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ORIGINAL ARTICLE

Early γGT and bilirubin levels as biomarkers for regeneration and outcomes in damaged bile ducts after liver transplantation

Henrik Junger[1] | Marco Mühlbauer[1] | Frank W. Brennfleck[1] | Leonhard A. Schurr[1] | Markus Goetz[1] | Elke Eggenhofer[1] | Gabriele Kirchner[1,2] | Katja Evert[3] | Stefan Fichtner-Feigl[1,4] | Edward K. Geissler[1,5] | Hans J. Schlitt[1] | Stefan M. Brunner[1]

[1]Department of Surgery, University Hospital Regensburg, Regensburg, Germany

[2]Department of Internal Medicine I, University Hospital Regensburg, Regensburg, Germany

[3]Department of Pathology, University Regensburg, Regensburg, Germany

[4]Department of Surgery, University Hospital Freiburg, Freiburg, Germany

[5]Fraunhofer Institute for Experimental Medicine and Toxicology, Regensburg, Germany

Correspondence
*Stefan M. Brunner, Department of Surgery, University Medical Center Regensburg, Franz-Josef-Strauss-Allee 11, 93053 Regensburg, Germany.
Email: stefan.brunner@ukr.de

Abstract

Background: Early patient and allograft survival after liver transplantation (LT) depend primarily on parenchymal function, but long-term allograft success relies often on biliary-tree function. We examined parameters related to cholangiocyte damage that predict poor long-term LT outcomes after donation after brain death (DBD).

Methods: Sixty bile ducts (BD) were assessed by a BD damage-score and divided into groups with "major" BD-damage ($n = 33$) and "no relevant" damage ($n = 27$) during static cold storage. Patients with "major" BD damage were further investigated by measuring biliary excretion parameters in the first 14 days post-LT (followed-up for 60-months).

Results: Patients who received LT showing "major" BD damage had significantly worse long-term patient survival, versus grafts with "no relevant" damage ($p = .03$). When "major" BD damage developed, low bilirubin levels ($p = .012$) and high gamma-glutamyl transferase (GGT)/bilirubin ratio ($p = .0003$) were evident in the early post-LT phase (7–14 days) in patients who survived (> 60 months), compared to those who did not. "High risk" patients with bile duct damage and low GGT/bilirubin ratio had significantly shorter overall survival ($p < .0001$).

Conclusions: Once "major" BD damage occurs, a high GGT/bilirubin ratio in the early post-operative phase is likely indicator of liver and cholangiocyte regeneration, and thus a harbinger of good overall outcomes. "Major" BD damage without markers of regeneration identifies LT patients that could benefit from future repair therapies.

KEYWORDS
bile duct damage, biliary tree regeneration, liver transplantation

1 | INTRODUCTION

Since many years liver transplantation (LT) has advanced from an experimental procedure to a standard therapeutic option for patients with end-stage liver disease and acute liver failure.[1] After LT, biliary complications occur in 6%–35% of patients and are a major cause of morbidity and mortality.[2–5] Although patient and allograft survival during the first weeks after LT are dependent primarily on parenchymal function, long-term allograft viability hinges also on biliary wound healing and adequate bile drainage.[5,6]

In a previous study we found that most bile ducts (BDs) from transplanted donation after brain death (DBD) livers show evidence of epithelial damage immediately after cold storage, and quantified this damage by establishing a "Bile Duct Damage Score" (BDDS) that predicts biliary complications after LT and graft survival[7]; this finding has since been confirmed by others.[8,9] An increase in tissue repair activity associated with maintaining epithelial integrity appears to be associated with reduced biliary complications.[10] This now raises questions including: (1) does the observed BD damage during static cold storage conditions affect long-term LT outcomes, and (2) are there routine parameters to identify patients with graft/life-threatening bile duct damage.

In clinical practice, elevated serum gamma-glutamyl transferase (GGT) is generally used as an indicator of liver disease, such as biliary obstruction, alcohol consumption or exposure to certain medications.[11] However, it has been shown that after surgery for ruptured abdominal aortic aneurysm,[12] or after liver resection,[13] GGT is transiently increased in patients who had a good outcome. In these short-term observational studies, GGT level was inversely related to other liver laboratory parameters such as aspartate aminotransferase (ALT), alanine aminotransferase (AST), as well as total bilirubin.[12,13] After liver resection, elevated GGT levels have also been reported in patients with indications of good tissue regeneration and outcomes.[13] Therefore, an immediate postoperative elevation of GGT after LT appears to indicate a physiological liver regeneration response, while chronic GGT elevation reflects a pathological response to tissue stress later after LT.[14]

Indeed, little is known about the long-term effects of biliary epithelial damage occurring during static cold liver allograft storage conditions. We hypothesize that "major BDD" has a negative effect on long-term (1–5 year) patient outcome, including overall survival and that serum markers can indicate bile duct regeneration after LT. We expect to establish a score based on serum markers and the BDDS to specifically identify patients with graft/life-threatening bile duct damage

2 | MATERIAL AND METHODS

2.1 | Study setting

This was a retrospective analysis of patients where a BD tissue sample was collected from transplanted livers in the years 2009 – 2013 and 2018-2020, as standard procedure (University Hospital Regensburg). All transplant patients during this period were considered for inclusion, but if the common BD was too short to take samples, patients were excluded from this study; in patients with adequately long BD, two-mm long circular specimens of common BDs were taken. The study protocol conformed to ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the local Institutional Review Board at the University Hospital Regensburg (IRB, Ethics Committee no. 11-160-0183).

2.2 | Patient selection and data collection

From 65 LT patients, five were lost during follow up. Thus, BDs from 60 donors (DBD) were assessed by the "Bile Duct Damage Score" and divided into groups with either "major" bile duct damage (BDD, n = 33) or "no relevant" bile duct damage (no-BDD, n = 27) after cold donor liver storage (Suppl. Figure 1A).

BDDS was evaluated as follows: BD samples with a regular monolayer of high prismatic cylinder epithelium were categorized as "grade 0." Specimens with flattened, but still present, epithelial cells were classified as "grade 1" damage. Destroyed biliary epithelium, with preserved subepithelial connective tissue, was graded as "grade 2" damage. "Grade 3" damage was defined as destroyed biliary epithelium combined with disrupted connective tissue without nuclei, indicating necrosis of the BD; BDD samples with grade 0, grade 1 and less than 10% grade 2 or 3 damage were defined as BDs with "no relevant" damage. BD specimens with more than 10% grade 2 or 3 damage were classified as having "major" damage. The cut-off was set at 10% grade 2 or 3 damage to account for artefacts that might have occurred through the cutting and staining procedure.[7]

Liver graft patient records were analyzed to obtain patient and graft survival, including the cause of death or graft loss. Causes for death and/or graft loss were divided into multi-organ failure as a result of biliary complications (biliary leakage, BD necrosis (defined as histologically proven BD wall necrosis), biliary casts/sludge, ischemic type biliary lesions (defined as progressive sclerosis with formation of multiple intrahepatic BD strictures, or anastomotic/non-anastomotic biliary tract strictures requiring intervention or surgery), disease reoccurrence (HCC, re-cirrhosis), vascular complications and infection/cardiac arrest (Table 1). Biliary drainage was assessed by cholestasis-related parameters GGT and bilirubin (Clinical Chemistry Laboratories, University Hospital Regensburg). The post-LT follow-up time was 60 months.

2.3 | Statistical analysis

Statistical analyses were performed using the statistical software package SPSS 23 (IBM SPSS, Chicago, IL, USA) and Graph Pad Prism 9 (GraphPad Software, San Diego, CA, USA). Kaplan–Meier graphs were used for survival analyses. Donor and patient data are presented as mean with standard deviation (SD) or as n (number) with

TABLE 1 Major cause of mortality

Pat. #	BD damage	Diagnose	Biliary complication & treatment	Survival months	Cause of death or re-Tx	Specification
					Biliary complications	
1	1	HepC, HCC	BD stenting due to stenosis	26		Cholangitis, SBP, sepsis, MOV
2	1	LC HepC	BD stenting due to sludge	1.5		Cholangitis/sepsis due to clogged stent with MOV
3	1	M. Osler-Rendu	BDA due to necrosis and new BD due to insufficiency	0.4		Occlusion of A. hepatica after BDA due to necrosis and new BDA
4	1	a. LV HCC	BD stenting due to stenosis	4.9		Transplant failure with MOV after liver biopsy due to increased cholestasis parameters
5	1	LC C2	New BDA due to bile leakage	2.2		Bile leakage and intestinal perforation with MOV
6	1	LC C2	Overstitching of BD-anastomosis due to anastomosis insufficiency	2.1		Bile leakage with erosions bleeding, sepsis with MOV
7	1	LC C2	New BDA due to necrosis, recurrent insufficiency of the anastomosis	1.7		BD necrosis with cholangitis with sepsis associate erosions bleeding
8	1	LC C2	BD necrosis with PTCD and Re-Tx	12.5		Secondary sclerosing cholangitis
9	1	LC C2	BD necrosis and Re-Tx	3		BD necrosis and transplant failure
10	1	LC C2	BD necrosis and Re-Tx	1		BD necrosis and transplant failure
11	1	LC C2	BD necrosis and Re-Tx	0.8		BD necrosis and transplant failure
12	1	Cryptogenic LC	BD necrosis and Re-Tx	0.2		BD necrosis and transplant failure
13	1	LC, C2	BD necrosis	0.1		BD necrosis and transplant failure
14	0	LC C2	BD stenting due to sludge	2.6		Cholangitis with liver abscess with MOV
					Disease recurrence	
15	1	LC C2		10		HCC recurrence
16	1	LC HepC	BD stenting due to stenosis	20.5		HCV reactivation
17	1	HCC	BD Stenting due to stenosis	12.7		HCC recurrence
18	0	LC C2 HCC	New BDA due to necrosis	4.4		HCC recurrence
19	0	HepC, HCC		18.4		HCC recurrence

(Continues)

TABLE 1 (Continued)

Pat. #	BD damage	Diagnose	Biliary complication & treatment	Survival months	Cause of death or re-Tx	Specification
20	0	LC C2		16.2		Re-cirrhosis with pneumoniae and acute on chronic LV with MOV
					Vascular complications	
21	1	HCC	Overstitching of BD-anastomosis due to anastomosis insufficiency	0.9		Intra cerebral bleeding after seizure
22	1	LC C2		0.1		Disseminated intravascular coagulation failure during LTX
23	0	HepC, HCC		3.5		Acute bleeding, hypoxic brain damage
24	0	SSC		0.2		Bleeding and acute occlusion of A. hepatica
					Infections/Cardiac arrest	
25	1	M. Wilson	BD stenting due to stenosis	0.1		Pneumoniae with MOV
26	1	LC, C2		1.7		Cardiac arrest due to respiratory insufficiency due to pneumonia

Abbreviations: aLV, acute liver failure; BD, bile duct; BDA, biliodigestive anastomosis; C2, alcohol induced; HCC, hepatocellular carcinoma; HepC, Hepatitis C virus infection; LC, liver cirrhosis; MOV, multi organ failure; SBP, spontaneous bacterial peritonitis; SSC, secondary sclerosing cholangitis.

percentages. Comparisons between histological and laboratory values were performed using a two-sided *t*-test or Mann-Whitney test where applicable, and data are presented as mean ± SD (14). ROC analysis was done to distinguish between survivors and non-survivors; to identify cut-off values for laboratory parameters, time of assessment was at 5 years for the first 48 patients (collected between 2009 and 2013), and 2–4 years for the most recent collective of 12 patients (between 2018 and 2020). Mean values of each patient from days 7 to 14 post-transplant were used for the ROC analysis. Day 7–14 post-transplant was chosen to better reflect the IRI damage-repair window after liver transplant. Multivariate Cox Regression Hazard analysis was performed to identify possible predictors of survivors versus non-survivors out of the following candidate variables: GGT/bilirubin ratio, "major" bile duct damage, DRI (donor impact) and Lab-MELD (patient impact). Under the rule of thumb of 8-10 events per variable, including four variables for 25 events are barely adequate. The level of significance was set at a probability of $p < .05$.

Bile Duct Risk Score. A score that considers factors that significantly influence patient survival in Multivariate Cox Regression Hazard analysis was developed to enable patient risk stratification. A score of 1 accounted for a "major" BDD and low GGT/bilirubin ratio (< 120). Patients with 0 and/or 1 point were defined as "low-risk," and those with two point as "high-risk" patients.

3 | RESULTS

3.1 | Patient data

Out of 65 LT patients, five patients were lost during follow-up (60 months), leaving 60 for evaluation in this study. A duct-to-duct anastomosis was performed in 54 patients (90%), while six patients (10%) received a biliodigestive anastomosis. No stents or T-tubes were used during implantation for biliary anastomosis. During LT surgery, a sequential first portal, then arterial, reperfusion was performed in all cases. The most frequent diagnoses leading to LT were alcoholic ($n = 26$; 43%) and viral liver cirrhosis ($n = 8$; 13%), followed by cryptogenic liver cirrhosis ($n = 6$; 8%), hepatocellular carcinoma (HCC) in alcoholic or viral liver cirrhosis ($n = 5$; 8%) and acute liver failure ($n = 4$; 7%). Budd–Chiari syndrome ($n = 3$; 5%), cystic fibrosis ($n = 3$; 5%) and secondary sclerosing cholangitis ($n = 3$; 5%) presented less frequently. Rare diagnoses included Morbus Wilson ($n = 1$), Morbus Osler ($n = 1$),

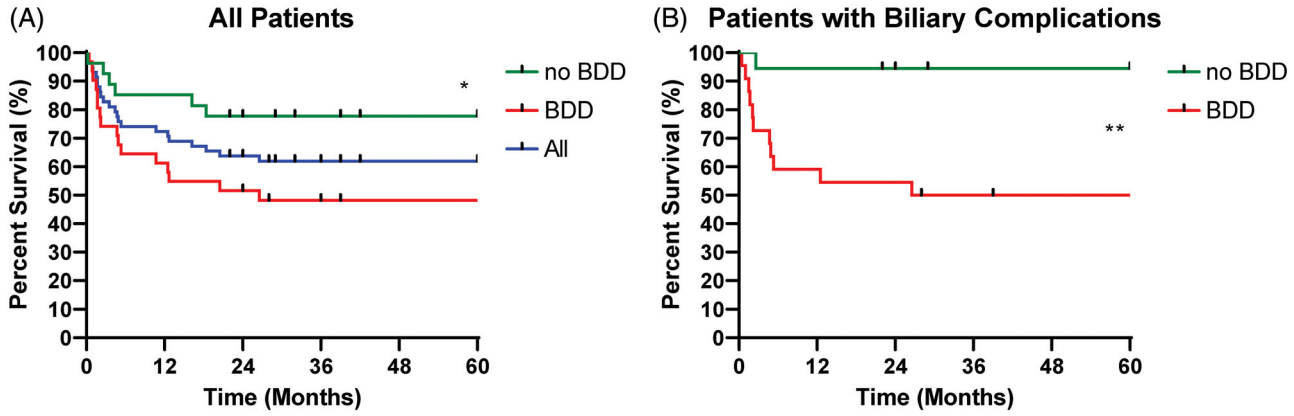


FIGURE 1 Bile duct damage score as prognostic parameter for patient survival. (A) Patient survival. (B) Biliary complication-associated deaths and graft losses (Kaplan Meier analysis, $^{*}p < .05$, $^{**}p = .003$, patient survival in %)

autoimmune hepatitis ($n = 1$) (Supp. Table 1). Patient sex, mean age, laboratory Model for End-Stage Liver Disease (Lab MELD), liver graft type (whole/split) and type of anastomosis (end-to-end/biliodigestive anastomosis) did not differ significantly between the study groups ($p = .19$, $p = .09$, $p = .09$, $p = .85$, $p = .08$; Supp. Table 2). Based on the BDDS, patients were grouped into those with "major" BD damage (> 10% grade 2 and 3 damage) or "no relevant" damage (< 10% grade 2 and 3 damage).[7]

3.2 | Donor data

Patients in the two different groups ("major" versus "no relevant" damage) received organs from donors with similar characteristics. No appreciable differences in donor age, sex, DRI, bilirubin, ALT, AST, or sodium, cold ischemia time (CIT), warm ischemia time (WIT), WIT explant, time on intensive care unit (ICU) or time of cardiac arrest was observed (Supp. Table 3).

3.3 | "Major" bile duct damage and long-term patient survival after LT

The overall median survival was 83 months post LT. Notably, when using our BDDS system, which has shown that "major" bile duct is a prognosticator for the occurrence of early and late biliary complications and for graft survival,[7] we could demonstrate in the current study that patients receiving transplants with "major" BD damage had markedly worse long-term median survival (13 months), versus patients receiving allografts with "no relevant" damage (> 60 months, $p = .03$, Figure 1A).

The most frequent reason for death and/or graft loss was multiorgan failure as a result of biliary complications ($n = 14$; 54%), followed by disease reoccurrence ($n = 6$; 23%), vascular complications ($n = 4$; 15%) and infection/cardiac arrest ($n = 2$; 8%; Table 1). Next, we looked at the long-term survival of patients and grafts together, focusing only on biliary complication-associated deaths and/or graft loss. Consistent with our hypothesis, median survival of the BDD group (46 months) was significantly shorter compared to the no BDD group (> 60 months, $p = .002$; Figure 1B).

Immunosuppression induction was performed in all patients with a Basiliximab (day 0 + day 4) plus Tacrolimus and Prednisolone regimen. Immunosuppression maintenance was based on either Tacrolimus, Cyclosporin A or an mTOR inhibitor (Sirolimus/Erverolimus). Depending on the immune status of the patient, this treatment (Tacrolimus, Cyclosporin A or mTOR inhibitor) was combined with low doses of Prednisolone and Mycophenolat-Mofetil. No appreciable differences between the "major" and the "no-relevant" damage group were observed ($p = .46$; Supp. Table 4).

3.4 | BDDS and survival

Not all patients with "major" damage suffered death or graft loss, and not all patients of the "no relevant" damage group survived. Thus, a subgroup analysis of patients with "major" and "no relevant" BD damage was performed. Therefore, patients were separated based on the BDDS into LT patients that survived or suffered death/graft loss (BDD-survivors vs. BDD-non-survivors and no-BDD-survivors vs. no-BDD-non-survivors). Since "major" BD damage was defined as 10% or more grade 2 and grade 3 damage, we examined whether the degree of biliary epithelial damage was different between the groups (Figure 2A). The "major" BDD damage groups combined had significantly worse epithelial damage compared to the "no relevant" BDD damage group (grade 0, 1 & 2: $p < .0001$, grade 3: $p = .003$). However, we found no significant difference between BDD-survivors and BDD-non-survivors, with both groups having similar amounts of intact epithelium (4% vs. 5%, $p = .4$), grade 1 (50% vs. 39%, $p = .3$), grade 2 (35% vs. 33%, $p = .7$) and grade 3 damage (11% vs. 25%, $p = .3$). Furthermore, no significant difference between no-BDD-survivors and no-BDD-non-survivors were observed for intact epithelium (25% vs. 34%, $p = .6$), grade 1 (75% vs. 65%, $p = .6$), grade 2 (3% vs. 1%, $p = .4$) and grade 3 damage (0% vs. 0%, $p > .99$; Figure 2B). These data suggest that epithelial damage is not the only factor affecting late post LT outcomes.

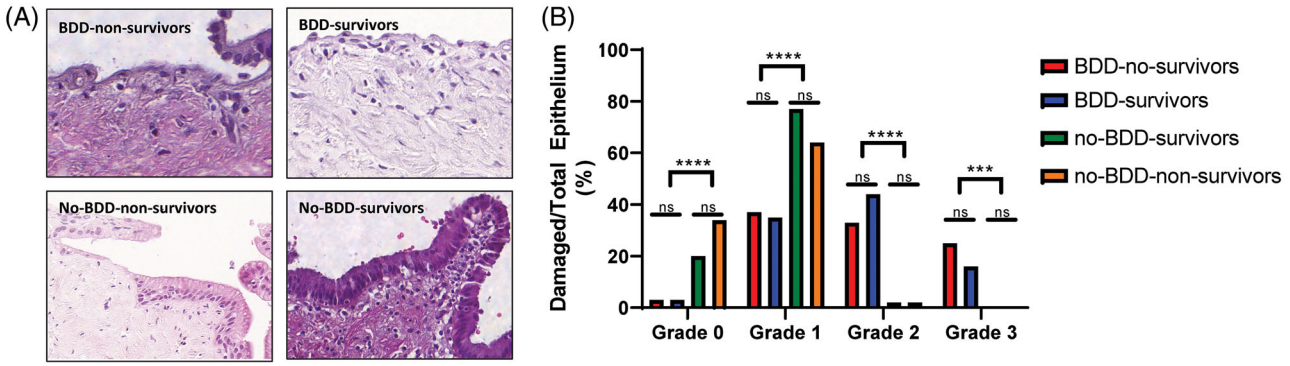


FIGURE 2 Bile duct damage scoring and mortality. (A) Representative H&E staining's (40×) of BDD-non-survivors (left top), BDD-survivors (right top), both with flattened epithelial cells and destroyed epithelium, but preserved subepithelial connective tissue. no-BDD-non-survivors (left bottom) and no-BDD-survivors (right bottom) showed both a regular epithelial layer with only small fractions of flattened epithelial cells. (B) BD damage (%) according to the BDDS in BDD-non-survivors (red), BDD survivors (blue), no-BDD-non-survivors (orange), no-BDD-survivors (green; ****$p < .0001$). Regular epithelium was categorized as "grade 0," with flattened epithelial cells as "grade 1," with destroyed epithelium, but preserved subepithelial connective tissue, as "grade 2," and with destructed biliary epithelium and connective tissue as "grade 3" damaged

3.5 | Early post-LT cholestasis parameters and mortality

In living donor LT, it has been shown that high postoperative bilirubin levels are predictors of graft loss.[15] An immediate postoperative elevation of GGT may indicate a physiological response reflecting liver regeneration, while evidence indicates that chronic GGT elevation after LT signifies a pathological response to tissue stress.[14] Thus, early postoperative cholestasis parameters were examined as measures of biliary excretion and liver regeneration after LT.

In our dataset of DBD donors, bilirubin was consistently lower in BDD-survivors and no-BDD-survivors following LT, versus those who did not survive (Figure 3 A, top). In patients with no-BDD, survivors and non-survivors, as well as in patients with BDD that survived, a decrease in bilirubin was observed during the first 14 days post-LT, expressed as delta bilirubin in %. The BDD-survivor group had a bilirubin clearance of at least 80-90%, compared to a very poor bilirubin clearance of only 10% in the BDD-non-survivor group at day 14 post-LT (Figure 3A, middle). When comparing bilirubin values during POD 7–14, no difference between the BDD-survivors and no-BDD-survivors was observed. However, BDD-survivors had a significantly lower bilirubin level of 2.44 mg/dl ± 1.82 during POD 7–14 compared to BDD-non-survivors at 7.18 mg/dl ± .79 $p = .013$; Figure 3A bottom).

Postoperatively, GGT levels increased gradually, reaching a maximum at 10 days post-LT in BDD-survivors at 645.8 U/l ± 510 U/l and then decreased thereafter. Notably, the increase in GGT levels was more pronounced during post-operative days 7-14, that is, deviating more from the normal range in patients with BD damage who survived, versus those who had no BD damage and/or died. After 11 days, GGT levels in BDD-survivors dropped and equalized compared to the rest (Figure 3B, top). BDD-survivors had a significantly higher GGT level of 417.0 U/l ± 295.0 during POD 7-14 compared to BDD-non-survivors at 238.4 U/l ± 176.3 U/l ($p = .03$; Figure 3B, bottom).

Next, GGT/bilirubin ratio was calculated to express the divergent changes in these parameters reflecting liver function/regeneration. Interestingly, BDD-survivors had a higher GGT/bilirubin ratio of 235 ± 270 versus BDD-non-survivors at a ratio of 84 ± 117 during post-LT days 7–14 (Figure 3C, p = .0003). Further, low GGT/bilirubin ration was associated with the occurrence of biliary complications, whereas patients with high GGT/bilirubin ration developed less biliary complications (Suppl. Table 5, $p = .002$). These results indicate good liver function and bile duct regeneration after the initial ischemia reperfusion injury post LT in this group.

3.6 | Identification of cut-off values indicating poor outcome

Mean patient values from post-LT days 7–14 were used in the ROC analysis. The ROC analysis was able to distinguish between survivors and non-survivors after LT and revealed a cut-off for bilirubin (2.3 mg/dl) with an AUC of .82 ($p < .0001$, sensitivity 79% & specificity 77%, Figure 3D). On the other hand, the GGT ROC analysis showed that GGT should not be used as a cut-off parameter to distinguish survivors from the non-survivors (AUC .63; $p = .08$, Figure 3D). Nevertheless, the ratio between GGT/bilirubin as an indicator for biliary and liver parenchymal regeneration showed a strong cut-off value (< 120) to distinguish between survivors and non-survivors with an AUC of .84 ($p < .0001$, sensitivity 88% & specificity 65%, Figure 3D).

3.7 | Bile duct damage and early cholestasis parameters are prognostic parameters for outcome of LT patients

To enable a risk stratification of LT patients based upon BDDS in combination with bilirubin and GGT values (POD 7-14), factors that might influence patient and graft survival were tested by Multivariate

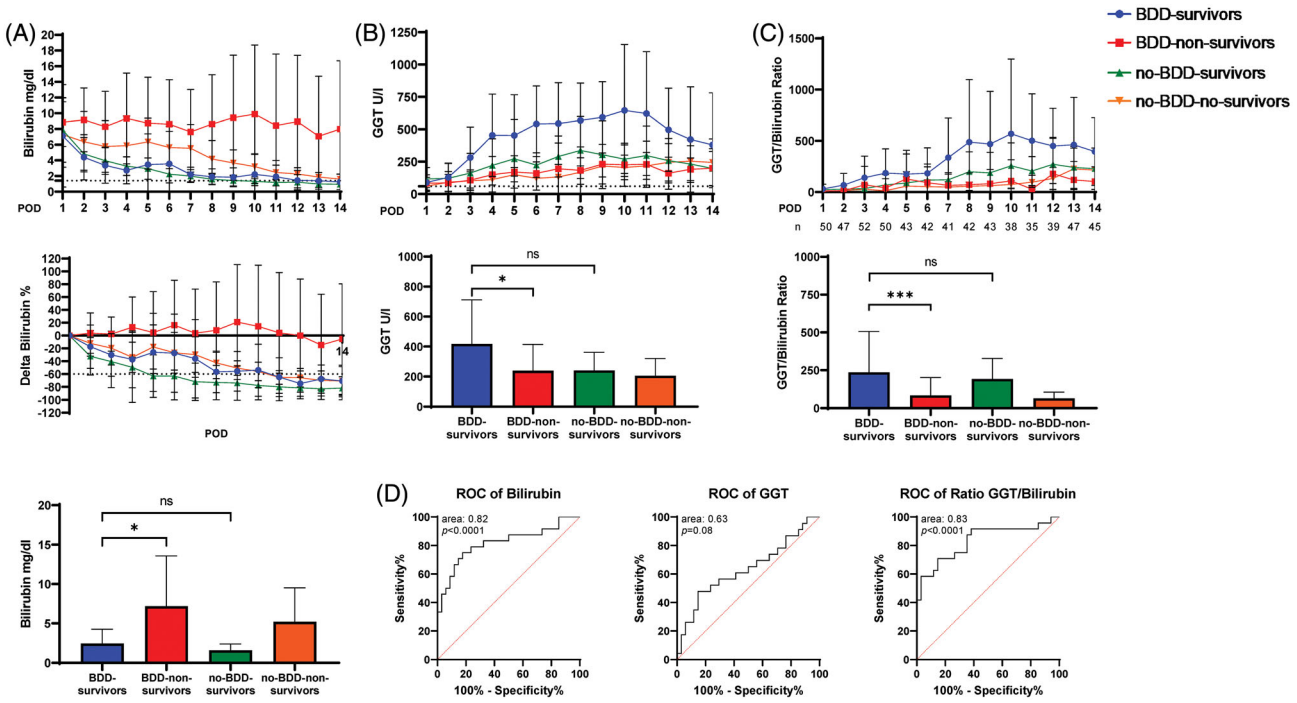


FIGURE 3 Early post-LT cholestasis parameters and mortality in patients with "major" and "no relevant" BD damage. (A) Bilirubin values (mg/dl) during the first 14 days post-LT (top), bilirubin clearance in the first 14 days post-LT (delta bilirubin in %, middle) and group comparisons of bilirubin values during POD 7-14 (bottom). (B) GGT values (U/l) during the first 14 days post-LT (top) and group comparisons (ns) of GGT values during POD 7-14 (bottom). (C) GGT/bilirubin ratio in the first 14 days post-LT (top) and, group comparisons of GGT/bilirubin ration during POD 7-14 (bottom, mean and SD for each group, $^{*}p < .05$, $^{**}p < .01$, not significant (ns)). (D) Receiver Operator Characteristics Curve for prediction of death/graft loss after LT. ROC analysis was able to distinguish between survivors and non-survivors and revealed a cut-off for bilirubin (2.3 mg/dl) with an AUC of .82 ($p < .0001$). ROC analysis for GGT revealed that GGT could not be used as a cut-off parameter (AUC .63; $p = .08$). GGT/bilirubin ration is a good indicator for biliary regeneration and liver function and predictor of post-LT outcome with a clear cut-off GGT/bilirubin ratio (< 120), with an AUC of .84 ($p < .0001$)

Cox Proportional Hazard analysis. The following candidate variables were tested: GGT/bilirubin ratio (with a greater AUC and a more balanced sensitivity and specificity than bilirubin alone), "major" bile duct damage, DRI (donor impact) and Lab-MELD (patient impact). Starting with four variables that might be good predictors of patient survival, we were able to reduce them to two independent variables predicting fatal post-LT outcome, which were: "major" bile duct damage ($p = .0003$, Hazard Ratio 4.1, 95% CI 1.59–10.40) and the GGT/bilirubin ratio ($p < .001$, Hazard Ratio 8.6, 95% CI 2.93–25.12), meaning "major" bile duct damage during static cold storage condition and low GGT/bilirubin ratio increases the post-LT risk for death/graft loss (Figure 4A).

For risk stratification, patients were then divided into two groups according to the BDDS combined with GGT/bilirubin ratio at POD 7-14 (Bile Duct Damage and Laboratory Score; BDDL). The "low-risk" (BDDL 0 and 1) patients showed a significantly longer survival (undefined) compared to "high-risk" (BDDL 2) patients (2 months, $p < .0001$; Figure 4B). Therefore, our results indicate that histological analysis (BDDS) and laboratory values (GGT/bilirubin ratio), combined in the BDDL-score, represents a new independent scoring system that has potential to predict survival for LT patients (Figure 4B).

4 | DISCUSSION

In this study we investigated whether "major" biliary epithelial damage during static cold storage conditions has an effect on long-term LT patient outcomes after DBD. Our results show that once "major" biliary epithelial damage occurs, patients have a greater risk of a fatal outcome due to biliary complications. Importantly, subgroup analysis of patients with "major" BD damage revealed that high GGT and low bilirubin levels during the day 7-14 post-LT identify those patients with a good outcome in terms of overall survival. The subgroup of patients with "major" damage showing an opposite trend in these two biomarkers had poor outcomes, including particularly death due to biliary complications; patients with "no relevant" BD damage died for other reasons. Multivariate analysis revealed that BDDS and low GTT/bilirubin ratio are the only independent risk factors predicting death/graft loss after LT.

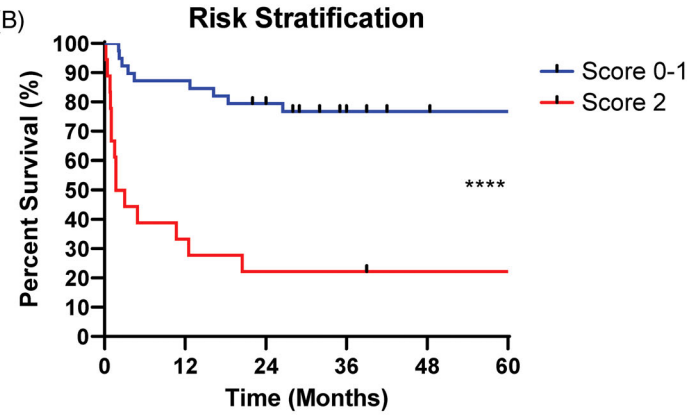
Since not all patients with "major" BD damage have a fatal outcome, our study focused on identifying the most vulnerable patients. In rodent models of liver injury and regeneration, it has previously been shown that GGT is a good marker of liver regeneration.[13,14,16–18] Furthermore, in living donor LT it has been shown that high postoperative bilirubin levels are predictors of graft loss.[15] It is therefore logical

(A)

Variable	Hazard Ratio	95% CI	*P* Value
„Major" Bile Duct Damage	4.1	1.59 – 10.40	0.003*
GGT/Bilirubin Ration	8.6	2.93 – 25.12	<0.001*
Lab MELD	0.96	0.96 – 1.03	0.07
Donor Risk Index	0.24	0.11 – 1.74	0.24

Multivariate Cox Proportional Hazard analysis. *significance p<0.05

(B)



Variable	Median Survival (months)	5-years survival rate
BDDL 0-1 – low risk	undefined	77 %
BDDL 2 – high risk	2	22 %

FIGURE 4 The Bile Duct Damage and Laboratory Score (BDDL) is a prognosticator for patient survival after LT. (A) Multivariable analysis of factors that might have influenced patient survival (in %) after LT. "Major" BD damage and low GTT/bilirubin ratio (< 120) independently correlate with shorten patient survival ($p = .003$, $p < .001$. (B) For risk stratifying BDD, "low" GGT/bilirubin ratio (< 120) were graded with 1 point each. Patients were divided into "low-risk" (BDDL 0-1), and "high-risk" (BDDL 2) groups. This BDDL-Score provided a significant risk stratification between the two different risk groups (Kaplan Meier analysis, $^{****}p < .0001$, patient survival in %)

that the increased GGT levels among those patients with "major" BD damage likely signal regeneration of hepatocytes and cholangiocytes.

Biliary complications (both anastomotic and ITBL) are typically based on ischemia phenomena observable typically > 6 months after LT.[4,5,19–21] However, 11/14 deaths/transplant failures happened within the first 3 months after LT. All 11 patients died due to early biliary complications (cholangiosepsis, extra- or intra-hepatic bile duct necrosis, or bile leakage with consecutive fatal complications; see Table 1). We argue that especially in these early deaths/transplant failures, the bile duct damage did play a major role in affecting outcome. Robert Porte et. al showed that damage observed at the common bile duct during cold storage conditions is also seen in the intrahepatic BD.[9] Since not all patients with "major" bile duct damage suffered a fatal outcome, we and others think that some patients are likely to have recovered from the "major" bile duct damage.[8] This idea leads to our contention that good bilirubin clearance in combination with elevated GGT values might indicate a favorable outcome. Thus, we combined both parameters and expressed them as the GGT/bilirubin ratio, which we suggest is a marker for biliary regeneration and good liver function in the early post LT period. That contention was confirmed by the finding, that a low GGT/bilirubin ration was significantly associated with the occurrence of biliary complications. Indeed, using the GGT/bilirubin ratio and "major" BDDS for risk stratification, we show that patients with "high risk" have a lower survival rate compared to patients with low/medium risk. Although the GGT/bilirubin ratio seems more impactful in the multivariate analysis, we gave both the BDDS and the GGT/bilirubin ratio the same weight in the BDDL score. The BDDS is a static parameter measured during static cold storage condition, thus we have no insights regarding the epithelium after the transplantation. On the other hand, we have GGT/bilirubin ration, easily accessible serum parameters, which are a dynamic parameter that changes over time after the transplantation.

Based on grouping patients with the BDDL-score in "low-risk" and "high-risk" LT patients, we could identify patients at risk for poorer long-term patient survival. Recently, multiple scores have been introduced for liver graft assessment. Known scoring systems are the liver graft assessment following transplantation (L-GrAFT) score,[22] model for early allograft function (MEAF) score,[23] and early allograft dysfunction (EAD)[24] parameter for prediction of early allograft failure. However, these scores only focus on early liver graft function (90 days) and only take laboratory values into account. Rather, we suggest that the application of this combined histological and biomarker approach (BDDL score) could identify the most vulnerable LT patients who show signs of substantial BD damage.

While the 5-year overall patient survival after LT is reported to be approximately 59%–72%,[25,26] this depends largely on the underlying disease and on organ allocation systems. Notably, survival in the cohort we analyzed in this study was exceptionally low due to the inclusion of a disproportionally high number of high-risk cases. For example, we had patients from the SiLVER Study[27] in our cohort, which were

high risk HCC patients. Also, introduction of the MELD system has resulted in survival rates in Germany below the international average, as published by Weismüller et al. with data from seven transplant centers, including ours.[28] In addition, in this study cohort there were a significant number of patients with biliary complications that could only be solved with re-transplantation; unfortunately, due to Eurotransplant allocation policies, many of these patients were not able to be prioritized for life-saving transplants. Together, multiple factors like these explain the relatively low survival rates in the present study cohort.

Our study does have limitations, including that it was performed as a retrospective analysis and is based on historical histology/laboratory data; notably, patient data is not entirely complete, including some laboratory results. Nonetheless, only five patients were lost to follow-up out of a total of 60. We do recognize that further prospectively planned LT studies are needed to confirm our findings, but validation of the predictive value of this combined pre-transplant BD histology and post-transplant biomarker approach could prove to be valuable for testing future treatment options in LT patients most at risk for life-threatening biliary complications.

AUTHOR CONTRIBUTIONS

HJ participated in study conception and design, performance of research, acquisition of data, analysis and interpretation of data and drafting of manuscript. LAS and MM participated in acquisition of data. FWB, MG, EE, KE, GIK, HJS, EKG participated in critical revision and writing of the manuscript. SFF participated in study conception, design and critical revision. SMB participated in study conception, design, analysis and interpretation of data and critical revision.

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CONFLICTS OF INTEREST

The authors disclose no funding or conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Henrik Junger https://orcid.org/0000-0002-6091-7166

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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