ORIGINAL ARTICLE

Prediction of biliary anastomotic stricture after deceased donor liver transplantation: the impact of platelet counts – a retrospective study

Kazuhiro Takahashi[1], Shunji Nagai[1], Krishna G. Putchakayala[1], Mohamed Safwan[1], Masahiko Gosho[2], Amy Y. Li[1], William J. Kane[1], Priyanka L. Singh[1], Michael D. Rizzari[1], Kelly M. Collins[1], Atsushi Yoshida[1], Marwan S. Abouljoud[1] & Gabriel T. Schnickel[1]

1 Department of Transplant and Hepatobiliary Surgery, Henry Ford Hospital, Detroit, MI, USA
2 Department of Clinical Trial and Clinical Epidemiology, Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan

Correspondence
Shunji Nagai, Department of Transplant and Hepatobiliary Surgery, Henry Ford Hospital, 2799 West Grand Boulevard, Detroit, MI 48202, USA.
Tel.: +1 313 916 2941;
fax: +1 313 916 4353;
e-mail: snagai1@hfhs.org

SUMMARY

Biliary stricture is a common cause of morbidity after liver transplantation (LT). This study aimed to determine the risk factors for post-transplant biliary anastomotic strictures (BAS), focusing on perioperative platelet counts. We enrolled 771 consecutive recipients who underwent ABO-identical/compatible deceased donor LT with duct-to-duct biliary reconstruction from January 2000 to June 2012. BAS was identified in 142 cases. The median time for stricture development was 176 days. Preoperative and postoperative platelet counts within 5 days after LT were significantly lower in patients with BAS than those without BAS. Using cutoff values acquired by the receiver operating characteristic curve analysis, persistent postoperative thrombocytopenia was defined as platelet counts $<41 \times 1000/\mu l$ and $<53 \times 1000/\mu l$ on postoperative day (POD) 3 and POD 5, respectively. Multivariate analysis indicated persistent postoperative thrombocytopenia (OR = 2.38) was the only independent risk factor for BAS. No significant associations were observed in terms of donor and surgical factors. Multivariate analysis demonstrated estimated blood loss (OR = 1.01, per 100 ml) was an independent contributing factor for persistent postoperative thrombocytopenia. We demonstrated low platelet count was associated with progression of post-transplant BAS. Minimizing intraoperative blood loss potentially contributes to maintain post-transplant platelet count, which may reduce incidence of BAS.

Transplant International 2017; 30: 1032–1040

Key words
biliary anastomotic stricture, duct to duct, liver transplant, platelet

Received: 17 December 2016; Revision requested: 13 February 2017; Accepted: 1 June 2017;
Published online: 13 July 2017

Introduction

Biliary strictures are one of the most common causes of morbidity after liver transplantation (LT) [1]. Despite progress in surgical technique and preservation methods, biliary strictures remain the most common complication after deceased donor LT, with an incidence of 5–15% [1,2]. Biliary strictures are classified as biliary anastomotic stricture (BAS) or non-BAS. BAS is an isolated stricture at the biliary anastomosis, commonly observed 5–8 months after transplant [3]. Potential causes of BAS include suboptimal surgical

doi:10.1111/tri.12996

technique, suture material, anastomotic tension, and infections, leading to fibrotic scarring of the anastomosis [1–3].

Thrombocytopenia is a frequent complication after LT [4]. Common etiologies include sequestration in the liver graft, immunologic reaction, increased consumption, decreased production, and medication side effects [4–6]. Although severe thrombocytopenia has been reported to increase mortality in several studies [4,7,8], pathophysiology remains unclear. In recent years, platelets have garnered attention for promoting liver regeneration and attenuating liver fibrosis [9–11]. A prospective clinical trial demonstrated that platelet transfusion in patients with liver cirrhosis attenuated liver fibrosis [11]. In this retrospective clinical study, we investigated the risk factors for progression of post-transplant BAS, focusing on peritransplant platelet counts.

Materials and methods

Study population

Between January 2000 and June 2012, 1044 patients underwent ABO-identical/compatible orthotopic LT from deceased donors at Henry Ford Hospital (Detroit, MI, USA). Patient records were identified by an administrative database. Twenty-eight patients were excluded for incomplete records. Another 204 patients with biliary reconstruction by hepaticojejunostomy were excluded due to the difference in the approach for BAS diagnosis. Forty-one patients with deceased donor LT from donation after cardiac death (DCD) donors were not enrolled because of the well-accepted increased risk of biliary stricture in DCD grafts [12]. Thus, a final population of 771 recipients of brain death donor LT with duct-to-duct biliary reconstruction was enrolled. All data for the current study were collected in accordance with the Henry Ford Hospital Internal Review Board.

Operative procedures

Organ procurement was performed by aortic perfusion, using a standardized technique with the exception of the preservation solution. We used University of Wisconsin solution until 2003 and then switched to histidine–tryptophan–ketoglutarate (HTK) solution thereafter. All patients underwent transplantation according to common implantation techniques. Biliary reconstruction was performed with careful dissection of the hilum to preserve adequate blood supply to the bile duct. After hepatic arterial anastomosis, a biliary reconstruction was performed. The duct-to-duct anastomosis was performed using 6-0 polydioxanone. Hepatic arterial (HA) flow was measured by a flowmeter, Optima Flow-QC Meters® (Transonic Systems Inc, Ithaca, NY, USA). HA flow/graft weight ratio was calculated by HA flow (ml/min)/graft weight (g).

Platelet counts

Peritransplant platelet counts were recorded daily from admission until discharge and followed up at the outpatient clinic. We classified patients as having either a low or high platelet count on each postoperative day (POD), using the cutoff value calculated by the receiver operating characteristic (ROC) curve analysis and the Youden Index. Persistent postoperative thrombocytopenia was defined as low platelet counts on both POD 3 and POD 5. This was based on previous reports describing that low platelet counts on POD 5 were related to graft loss and severe postoperative morbidity [8,13].

Outcome parameters

Endoscopic retrograde cholangiopancreatogram (ERCP) was performed for clinical suspicion of stricture. Indications for ERCP included abdominal pain, fever, jaundice, and abnormal liver function tests. Clinically significant BAS was defined as a focal stenosis more than 50% at the anastomosis detected by ERCP, which was considered as incidence of BAS in the analysis. When the recipient bile duct was narrower than the donor bile duct, but there was no focal stenosis at the anastomosis, this was considered as a donor to recipient size mismatch, but not BAS. Early allograft dysfunction (EAD) was defined by bilirubin $\geq$10 mg/dl on day 7, international normalized ratio $\geq$1.6 on day 7, and AST or ALT >2000 IU/l within the first 7 days, according to a modified version of the original EAD definition in the Model for End-Stage Liver Disease (MELD) era of organ sharing [14].

Statistical analysis

Discrete variables were compared using the chi-square test or Fisher's exact test. Continuous variables are expressed as the median, minimum, and maximum and were compared using *t*-test. In the ROC analysis, the Youden Index and its 95% confidence intervals (CI) were used for deciding the cutoff value of postoperative

platelet count. The 95% CI was estimated using a nonparametric bootstrap approach (5000 times of resampling). Statistical analyses were performed using the SPSS 21.0 (SPSS Inc., Chicago, IL, USA) and SAS software version 9.4 (SAS Institute, Cary, NC, USA). Variables in the univariate analyses were included in a multivariate logistic regression analysis by our clinical expertise. $P < 0.05$ was considered statistically significant.

Results

Demographics and clinical outcomes

The median follow-up was 5.6 years. Of the 771 patients, ERCP was performed on 424 patients with clinical suspicion of biliary stricture. One hundred cases were found to be size mismatches. A total of 142 cases were finally diagnosed as BAS (18.4%). The median diagnosis time of stricture was 176 days. The baseline characteristics of recipient, donor, surgical, and postoperative factors in patients with and without BAS are listed in Table 1. In the BAS group, more patients were male and had higher MELD scores. No differences were observed in recipient age, ethnicity, type of liver disease, donor, surgical, and post-transplant variables.

Peritransplant platelet counts in patients with and without BAS

Peritransplant platelet dynamics are presented in Fig. 1. Platelet counts in both groups reached minimum values on POD 3. Pre- and post-transplant platelet counts within 5 days after LT were significantly lower in patients with BAS than those without BAS.

An optimal cutoff value of postoperative platelet count on POD 3 and 5 for progression of BAS was acquired by ROC analysis. Sensitivity, specificity, sensitivity + specificity − 1, Youden Index, 95% CI of Youden Index, and area under curve for several cut points on POD 3 and 5 are shown in Table 2. Youden Indexes showed optimal cutoff values of 41 × 1000/μl on POD 3 and 53 × 1000/μl on POD 5. Persistent postoperative

Table 1. Demographics (total $n = 771$).

	BAS yes ($n = 142$)	BAS no ($n = 629$)	P
Recipient variables			
Age	55 (24–70)	53 (16–73)	0.09
Gender, male	104 (73.2%)	401 (63.8%)	0.03
Ethnicity, Caucasian	98 (69.0%)	461 (73.3%)	0.30
Disease type			
Hepatitis C	74 (52.1%)	264 (41.9%)	0.10
Alcohol	23 (16.2%)	104 (16.5%)	
Hepatocellular carcinoma	15 (10.6%)	55 (8.7%)	
Others	30 (21.1%)	206 (32.9%)	
BMI (kg/m^2)	29.6 (20–48)	29.1 (12–51)	0.43
MELD score	21 (7–40)	19 (6–40)	0.003
Previous LT history, yes	6 (4.2%)	24 (3.8%)	0.83
Donor variables			
Age	43 (13–85)	43 (17–83)	0.80
CMV high risk, yes	32 (22.5%)	110 (17.5%)	0.17
Liver donor risk index	1.28 (0.80–2.44)	1.32 (0.84–2.44)	0.98
Surgical variables			
Hepatic arterial flow (ml/min)	401 (100–1750)	367 (25–2400)	0.15
Warm ischemia time (min)	44 (16–90)	43 (9–103)	0.73
Cold ischemia time (min)	340 (164–545)	347 (90–720)	0.55
Estimated blood loss (ml)	2000 (300–14,000)	1730 (200–58,000)	0.96
Postoperative variables			
Length of stay after LT (days)	15 (2–171)	14 (2–171)	0.45
Biliary leak history, yes	5 (3.5%)	32 (5.1%)	0.43
Early allograft dysfunction, yes	48 (33.8%)	221 (35.1%)	0.75
Reoperation within 30 days after LT	26 (18.3%)	139 (22.1%)	0.32
Acute cellular rejection, yes	23 (16.9%)	113 (17.9%)	0.61
30-day mortality	0 (0%)	13 (4.8%)	0.08

BAS, biliary anastomotic stricture; BMI, body mass index; MELD, Model of End-Stage Liver Disease; LT, liver transplantation; CMV, cytomegalovirus.

Transplant International 2017; 30: 1032–1040

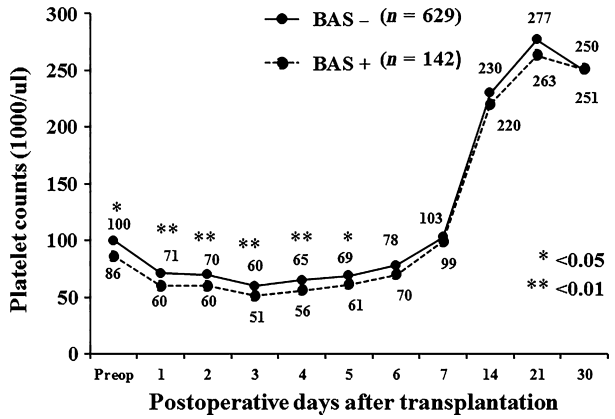

Figure 1 Perioperative platelet dynamics in patients with or without biliary anastomotic stricture (BAS). Platelet counts in both groups reached minimum counts on the postoperative day 3. Preoperative and postoperative platelet counts within 5 days after liver transplantation were significantly lower in patients with BAS. Median values are shown. *$P < 0.05$. **$P < 0.01$.

thrombocytopenia was defined as platelet counts <41 × 1000/μl and <53 × 1000/μl on POD 3 and POD 5, respectively. Persistent postoperative thrombocytopenia was found in 203 patients.

Prediction of BAS

The risk factors for the development of BAS were compared by a logistic regression model (Table 3). Recipient male gender [odds ratio (OR) = 1.56, $P = 0.03$], disease type viral hepatitis C (OR = 1.46, $P = 0.04$), MELD score ≥30 (OR = 2.20, $P < 0.001$), HTK solution (OR = 1.71, $P = 0.03$), and persistent postoperative thrombocytopenia (OR = 2.49, $P < 0.001$) were significantly associated with the development of BAS on univariate analysis. A multivariate logistic regression analysis was performed with 14 variables [recipient age ≥60, recipient male gender, disease type of viral hepatitis C, MELD score ≥30, preoperative refractory ascites (grade ≥3), macrosteatosis ≥25%, liver donor risk index (LDRI) ≥1.7, HA flow/graft weight ratio, suture type of biliary reconstruction, HTK solution, biliary stent, EAD, bile leak, and persistent postoperative thrombocytopenia]. Persistent postoperative thrombocytopenia (OR = 2.38, $P = 0.01$) became the only independent risk factor for development of BAS.

Prediction of persistent postoperative thrombocytopenia

The contributing factors for persistent postoperative thrombocytopenia were compared using a logistic regression model (Table 4). Body mass index (BMI) ≥30 (OR = 1.40, $P = 0.04$), MELD score ≥30 (OR = 2.64, $P < 0.001$), preoperative encephalopathy (OR = 1.92, $P = 0.02$), estimated blood loss (OR = 1.01, per 100 ml up, $P = 0.04$), HTK solution (OR = 1.70, $P = 0.005$), red blood cell transfusion (OR = 1.08, $P < 0.001$), fresh frozen plasma transfusion (OR = 1.04, $P = 0.001$), platelet transfusion (OR = 1.02, $P < 0.001$), EAD (OR = 1.54, $P = 0.01$), and postoperative platelet transfusion (OR = 4.57, $P < 0.001$) were associated with persistent postoperative thrombocytopenia. Estimated blood loss, red blood cell transfusion, fresh frozen plasma transfusion, and perioperative platelet transfusion were highly correlated as calculated by the Pearson correlation coefficient. Therefore, estimated blood loss was included in the final multivariate model. Because donor age and cold ischemia time are components of LDRI, LDRI ≥1.7 was included in the multivariate model instead of these two variables. A multivariate logistic regression analysis was performed with 20 variables (recipient age ≥60, recipient male gender, Caucasian, disease type viral

Table 2. ROC curve analyses of platelet counts on POD 3 and 5.

	Cutoff value (×1000/μl)	Rank	Sensitivity	Specificity	Sensitivity + specificity − 1	Youden Index	95% CI of Youden Index*	AUC (95% CI)
POD 3	41	1	0.465	0.691	0.156	0.156	0.086, 0.250	0.57 (0.52, 0.62)
	42	2	0.465	0.682	0.146	–	–	
	43	3	0.479	0.664	0.143	–	–	
POD 5	53	1	0.540	0.596	0.136	0.136	0.068, 0.232	0.55 (0.50, 0.60)
	54	2	0.540	0.581	0.121	–	–	
	65	3	0.669	0.450	0.119	–	–	

ROC, receiver operating characteristic; POD, postoperative; CI, confidence interval; AUC, area under curve.

Table 3. Univariate and multivariate analysis of risk factors for biliary anastomotic stricture (n = 142 cases/771 cases total).

	Univariate analysis			Multivariate analysis		
	OR	CI	*P*	OR	CI	*P*
Recipient variables						
Age ≥60, yes	1.28	0.86–1.91	0.22	1.90	0.95–3.78	0.07
Gender, male	1.56	1.04–2.33	0.03	2.00	0.96–4.16	0.06
Ethnicity, Caucasian	0.81	0.55–1.21	0.30	–	–	–
Disease type, virus hepatitis C, yes	1.46	1.02–2.11	0.04	1.23	0.63–2.41	0.54
BMI ≥30, yes	1.30	0.90–1.87	0.16	–	–	–
MELD score ≥30, yes	2.20	1.42–3.43	<0.001	1.83	0.84–4.00	0.13
Preoperative refractory ascites (grade ≥3), yes	1.35	0.74–2.46	0.32	1.03	0.45–2.34	0.95
Preoperative encephalopathy (grade ≥3), yes	1.00	0.47–2.15	0.99	–	–	–
TIPS prior to transplantation, yes	0.56	0.22–1.45	0.23	–	–	–
Dialysis prior to LT, yes	0.84	0.38–1.84	0.66	–	–	–
Previous history of LT, yes	1.11	0.45–2.76	0.83	–	–	–
Preoperative albumin (per 1 mg/dl up)	0.84	0.62–1.13	0.25	–	–	–
Donor variables						
Age ≥40, yes	0.87	0.60–1.26	0.46	–	–	–
Gender mismatch, yes	1.02	0.70–1.47	0.93	–	–	–
CMV high risk, yes	0.73	0.47–1.14	0.17	–	–	–
Macrosteatosis ≥25%, yes	1.29	0.28–5.96	0.75	2.25	0.31–16.14	0.42
Liver donor risk index ≥1.7, yes	1.23	0.76–1.99	0.39	1.12	0.46–2.73	0.81
Surgical variables						
HA flow (per 100 ml/min up)	1.07	0.98–1.17	0.15	–	–	–
HA flow/graft weight ratio (ml/min/g, per 0.01 up)	1.24	0.98–1.58	0.07	1.24	0.93–1.67	0.15
Warm ischemia time ≥60 min, yes	1.01	0.57–1.76	0.99	–	–	–
Cold ischemia time ≥360 min, yes	1.12	0.77–1.62	0.56	–	–	–
Venous anastomosis, bicaval, yes	0.84	0.56–1.26	0.39	–	–	–
Suture type of biliary reconstruction, running	1.0	Reference	–	1.0	Reference	–
Interrupted	1.03	0.61–1.75	0.90	2.44	0.82–7.26	0.11
Combination	1.05	0.65–1.71	0.83	0.75	0.32–1.74	0.50
Revision of HA anastomosis, yes	0.45	0.13–1.49	0.19	–	–	–
HTK solution, yes	1.71	1.05–2.79	0.03	1.99	0.86–4.57	0.09
Biliary stent, yes	0.67	0.28–1.62	0.37	0.89	0.18–4.42	0.88
Estimated blood loss (per 100 ml up)	1.00	0.99–1.01	0.96	–	–	–
Splenic arterial ligation, yes	0.45	0.08–2.47	0.36	–	–	–
Red blood cell transfusion (per 1 unit up)	0.99	0.95–1.02	0.45	–	–	–
Fresh frozen plasma transfusion (per 1 unit up)	0.99	0.98–1.02	0.79	–	–	–
Platelet transfusion (per 1 unit up)	0.99	0.96–1.01	0.32	–	–	–
Postoperative variables						
Early allograft dysfunction	1.06	0.72–1.56	0.75	1.02	0.49–2.11	0.97
Platelet transfusion, yes	0.97	0.67–1.41	0.89	–	–	–
Biliary leak history, yes	0.68	0.26–1.78	0.43	0.74	0.08–7.23	0.79
Acute cellular rejection, yes	0.88	0.54–1.44	0.48	–	–	–
Reoperation 30 days after transplantation, yes	0.79	0.50–1.26	0.32	–	–	–
Persistent postoperative thrombocytopenia*	2.49	1.70–3.64	<0.001	2.38	1.22–4.65	0.01

OR, odds ratio; CI, confidence interval; BMI body mass index, MELD, Model of End-Stage Liver Disease; LT, liver transplantation; TIPS, transjugular intrahepatic portosystemic shunt; CMV cytomegalovirus; HA, hepatic arterial; HTK, histidine–tryptophan–ketoglutarate.

*Platelet count <41 × 1000/μl on POD 3 and platelet count <53 × 1000/μl on POD 5.

hepatitis C, BMI ≥30, MELD score ≥30, preoperative refractory ascites, preoperative encephalopathy, dialysis prior to LT, previous history of LT, gender mismatch, cytomegalovirus high risk, macrosteatosis ≥25%, LDRI ≥1.7, HA flow/graft weight ratio, warm ischemia time ≥60 min, splenic arterial ligation, estimated blood loss,

Transplant International 2017; 30: 1032–1040

Table 4. Univariate and multivariate analysis for persistent postoperative thrombocytopenia (n = 203 cases/771 cases total).

	Univariate analysis			Multivariate analysis		
	OR	CI	*P*	OR	CI	*P*
Recipient variables						
Age ≥60, yes	0.83	0.58–1.19	0.31	0.90	0.54–1.49	0.67
Gender, male	0.75	0.54–1.04	0.09	0.66	0.40–1.10	0.11
Ethnicity, Caucasian	0.82	0.57–1.16	0.26	0.82	0.49–1.38	0.82
Disease type, virus hepatitis C, yes	0.89	0.65–1.23	0.48	0.83	0.51–1.36	0.83
BMI ≥30, yes	1.40	1.01–1.93	0.04	1.19	0.74–1.91	0.47
MELD score ≥30, yes	2.64	1.76–3.95	<0.001	1.56	0.83–2.93	0.17
Preoperative refractory ascites (grade ≥3), yes	1.27	0.90–2.08	0.14	1.54	0.82–2.86	0.18
Preoperative encephalopathy (grade ≥3), yes	1.92	1.14–3.23	0.02	1.57	0.74–3.32	0.24
Dialysis prior to LT, yes	0.89	0.45–1.75	0.73	0.66	0.23–1.90	0.44
Previous history of LT, yes	0.69	0.28–1.71	0.42	0.45	0.12–1.63	0.23
Donor variables						
Age ≥40, yes	0.88	0.63–1.22	0.44	–	–	–
Gender mismatch, yes	1.11	0.80–1.54	0.54	1.20	0.74–1.95	0.46
CMV high risk, yes	1.10	0.72–1.68	0.66	1.01	0.55–1.87	0.96
Macrosteatosis ≥25%, yes	0.54	0.06–4.83	0.58	0.82	0.21–3.21	0.78
Liver donor risk index ≥1.7	0.99	0.64–1.55	0.98	1.13	0.61–2.12	0.70
Surgical variables						
HA flow (per 100 ml/min up)	0.98	0.90–1.05	0.74	–	–	–
HA flow/graft weight* (ml/min/g, per 0.01 up)	1.01	0.99–1.02	0.36	1.08	0.84–1.41	0.53
Warm ischemia time ≥60 min, yes	1.12	0.69–1.82	0.65	1.05	0.34–3.22	0.94
Cold ischemia time ≥360 min, yes	1.02	0.73–1.41	0.92	–	–	–
Estimated blood loss (per 100 ml up)*	1.01	1.00–1.01	0.04	1.01	1.00–1.03	0.04
Splenic arterial ligation, yes	0.59	0.14–2.50	0.47	0.83	0.10–7.21	0.86
HTK solution, yes	1.70	1.17–2.47	0.005	1.60	0.66–3.87	0.30
Red blood cell transfusion (per 1 unit up)	1.08	1.05–1.11	<0.001	–	–	–
Fresh frozen plasma transfusion (per 1 unit up)	1.04	1.01–1.06	0.001	–	–	–
Platelet transfusion (per 1 unit up)	1.04	1.02–1.06	<0.001	–	–	–
Postoperative variables						
Early allograft dysfunction, yes	1.54	1.11–2.14	0.01	1.67	0.92–2.99	0.09
Platelet transfusion, yes	4.57	3.22–6.49	<0.001	–	–	–

OR, odds ratio; CI, confidence interval; BMI, body mass index; MELD, Model of End-Stage Liver Disease; LT, liver transplantation; CMV, cytomegalovirus; HA, hepatic arterial; HTK, histidine–tryptophan–ketoglutarate.

Estimated blood loss, red blood cell transfusion, fresh frozen plasma transfusion, and perioperative platelet transfusion were highly correlated. Therefore, estimated blood loss was included in the final multivariate model.

HTK solution, and EAD). This multivariable analysis demonstrated that estimated blood loss became the only independent contributing factor for persistent postoperative thrombocytopenia (OR = 1.01, per 100 ml up, P = 0.04).

Discussion

Despite recent advances in organ preservation, surgical technique and immunosuppression, the incidence of BAS continues to range from 5% to 15% after deceased donor LT [1]. The pathogenesis of BAS is known to be due to suboptimal techniques, local ischemia, increased donor age, graft steatosis, cytomegalovirus infection, male gender recipient, acute cellular rejection, high MELD score, etc. [2,15–18]. Several studies have reported bile leak to be an independent risk factor and the internal stent to be independent protective factors [19,20]. Further, DCD grafts and living donor grafts bear markedly higher risk [12,21]. In contrast, whether duct-to-duct anastomosis, or hepaticojejunostomy are risk factors for progression of BAS remains unknown, and no significant differences have been observed between interrupted versus continuous suture [19]. We identified persistent postoperative thrombocytopenia to be the only risk factor for progression of BAS. No direct

associations were observed in terms of recipient, donor, and surgical factors including suture type of biliary reconstruction and use of internal stent. It was notable that HTK solution tended to relate with progression of BAS. This result was compatible with a recent study from the European Liver Transplant Registry, reporting that HTK solution was independently associated with a 10% increased risk of graft loss with higher incidence of primary nonfunction, biliary complications, and infectious complications than other solutions [22]. Further, in our series, HA flow/graft weight ratio and HA flow/body weight ratio (data not shown) did not relate with BAS or persistent postoperative thrombocytopenia. It is known that the bile duct is mainly supplied by the hepatic artery, and a decrease in HA flow can cause biliary complications. Kim *et al.* [23] described HA flow/body weight ratio of <5 ml/min/kg to be associated with higher rates of biliary complications. Hashimoto *et al.* [24] reported a relationship between impaired HA buffer response and early development of biliary complications. In our study, the timing of HA flow measurement was dependent on each surgeon's judgment and it was not unified. In some cases, flow was measured immediately after HA anastomosis, while in other cases it was delayed until later in the case. This might result in a difference from previous articles.

Platelets are the smallest blood components that contain biophysiological substances, including hepatocyte growth factor (HGF), tumor necrosis factor-α, interleukin-6, serotonin, and insulin growth factor-1 [25]. Following activation, platelets release these substances to either enhance or limit tissue injury. The relationship between platelets and LT was reported from the late 1990s, and thrombocytopenia after LT was found to be associated with poor graft and overall survivals [4,7]. However, this relationship has remained unelucidated. Recently, Lesurtel *et al.* [8] proposed the 60-5 criteria, in which a platelet count <60,000/μl on POD 5 was an independent risk factor associated with severe postoperative complications, early graft failure, and patient mortality. In recent years, experimental studies have demonstrated that platelets play an important role in attenuating fibrosis in the liver [26]. Murata *et al.* [10] reported that platelets accumulated in the fibrotic liver immediately after hepatectomy, promoted liver regeneration and decreased liver fibrosis. Takahashi *et al.* [27] found that platelets enhanced HGF release from Kupffer cells and suppressed activation of hepatic stellate cells, the cell primarily responsible for liver fibrosis progression. Ikeda *et al.* [28] stated that adenine nucleotides enriched in platelets suppressed hepatic stellate cell activation via the adenosine–cyclic adenosine monophosphate signaling pathway.

In this study, we focused on thrombocytopenia within 5 days after LT based on the results from previous reports showing that low platelet counts on POD 5 were a risk factor for graft loss and severe postoperative morbidity [8,13]. We defined persistent postoperative thrombocytopenia as platelet count $<41 \times 1000/\mu l$ on POD 3 and platelet count $<53 \times 1000/\mu l$ on POD 5, according to the result from ROC analyses and Youden indexes. There are several considerations regarding our results. First, it remained controversial whether low platelet counts were merely sequelae of worse post-transplant graft function. In our study, there was no association between BAS and EAD, which is a well-accepted surrogate marker of early graft function [14]. Second, peritransplant thrombocytopenia can be a consequence of splenic sequestration, reflecting severity of portal vein hypertension. Our results showed no association between persistent postoperative thrombocytopenia and preoperative clinical signs of portal hypertension, including existence of refractory ascites or preoperative encephalopathy. Considering previous experimental evidence [10,26–28], we hypothesize that more platelets accumulate in the bile duct in patients with high platelet counts. This may result in release of higher concentrations of growth factors, such as HGF and adenine nucleotides, which suppress fibroblast function and attenuate excessive fibrotic healing at the anastomosis.

Our results suggest that keeping post-transplant platelet counts higher by minimizing intraoperative blood loss could indirectly avoid progression of post-transplant BAS. These results are compatible with the study from Selvalkular *et al.* [29] who reported that increased intraoperative blood loss was a predictor for post-transplant biliary complications including BAS after LT. The current options to increase platelet counts include platelet transfusion, thrombopoietin mimetic agents, thrombopoietin-receptor agonists, splenectomy, and ligation of the splenic artery. Recent reports demonstrate that platelet transfusion promotes graft regeneration after living donor LT [30,31], whereas our results showed that platelet transfusion was found to be a risk factor for persistent postoperative thrombocytopenia, which correlated with progression of BAS. This might be because patients who required platelet transfusion were more likely to have a complicated surgery, leading to larger amount of blood loss and intraoperative coagulopathy, which might mask the positive effect of

platelets. Further, in our study, splenic arterial ligation did not play a role in preventing BAS or persistent postoperative thrombocytopenia. We recently reported that postoperative thrombocytopenia might be associated with poor liver graft survival, and suggested that medical therapies to enhance peritransplant platelet count would be beneficial to improve liver graft outcomes [13]. Currently, we are planning to investigate a possible prognostic impact of thrombopoietin-receptor agonists on liver graft survival. Similarly, maintaining peritransplant platelet count by thrombopoietin-receptor agonists or other medical and surgical therapies may decrease a risk of BAS after LT. Further investigations would be warranted to determine possible effects of these platelet therapies on incidence of BAS.

This study has some limitations. First, the data was retrospective in nature. In addition, despite the relatively large number of cases, the study is from a single center, and the results were influenced by the internal practice pattern by each transplant anesthesia and surgical team, which may limit generalizability. Second, our study did not demonstrate the direct evidence that platelets suppress BAS after LT. Despite these limitations, platelets appear to play an important role in LT and additional studies may translate into viable methods for reducing risk and improving outcomes in this patient population.

In conclusion, we demonstrated for the first time that low platelet count is associated with progression of BAS after LT. Keeping platelet count high by minimizing intraoperative blood loss could potentially reduce incidence of post-transplant BAS.

Authorship

KT, SN, AYL, WJK, PLS, MS, MDR, KMC, AY, MSA and GTS: contributed equally to this work. KT, SN, KGP, AYL, PLS and MS: collected data. KT, SN and MG: analyzed the data. KT, SN and GTS: wrote the manuscript. MDR, KMC, AY and MSA: provided critical comments on the manuscript.

Funding

This study was supported by research grants from the Henry Ford Hospital Transplant Institute, H10097.

Conflict of interest

The authors declare no conflict of interests.

REFERENCES

1. Kochhar G, Parungao JM, Hanouneh IA, Parsi MA. Biliary complications following liver transplantation. *World J Gastroenterol* 2013; **19**: 2841.
2. Gastaca M. Biliary complications after orthotopic liver transplantation: a review of incidence and risk factors. *Transplant Proc* 2012; **44**: 1545.
3. Jagannath S, Kalloo AN. Biliary complications after liver transplantation. *Curr Treat Options Gastroenterol* 2002; **5**: 101.
4. Chatzipetrou MA, Tsaroucha AK, Weppler D, *et al.* Thrombocytopenia after liver transplantation. *Transplantation* 1999; **67**: 702.
5. Kaneko K, Shirai Y, Wakai T, Yokoyama N, Akazawa K, Hatakeyama K. Low preoperative platelet counts predict a high mortality after partial hepatectomy in patients with hepatocellular carcinoma. *World J Gastroenterol* 2005; **11**: 5888.
6. Okano E, Ko S, Kanehiro H, Matsumoto M, Fujimura Y, Nakajima Y. ADAMTS13 activity decreases after hepatectomy, reflecting a postoperative liver dysfunction. *Hepatogastroenterology* 2010; **57**: 316.
7. Chang FY, Singh N, Gayowski T, *et al.* Thrombocytopenia in liver transplant recipients: predictors, impact on fungal infections, and role of endogenous thrombopoietin. *Transplantation* 2000; **69**: 70.
8. Lesurtel M, Raptis DA, Melloul E, *et al.* Low platelet counts after liver transplantation predict early posttransplant survival: the 60-5 criterion. *Liver Transpl* 2014; **20**: 147.
9. Lesurtel M, Graf R, Aleil B, *et al.* Platelet-derived serotonin mediates liver regeneration. *Science* 2006; **312**: 104.
10. Murata S, Hashimoto I, Nakano Y, Myronovych A, Watanabe M, Ohkohchi N. Single administration of thrombopoietin prevents progression of liver fibrosis and promotes liver regeneration after partial hepatectomy in cirrhotic rats. *Ann Surg* 2008; **248**: 821.
11. Maruyama T, Murata S, Takahashi K, *et al.* Platelet transfusion improves liver function in patients with chronic liver disease and cirrhosis. *Tohoku J Exp Med* 2013; **229**: 213.
12. Seehofer D, Eurich D, Veltzke-Schlieker W, Neuhaus P. Biliary complications after liver transplantation: old problems and new challenges. *Am J Transplant* 2013; **13**: 253.
13. Takahashi K, Nagai S, Putchakayala KG, *et al.* Prognostic impact of postoperative low platelet count after liver transplantation. *Clin Transplant* 2017; **31**: e12891.
14. Olthoff KM, Kulik L, Samstein B, *et al.* Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. *Liver Transpl* 2010; **16**: 943.
15. Chang TI, Ho MC, Wu YM, Lee PH, Hu RH. Biliary complications after liver transplantation: an 18-year single-center experience. *J Formos Med Assoc* 2011; **110**: 183.
16. Luthold SC, Kaseje N, Jannot AS, *et al.* Risk factors for early and late biliary complications in pediatric liver transplantation. *Pediatr Transplant* 2014; **18**: 822.

17. Chok KS, Chan SC, Cheung TT, *et al.* Bile duct anastomotic stricture after adult-to-adult right lobe living donor liver transplantation. *Liver Transpl* 2011; **17**: 47.
18. Mocchegiani F, Vincenzi P, Lanari J, *et al.* Immunological risk factors in biliary strictures after liver transplantation. *Ann Transplant* 2015; **20**: 218.
19. Welling TH, Heidt DG, Englesbe MJ, *et al.* Biliary complications following liver transplantation in the model for end-stage liver disease era: effect of donor, recipient, and technical factors. *Liver Transpl* 2008; **14**: 73.
20. Verdonk RC, Buis CI, Porte RJ, *et al.* Anastomotic biliary strictures after liver transplantation: causes and consequences. *Liver Transpl* 2006; **12**: 726.
21. Abou Abbass A, Abouljoud M, Yoshida A, *et al.* Biliary complications after orthotopic liver transplantation from donors after cardiac death: broad spectrum of disease. *Transplant Proc* 2010; **42**: 3392.
22. Adam R, Delvart V, Karam V, *et al.* Compared efficacy of preservation solutions in liver transplantation: a long-term graft outcome study from the European Liver Transplant Registry. *Am J Transplant* 2015; **15**: 395.
23. Kim PT, Saracino G, Jennings L, *et al.* Ratio of hepatic arterial flow to recipient body weight predicts biliary complications after deceased donor liver transplantation. *HPB (Oxford)* 2014; **16**: 1083.
24. Hashimoto K, Miller CM, Quintini C, *et al.* Is impaired hepatic arterial buffer response a risk factor for biliary anastomotic stricture in liver transplant recipients? *Surgery* 2010; **148**: 582.
25. Holmsen H. Physiological functions of platelets. *Ann Med* 1989; **21**: 23.
26. Watanabe M, Murata S, Hashimoto I, *et al.* Platelets contribute to the reduction of liver fibrosis in mice. *J Gastroenterol Hepatol* 2009; **24**: 78.
27. Takahashi K, Murata S, Fukunaga K, Ohkohchi N. Human platelets inhibit liver fibrosis in severe combined immunodeficiency mice. *World J Gastroenterol* 2013; **19**: 5250.
28. Ikeda N, Murata S, Maruyama T, *et al.* Platelet-derived adenosine 5′-triphosphate suppresses activation of human hepatic stellate cell: in vitro study. *Hepatol Res* 2012; **42**: 91.
29. Selvakumar N, Saha BA, Naidu SC. Is duct to duct biliary anastomosis the rule in orthotopic liver transplantation? *Indian J Surg* 2013; **75**: 368.
30. Han S, Park HW, Song JH, *et al.* Association between intraoperative platelet transfusion and early graft regeneration in living donor liver transplantation. *Ann Surg* 2016; **264**: 1065.
31. Kim J, Yi NJ, Shin WY, Kim T, Lee KU, Suh KS. Platelet transfusion can be related to liver regeneration after living donor liver transplantation. *World J Surg* 2010; **34**: 1052.