods

This was a retrospective single-center study of consecutive patients who underwent whole single organ DBD liver transplantation for chronic liver disease between January 2007 and June 2011. In our unit, we allocate DCD livers to older more stable recipients, preferring early cirrhotics with hepatocellular carcinoma [4]. Split livers are reserved for smaller recipients, often with cholestatic disease [17]. During the study time period, 302, 101, and 78 single organ DBD, DCD, and split liver transplants were performed for chronic liver disease, respectively. Four DBD recipients were excluded from the analysis because the AST peaked >72 h after transplantation, suggesting that hepatic ischemia-reperfusion injury was not the cause. Therefore, the study cohort comprised of 296 patients who underwent 298 DBD liver transplants.

Data were collected on the following donor and graft variables: age, gender, height, AST, inotropes, warm ischemic time, and cold ischemic time. Donor risk index (DRI) was calculated as previously described [18]. An allograft biopsy was performed immediately after reperfusion (time zero) in 216 patients (72.5%) and was graded by an independent transplant histopathologist.

The following recipient characteristics at the time of admission for transplantation were recorded (baseline): age, gender, ethnicity, body mass index, additional comorbidity including need for hemodialysis, international normalized ratio, serum bilirubin, serum creatinine, serum sodium, and presence of ascites (past history or ultrasonographic evidence). Refractory ascites was defined according the International Ascites Club criteria [19,20]. The MELD (Model for End-Stage Liver Disease) score was determined [21]. The UK Score for Patients with End-Stage Liver Disease (UKELD), a recently devised scoring system that incorporates serum sodium in addition to the MELD variables that is now used routinely in the UK to prioritize graft allocation, was also calculated [22]. Intra-operative red cell concentrate, fresh frozen plasma and platelet transfusion requirements, intra-operative use of cryoprecipitate, and intra-operative inotropes (noradrenaline/adrenaline infusion at the time of admission to the Intensive Care Unit) were noted. Documented peri-operative variables (following transplantation but prior to hospital discharge) were peak serum AST, peak serum creatinine, need for renal replacement therapy, and sepsis. Renal function was then recorded at 1-, 3-, 6-, 9-, 12-, 18-, 24-, 30-, 36-, 42-, and 48 months following transplantation. Patients receiving renal replacement therapy during the immediate postoperative period were given a peak serum creatinine of three times baseline if the actual recorded value was less [23]. Similarly, beyond the peri-operative period patients on hemodialysis were given an estimated glomerular filtration rate (eGFR) of 15 ml/min/1.73 m^2 [24].

Peri-operative acute renal dysfunction (following transplantation but prior to hospital discharge) was defined according to the RIFLE criteria for AKI: peak serum creatinine ≥2 times the baseline level [23]. The main measure of renal function thereafter was eGFR, determined using the Modification of Diet in Renal Disease Study 4-variable equation [25]:

$$(\text{eGFR} = 186 \times \text{creatinine}(\text{mg/dl})^{-1.154} \times \text{age}(\text{years})^{-0.203} \times 1.212(\text{if black}) \times 0.742(\text{if female})).$$

Chronic kidney disease was defined as eGFR <60 ml/min/1.73 m^2 on at least two occasions and sustained from 6 months post-transplant onward: stage 3, stage 4, and stage 5 chronic kidney disease were defined as eGFR 30–59 ml/min/1.73 m^2, 15–29 ml/min/1.73 m^2, and <15 ml/min/1.73 m^2 or on dialysis, respectively [24].

Standard immunosuppressant was tacrolimus aiming for a trough level of 8–10 within the first 3 months of transplantation, azathioprine, and reducing dose prednisolone discontinued by 3 months (216 patients, 72.5%). Deviation from the protocol was physician and surgeon dependent and determined by perceived risk of AKI, occurrence of acute cellular rejection during the peri-operative period, previous graft rejection if regraft, autoimmune hepatitis as the indication for transplantation, adverse effects or, for a small number of patients, drug trial participation. The mean tacrolimus trough levels of the entire cohort on day 1, day 2, day 3, and day 7 post-transplant were 4.6 (SD 4.6) μg/l, 6.7 (SD 5.0) μg/l, 7.8 (SD 5.7) μg/l, and 8.2 (SD 3.9) μg/l, respectively.

All transplants involved the piggyback cava-preserving technique. At least 80% of transplants involved a temporary porto-caval shunt. Hepatic ischemia-reperfusion injury minimizing strategies were not used in any donor. Intravenous *N*-acetylcysteine was administered to 33 recipients (11.1%) following admission to the Intensive Care Unit. This decision was surgeon dependent and in all cases precipitated by clinical evidence of initial poor graft function such as hemodynamic instability, lactic acidosis, and/or high serum AST.

Statistical analyses

Normally distributed continuous variables and nonparametric continuous variables were compared using the Student's *t*-test and Mann–Whitney test, respectively. Chi-squared analysis or Fisher's exact test were used for the comparison of categorical data. Survival was estimated using a Kaplan–Meier plot with log-rank test for differences, and adjusted survival was determined using Cox proportional hazards analysis. Cumulative incidence of chronic kidney disease was estimated using the Kaplan–Meier method. A multivariate linear regression analysis was performed to explore the relationship between donor and graft variables and hepatic ischemia-reperfusion injury. Clinically relevant variables were included simultaneously with log peak AST as the dependent variable. To identify variables associated with AKI, a logistic regression analysis was performed including all clinically relevant factors simultaneously. $P < 0.05$ was considered statistically significant unless otherwise stated.

Data were analyzed using the SPSS (SPSS Inc., Chicago, IL, USA) 18 package. All values are expressed as mean and standard deviation (SD), and median and inter-quartile range (IQR) as appropriate.

Results

Baseline patient, donor, and graft characteristics

Patient characteristics at the time of hospital admission for transplantation are outlined in Table 1. The median time from listing to transplantation was 71 (IQR 25–185) days. The median follow-up time from transplantation was 3.0 (IQR 1.8–4.3) years.

Donor and graft characteristics are documented in Table 2.

Immunosuppression

Renal sparing immunosuppression was prescribed immediately after transplantation in 66 patients (22.1%) who had a perceived greater risk of AKI (Table S1). Fifty-four patients (18.1%) received low dose tacrolimus (trough level of 5–8) plus mycophenolate and steroid, seven patients (2.3%) received an IL-2 receptor antagonist plus mycophenolate and steroid with tacrolimus from day 5, four patients (1.3%) received mycophenolate and steroid alone and tacrolimus from day 5, and one patient (0.3%) received azathioprine and steroid alone with tacrolimus from day 5. The tacrolimus trough levels on day 2 [renal sparing, 4.6 (3.7) μg/l; no renal sparing, 7.1 (5.1) μg/l, mean (SD); $P = 0.006$], day 3 [renal sparing, 6.0 (4.4) μg/l; no renal sparing, 8.3 (5.9) μg/l, mean (SD); $P = 0.013$], and day 7 [renal sparing, 6.4 (3.1) μg/l; no renal sparing, 8.7 (4.0) μg/l, mean (SD); $P < 0.001$] were lower in the renal sparing immunosuppression group ($P < 0.017$ considered significant). Long-term renal sparing immunosuppression was prescribed to 91 of the 282 surviving patients by the time of hospital discharge (32.3%).

Peri-operative renal function

The baseline serum creatinine was 85 (IQR 66–99) μmol/l and the baseline eGFR was 89 (SD 35) ml/min/1.73 m^2. One hundred and eleven patients (37.2%) had a baseline eGFR ≥90 ml/min/1.73 m^2, 131 patients (44.0%) had an eGFR 60–89 ml/min/1.73 m^2, 55 patients (18.5%) had an eGFR 30–59 ml/min/1.73 m^2 and one patient (0.3%) had an eGFR <15 ml/min/1.73 m^2. One hundred and seventy-six patients (59.1%) had ascites, 60 patients (20.1%) had refractory ascites, and 78 patients (26.2%) were hyponatremic. Eleven patients (3.7%) fulfilled the diagnostic criteria for type 2 hepatorenal syndrome. No patient was receiving renal replacement therapy prior to transplantation.

Immediately after transplantation, acute renal dysfunction was a common complication. The median peak peri-operative serum creatinine was 133 (IQR 92–223) μmol/l and the median change in creatinine from baseline was +55 (IQR 13–169)%. One hundred and seven patients (35.9%) developed AKI, of whom 63 (58.9%) required renal replacement therapy. The median time to onset of AKI was 40 (IQR 24–61) h, and the median duration of AKI was 7 (IQR 3–14) days.

Association between AKI and morbidity and mortality

Patients with AKI had a prolonged Intensive Care Unit admission [AKI, 5 (3–7) days; no AKI, 2 (2–3) days, median (IQR), $P < 0.001$] and hospital stay [AKI, 15 (11–22) days; no AKI, 9 (8–12) days, median (IQR), $P < 0.001$].

The estimated 3-year cumulative incidence of chronic kidney disease in patients with AKI was 43.7%, and in patients with no AKI was 31.6% (log-rank $P = 0.173$). After adjusting for relevant clinical variables (multivariate model including recipient age, gender, pretransplant eGFR, pretransplant diabetes mellitus, hepatitis C virus status simultaneously; data not shown) peri-operative AKI was a predictor of chronic kidney disease (HR 1.58; 95% CI 1.03–2.44, $P = 0.037$).

Patient survival was reduced in the AKI group (AKI, 80.5%; no AKI, 90.8%, estimated 3-year survival, log-rank $P = 0.006$). In a multivariate model (including the relevant clinical variables age, gender, pretransplant MELD score, pretransplant eGFR, pretransplant diabetes mellitus simultaneously; data not shown) AKI had a hazard ratio for death of 3.22 (95% CI 1.55–6.71, $P = 0.002$).

Table 1. Clinical characteristics of donation after brain death recipients at the time of hospital admission for transplantation (pretransplant), intra-operative and during the immediate postoperative period, and univariate analyses of variables associated with peri-operative acute kidney injury.

	All patients (n = 298)	AKI (n = 107)	No AKI (n = 191)	AKI versus no AKI *P* value
Pretransplant				
Age (years)	52.6 (11.0)	51.1 (11.2)	53.5 (10.8)	0.082
Gender (male: female)	1.9:1	2.3:1	1.7:1	0.239
Ethnicity				
White	260 (87.2)	86 (80.4)	174 (91.1)	
Asian	29 (9.7)	15 (14.0)	14 (7.3)	
Black	9 (3.0)	6 (5.6)	3 (1.6)	**0.021**
Body mass index	27.5 (5.0)	27.7 (4.5)	27.3 (5.3)	0.496
Etiology of liver disease				
Alcohol	76 (25.5)	29 (27.1)	47 (24.6)	
Hepatitis C	60 (20.1)	22 (20.6)	38 (19.9)	
Primary biliary cirrhosis	39 (13.1)	13 (12.1)	26 (13.6)	
Primary sclerosing cholangitis	24 (8.1)	10 (9.3)	14 (7.3)	
Nonalcoholic fatty liver disease	19 (6.4)	8 (7.5)	11 (5.8)	
Hepatitis B	15 (5.0)	5 (4.7)	10 (5.2)	
Autoimmune hepatitis	11 (3.7)	2 (1.9)	9 (4.7)	
Other	54 (18.1)	18 (16.8)	36 (18.8)	0.915
Hepatocellular carcinoma	77 (25.8)	21 (19.6)	56 (29.3)	0.067
Regraft	12 (4.0)	4 (3.7)	8 (4.2)	0.557
Inpatient	18 (6.0)	7 (6.5)	11 (5.8)	0.786
MELD score	16 (7)	17 (6)	15 (7)	**0.010**
UKELD score	51 (6)	53 (6)	50 (6)	**0.001**
Creatinine (μmol/l)	85 (66–99)	87 (66–103)	82 (67–99)	0.332
eGFR (ml/min/1.73 m^2)	89 (35)	88 (34)	90 (36)	0.708
Sodium (mmol/l)	138 (134–140)	136 (132–139)	138 (135–141)	**<0.001**
Ascites				
None	122 (40.9)	32 (29.9)	90 (47.1)	
Diuretic controlled	166 (38.9)	44 (41.1)	72 (37.7)	
Refractory	60 (20.1)	31 (29.0)	29 (15.2)	**0.003**
Renal replacement therapy	0	0	0	
Diabetes mellitus				
No	225 (75.5)	75 (70.1)	150 (78.5)	
Noninsulin dependent	39 (13.1)	16 (15.0)	23 (12.0)	
Insulin dependent	34 (11.4)	16 (15.0)	18 (9.4)	0.231
Hypertension	42 (14.1)	12 (11.2)	30 (15.7)	0.285
Intra-operative				
RCC transfusion (units)	2 (0–4)	3 (2–6)	1 (0–3)	**<0.001**
FFP transfusion (units)	8 (4–12)	11 (7–17)	6 (4–10)	**<0.001**
Platelet transfusion (units)	10 (0–15)	10 (0–16)	5 (0–10)	**0.018**
Received cryoprecipitate	12 (4.0)	10 (9.4)	2 (1.0)	**0.001**
Inotropes	217 (72.8)	95 (88.8)	122 (63.9)	**<0.001**
Peri-operative				
N-acetylcysteine	33 (11.1)	23 (21.5)	10 (5.2)	**<0.001**
Peak AST (U/l)	1260 (761–2188)	1665 (905–3040)	1153 (679–1675)	**<0.001**
Sepsis	48 (16.1)	30 (28.0)	18 (9.4)	**<0.001**
Renal sparing immunosuppression	66 (22.1)	36 (33.6)	30 (15.7)	**<0.001**

Values expressed as mean (standard deviation), median (inter-quartile range), and number (%) where appropriate.
AKI, acute kidney injury; AST, aspartate amino-transferase; eGFR, estimated glomerular filtration rate; FFP, fresh frozen plasma; MELD, model for end-stage liver disease; RCC, red cell concentrate; UKELD, UK score for patients with end-stage liver disease.
Bold represents statistical significance.

Table 2. Donor and graft characteristics of donation after brain death recipients, and univariate analyses of variables associated with peri-operative acute kidney injury.

	All patients (n = 298)	AKI (n = 107)	No AKI (n = 191)	AKI versus no AKI P value
Donor characteristics				
Age (years)	49.9 (13.8)	51.1 (15.2)	49.2 (12.9)	0.251
Age ≥65 years	40 (13.5)	21 (19.8)	19 (9.9)	**0.017**
Gender (male: female)	1.1:1	1.1:1	1.1:1	0.802
Height (m)	1.69 (0.15)	1.69 (0.12)	1.69 (0.16)	0.981
AST (U/l) (n = 145)	33 (22–54)	33 (20–66)	34 (22–51)	0.763
Inotropes	250 (85.3)	88 (82.2)	162 (87.1)	0.258
Graft characteristics				
>30% macrovesicular steatosis (n = 216)	18 (8.3)	10 (14.1)	8 (5.6)	**0.032**
>30% microvesicular steatosis (n = 216)	52 (24.1)	33 (45.8)	19 (13.2)	**<0.001**
Cold ischemic time (h)	8.5 (2.4)	8.8 (2.5)	8.3 (2.3)	0.088
Cold ischemic time >12 h	24 (8.1)	13 (12.3)	11 (5.8)	0.050
Warm ischemic time (min)	40.7 (7.0)	41 (7)	41 (7)	0.788
Warm ischemic time >45 min	71 (24.0)	26 (24.5)	45 (23.7)	0.870
Donor risk index	1.44 (1.28–1.67)	1.52 (1.30–1.77)	1.42 (1.25–1.58)	**0.041**

Values expressed as mean (standard deviation), median (inter-quartile range), and number (%) where appropriate.
AKI, acute kidney injury; AST, aspartate amino-transferase.
Bold represents statistical significance.

Severity of hepatic ischemia-reperfusion injury

During the immediate postoperative period, serum AST peaked within 24 h of admission to the Intensive Care Unit in 94.3% of patients. The median peak AST was 1260 (IQR 761–2188) U/l. One hundred and seventy-six patients (59.1%), 83 patients (27.9%), and 39 patients (13.1%) had a peak AST of <1500, 1500–2999 and ≥3000 U/l, respectively. Peak AST related well to the histological grading of injury on 'time zero' allograft biopsy (n = 210, mild, 1103 U/l; mild-to-moderate, 1350 U/l; moderate, 1568 U/l; moderate-to-severe, 2513 U/l, median; P = 0.005).

Donor and graft variables associated with hepatic ischemia-reperfusion injury

On univariate analysis, peak AST was associated with donor gender (male, 1357 U/l; female, 1166 U/l, P = 0.014), warm ischemic time (Spearman's r = 0.276, P < 0.001), and the presence of >30% macrovesicular steatosis (n = 216, >30%, 2134 U/l; ≤30%, 1241 U/l, P = 0.001) and >30% microvesicular steatosis (n = 216, >30%, 1544 U/l; ≤30%, 1203 U/l, P = 0.035). Peak AST did not relate to donor age (Spearman's r = 0.041, P = 0.481), donor height (Spearman's r = 0.100, P = 0.087), donor AST (n = 145, Spearman's r = 0.126, P = 0.130), donor inotropes (inotropes, 1257 U/l; no inotropes, 1346 U/l, P = 0.512), cold ischemic time (Spearman's r = 0.073, P = 0.209), or donor risk index (Spearman's r = 0.015, P = 0.801).

In a multivariate model adjusting for recipient factors, male donor (P = 0.016), increasing warm ischemic time (P = 0.002) and the presence of >30% macrovesicular steatosis on 'time zero' biopsy (P < 0.001) were independently associated with increasing severity of hepatic ischemia-reperfusion injury (Table 3).

Recipient factors associated with peri-operative AKI

Recipient factors associated with peri-operative AKI on univariate analysis are outlined in Table 1. There was no difference in the pretransplant serum creatinine (P = 0.332) or eGFR (P = 0.708) of DBD patients who did and did not develop AKI. Instead, the presence of ascites (P = 0.003) and hyponatremia (AKI, 39.3%; no AKI, 18.8%; P < 0.001) were more common in the AKI group. AKI patients had a higher MELD (P = 0.010) and UKELD score (P = 0.001) than those who did not develop renal injury. The prevalence of hepatitis C (AKI, 20.6%; no AKI, 19.9%; P = 0.891), diabetes (P = 0.231), and hypertension (P = 0.285) was not different between the AKI and non-AKI patients.

Thirty-four percent of patients who developed AKI compared to 15.7% of patients who did not develop AKI received renal sparing immunosuppression from immediately following liver transplantation (P < 0.001).

A multivariate model including all clinically relevant variables simultaneously (Table 4) identified that refractory ascites (P = 0.019), ≥5 units red cells intra-operatively (P = 0.005), intra-operative inotropes (P = 0.008), and sepsis during the postoperative period (P = 0.007) were associated with the development of AKI.

Table 3. Multivariate linear regression analysis of variables associated with log peak aspartate amino-transferase following donation after brain death liver transplantation.

	B	95% CI	*β*	*P* value
Donor characteristics				
Age (years)	0.001	−0.006, 0.009	0.025	0.708
Male gender	0.271	0.051, 0.492	0.170	**0.016**
Height (m)	−0.365	−1.052, 0.322	−0.073	0.296
Inotropes	−0.127	−0.422, 0.168	−0.056	0.398
Graft characteristics				
Cold ischemic time (h)	0.030	−0.016, 0.076	0.088	0.195
Recipient warm ischemic time (min)	0.024	0.009, 0.039	0.207	**0.002**
>30% macrovesicular steatosis	0.693	0.314, 1.072	0.243	**<0.001**
>30% microvesicular steatosis	0.111	−0.142, 0.363	0.060	0.389
Recipient characteristics				
MELD score	−0.007	−0.024, 0.009	−0.059	0.388
Intra-operative RCC transfusion ≥5 units	−0.270	−0.552, 0.012	−0.130	0.060
Intra-operative inotropes	0.194	−0.048, 0.436	0.107	0.115
Postoperative sepsis	0.276	−0.037, 0.588	0.117	0.084

Reference group (relative risk 1.000): donor female gender, no donor inotropes, ≤30% macrovesicular steatosis, ≤30% microvesicular steatosis, intra-operative RCC <5 units, no intra-operative inotropes, no postoperative sepsis.
B, unstandardized regression coefficient; *β*, standardized regression coefficient; CI, confidence interval; MELD, model for end-stage liver disease; RCC, red cell concentrate.
Bold represents statistical significance.

Table 4. Multiple logistic regression analysis of variables associated with peri-operative acute kidney injury following donation after brain death liver transplantation.

	OR	95% CI	*P* value
Age (decade)	0.90	0.68–1.18	0.448
Female gender	0.90	0.47–1.73	0.754
Ethnicity			
White	1.00		0.099
Asian	2.29	0.86–6.14	0.160
Black	3.80	0.59–24.43	0.554
Hepatitis C	1.28	0.57–2.88	0.554
Pretransplant			
Diabetes			
None	1.00		
Noninsulin	1.44	0.60–3.46	0.417
Insulin dependent	1.82	0.72–4.62	0.208
Hypertension	0.54	0.21–1.42	0.214
eGFR (ml/min/1.73 m^2)			
≥90	1.00		
60–89	0.98	0.51–1.89	0.953
<60	0.93	0.40–2.17	0.870
MELD score	1.01	0.96–1.06	0.718
Ascites			
None	1.00		
Diuretic controlled	1.33	0.64–2.78	0.445
Refractory	2.75	1.18–6.39	**0.019**
Intra-operative			
RCC transfusion (U)			
0	1.00		
1–4	1.68	0.81–3.49	0.164
≥5	3.57	1.47–8.67	**0.005**
Inotropes	2.88	1.31–6.33	**0.008**
Post-operative			
Peak AST (U/l)			
<1500	1.00		
1500–2999	2.02	1.04–3.94	**0.039**
≥3000	8.02	3.28–19.64	**<0.001**
Sepsis	2.86	1.34–6.13	**0.007**
Renal sparing immunosuppression	1.37	0.68–2.78	0.381

Reference group (relative risk 1.00): male gender, no hepatitis C, no hypertension, no inotropes, no sepsis, no RS immunosuppression.
AST, aspartate amino-transferase; CI, confidence interval; eGFR, estimated glomerular filtration rate; MELD, model for end-stage liver disease; OR, odds ratio; RCC, red cell concentrate.
Bold represents statistical significance.

Donor and graft variables associated with peri-operative AKI

Donor and graft variables associated with peri-operative AKI on univariate analysis are outlined in Table 2. AKI patients were more likely to have a donor ≥65-year old ($P = 0.017$), >30% macrovesicular steatosis on 'time-zero' biopsy ($P = 0.032$) or a cold ischemic time >12 h ($P = 0.050$). The donor risk index was higher in patients who developed AKI compared to patients who did not ($P = 0.041$).

Association between hepatic ischemia-reperfusion injury and AKI

Peak serum AST demonstrated a significant correlation with peak peri-operative serum creatinine (Spearman's $r = 0.283$, $P < 0.001$) and peak peri-operative change in serum creatinine from baseline (Spearman's $r = 0.268$, $P < 0.001$). Peak AST was higher in AKI patients [AKI, 1665 (905–3040) U/l; no AKI, 1153 (679–1675) U/l, median (IQR); $P < 0.001$, Fig. 1]. The incidence of AKI in patients with a peak serum AST of <1500, 1500–2999 and ≥3000 U/l was 26.1%, 39.8% and 71.2%, respectively ($P < 0.001$, Fig. 2).

On multivariate analysis, after adjusting for all clinically relevant variables, hepatic ischemia-reperfusion injury was strongly associated with the development of AKI ($P < 0.001$, Table 4).

Discussion

In this large contemporary single-center study of patients undergoing DBD liver transplantation, we have examined

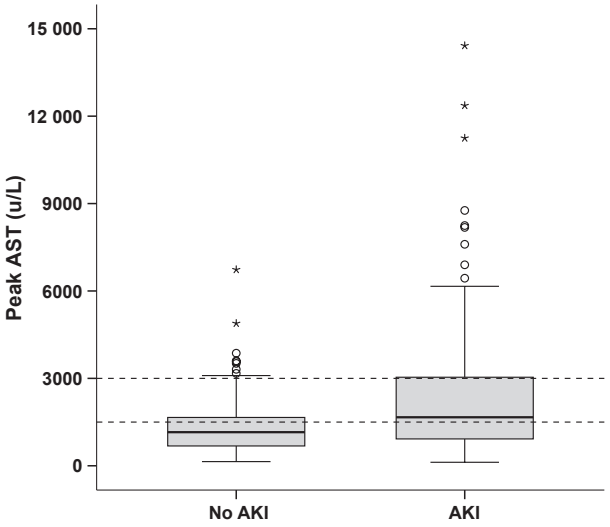

Figure 1 Boxplot of peak peri-operative aspartate amino-transferase (AST) in patients who did and did not develop acute kidney injury (AKI). Demonstrates median, inter-quartile range, outliers (○) and extreme cases (⋆).

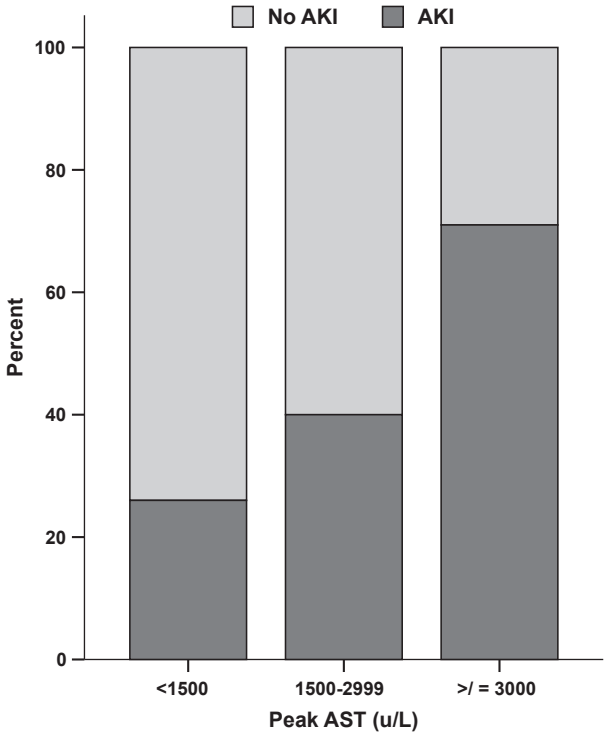

Figure 2 Percentage of patients with a peak aspartate amino-transferase (AST) <1500, 1500–2999 and ≥3000 U/l who did and did not develop acute kidney injury (AKI).

for the first time the relationship between hepatic ischemia-reperfusion injury and renal outcomes. We have shown that peak peri-operative serum AST, a surrogate marker of hepatic ischemia-reperfusion injury, is strongly associated with AKI in this setting. On univariate analysis peak AST correlated with peak serum creatinine and peak change in serum creatinine from baseline and was higher in AKI patients than those with maintained renal function. In a multivariate model, peak AST remained strongly associated with renal injury. The development of AKI after DBD liver transplantation had significant implications for morbidity and mortality, as highlighted by the longer duration of hospitalization, increased likelihood of chronic kidney disease and worse survival of AKI patients.

Acute kidney injury after liver transplantation is multifactorial in origin. Pretransplant neuro-humoral and circulatory derangement, and intrinsic chronic kidney disease, predisposes patients with end-stage liver failure to acute renal dysfunction [26]. Intra-operatively, hemodynamic insults including surgical technique and hemorrhage culminate in renal ischemia, inflammation, and injury [1,2,27]. Thereafter, the administration of a calcineurin inhibitor further compromises renal perfusion and function [28].

The role of graft injury in the pathogenesis of AKI following liver transplantation is less well recognized. Hepatic ischemia-reperfusion injury is associated with a systemic inflammatory response, which may cause AKI through hemodynamic mechanisms and direct tubular cell death [10,14,29–31]. In a previous study examining the impact of hepatic ischemia-reperfusion injury on clinical outcomes, liver transplant recipients with severe graft injury were more likely to require peri-operative hemodialysis [13]. Moreover, initial liver graft dysfunction as defined by the Toronto group (largely determined by AST) has been observed to be a risk factor for a 50% increase in serum creatinine [32]. We have previously shown that the greater graft injury of DCD liver transplants is associated with an increased frequency of AKI [4]. In this population, peak serum AST is the only variable associated with renal injury [4]. It follows that hepatic ischemia-reperfusion injury, by driving a systemic inflammatory response, plays an important role in the pathogenesis of AKI after liver transplantation.

The key mediator of the greater graft injury of DCD organs is hypothesized to be the added donor warm ischemic time [33–35]. DCD liver transplant recipients who develop AKI have a longer recipient warm ischemic time [4]. Furthermore, Chen *et al.* found that longer recipient warm ischemic time was the only donor or graft variable associated with postoperative acute renal failure [2]. In our study of DBD liver transplant recipients, increasing warm ischemic time was independently associated with increasing severity of hepatic ischemia-reperfusion injury. Warm ischemia duration correlates with the postoperative systemic inflammatory response

[9,11,33]. Therefore, warm ischemic time may be a critical factor in the development of renal complications after liver transplantation.

Additional graft variables related to AKI were donor age and the presence of significant hepatic steatosis. AKI patients were more likely to have received a graft from an older donor than non-AKI patients. The presence of >30% hepatic steatosis was associated with greater hepatic ischemia-reperfusion injury, and was more prevalent in the AKI group. These findings are in agreement with the well recognized age- and steatosis-related increased susceptibility to hepatic ischemia-reperfusion injury [36,37]. The DRI was slightly higher in patients with AKI, but not associated with peak AST. The DRI was originally derived from the Scientific Registry of Transplant Recipients and may not be as applicable in other donor populations. Therefore, this may have contributed to any inconsistency in the results. Graft quality has evolved in recent years, in parallel with the discrepancy between supply and demand for liver transplantation [38]. We postulate that the increasing acceptance of marginal DBD grafts may have negative consequences for postliver transplant renal function.

In this study, hepatic ischemia-reperfusion injury was estimated by peak serum AST within 72 h of liver transplantation, an accepted measure of hepatocellular damage [13,41]. It is noteworthy that AST is not specific to the liver being present in many tissues including the kidneys [39]. Therefore, an alternative explanation for the relationship between peak AST and renal outcomes could be increased release as a consequence of kidney injury [40]. Similarly, serum AST may rise following skeletal and cardiac muscle damage, a degree of which is expected in patients with multiorgan failure [39]. However, serum AST levels have been shown to be only marginally elevated in nonliver injury [39,40]. Moreover, peak AST correlated well with the histological grade of hepatic preservation injury on time zero biopsy as previously described [41]. Urinary excretion does not participate in the clearance of AST [39].

The study has some additional potential limitations that should be mentioned. Firstly, the frequency of perioperative AST and creatinine measurement was variable. All patients had blood sampling immediately on arrival to the Intensive Care Unit and, in most cases, 12-hourly for the first 24–48 h. It is possible, for example, that the peak AST underestimated the severity of graft injury. However, correlation with biopsies does suggest that values were representative. Secondly, the lack of pretransplant renal impairment may raise some concerns about the generalizability of the results for some populations of liver transplant recipients. Nevertheless, the study cohort is typical of recipients who do not undergo combined liver–kidney transplantation in many countries.

Prospective studies are necessary to elucidate further the cause-and-effect relationship between hepatic ischemia-reperfusion injury and AKI. However, our findings do allow the following suggestions to be made. Patients who undergo DBD liver transplantation and demonstrate greater hepatic ischemia-reperfusion injury have an increased incidence of AKI, with long-term implications for renal function. Lower quality or marginal DBD grafts may be an important risk factor for the development of peri-operative AKI and chronic kidney disease. Consequently, renal sparing immunosuppression may be appropriate particularly in those individuals with greatly increased transaminases [42,43]. Treatments that target the hepatic ischemia-reperfusion injury and the systemic inflammatory response may be future therapeutic options that require further study [9,44–47].

In conclusion, in this large contemporary single-center study, we have shown for the first time that hepatic ischemia-reperfusion injury demonstrates a strong independent relationship with peri-operative AKI in DBD liver transplant recipients. Hepatic ischemia-reperfusion injury may play a critical and modifiable role in the pathogenesis of AKI in this setting.

Authorship

JAL: designed research study, performed research study, collected data, analyzed data, and wrote paper. MJA, CC, MA, CK and BKG: collected data. PM and JWF: designed research study.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1. Clinical characteristics of donation after brain death recipients who did and did not receive renal sparing immunosuppression immediately after transplantation for a perceived greater risk of acute kidney injury.

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