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# Elevated Preoperative Serum Bilirubin Improves Reperfusion Injury and Survival Postliver Transplantation

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**Background.** The cytoprotective effects of hemeoxygenase-1 and its product biliverdin/bilirubin are widely acknowledged in experimental transplant medicine. However, its potentially beneficial effect during organ reperfusion is not established. **Methods.** In a matched study, we compared markers of reperfusion injury (alanine aminotransferase/aspartate aminotransferase) and transplantation outcome (complication rates, liver function, and survival) between recipient groups with “normal” versus “increased” preoperative bilirubin values. Groups were matched for donor and recipient age, liver disease, year of transplantation, and recipient’s preoperative condition (modified model for end-stage liver disease score excluding bilirubin). **Results.** The postoperative transaminase peak was significantly higher when comparing the “normal” to the “increased” bilirubin group (maximum aspartate aminotransferase “normal” 2013 [325-13 210] U/L vs “increased” 1360 [221-15 460] U/L,  $P = 0.006$ ; maximum alanine aminotransferase “normal” 1151 [82-6595] U/L vs “increased” 820 [66-5382] U/L,  $P = 0.01$ ). Grafts in the “increased” bilirubin group had faster recovery of graft function with faster decrease in international normalized ratio at days 3 and 7 posttransplantation in the “increased” vs “normal” bilirubin group. Although long-term functional parameters (international normalized ratio and bilirubin posttransplantation) as well as surgical and biliary complication rates were similar in both groups, 1-year survival rates were significantly higher in the group with increased preoperative bilirubin (graft survival, “normal” 86% vs “increased” 97%;  $P = 0.006$ ). **Conclusions.** Increased bilirubin levels of liver graft recipients before transplantation are associated with reduced reperfusion injury and improved survival after transplantation.

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Liver grafts from expanded-criteria donors are associated with inferior outcome. However, the severe organ shortage has resulted in expansion of donor selection criteria and

increasing numbers of expanded-criteria donor organs are accepted for transplantation.<sup>1-3</sup> Liver transplantation with expanded-criteria grafts is often associated with severe ischemia and reperfusion injury (IRI), resulting in primary nonfunction or delayed graft function.<sup>4</sup> The extent of IRI is an important determinant for the severity of postoperative hepatocyte dysfunction and cholangiopathy.<sup>5-8</sup> Reperfusion injury also induces an inflammatory cascade that results in the long-term immunological changes and chronic graft rejection.<sup>9</sup> Minimizing IRI is important, and various strategies have been proposed for its attenuation.<sup>10,11</sup> The translation of such strategies into clinical practice, however, remains often unsuccessful either due to practical inapplicability or lack of a clinical effect.

Hemeoxygenase-1 (HO-1) has been proposed as a potent mediator for reducing IRI and its cytoprotective, antioxidant, anti-inflammatory, immune-modulative, and vasorelaxation effects have been studied extensively.<sup>12-15</sup> HO-1 cleaves heme to biliverdin, carbon monoxide, and free iron. Biliverdin, in turn, is reduced to bilirubin, which is known for its antioxidative properties.<sup>16-18</sup> 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.<sup>19</sup> This redox cycle is believed to be—among others—one of the pivotal and inducible mechanisms of bilirubin induced cytoprotection in hepatic

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IRI, rejection, and inflammation.<sup>18,20</sup> Moreover, the beneficial effects of both HO-1 and bilirubin are not limited to hepatic cytoprotection after liver transplantation. The protective effects also extend to other systems, such as in gastrointestinal, cardiovascular, and autoimmune diseases.<sup>21-24</sup>

On the other hand, bilirubin is known as a marker of cholestasis and liver dysfunction. It is 1 of the 3 variables used to calculate the model for end-stage liver disease score (MELD), which is widely used to estimate a patient's short-term survival while awaiting liver transplantation.<sup>25</sup> However, increased bilirubin levels before liver transplantation do not predict posttransplant outcome because the liver is replaced during surgery. In fact, high bilirubin levels during transplantation could offer a protection against IRI. Recently, our group demonstrated protective effects of increased pretransplant bilirubin levels against IRI in the setting of living donor liver transplantation.<sup>26</sup> In contrast, the effects of high bilirubin levels for deceased donor liver transplantation remain unclear.<sup>27</sup> The deceased donor population is generally very heterogeneous and many recipient and donor-related factors like age or steatosis confounded earlier analysis. Therefore, we evaluated the impact of preoperative bilirubin values on IRI, and early outcome after liver transplantation in our deceased donor transplant population, controlling recipient and donor variables in a matched study design.

## MATERIALS AND METHODS

### Study Design

The study was approved by the University Health Network's Research Ethics Board (14-8069-BE) and conformed with the ethical guidelines of the 1975 Declaration of Helsinki. Between January 2000 and December 2013, all adult patients receiving a full size heart beating donor (HBD) graft at our institution were prospectively entered into our transplant database ( $n = 854$ ). Patients suffering from acute liver failure or receiving a retransplantation were excluded from the study population.<sup>27</sup> Patients were divided into a "normal" ( $n = 215$ ) and an "increased" bilirubin group ( $n = 639$ ). The cutoff value dividing both groups was 1.2 mg/dL (21  $\mu\text{mol/L}$ ), which is the upper limit of the normal range for total serum bilirubin<sup>28</sup> and the 25th percentile of the preoperative total bilirubin levels in our orthotopic liver transplantation (OLT) population group. To obtain 2 comparable groups for the influence of preoperative bilirubin values on IRI, patient matching was performed 1:1 for the following criteria: donor age ( $\pm 3$  years), recipient age ( $\pm 3$  years), recipient's etiology of liver disease (hepatitis B virus and/or hepatitis C virus [HCV], ethyl-toxic liver cirrhosis, nonalcoholic steatohepatitis, primary sclerosing cholangitis, others, other etiologies; hepatocellular carcinoma [HCC]), recipient MELD score subtracted by the bilirubin fraction ( $\text{MELD ex bilirubin} = 11.2 \times \ln \text{international normalized ratio [INR]} + 9.57 \times \ln[\text{creatinine (mg/dL)}]$ ),<sup>26</sup> and period of transplantation (year of OLT  $\pm 3$  years). The matching for a MELD score ex bilirubin (calculated by serum creatinine and INR only) is supposed to provide a similar preoperative clinical condition in both groups independent of the serum bilirubin. In case more than 1 patient from the "increased bilirubin" group fit the criteria of a patient from the "normal bilirubin" group, the patient with the closed match was selected for the analysis. Sufficient matches in both groups were

found in 116 paired cases. The high number of matching failures was mainly caused by the unequal distribution of patients with a biliary etiology between both groups. Furthermore, bilirubin values often correlated with INR and creatinine values causing a discrepancy of the modified MELD score (ex bilirubin) in both groups.

The 2 groups were compared for differences in IRI, postoperative liver function, and complications. Subanalyses were performed within the "increased bilirubin" group for "mildly" ( $>1.2 \text{ mg/dL} \leq 3 \text{ mg/dL}$ ,  $>21 \mu\text{mol/L} \leq 51 \mu\text{mol/L}$ ), "moderately" ( $>3 \text{ mg/dL} \leq 6 \text{ mg/dL}$ ,  $>51 \mu\text{mol/L} \leq 102 \mu\text{mol/L}$ ), and "severely increased" ( $>6 \text{ mg/dL}$ ,  $> 102 \mu\text{mol/L}$ ) preoperative bilirubin values.

### OLT Procedure and Immunosuppression

Organ procurement and full size graft OLT was performed as previously described.<sup>29,30</sup> Cava replacement transplantation was the procedure of choice. The immunosuppression regimen consisted primarily of calcineurin inhibitors (CNI), either cyclosporine or tacrolimus, and steroids. Antiproliferative drugs (mycophenolate, mammalian target of rapamycin inhibitors or Azathioprine) were used in CNI-sparing regimens when there was concern about CNI-induced side effects. Steroids were tapered during the first 3 months after transplantation and consecutively stopped if no immunological event had occurred.

### Donor, Preoperative Recipient, and Perioperative Data

Donor age, gender, and body mass index (BMI) were documented at the time of organ procurement. For the recipients, the following preoperative data were collected: age, gender, preoperative biochemical profile, calculated MELD score, and etiology of liver disease. Cold ischemia time (CIT), warm ischemia time (WIT), and type of biliary anastomosis were documented as perioperative variables. Grafts from donor older than 65 years, donor BMI greater than 35  $\text{kg/m}^2$ , or CIT greater than 12 hours were considered to be expanded criteria organs.<sup>31</sup> Primary nonfunction of the graft was defined as death or retransplantation within 7 days after OLT in the absence of any infectious or vascular problems.<sup>32</sup>

### Postoperative Outcome

Liver graft function was evaluated clinically and by biochemical markers measured daily after transplantation until discharge and afterwards at regular intervals. Reperfusion injury was determined by maximum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) within 48 hours of transplantation.<sup>6,33</sup> To assess graft function, we evaluated INR shifts within the first week as compared with the pretransplant values (as marker of initial function), absolute INR levels 14 days after transplantation (as early marker of liver function), as well as total serum bilirubin 3 months after transplantation (as marker of liver function after bilirubin stabilization).

Patient's postoperative intensive care unit (ICU) and hospital stay was recorded for each case. Surgical complications were documented according to the Clavien-Dindo classification.<sup>34</sup> Scores greater than 3 were considered as relevant 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 retransplantation.

## Statistical Analysis

The data were analyzed with the SPSS 20 statistical package (IBM, Chicago, IL). Data were presented as median (range). Mann-Whitney *U* test and Kruskal-Wallis test were used for all continuous variables for comparison of the study groups. Differences between categorical variables were tested with  $\chi^2$ -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.

Normal distribution was tested by Kolmogorov-Smirnov test. For further analysis, bilirubin and transaminases values were logarithmically transformed to approach normal distribution for these data sets, minimizing the statistically distorting effect of outliers. In a multivariate linear regression analysis, the preoperative total bilirubin (logarithmized) was analyzed together with donor BMI, as well as CIT and WIT as independent variables to evaluate their impact on the maximum AST (logarithmized) as dependent variable and indicator for IRI.

## RESULTS

From January 2000 until December 2013, 854 adult patients (excluding cases of acute liver failure) received a full size HBD liver graft as their 1st transplantation at our institution. Out of this patient population, 116 matches were found between the “normal” (preoperative total serum bilirubin  $\leq 1.2$  mg/dL) and the “increased bilirubin” groups (in total 232 patients).

### Donor Data, Recipient Demographics, and Perioperative Status

The matching variables (donor age, recipient age, etiology of liver disease, preoperative MELD ex bilirubin, transplant period) were distributed equally in both groups (Table 1). The remaining donor characteristics, gender and BMI, were similar in both groups. Recipient characteristics that were not covered by the matching criteria were also comparable in both groups.

As expected, the preoperative medical MELD score was significantly higher in the “increased bilirubin” group (“normal” 9 [6-23] vs “increased” 14 [7-37], *P* < 0.001). The preoperative transaminases were significantly higher in the “increased bilirubin” group (ALT, “normal” 35 [3-274] U/L vs “increased” 46 [4-327] U/L, *P* = 0.045; AST: “normal” 42 [10-418] U/L vs “increased” 77 [19-253], *P* < 0.001).

WIT and CIT during transplantation were comparable in both groups. Also, the frequency of Roux-en-Y anastomosis compared with end-to-end bile duct anastomosis was comparable in both groups.

In the “normal bilirubin” group, 19.8% of transplanted organs met our criteria for expanded criteria grafts versus 25.0% in the “increased bilirubin” group (*P* = 0.34).

### Ischemia-Reperfusion Injury and Liver Function

The postoperative transaminase peak was significantly higher when comparing the “normal” to the “increased bilirubin” group (maximum AST “normal” 2013 [325-13 210] U/L vs “increased” 1360 [221-15 460] U/L, *P* = 0.006; maximum ALT “normal” 1151 [82-6595] U/L vs “increased” 820 [66-5382] U/L, *P* = 0.01; see Figure 1).

**TABLE 1.**

### Donor and recipient characteristics

Preoperative perioperative values	Normal bilirubin (n = 116)	Increased bilirubin (n = 116)	<i>P</i>
Period of transplantation			
2000-2002	7.9%	8.6%	0.76
2003-2005	25.9%	27.6%	
2006-2008	29.3%	26.7%	
2009-2011	26.7%	21.6%	
2012-2013	11.2%	15.5%	
Donor age, y	53 (10-79)	54 (13-84)	0.64
Donor BMI, kg/m <sup>2</sup>	25.6 (15.3-42.6)	25.7 (17.6-44.9)	0.67
Donor sex (female)	45.2%	34.5%	0.11
Expanded criteria grafts	19.8%	25.0%	0.34
Recipient age, y	58.1 (27.5-69.7)	58.6 (26.5-68.3)	1.0
Recipient BMI, kg/m <sup>2</sup>	26 (16.5-37.6)	27.2 (17.2-39.3)	0.23
Recipient sex (female)	15.5%	13.5%	0.44
Recipient etiology			
Viral	75.8%	75.8%	1
HCC	81.9%	81.9%	
EtOH	19.0%	19.0%	
NASH	6.9%	6.9%	
PSC	0.8%	0.8%	
Other	2.6%	2.6%	
Recipient MELD (preoperative)	9.1 (6-23)	14.0 (7-37)	<0.001
Recipient MELD without TBIL	10.0 (6-23)	10.6 (6-26)	0.47
Recipient TBIL (preoperative), mg/dL	0.7 (0.2-1.2)	2.4 (1.3-37.3)	<0.001
Recipient transaminases (preoperative)			
ALT, U/L	35 (3-274)	46 (4-327)	0.045
AST, U/L	42 (10-418)	77 (19-253)	<0.001
CIT, min	451 (133-1092)	476.5 (139-1118)	0.36
WIT, min	49 (23-99)	50 (29-105)	0.46
Roux-en-Y biliary reconstruction	42%	41%	0.96

Adequate matching of both groups was successful as demonstrated by equivalent values for donor and recipient age, periods of transplantation, etiology of liver disease, and recipient MELD ex bilirubin. The remaining characteristics, such as sex and BMI, were similar in both groups. The preoperative MELD score was significantly higher in the “increased bilirubin” group. Also, preoperative transaminases were slightly higher in the “increased bilirubin” group. Ischemia times as well as the method of biliary reconstruction were similar.

Data presented as median with minimum and maximum for continuous variables, percentage for categorical variables.

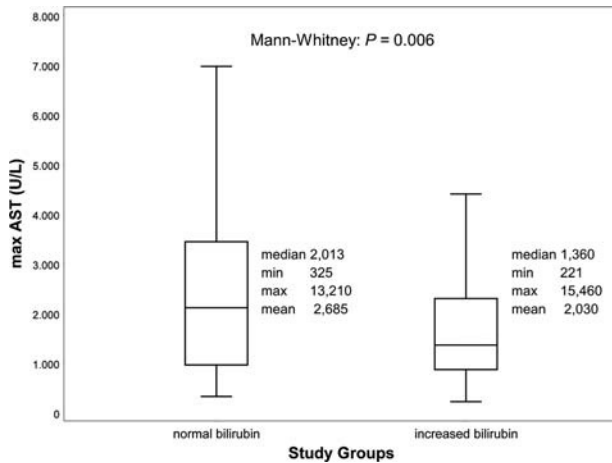
MELD score =  $3.78 \times \ln[\text{bilirubin (mg/dL)}] + 11.2 \times \ln[\text{INR}] + 9.57 \times \ln[\text{creatinine (mg/dL)}]$ .

MELD ex bilirubin =  $11.2 \times \ln[\text{INR}] + 9.57 \times \ln[\text{creatinine (mg/dL)}]$ .

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.

EtOH, ethyl-toxic liver cirrhosis; NASH, nonalcoholic steatohepatitis; PSC, primary sclerosing cholangitis; TBIL, total serum bilirubin; viral, hepatitis B and/or hepatitis C infection.

In a multivariate linear regression analysis, we investigated if the preoperative bilirubin value had an independent influence on the peak of transaminases. Preoperative bilirubin values and peak AST were logarithmically transformed to achieve a normal distribution. AST was used as a surrogate marker of IRI (dependent variable), whereas CIT, WIT, donor BMI, and preoperative bilirubin values were investigated as independent variables. Preoperative bilirubin had a significantly attenuating impact on peak AST values ( $\beta$ , -0.15; *P* = 0.04; 95% confidence interval [CI], -0.299 to -0.008), whereas both CIT and donor BMI significantly increased AST levels (CIT  $\beta$ , 0.18; *P* = 0.01; 95% CI, 0.0-0.002; BMI  $\beta$ , 0.27; *P* < 0.001; 95% CI, 0.025-0.088). Prolonged WIT appeared to increase IRI as well, but did



**FIGURE 1.** Peak AST after transplantation. Box-plot (median with 25th and 75th percentile, whiskers represent inner fences, outliers were excluded from plot for clearer visualization) for maximal aspartate aminotransferase (AST) values within 2 days after transplantation. The postoperative rise in transaminases was significantly lower when comparing the “increased” (>1.2 mg/dL, >21  $\mu\text{mol/l}$ ) to the “normal bilirubin” ( $\leq 1.2$  mg/dL,  $\leq 21$   $\mu\text{mol/l}$ ) group.

not reach significance ( $\beta$ , 0.13;  $P = 0.08$ ; 95% CI,  $-0.001$  to 0.022).

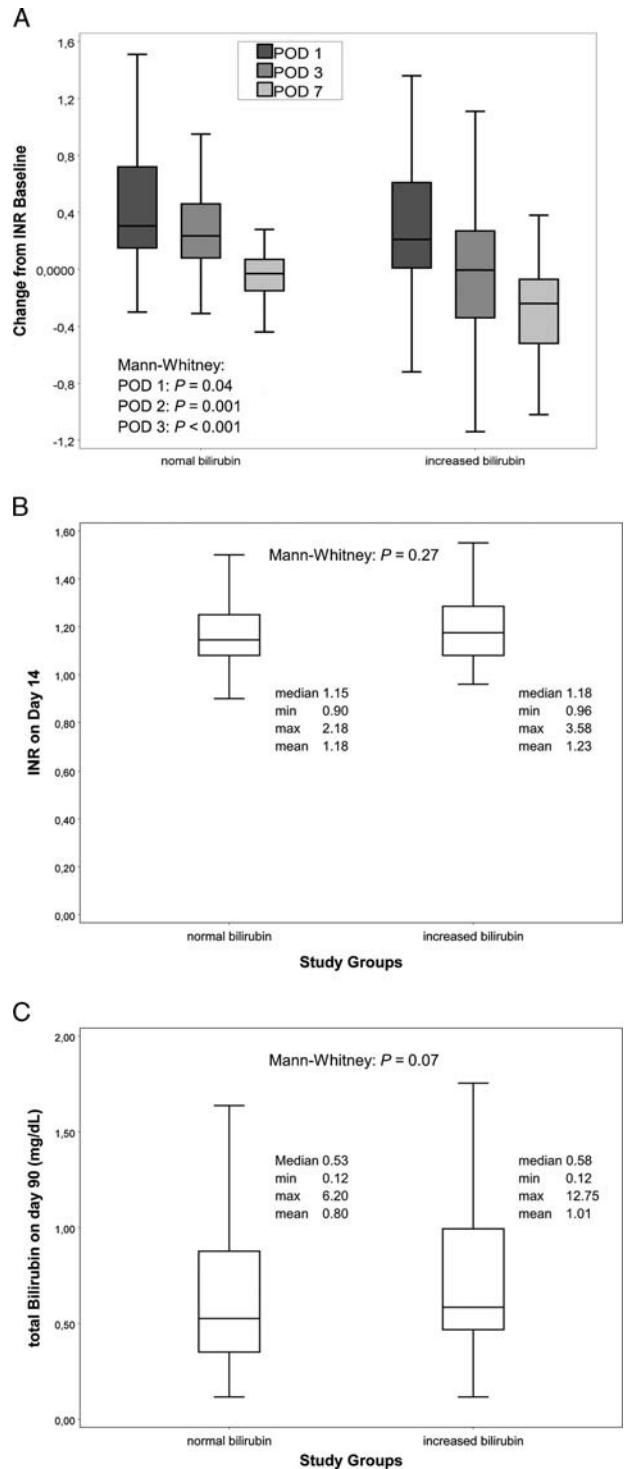
Grafts in the “elevated bilirubin” grouped picked up liver function faster than those from the “normal bilirubin” group. Within the first 24 hours, INR values had increased by a median of only 0.21 ( $-4.1$  to 2.9) in the “increase bilirubin” group versus 0.31 ( $-0.3$  to 2.5) in the “normal bilirubin” group ( $P = 0.04$ ). By day 3, median INR values in the “normal bilirubin” group were still increased when compared to the pretransplant values (0.24 [ $-0.71$  to 2.1]), whereas the median INR values in the “increased bilirubin” group had recovered the baseline value ( $-0.01$  [ $-3.95$  to 3.1],  $P < 0.001$ ). This faster adjustment of the INR in the “increased bilirubin” group was even more pronounced at day 7 (“normal”  $-0.03$  [ $-0.87$  to 0.28] vs “increased”  $-0.24$  [ $-5.27$  to 0.99],  $P < 0.001$ ; see Figure 2A). Fourteen days after transplantation, when INR values had adjusted to stable level as early marker of liver function, absolute INR values were similar in both “normal” and “increased bilirubin” groups (“normal” 1.2 [0.9-2.2] vs “increased” 1.2 [1.0-3.6],  $P = 0.27$ ; see Figure 2B). The total bilirubin levels 90 days after transplantation as later marker of liver function were comparable in both groups (“normal” 0.5 [0.1-6.2] mg/dL vs “increased” 0.6 [0.1-12.8] mg/dL,  $P = 0.07$ ; see Figure 2C).

### Subgroup Analysis, Dose Dependency of Bilirubin Effect

To evaluate a potential dose dependency of the protective bilirubin effect, we further divided the “increased bilirubin” group. Analyzing “mildly,” “moderately,” and “severely increased” subgroups, the IRI appeared to be lowest in the first 2 groups. However, the “severely increased” group was rather small ( $n = 13$ ) and the subgroups were otherwise not matched to each other. Accordingly, a dose-dependent, protective effect of bilirubin was not verifiable. Furthermore, no functional differences (INR at 14 days, bilirubin at 30 days) were observed in between the subgroups.

### Postoperative Outcome

Postoperative ICU stay (“normal” 1 [0-115] vs “increased” 2 [0-53],  $P = 0.09$ ) as well as length of hospital stay (“normal” 10.5 [4-196] vs. “increased” 12 [5-182],  $P = 0.1$ )



**FIGURE 2.** Postoperative Liver Function. Box-plot (median with 25th and 75th percentile, whiskers represent inner fences, outliers were excluded from plot for clearer visualization). A, Changes from pretransplant INR 1, 3, and 7 days after transplantation—liver grafts picked up function more quickly as demonstrated by a more rapid drop of INR values after transplantation. INR 14 days after transplantation (B) and total bilirubin 90 days after transplantation (C), both groups have equivalent marker of postoperative liver function.



were similar in both groups (Table 2). However, 1-year graft (“normal” 86% vs “increased” 97%,  $P = 0.006$ ; see Figure 3) and patient survival (“normal” 87% vs “increased” 97%,  $P = 0.01$ ) was significantly better in the “increased bilirubin” group. After the first year, survival curves decreased parallel resulting in a 3-year graft survival of 75% in the “normal bilirubin” group versus 85% in the “increased bilirubin” group ( $P = 0.06$ , see Figure 3). The patient survival behaved accordingly (“normal” 76% vs “increased” 85%,  $P = 0.08$ ).

None of the patients lost their graft due to primary nonfunction or rejection. Only 1 patient was retransplanted within the first year after transplantation (patient from the “normal bilirubin” group due to a thrombosis of the hepatic artery). The most common cause of graft failure within 1 year was death due to multiorgan failure after a prolonged ICU stay (6 patients in “normal” and 3 patients in “increased bilirubin” group). Four patients of the “normal bilirubin” group died within 1 year of fulminant cancer (HCC) recurrence and 1 because of hepatitis C progression. Cardiac causes accounted for 2 deaths in the “normal” and 1 death in the “increased” bilirubin group. Two more patients from the “normal” group died of aggressive, nonhepatic malignant diseases that were diagnosed for the first time after transplantation (Table 3).

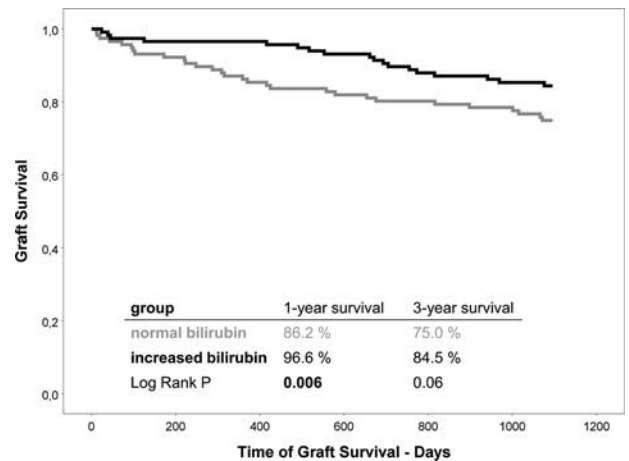
The rate of severe surgical complications (Clavien-Dindo score 3b-5) was similar in both groups (“normal” 17.2% vs “increased” 20.7%,  $P = 0.5$ ). The in-hospital mortality (Clavien-Dindo score 5) was lower in the “increased” group, however, without a significant difference (“normal” 5.2% vs. “increased” 2.6%,  $\chi^2$ -test  $P = 0.3$ ). Biliary complications within the first year were also similarly distributed between both groups (“normal” 10.3% vs “increased” 7.8%,  $P = 0.49$ ). Bile duct strictures occurred in 7.8% of the “normal bilirubin” patients, compared to 6.0% in the “increased bilirubin” group ( $P = 0.60$ ). The rate of bile leaks within the

**TABLE 2.****Complications and survival rates**

Follow-up	Normal bilirubin (n = 116)	Increased bilirubin (n = 116)	P
Length of hospital stay	10.5 (4-196)	12 (5-182)	0.1
Time on ICU	1 (0-115)	2 (0-53)	0.09
Surgical complications	17.2%	20.7%	0.5
Dindo III + IV			
Hospital lethality (Dindo V)	5.2%	2.6%	0.3
Biliary complication 1 y			
Total	10.3%	7.8%	0.49
Stricture	7.8%	6.0%	0.6
Leak	2.6%	1.7%	0.65
Graft rejection 1 y ( $\geq$ RAI 3)	23.2%	20.7%	0.63
Survival, 1 y			
Graft	86.2%	96.6%	0.006
Patient	87.1%	96.6%	0.01
Survival 3 y			
Graft	75.0%	84.5%	0.05
Patient	75.9%	84.5%	0.09

Postoperative complications and length of hospital stay were comparably distributed in both groups. Data presented as median with minimum and maximum for continuous variables, percentage for categorical variables.

RAI, rejection activity index.



**FIGURE 3.** Liver graft survival after transplantation. Kaplan-Meier graphs for graft survival 3 years after transplantation. Survival after 1 year was significantly better in the “increased bilirubin” group. Afterward, survival curves of both groups decrease in a parallel manner.

first year after transplantation was 2.6% in the “normal bilirubin” and 1.7% in the “increased bilirubin” group ( $P = 0.65$ ). Episodes of biopsy proven cellular rejection within the 1st posttransplantation year occurred in 23.2% of “normal” and 20.7% of “increased bilirubin” patients ( $P = 0.63$ ).

## DISCUSSION

In this study, we analyzed the effects of preoperative bilirubin levels on outcome after deceased donor liver transplantation. Two groups of recipients with either normal or increased preoperative bilirubin values were matched, minimizing confounding differences due to preoperative patient condition, disease etiology, donor age, and period of transplantation. As indicator of IRI, we demonstrated significantly increased peak transaminases after OLT in the “normal bilirubin” group when compared with recipients with increased bilirubin values before transplantation. Although stabilized marker of liver function after the initial posttransplant phase and surgical complication rates were similar in both groups, “increased bilirubin” levels were associated with a significantly better 1-year graft survival rate, suggesting a clinically relevant protective effect of increased perioperative bilirubin values. Additionally, liver grafts appeared to pick up liver

**TABLE 3.****Cause of death**

Cause of death	Normal bilirubin	Increased bilirubin
Total number of death 1-year post-LTx	15 (13%)	4 (3%)
Cause of death		
Multi organ failure on ICU	6	3
Fulminant HCC recurrence	4	0
Fulminant HCV progression	1	0
Cardiac cause of death	2	1
None hepatic malignancy	2	0

Cause of patient death within 1 year after Transplantation. Multiorgan failure on ICU as the most common cause of death occurred twice as many times in the “normal bilirubin” group than in the other group. The other causes of death were also more often in the “normal bilirubin” group. Total number of patients per group presented.

LTx, liver transplantation.

function more quickly in “increased bilirubin” recipients, as demonstrated by the more pronounced early posttransplant INR changes.

Our results demonstrate in a transplantation setting that the cytoprotective effect of bilirubin might be clinically relevant. Bilirubin has been known to be a powerful physiological anti-oxidant for a long time.<sup>16-18,20</sup> Furthermore, several animal studies have suggested beneficial effects of bilirubin on hepatic reperfusion injury. In a rodent model, Kato et al<sup>35</sup> could demonstrate that a bilirubin rinse of liver grafts before transplantation is a simple strategy to ameliorate oxidative stress and hepatobiliary dysfunction. Similarly, Fondevila et al<sup>36</sup> demonstrated a cytoprotective effect of biliverdin in a 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. Similar effects have been described in animal studies for IRI after other solid organ transplantation.<sup>20</sup> Despite these favorable outcomes, a previous retrospective analysis of a deceased donor population by Manzinate et al<sup>27</sup> failed to reproduce the results of these animal studies in a clinical setting. In this group of liver transplant recipients, no correlation was found between preoperative bilirubin and postoperative IRI as well as graft survival. However, especially the heterogeneity of the donor group likely confounded these findings. In contrast, in a study investigating kidney transplantation, Lee et al<sup>37</sup> were able to demonstrate a protective effect of postoperatively increased serum bilirubin levels on graft survival and rates of acute rejection after kidney transplantation. In a previous study, we analyzed the protective effects of increased preoperative bilirubin values on IRI after living donor liver transplantation representing a homogeneously healthy donor population.<sup>26</sup> We found significantly lower transaminase peak after transplantation in recipients with preoperatively increased bilirubin values. However, because of the limited preservation injury during live donation liver transplantation, we were not able to demonstrate a clinical benefit. Now, in a group of HBD graft recipients, about 20% of all transplanted organs met expanded criteria graft standards, increasing the relevance of IRI in this population.

In our study population, primary non function as the maximal manifestation of IRI did not occur. However, patient survival was mainly compromised by hepatic dysfunction and failure after prolonged ICU treatment and HCC as well as HCV recurrence. Recently, the extent of IRI has been suggested to be a major risk factor for an aggressive recurrence of both HCC and HCV.<sup>38-40</sup> Thus, an association between decreased IRI due to bilirubin cytoprotection and reduced numbers of patient mortality for fulminant HCC and HCV recurrence appears possible.

Our results indicate that serum bilirubin levels have a relevant effect on IRI after human OLT. Several authors have suggested using this protective effect by applying recombinant bilirubin/biliverdin perioperatively.<sup>20</sup> However, a clinical application has not been established, and treatment protocols have not been developed thus far. Further prospective clinical studies are mandatory to develop strategies to implement bilirubin administration and HO-1 induction during and after transplantation. In addition, the indication of bilirubin-lowering treatment strategies like biliary stenting in patients awaiting an OLT might have to be reconsidered, bearing in mind the protective effect during the transplantation.

Our study had several limitations. To minimize the heterogeneity of our HBD graft recipients, we used a matched study design. During the matching process, many cases had to be excluded because no adequate match was available. Thus, the study groups had a rather small sample size. Also, additional donor risk factors influencing reperfusion injury (eg, use of vasoactive drugs, long-standing hypotension, length of ICU stay, laboratory values, and so on) were not taken into account in our analysis. The validity of our results was still conclusive because of the coherent posttransplant treatment regimen we used for all patients and because of the balanced matching result of our study groups. However, the sample size was too small to effectively analyze the dose dependency of the bilirubin effect. Subanalyses of certain subgroups were also not possible because of the low case numbers, for example, analyzing a potentially protective effect of bilirubin and HO-1 on HCV and HCC recurrence after transplantation. Finally, we assume that the protective effect of bilirubin in reperfusion injury as described in many experimental cell culture and animal studies has had a positive impact on posttransplant outcome. Our retrospective clinical analysis however lacks mechanistic evaluation of this effect (eg, quantification of oxidative stress).

Although 1-year graft and patient survival is significantly higher in the increased bilirubin group, the cause of death in 40% of patients in the normal bilirubin group was related to recurrence of HCC or other malignancies which may not be directly related to the protective effect of high bilirubin. Further studies with larger number of patients are necessary to elucidate these aspects.

Our matching strategy was based on modified MELD score using only INR and creatinine values (excluding bilirubin). The modified MELD score allows to adequately evaluate and quantify the preoperative degree of liver failure and patient condition. Using other scoring system such as Child Pugh score for matching would have only added the potential value of serum albumin as another marker of liver synthetic function in addition to INR.

In conclusion, high bilirubin levels of OLT recipients before transplantation are associated with reduced IRI and improved survival after transplantation. Inducible HO-1, as the likely mechanism of protection of bilirubin, represents a clinical relevant target for future studies.

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