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High preoperative bilirubin values protect against reperfusion injury after live donor liver transplantation

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Summary

Heme Oxygenase-1 and its product biliverdin/bilirubin have been demonstrated to protect against ischemia/reperfusion injury (IRI). We investigated whether increased preoperative bilirubin values of transplant recipients decrease IRI. Preoperative bilirubin levels of live donor liver recipients were correlated to postoperative liver transaminase as a marker of IRI. Additionally, two recipient groups with pretransplant bilirubin levels >24 μmol/l ($n = 348$) and ≤24 μmol/l ($n = 118$) were compared. Post-transplant liver function, complications, length of hospital stay, and patient and graft survival were assessed. Preoperative bilirubin levels were negatively correlated to the postoperative increase in transaminases suggesting a protective effect against IRI. The maximal rise of ALT after transplantation in high versus low bilirubin patients was 288 (−210–2457) U/l vs. 375 (−11–2102) U/l, $P = 0.006$. Bilirubin remained a significant determining factor in a multivariate linear regression analysis. The MELD score and its individual components as a marker of severity of chronic liver disease were significantly higher in the high versus low bilirubin group ($P < 0.001$). Despite this, overall complication rate (21.0% vs. 21.2%, $P = 0.88$), hospital stay [13 (4–260) vs. 14 (6–313) days, $P = 0.93$), and 1-year graft survival (90.8% vs. 89.0%, $P = 0.62$) were similar in both groups. High bilirubin levels of liver recipients before live donor transplantation is associated with decreased postoperative IRI.

Introduction

The severe organ shortage has resulted in an increased use of marginal donor organs, such as steatotic livers, grafts obtained after cardiac death, or liver grafts from older donors. Liver transplantation with marginal grafts is often associated with severe ischemia–reperfusion injury (IRI) with significant impact on the success of orthotopic liver transplantation (OLT). The extent of IRI is an important determinant for the degree of postoperative liver cell dysfunction and cholangiopathy [1–4]. Reducing IRI is important and numerous strategies have been proposed for its attenuation [5]. The transfer of such strategies from bench to bedside, however, remains often unsuccessful either due to practical inapplicability or lack of a clinical effect.

The antioxidant enzyme Heme Oxygenase-1 (HO-1) has been proposed as a potent player in the attenuation of IRI and its antioxidative, immunomodulative, and vasorelaxive effects have been studied extensively [6–9]. HO-1 cleaves heme to form biliverdin, carbon monoxide, and free iron. Biliverdin, in turn, is enzymatically reduced to bilirubin, which is known for its antioxidative properties [10,11]. Bilirubin scavenges reactive oxygen species, a process in which it is being oxidized back to biliverdin. This oxidation reaction is effective for both unconjugated and conjugated bilirubin [12]. This redox cycle is believed to play a pivotal and

inducible role as protective mechanism in IRI and rejection [13].

On the other hand, bilirubin is known as a marker of cholestasis and liver dysfunction. It is one of the three variables of the model for end-stage liver disease score (MELD), which is widely used to quantify the severity of end-stage liver disease [14]. In contrast, during transplantation an increased preoperative bilirubin level could offer a protective effect against IRI. In a clinical setting, high preoperative bilirubin values of liver recipients failed to prove a beneficial effect on hepatic IRI after orthotopic liver transplantation using grafts recovered from brain dead donors [15]. However, the deceased donor group is very heterogeneous and many donor-related factors like donor age or steatosis might have confounded these results. Therefore, we analyzed the effect of preoperative bilirubin levels on hepatic IRI in our more homogeneous live donor liver transplantation (LDLT) population.

Materials and methods

Study design

The study was approved by the University Health Network's Research Ethics Board (# 14-8069-BE). Between January 2000 and April 2014, all adult LDLT at our institution were prospectively entered into our transplant database and analyzed retrospectively. Patients suffering from acute liver failure were excluded from the analysis [15]. Preoperative total bilirubin levels were correlated with the postoperative rise of liver transaminases as a marker of IRI. In addition, patients were split into 'low' and 'high bilirubin' groups. As a critical value dividing both groups, we used 24 μmol/l (1.4 mg/dl), which was the 25th percentile of the preoperative total bilirubin levels in the whole LDLT group. This value is also close to the upper limit of normal for total bilirubin of 26 μmol/l [16]. To evaluate whether higher bilirubin cut-off levels have different and a more pronounce effect, the 'high bilirubin' group was further divided into 'mildly' (>24 μmol/l ≤ 100 μmol/l, >1.4 mg/dl ≤ 5.8 mg/dl, 25th percentile) and 'severely increased' (>100 μmol/l, >5.8 mg/dl, 75th percentile) subgroups. Groups were compared for differences in IRI, postoperative liver function, and complications.

In an additional subgroup analysis, all cases with a biliary cause of liver disease (primary sclerosing cholangitis/PSC and primary biliary cirrhosis/PBC) were eliminated from the analysis to avoid a confounding effect from these etiologies.

Listing practices for live donation at the University of Toronto

At our institution, all patients on the liver transplant waiting list along with their next of kin are systematically encouraged to consider live donation, irrespectively of their MELD score or disease etiology and severity. Donors and recipients are thoroughly informed about the morbidity and mortality rate based on the program experience [17,18]. At our institution, the overall survival rates for recipients are similar for both LDLT and deceased donation liver transplantation [19]. Potential donors undergo a careful medical evaluation process to reliably guarantee a satisfactory health status. Steatosis was excluded in all cases prior to live liver donation.

Donor, preoperative recipient, and perioperative data

Donor age, gender, and body mass index (BMI) were documented at the time of donation. For the recipients, the following preoperative data were collected: age, gender, preoperative biochemical profile, calculated MELD score, and underlying liver disease. The diagnoses were further divided into primary hepatocellular and primary biliary etiology.

Organ procurement for live donor hepatectomies was performed as previously described [17]. All patients received a right liver graft (segments V–VIII). Cold ischemia time (CIT), warm ischemia time (WIT), and type of biliary anastomosis were documented as perioperative variables. In patients receiving LDLT with multiple bile ducts a Roux-en-Y biliary reconstruction was performed. In all other cases, a duct-to-duct reconstruction was the preferred choice.

We identified patients receiving antibody induction therapy, the type of agent used for the induction and the postoperative immunosuppressive regimen in each case. The baseline immunosuppression regimen was based on either tacrolimus or cyclosporine.

Postoperative outcome

Liver graft function was evaluated clinically and through biochemical markers measured daily after transplantation until discharge and afterward at regular intervals. Reperfusion injury was determined by maximum increase of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) within 48 h of transplantation [2,20]. To assess graft function, we evaluated international normalized ratio (INR) levels 14 days after transplantation (as early marker of liver function), as well as bilirubin 3 months after transplantation (as marker of liver function after bilirubin stabilization).

Patient's postoperative ICU and hospital stay was recorded for each case. Surgical complications were documented according to the Clavien–Dindo classification [21]. Scores greater than 3b were considered as 'severe' complications. The first year incidence of biliary complications and cellular rejection confirmed by core biopsy were

identified for comparison between both groups. Additionally, graft and patient survival at 1-year was compared, and the cause of graft loss was analyzed. Graft failure was defined as death or re-transplantation.

Statistical analysis

The data were analyzed with the SPSS 20 statistical package (IBM, Chicago, IL, USA). Data were presented as median (range). Mann–Whitney *U*-test and Kruskal–Wallis *t*-test were used for all continuous variables for comparison of the study groups. Differences between categorical variables were tested with chi-squared test. Graft and patient survivals were calculated by Kaplan–Meier method and compared by log-rank test. A *P*-value below 0.05 was considered as significant.

For further analysis, bilirubin and transaminases values were logarithmically transformed in order to approach normal distribution for these data sets, to minimize the statistically distorting effect of outliers, and to achieve better data visualization. Linear regression and Spearman correlation analyses were performed between preoperative total bilirubin and both maximum absolute AST and ALT increase within 48 h [15]. Permutation test was used to confirm significance of the correlation.

In a multivariate linear regression analysis, the preoperative total bilirubin (logarithmized) was analyzed together with donor and recipient age, as well as CIT and WIT as independent variables to evaluate their impact on the maximum absolute ALT increase (logarithmized) as dependent variable and indicator for IRI.

Results

From January 2000 until March 2014, 476 adult patients received a LDLT at the Multi Organ Transplant Program of the Toronto General Hospital, University of Toronto. Ten cases were excluded from the data analysis, as per protocol, because the indication for transplantation was fulminant hepatic failure. Of the remaining 466 patients, 118 had a preoperative total bilirubin value ≤24 μmol/l (1.4 mg/dl, 25th percentile) and were thus assigned to the 'low bilirubin' group, as opposed to the remaining 348 patients, who were part of the 'high bilirubin' group.

Donor data, recipient demographics, and perioperative status

Live donor characteristics, such as age, gender, and BMI were similar in both groups (see Table 1). Steatosis (>10%) was absent in all donor grafts, as this is considered a contraindication for live liver donation in our program. Recipient characteristics were generally similar in both groups (see Table 1). Viral infections, alcoholic liver disease (EtOH), nonalcoholic steato-hepatitis (NASH), autoimmune hepatitis (AIH), and other etiologies of liver disease (mainly metabolic or polycystic etiologies) were similarly distributed between 'low' and 'high bilirubin' groups. Only PSCs were significantly more frequent in the 'high bilirubin' group ('low' 8.5% vs. 'high' 16.4%, $P = 0.004$), whereas hepatocellular carcinomas (HCC) occurred more often in the 'low bilirubin' group ('low' 34.7% vs. 'high' 25.0%, $P = 0.04$, see Table 2).

As expected, the preoperative MELD score was significantly higher in the 'high bilirubin' group ['low' 10 (6–22) vs. 'high' 17 (8–40), $P < 0.001$]. However, even when the bilirubin fraction was removed from the MELD calculation

Table 1. Donor and recipient characteristics, peri- and postoperative data.

Total patients (n = 466)	Low TBIL (n = 118)	High TBIL (n = 348)	*P*-value
Age, Donor (years)	36.8 (19–69)	37.5 (17–61)	0.88
Male gender, Donor	47.4%	48.5%	0.83
BMI, Donor (kg/m^2)	26.2 (17.1–38.6)	26.2 (15.4–39.7)	0.92
Age, Recipient (years)	54.3 (20–71)	53.2 (18–72)	0.08
Male gender, Recipient	65.3%	62.6%	0.611
BMI, Recipient (kg/m^2)	26.3 (14.5–48.7)	25.9 (14.5–45.2)	0.42
Preop total bilirubin (mmol/dl)	16 (4–24)	58 (25–1388)	<0.001
Preop ALT (U/l)	35 (7–185)	42 (7–659)	0.11
Preop AST (U/l)	47 (11–247)	69 (19–642)	<0.001
Preop MELD [14]	10.0 (6–22)	17.0 (8–40)	<0.001
MELD ex bilirubin	9.7 (6–22)	12.0 (4–33)	<0.001
Cold ischemia time (min)	93 (25–300)	88 (13–362)	0.68
Warm ischemia time (min)	49 (20–89)	48 (15–101)	0.45
Roux-en-Y	47.7%	54.8%	0.20
Length of hospital stay (days)	14 (6–313)	13 (4–260)	0.93
Length of ICU stay (days)	1 (0–29)	1 (0–159)	0.68

Live donor characteristics, such as age, gender, and BMI were similar in both groups beside the preoperative bilirubin values, only the preoperative MELD score and the AST values were significantly higher in the 'high bilirubin' group. Even after the bilirubin fraction was subtracted from the MELD score (modified MELD ex bilirubin), the value was still higher in the 'high bilirubin' group, suggesting a poorer preoperative clinical condition and liver function unrelated to the bilirubin levels.
BMI, body mass index; MELD, model of end-stage liver disease.
MELD score = 3.78 × ln[bilirubin (mg/dl)] + 11.2 × ln[INR] + 9.57 × ln[creatinine (mg/dl)].
MELD ex bilirubin = 11.2 × ln[INR] + 9.57 × ln[creatinine (mg/dl)].
If the patient was dialyzed twice within the last 7 days before transplantation, then the value for serum creatinine was corrected 4.0 mg/dl; any value less than 1 is given a value of 1.

Table 2. Etiology of terminal liver disease.

Total patients (n = 466)	Low TBIL (n = 118)	High TBIL (n = 348)	P-value
Hep B	7.6%	6.3%	0.67
Hep C	35.6%	32.8%	0.57
HCC	34.7%	25%	0.04
EtOH	23.7%	19.8%	0.37
NASH	10.2%	9.2%	0.76
PSC	8.5%	16.4%	0.03
PBC	5.9%	10.3%	0.15
AIH	6.8%	4.9%	0.43
Other	11.0%	7.2%	0.19

Viral infections, ethyl-toxic (EtOH), nonalcoholic steato-hepatitis (NASH), autoimmune hepatitis (AIH), and other etiological causes of liver disease (mainly metabolic or polycystic etiologies) were similarly distributed between 'low' and 'high bilirubin' groups. Only primary sclerosing cholangitis (PSC) was significantly more frequent in the 'high bilirubin' group, whereas hepatocellular carcinomas (HCC) occurred more often in the 'low bilirubin' group. PBC, primary biliary cirrhosis.

(MELD score calculated with INR and creatinine values only), the values remained significantly higher in the 'high bilirubin' group ['low' 9.7 (6–22) vs. 'high' 12.0 (4–33), $P < 0.001$], suggesting a poorer liver function and overall health status in this patient group that is not only explained by the higher frequency of primary biliary disease.

Warm ischemia time and CIT during transplantation were comparable in both groups. Also, the frequency of Roux-en-Y anastomosis compared to end-to-end bile duct anastomosis was close to 50% in both groups (see Table 1).

In both groups, the identical percentage of recipients received an induction therapy with either thymoglobulin or basiliximab (both 78.3%, $P = 0.99$). In both groups, the majority of recipients received tacrolimus as calcineurin inhibitor ('low' 70.4% vs. 'high' 65.3%, $P = 0.69$). The remaining recipients received either cyclosporin only ('low' 17.4% vs. 'high' 17.9%, $P = 0.63$), or they were started on one of both immunosuppressive drugs and converted to the other later. A little less than half of the patient population received mycophenolate mofetil after transplantation in both groups ('low' 43.5% vs. 'high' 48.3%, $P = 0.42$).

Ischemia–reperfusion injury and liver function

Linear regression and Spearman correlation analyses between logarithmically transformed preoperative total bilirubin levels and postoperative rise in transaminases as a marker of IRI showed significant negative coefficients for both ALT ($\beta = -0.128$, $P = 0.007$; $\rho = -0.115$, $P = 0.013$, see Fig. 1a) and AST ($\beta = -0.104$, $P = 0.027$; $\rho = -0.103$, $P = 0.027$, see Fig. 1b). Furthermore, the postoperative rise in transaminases was significantly lower when comparing the 'high' to the 'low bilirubin' group [AST 'low' 443.5 (−46–2585) U/l vs. 'high' 349 (−42–2802) U/l, $P = 0.006$; ALT 'low' 375 (−11–2102) U/l vs. 'high' 288 (−210–2457) U/l, $P = 0.006$; see Fig. 2a,b). Analyzing the 'mildly' and 'severely increased' subgroups, the IRI appeared to further decrease with higher bilirubin levels suggesting a concentration depending protective effect (Fig. 2c,d). When the biliary etiologies (PSC and PBC) were excluded from the analysis, this protective effect persisted [AST 'low' 438 (−46–1543) U/l vs. 'high' 341 (−3–2563) U/l, $P = 0.03$; ALT 'low' 313 (−11–1376) U/l vs. 'high' 281 (−210–2109) U/l, $P = 0.05$).

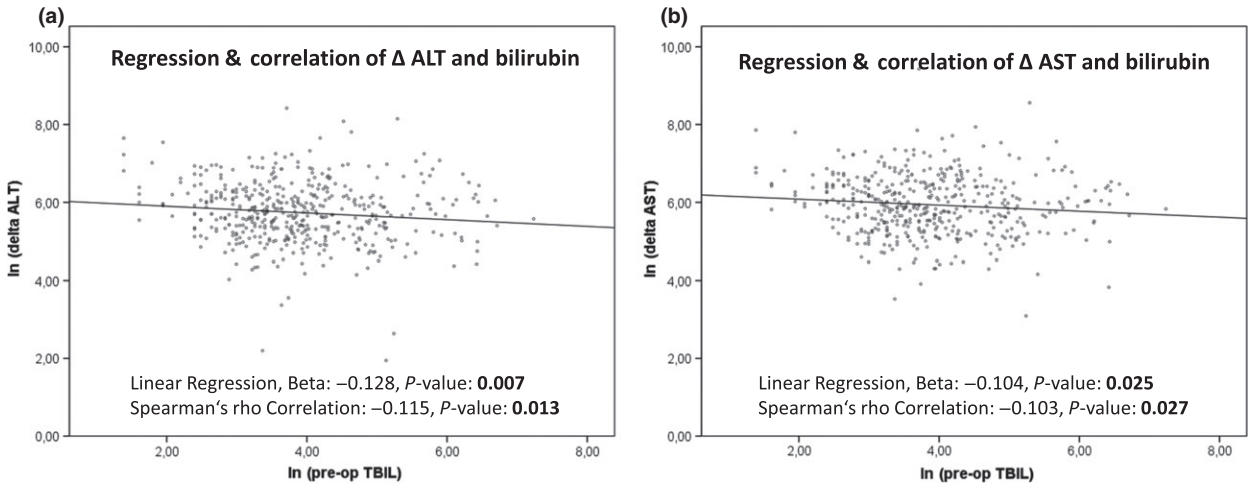

Figure 1 Linear regression and correlation between preoperative bilirubin and postoperative rise of transaminases. The preoperative bilirubin and postoperative rise of transaminases were logarithmically transformed for easier visualization on a scattered dot chart with regression line. Linear regression and Spearman's correlation analyses demonstrated negative coefficients suggesting a mildly protective effect against IRI.

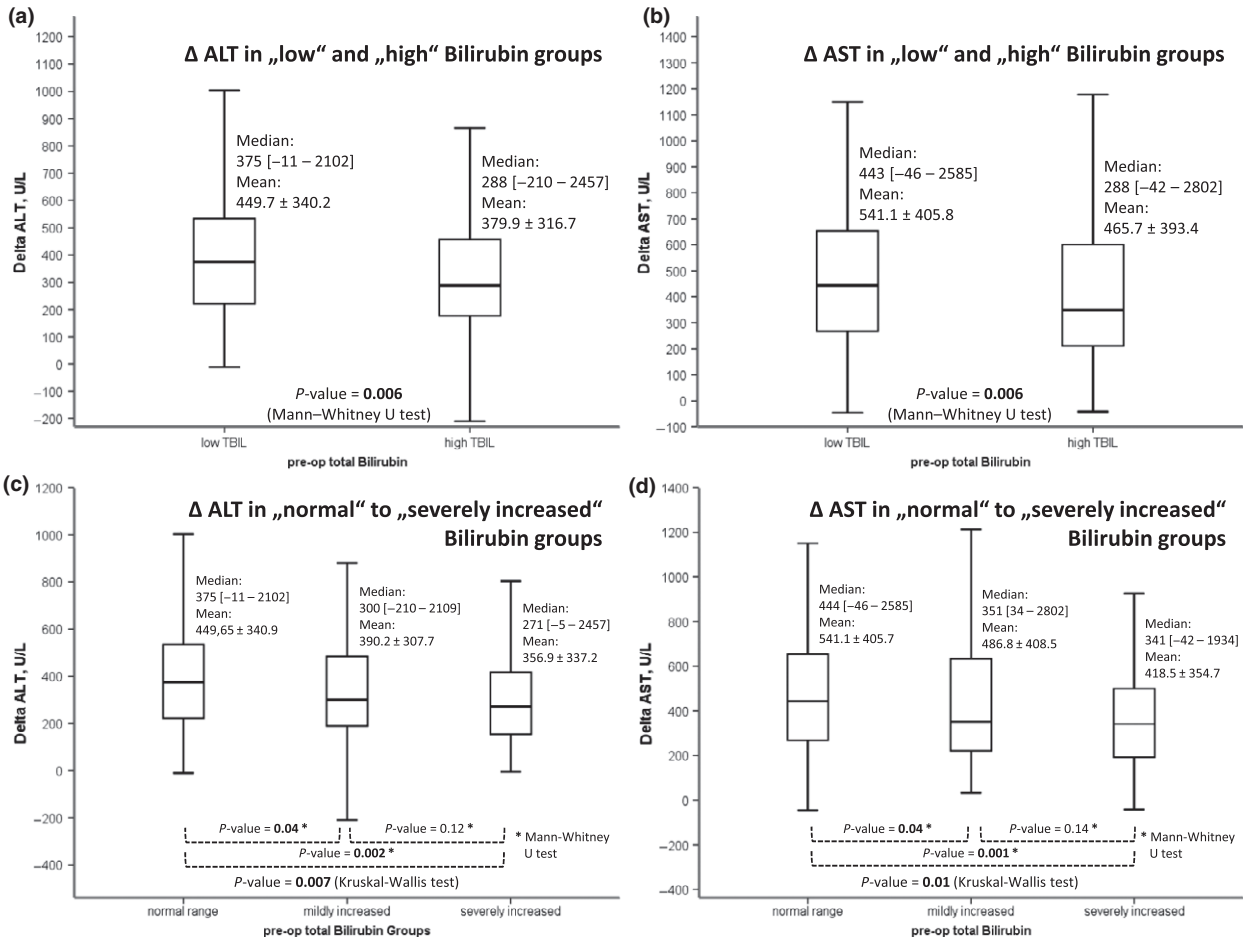

Figure 2 Postoperative transaminase increase. Box-plot (median with 25th and 75th percentile, whiskers represent inner fences, outliers were excluded from plot for clearer visualization) for absolute postoperative rise of both alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The postoperative rise in transaminases was significantly lower when comparing the 'high' (>24 μmol/l, >1.4 mg/dl) to the 'low bilirubin' (≤24 μmol/l, ≤1.4 mg/dl) group (A and B). When dividing the 'high bilirubin' group into 'mildly' (>24 μmol/l ≤ 100 μmol/l, >1.4 mg/dl ≤5.8 mg/dl) and 'severely increased' (>100 μmol/l, >5.8 mg/dl) subgroups, the effect appears to be concentration dependent (C and D).

International normalized ratio values 14 days after transplantation, as early marker of liver function, were almost identical in both 'low' and 'high bilirubin' groups ['low' 1.21 (0.82–1.95) vs. 'high' 1.22 (0.89–2.21), $P = 0.63$; see Fig. 3a]. Similarly, the total bilirubin levels 90 days after transplantation as a later marker of liver function were equally distributed in both groups ['low' 10 (2–226) μmol/l vs. 'high' 10 (2–230) μmol/l, $P = 0.12$; see Fig. 3b]. These findings held true in the nonbiliary etiology subgroup analysis [INR 'low' 1.24 (0.82–1.95) vs. 'high' 1.22 (0.89–2.21), $P = 0.91$; total bilirubin 'low' 10 (2–226) μmol/l vs. 'high' 11 (2–230) μmol/l, $P = 0.11$]. No functional differences were observed in the more detailed analysis using the divided 'high bilirubin' subgroups ('mildly' and 'severely increased').

Multivariate linear regression

To evaluate whether preoperative bilirubin values were an independent influence factor on IRI, we included logarithmically transformed preoperative total bilirubin levels in a multivariate linear regression analysis together with donor age, recipient age, CIT, and WIT as independent variables. The logarithmically transformed postoperative rise in ALT was used as dependent variable and indicator for the degree of IRI. As in the correlation analysis, preoperative bilirubin was negatively associated with the postoperative rise of ALT levels [standardized regression coefficient −0.12, $P = 0.046$, confidence interval (CI) −0.165 to −0.001], suggesting a protective effect. In this multivariate analysis, bilirubin was the only independently significant variable

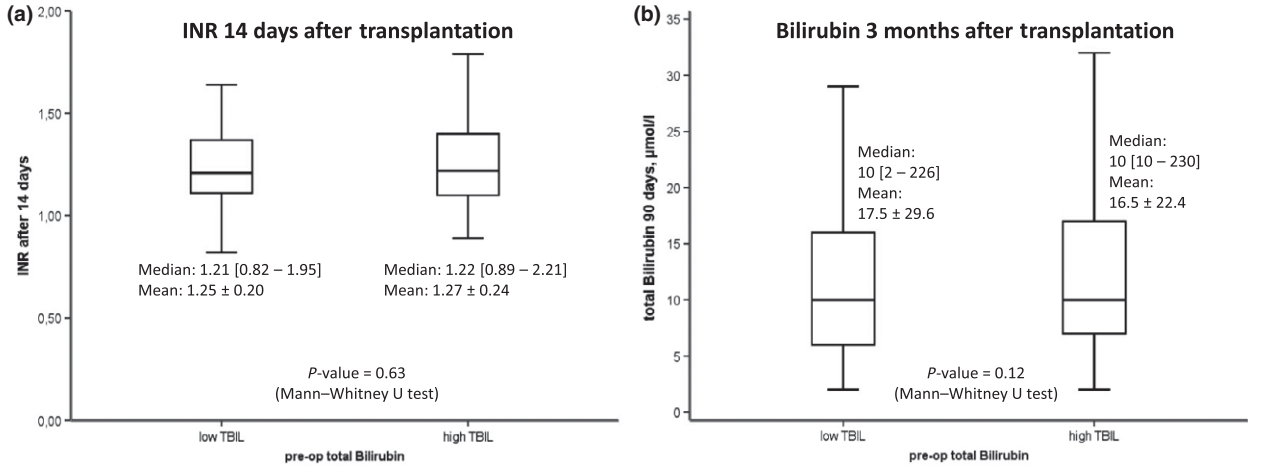

Figure 3 Postoperative liver function. Box-plot (median with 25th and 75th percentile, whiskers represent inner fences, outliers were excluded from plot for clearer visualization) for international normalized ratio (INR) 14 days after transplantation and total bilirubin 90 days after transplantation. Both groups have equivalent marker of postoperative liver function.

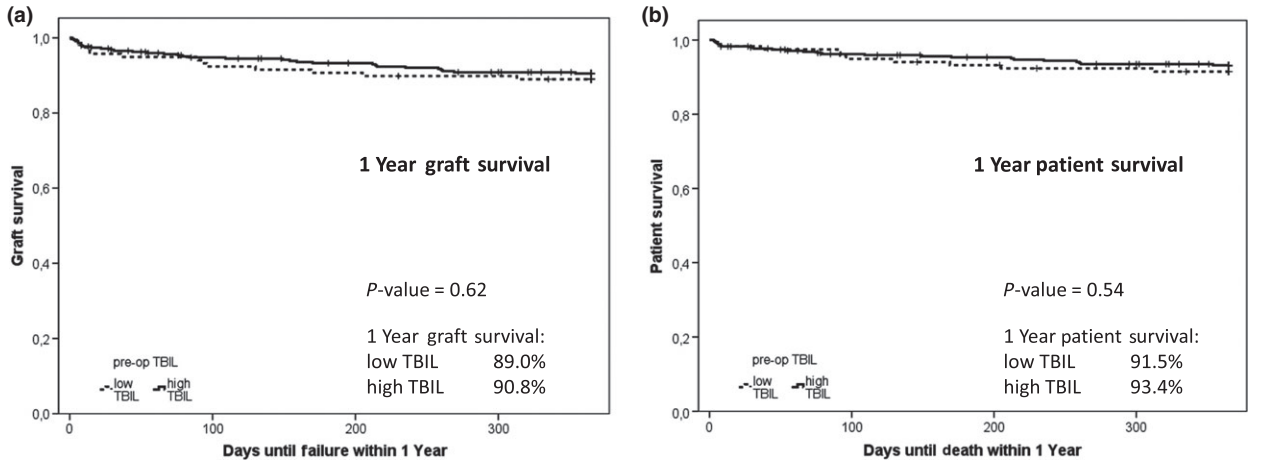

Figure 4 Graft and patient survival. Kaplan–Meier graphs for graft and patient survival 1 year after transplantation. Survival was similar in both groups.

besides donor age (coefficient 0.16, $P = 0.006$, CI 0.003–0.016). Recipient age (coefficient 0.01, $P = 0.846$, CI −0.007 to 0.008), CIT (coefficient 0.11, $P = 0.07$, CI 0–0.003), and WIT (coefficient −0.06, $P = 0.29$, CI −0.007 to 0.002) were each not significantly associated with postop ALT values.

Postoperative course

Although the 'high bilirubin' group had an overall poorer preoperative condition, as indicated by the MELD score (see Table 1), postoperative intensive care unit stay ['low' 1 (0–29) vs. 'high' 1 (0–159), $P = 0.68$; see Table 1] as well as length of hospital stay ['low' 14 (6–313) vs. 'high' 13 (4–260), $P = 0.93$; see Table 1] were similar in both groups. Also, 1-year graft ('low' 89.0% vs. 'high' 90.8%, $P = 0.62$; see Fig. 4a) and patient survival ('low' 91.5% vs. 'high' 93.4%, $P = 0.54$; see Fig. 4b) did not show significant differences. These findings were similar even when the biliary etiologies were eliminated from the analysis (1-year graft survival 'low' 87.1% vs. 'high' 89.2%, $P = 0.61$; 1-year patient survival 'low' 90.1% vs. 'high' 92.0%, $P = 0.59$).

The most common course for graft failure within 1 year was death due to a septic multi-organ failure after a

Table 3. Complications.

Complications	Low TBIL (n = 118)	High TBIL (n = 348)	P-value
Cause of failure, 1 year			
Graft unrelated	5 (4.2%)	6 (1.8%)	0.12
Septic MOF	4 (3.4%)	13 (3.7%)	0.86
Vascular	3 (2.5%)	5 (1.4%)	0.42
Hemorrhage	0	1 (0.3%)	0.56
Liver dysfunction	0	3 (8.6%)	0.31
Biliary complication	1 (0.8%)	1 (0.3%)	0.42
HCC	0	3 (8.6%)	0.31
Severe complications [21]	25 (21.2%)	73 (21.0%)	0.88
Biliary complications	15 (12.7%)	55 (15.8%)	0.42
Leak	7 (6.0%)	23 (6.6%)	0.80
Stricture	8 (6.8%)	35 (10.1%)	0.29
Cellular rejection, 1 year	20 (17.0%)	46 (13.2%)	0.28

Causes of graft failure and postoperative complications were distributed equally in both groups.

prolonged ICU-course (see Table 3). The frequency of multi-organ failure in the first year was similar in both 'low' and 'high bilirubin' groups ('low' 3.4% vs. 'high' 3.7%, $P = 0.86$). Death due to liver unrelated causes, such as cardiac ischemia, stroke, or intracranial hemorrhage, was the second most common cause of graft loss ('low' 4.2% vs. 'high' 1.7%, $P = 0.12$). Vascular causes such as arterial or portal venous thrombosis accounted for 2.5% of graft losses in the 'low' and 1.4% in the 'high bilirubin' group ($P = 0.42$). Although the indication of HCC as reason for transplantation was higher in the 'low bilirubin' group, the only three deaths from aggressive HCC recurrence within 1 year after transplantation occurred all in the 'high bilirubin' group ($P = 0.31$). Singular causes of graft failure included recurrent cholangitis, cryptogenic liver failure, and small-for-size syndrome. There was no graft loss within the first year due to primary nonfunction or rejection.

The rate of severe postoperative complications (Clavien–Dindo score ≥3b) was close to 21% in both groups (see Table 3). Biliary complications within the first year were also similarly distributed between both groups ('low' 12.7% vs. 'high' 15.8%, $P = 0.42$; see Table 3). Bile duct strictures occurred in 6.8% (5.9% anastomotic vs. 0.8% central strictures) of the 'low bilirubin' patients, compared to 10.1% (7.2% anastomotic vs. 2.9% central strictures) in the 'high bilirubin' group ($P = 0.29$). The rate of bile leaks within the first year after transplantation was 6.0% in the 'low bilirubin' and 6.6% in the 'high bilirubin' group ($P = 0.8$).

Discussion

In this study, we analyzed the effects of preoperative bilirubin levels of liver recipients on hepatic IRI in a homogeneous LDLT population. Live donors underwent a thorough medical evaluation process to minimize the risks for live liver donation. We used standardized recovery and transplantation protocols with a relatively small range of preservation time. Therefore, all recipients received consistently healthy liver grafts, minimizing confounding differences in graft quality, and emphasizing the effect of the recipient's preoperative condition on the transplantation outcome.

First, we demonstrated in the entire cohort a significant negative correlation between preoperative bilirubin values of transplant recipients and postoperative liver transaminases as a marker of IRI. The reduction of postoperative transaminase levels indicates a protective effect of preoperatively increased bilirubin levels against IRI. In a multivariate linear regression analysis, bilirubin was an independent protective factor for IRI. In a second step, we divided the LDLT population into both a 'high' and a 'low' bilirubin group. The critical total bilirubin value dividing both groups was 24 μmol/l (1.4 mg/dl, 25th percentile of all values), which is also close to the cut-off of the physiological range. Apart from the preoperative bilirubin levels and the MELD score, both groups were very homogeneous considering demographic data. We demonstrated that 'high' bilirubin values before transplantation led to significantly lower IRI as measured by liver transaminases. We performed additional analysis with 'mildly' and 'severely increased' subgroups (division cut-off values were 25th and 75th percentile). Indeed, reperfusion injury is more decreased with higher bilirubin levels suggesting a concentration-dependent protective effect. The protective effect of bilirubin on IRI was present both with and without inclusion of patients with cholestatic etiologies as underlying liver disease. In contrast to the noncholestatic group, these patients have a comparably high bilirubin levels in relation to the overall liver function and clinical situation. This indicated that the 'high bilirubin' group was not positively confounded because of a higher portion of cholestatic patient.

Ischemia/reperfusion injury as the predominant cause of primary graft dysfunction is an important factor for early graft survival [1,2,20]. Once a liver graft has survived a significant IRI impact, it has an equivalent survival rate as grafts with minor IRI [20]. Marginal grafts are especially susceptible to IRI indicating a particular need for protective strategies [5,22]. On the other hand, primary nonfunction in LDLT is rare as the extent of IRI is limited due to careful donor recruitment and short ischemia times. Nonetheless, this patient group presents an excellent chance to study the impact of recipient-related variables as the more homogeneous and healthy donor group presents fewer confounding factors.

Bilirubin is known to be a powerful physiological antioxidant [10,11,13,23]. In a rodent model, Kato *et al.* [24]

were able to show that a bilirubin rinse of liver grafts before transplantation is a simple strategy to ameliorate oxidative stress and hepatobiliary dysfunction. Similarly, Fondevila *et al.* [25] demonstrated a cytoprotective effect of biliverdin as a precursor of bilirubin in another rodent liver transplantation model. Different to Kato *et al.*, this group did not treat the liver graft before transplantation but rather during and after reperfusion in the recipient rat. Despite these favorable outcomes, a previous clinical study by Manzinate *et al.* using a deceased donor population failed to reproduce the results of these animal studies. In a retrospective study design comprising 608 orthotopic liver transplantations of the Birmingham transplant program, no correlation was apparent between preoperative bilirubin and postoperative IRI as well as graft survival [15]. However, especially the heterogeneity of the donor group might have confounded these findings. On the contrary, Lee *et al.* were able to demonstrate a protective effect of postoperatively increased serum bilirubin levels on graft survival and rates of acute rejection after kidney transplantation [26]. They correlated this effect to UDP-glucuronosyltransferase polymorphisms in the graft recipient that were presumably associated with postoperative changes of bilirubin serum levels. However, markers of IRI were not investigated in this study.

Our results represent the first study indicating that serum bilirubin levels have a relevant effect on IRI after human OLT. These findings are not only important for preoperative treatment regimens before transplantation but also before liver resection. Preoperative biliary stenting immediately before liver surgery, for example, might be indicated more restrictively if liver function is otherwise stable. But more importantly, further research is now legitimate in order to identify the role and inducibility of HO-1 in clinical transplant settings and in order to find strategies to implement the protective antioxidant effect of bilirubin during and after OLT.

One critical point of our study is the clinical disparity of the two recipient groups before transplantation as demonstrated by the diverging MELD scores. Total bilirubin as the main variable of this study is one of the values needed to calculate the MELD score, which predicts the survival of patients with chronic liver disease [14]. Therefore, the patient in the 'high bilirubin' group can be considered to have a poorer liver function and to be sicker than the patients of the 'low bilirubin' group. In fact, the two other values needed for the MELD score, INR and creatinine, were also significantly higher in the 'high bilirubin' group. However, the clinical condition before transplantation *per se* does not have an impact on IRI as our primary outcome variable. Although some groups suggested that a higher preoperative MELD score could affect the postoperative outcome [27–31], we found a comparable long-term survival in our own LDLT population comparing high versus low MELD patients [32]. However, it is possible that the protective effect of high bilirubin levels contributed to a favorable outcome despite a clinically more critical condition in high MELD patients. It is important to distinguish high bilirubin levels preoperatively that indicate the chance of survival without transplantation from postoperative bilirubin dynamics that indicate hepatobiliary injury of the transplanted graft.

In addition to the disparity of the pretransplant MELD scores of the liver recipients in both groups, this study has two more limitations. It consists of a relatively small patient population operated over a 14-year period of time. We compensated for these shortcomings using a homogenous donor group and a demographically uniform recipient population, treated with a consistent postoperative protocol. In addition, the single-center study design allowed for a detailed analysis of postoperative complications.

In summary, high bilirubin levels of liver recipient before transplantation are associate with reduced IRI after transplantation. While clinical relevance is limited for LDLT, this study is a proof of principle for the protective effect of bilirubin for hepatic IRI in a human transplant setting. Inducible HO-1, as the likely mechanism of protection of bilirubin, represents a clinical relevant target for future studies.

Authorship

VNS: participated in research design, participated in the performance of the research, participated in data analysis, participated in writing the manuscript. NG: participated in the performance of the research, participated in writing the manuscript. JMK: participated in writing the manuscript. MAM: participated in research design, participated in data analysis. NS, LL, GAL and ELR: participated in the performance of the research (patient follow up). MSC, PDG, IDM, AG and DRG: participated in the performance of the research (performing surgery). MS: participated in research design, participated in writing the manuscript, participated in the performance of the research (performing surgery).

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References

1. Fondevila C, Busuttil RW, Kupiec-Weglinski JW. Hepatic ischemia/reperfusion injury–a fresh look. *Exp Mol Pathol* 2003; **74**: 86.

2. Chen XB, Xu MQ. Primary graft dysfunction after liver transplantation. *Hepatobiliary Pancreat Dis Int* 2014; **13**: 125.
3. Mourad MM, Algarni A, Liossis C, Bramhall SR. Aetiology and risk factors of ischaemic cholangiopathy after liver transplantation. *World J Gastroenterol* 2014; **20**: 6159.
4. Cursio R, Gugenheim J. Ischemia-reperfusion injury and ischemic-type biliary lesions following liver transplantation. *J Transplant* 2012; **2012**: 164329.
5. Zhai Y, Petrowsky H, Hong JC, Busuttil RW, Kupiec-Weglinski JW. Ischaemia-reperfusion injury in liver transplantation–from bench to bedside. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 79.
6. Origassa CS, Camara NO. Cytoprotective role of heme oxygenase-1 and heme degradation derived end products in liver injury. *World J Hepatol* 2013; **5**: 541.
7. Richards JA, Wigmore SJ, Devey LR. Heme oxygenase system in hepatic ischemia-reperfusion injury. *World J Gastroenterol* 2010; **16**: 6068.
8. Sass G, Barikbin R, Tiegs G. The multiple functions of heme oxygenase-1 in the liver. *Z Gastroenterol* 2012; **50**: 34.
9. Wang CF, Wang ZY, Li JY. Dual protective role of HO-1 in transplanted liver grafts: a review of experimental and clinical studies. *World J Gastroenterol* 2011; **17**: 3101.
10. Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN. Bilirubin is an antioxidant of possible physiological importance. *Science* 1987; **235**: 1043.
11. Llesuy SF, Tomaro ML. Heme oxygenase and oxidative stress. Evidence of involvement of bilirubin as physiological protector against oxidative damage. *Biochim Biophys Acta* 1994; **1223**: 9.
12. Stocker R, Peterhans E. Antioxidant properties of conjugated bilirubin and biliverdin: biologically relevant scavenging of hypochlorous acid. *Free Radic Res Commun* 1989; **6**: 57.
13. Baranano DE, Rao M, Ferris CD, Snyder SH. Biliverdin reductase: a major physiologic cytoprotectant. *Proc Natl Acad Sci U S A* 2002; **99**: 16093.
14. Kamath PS, Wiesner RH, Malinchoc M, *et al.* A model to predict survival in patients with end-stage liver disease. *Hepatology* 2001; **33**: 464.
15. Manzinate F, McDaid J, Devey L, Gunson B, Wigmore SJ. Pretransplant bilirubin concentration does not correlate with early reperfusion injury following liver transplantation. *Transplantation* 2007; **83**: 103.
16. The Medical Council of Canada. Clinical Laboratory Tests – Normal Values. Objectives for the Qualifying Examination 2014 [cited 2014 July 15, 2014]; Available from: http://apps.mcc.ca/Objectives_Online/objectives.pl?lang=english&loc=values.
17. Cattral MS, Molinari M, Vollmer CM Jr, *et al.* Living-donor right hepatectomy with or without inclusion of middle hepatic vein: comparison of morbidity and outcome in 56 patients. *Am J Transplant* 2004; **4**: 751.
18. Adcock L, Macleod C, Dubay D, *et al.* Adult living liver donors have excellent long-term medical outcomes: the University of Toronto liver transplant experience. *Am J Transplant* 2010; **10**: 364.
19. Reichman TW, Katchman H, Tanaka T, *et al.* Living donor versus deceased donor liver transplantation: a surgeon-matched comparison of recipient morbidity and outcomes. *Transpl Int* 2013; **26**: 780.
20. Rosen HR, Martin P, Goss J, *et al.* Significance of early aminotransferase elevation after liver transplantation. *Transplantation* 1998; **65**: 68.
21. Clavien PA, Barkun J, de Oliveira ML, *et al.* The Clavien-Dindo classification of surgical complications: five-year experience. *Ann Surg* 2009; **250**: 187.
22. Busuttil RW, Tanaka K. The utility of marginal donors in liver transplantation. *Liver Transpl* 2003; **9**: 651.
23. Ollinger R, Wang H, Yamashita K, *et al.* Therapeutic applications of bilirubin and biliverdin in transplantation. *Antioxid Redox Signal* 2007; **9**: 2175.
24. Kato Y, Shimazu M, Kondo M, *et al.* Bilirubin rinse: a simple protectant against the rat liver graft injury mimicking heme oxygenase-1 preconditioning. *Hepatology* 2003; **38**: 364.
25. Fondevila C, Shen XD, Tsuchiyashi S, *et al.* Biliverdin therapy protects rat livers from ischemia and reperfusion injury. *Hepatology* 2004; **40**: 1333.
26. Lee JP, Kim do H, Yang SH, *et al.* Serum bilirubin affects graft outcomes through UDP-glucuronosyltransferase sequence variation in kidney transplantation. *PLoS One* 2014; **9**: e93633.
27. Weismuller TJ, Fikatas P, Schmidt J, *et al.* Multicentric evaluation of model for end-stage liver disease-based allocation and survival after liver transplantation in Germany–limitations of the 'sickest first'-concept. *Transpl Int* 2011; **24**: 91.
28. Klein KB, Stafinski TD, Menon D. Predicting survival after liver transplantation based on pre-transplant MELD score: a systematic review of the literature. *PLoS ONE* 2013; **8**: e80661.
29. Hoffmann K, Hinz U, Hillebrand N, *et al.* The MELD score predicts the short-term and overall survival after liver transplantation in patients with primary sclerosing cholangitis or autoimmune liver diseases. *Langenbecks Arch Surg* 2014; **399**: 1001.
30. Onaca NN, Levy MF, Netto GJ, *et al.* Pretransplant MELD score as a predictor of outcome after liver transplantation for chronic hepatitis C. *Am J Transplant* 2003; **3**: 626.
31. Onaca NN, Levy MF, Sanchez EQ, *et al.* A correlation between the pretransplantation MELD score and mortality in the first two years after liver transplantation. *Liver Transpl* 2003; **9**: 117.
32. Selzner M, Kashfi A, Cattral MS, *et al.* Live donor liver transplantation in high MELD score recipients. *Ann Surg* 2010; **251**: 153.