ORIGINAL ARTICLE

Association of thrombocytopenia with outcome following adult living donor liver transplantation

Viniyendra Pamecha, Shyam Sunder Mahansaria, Senthil Kumar, Kishore Gurumoorthy Subramanya Bharathy, Shridhar Vasantrao Sasturkar, Piyush Kumar Sinha, Niteen Kumar & Vaibhaw Kumar

Department of Liver Transplantation and Hepato Pancreatico Biliary Surgery, Institute of Liver & Biliary Sciences, New Delhi, India

Correspondence
Dr. Viniyendra Pamecha MBBS, MS, FEBS, FRCS, Liver Transplantation & Hepato Pancreatico Biliary Surgery, Institute of Liver & Biliary Sciences, D1, Vasant Kunj, New Delhi 110070, India.
Tel.: +91-9540946803;
fax: +91-11-92670-6746;
e-mail: viniyendra@yahoo.co.uk

[Correction added on 09 September 2016 after first online publication: Citation of author names have been corrected in this version.]

SUMMARY

This study aimed to evaluate the association of postoperative thrombocytopenia with outcome following adult living donor liver transplantation (LDLT) for end-stage liver disease (ESLD). It was a prospective study of 120 consecutive adult LDLT from September 2012 to May 2015. Preoperative platelet counts (PLTs) and postoperative PLTs were recorded at regular intervals till 3 months after LDLT. Univariate and multivariate analyses were performed. The median pretransplant PLT was 61 $\times$ 10^9/l. The lowest median PLT after LDLT was observed on POD 3. Patients were stratified into low platelet group (n = 83) with PLT <30 $\times$ 10^9/l and high platelet group (n = 37) with PLT ≥30 $\times$ 10^9/l. Patients with PLT <30 $\times$ 10^9/l had statistically significant higher grade III/IV complication (P = 0.001), early graft dysfunction (P = 0.01), sepsis (P = 0.001), and prolonged ascites drainage (P = 0.002). On multivariate analysis, PLT<30 $\times$ 10^9/l was identified as an independent risk factor for grade III/IV complications (P = 0.005). Overall, patients survival was significantly different between two groups (P = 0.04), but this predictive value was lost in patients who survived more than 90 days (P = 0.37). Postoperative PLT of <30 $\times$ 10^9/l was a strong predictor of major postoperative complications and is associated with early graft dysfunction, prolonged ascites drainage, and sepsis. The perioperative mortality rate was high in the thrombocytopenia group.

Transplant International 2016; 29: 1126–1135

Key words
end-stage liver disease, living donor liver transplantation, platelet counts

Received: 12 February 2016; Revision requested: 3 April 2016; Accepted: 15 July 2016; EV Pub Online: 29 August 2016

Introduction

Platelets have an important role in primary hemostasis, but are also known to have a role in various other non-hemostatic processes such as inflammation [1], antimicrobial defense [2], angiogenesis [3], ischemia/reperfusion injury [4], tissue repair, and liver regeneration [5,6]. All these properties, to some extent, are involved in the pathophysiological alterations that happen in patients undergoing liver transplantation (LT). Platelets may have a beneficial or detrimental impact on the outcomes of patients undergoing LT [7]. There are limited data regarding the association of postoperative thrombocytopenia with morbidity, liver allograft function, sepsis, and mortality in living donor liver transplantation (LDLT). Thrombocytopenia in immediate postoperative period after LDLT is associated with early graft dysfunction [8]. The post-transplant recovery

doi:10.1111/tri.12819

of thrombocytopenia in LDLT is assumed to be different from that in deceased donor liver transplantation (DDLT) as recovery of portal hypertension after partial graft is different from that of whole liver graft [9]. The aim of the study was to evaluate the association of postoperative thrombocytopenia with outcome following adult LDLT.

Patients and methods

Data were collected prospectively from September 2012 to May 2015 at the department of Liver Transplantation and Hepato Pancreatico Biliary Surgery, Institute of Liver and Biliary Sciences, New Delhi, India. Consecutive adult patients undergoing elective LDLT were enrolled in the study. The study was approved by institutional review board and ethics committee of the institute. Informed signed consents were obtained from all the participants. Patients with age <18 years, acute liver failure (ALF) patients undergoing emergency LDLT and those undergoing ABO-incompatible LDLT were excluded in the present study.

Platelet counts (PLTs) were recorded on the day before LT and thereafter once daily in the first postoperative week, then postoperative days (PODs) 10, 14, 18, 21, 1 month, 2 months, and 3 months. PLTs were performed by an automated hematology analyzer using VCS technology (Volume, Conductivity, Light Scatter, Beckman coulter LH 750). Demographic parameters included were age, sex, and body mass index (BMI). Clinical parameters evaluating the severity of liver disease included were preoperative platelet count, etiology of liver disease, model for end-stage liver disease (MELD) score, variceal bleeding, hepatorenal syndrome, hepatic encephalopathy, spontaneous bacterial peritonitis. Listing for transplantation is based on the standard criteria. Patients with high MELD scores are not denied transplant if they satisfy these criteria. If the patient is deemed too sick due to poor performance status, significant organ dysfunction, he or she may not be offered a transplant even if MELD scores are low. As per the protocol, all patients were to have negative preoperative cultures in the week preceding the transplant. Intraoperative parameters included were operative time, intra-operative blood loss, blood products transfusions (packed red blood cells, fresh frozen plasma, and platelet transfusions), graft-to-recipient body weight ratio (GRBWR), warm ischemia time (WIT), cold ischemia time (CIT).

Living donor liver transplantation was performed with a right lobe graft with reconstruction of segment 5 and 8 veins in 105 patients, and a left lobe graft in 15 patients. No patient underwent splenectomy or inflow modulation. Intra-operatively, we use continuous hemodynamic monitoring (Flo Trac®; Edward Life Sciences, Irvine, California, United States) to guide fluid therapy and point-of-care coagulation testing (TEG®; Haemonetics, Braintree, Massachusetts, United States) to guide transfusion. If there is an ongoing bleed, packed blood cells are transfused to maintain a hemoglobin level of around 7 g% and the TEG parameters are corrected with appropriate blood products. In the absence of bleeding, numbers on the TEG are not corrected even if they are abnormal. Post-transplant immunosuppression protocol consisted of steroid (methylprednisolone) induction in anhepatic phase followed by 5 days taper. Maintenance immunosuppression consisted of tacrolimus + mycophenolate mofetil + steroid (prednisolone). Steroids were tapered in the majority by the end of 3 months. Mycophenolate mofetil was avoided in patients with PLT<30 $\times$ 10^9/l post-transplant. The use of tacrolimus in the postoperative period was guided by serial liver function tests, rather than target drug levels. Usual target levels were 5–7 ng/ml in the first week. Basiliximab was not used for induction/renal sparing in this study population. Postoperative liver biopsies were reserved for unexplained causes of abnormal liver function tests, especially when the findings of biopsy were likely to alter the management strategy.

The outcome measures of interest were postoperative complications, infectious complications, allograft dysfunction, postoperative ascites drainage (>1 l on POD 14), and early postoperative mortality. Early allograft dysfunction (EAD) was defined as the presence of at least one or more of the following criteria after LT: a serum bilirubin level ≥10 mg/dl on day 7 and an international normalized ratio ≥1.6 on day 7 or an alanine or aspartate aminotransferase level >2000 IU/l within the first 7 days [10]. Postoperative complications were classified as per Clavien–Dindo classification [11]. Any complication occurring within the first 90 days postoperatively was defined as "early," and any complication occurring thereafter was defined as "late". Postoperative infectious complications were based on positive bacterial cultures of peripheral/central blood, urine, sputum, and intra-abdominal fluid. Infection was defined as per CDC (center for disease control) criteria [12]. Sepsis was defined as the presence (probable or documented) of infection together with systemic manifestations of infection [13]. Prolonged postoperative ascites drainage was defined as >1 l on

POD 14 [14]. Early postoperative mortality was defined as death occurring due to any cause within 90 days of LDLT.

Statistical analysis

Statistical analyses were performed with SPSS Statistics 21 for Mac (IBM). A receiver-operating characteristic (ROC) curve analysis was performed to calculate the optimum cutoff values for platelet counts to detect grade III/IV complications after LT. Continuous variables were expressed as medians and interquartile range (IQRs). Continuous variables were compared with the Student's *t*-test and Mann–Whitney *U*-test as appropriate. Differences between proportions derived from categorical data were compared with chi-square or Fisher's exact test. Variables that correlated with thrombocytopenia and major complications in the univariate analysis ($P < 0.05$) were included in the multivariate forward logistic regression analysis. For all tests, a P value of less than 0.05 was considered significant.

Results

During the study period, 152 patients underwent LDLT, of which 120 adult patients who were transplanted for ESLD were included in this study. Pediatric liver transplant (10 patients) and those transplanted for acute liver failure (22 patients) were excluded. The median pretransplant platelet count was 61 × 10^9/l (IQR = 42–88 × 10^9/l). The lowest median PLT after LT were observed on POD 3, the median being 22 × 10^9/l (IQR = 16–32 × 10^9/l). Based on ROC curve, POD 3 platelet count showed a good prediction ability (Fig. 1a) (area under the curve 0.68, $P < 0.001$) for grade III/IV complications. The optimal cutoff value on POD 3 for prediction of grade III/IV complications was 30 × 10^9/l. Based on this cutoff value, patients were divided into two groups: low platelet group, patients with PLT<30 × 10^9/l ($n = 83$), and high platelet group, patients with PLT ≥30 × 10^9/l ($n = 37$). The median preoperative PLT fell from 61 × 10^9/l (IQR = 42–88 × 10^9/l) to median nadir of 40 × 10^9/l (IQR = 23–57 × 10^9/l) in the first 7 days post-transplant. Then, they started to increase and exceeded the preoperative PLT by day 10; median PLTs on day 10 were 71 × 10^9/l (IQR = 41–123 × 10^9/l). Recovery of PLT after transplantation in both groups shown in Fig. 1b and statistically significant difference were obtained from both groups ($P < 0.001$).

Patient demographic details along with clinical and perioperative variables for two groups have been depicted in Table 1. A univariate logistic analysis of various factors showed that patients with PLT <30 × 10^9/l had significantly higher pretransplant thrombocytopenia, MELD score, intra-operative blood loss, intra-operative

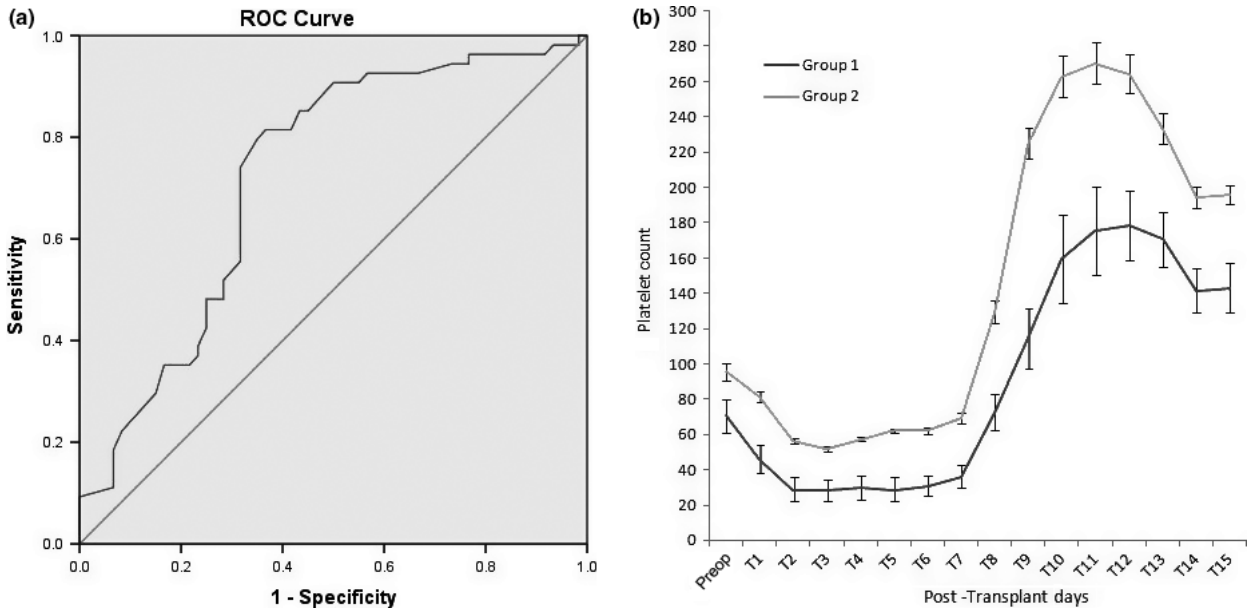


Figure 1 (a) Receiver-operating characteristic curve for postoperative platelet count in relation to major postoperative complications (area under the curve = 0.68). (b) Dynamics of platelet count in patients with platelet counts <30 × 10^9/l (Group 1) or ≥30 × 10^9/l (Group 2) after liver transplantation.

Transplant International 2016; 29: 1126–1135

Table 1. Comparison of patient characteristics and perioperative variables between low and high platelet group after liver transplantation.

Variables	All patients (n = 120)	Patients with platelet counts $<30 \times 10^9$/l (n = 83)	Patients with platelet counts $\geq 30 \times 10^9$/l (n = 37)	P value
Pretransplant variables				
Age in years*	46 (39–53)	47 (38–53)	45 (39–52)	0.89
Males, n (%)	108 (90)	76 (91.6)	32 (86.5)	0.51
BMI (kg/m^2)*	24.3 (21.6–28)	24.1 (21.6–29)	24.4 (21.7–27.6)	0.64
Pretransplant platelet count* $n \times 10^3$/mm^3	68 (45.3–97.3)	60 (42–87)	84 (59–125.5)	**0.005**
MELD score*	19 (16–24)	20 (18–26)	16 (13–20)	**<0.001**
Underlying liver disease n (%)				
Viral	20 (16.7)	14 (16.9)	6 (16.2)	0.86
Alcohol	44 (36.7)	34 (41)	10 (27)	0.21
Cryptogenic	27 (22.5)	19 (22.9)	8 (21.6)	0.93
ACLF	13 (10.8)	7 (8.4)	6 (16.2)	0.34
Others	16 (13.3)	9 (10.8)	7 (18.9)	0.36
Variceal bleeding n (%)	42 (35)	32 (38.6)	10 (27)	0.22
Hepatorenal syndrome n (%)	48 (40)	32 (38.6)	16 (43.2)	0.63
Hepatic encephalopathy n (%)	68 (56.7)	49 (59)	19 (51.4)	0.43
Spontaneous bacterial peritonitis n (%)	28 (23.3)	23 (27.7)	5 (13.5)	0.09
Donor age (years)*	29.5 (23–39)	27 (23–40)	30 (23.5–39)	0.92
Donor BMI*	24.3 (22–26.8)	24 (22.2–26.6)	25 (21.6–27.1)	0.99
Intra-operative variables				
Cold ischemia time (min)*	95 (74.5–120)	95 (73–120)	93 (77–121)	0.85
Warm ischemia time (min)*	42 (34–49)	40 (33–49)	42 (37.5–49)	0.87
Operative time (min)*	936 (840–1054)	940 (840–1055)	922 (820–1032)	0.37
GRBWR*	1.00 (0.85–1.1)	0.98 (0.86–1.1)	1.00 (0.84–1.1)	0.32
Intra-operative blood loss (ml)*	3000 (1670–4500)	3000 (1800–6000)	2200 (1450–3400)	**0.04**
Intra-operative red blood cell transfusion, number of units*	5 (3–10)	6 (3–13)	4 (2.5–7.5)	**0.02**
Intra-operative platelet transfusion, number of units*	1 (0–1)	1 (0–1)	0 (0–1)	**0.04**
Intra-operative fresh frozen plasma transfusion, number of units*	4 (2–6)	4 (2–7)	4 (2–4)	0.10
Intra-operative ascites drainage (ml)*	850 (100–5000)	1000 (100–5000)	500 (150–2250)	0.90

BMI, body mass index; MELD, model for end-stage liver disease; ACLF, acute on chronic liver failure; GRBWR, graft recipient body weight ratio.

*The data are presented as medians and interquartile ranges. P values highlighted in bold are significant.

packed red blood cell transfusion, and platelet transfusion in comparison with patients with PLT $\geq 30 \times 10^9$/l (Table 1).

Postoperative thrombocytopenia and outcome

Overall, the incidence of major postoperative complications (grade III/IV) was 49.2% and that of EAD was 25.8%. The incidence of major postoperative complications (grade III/IV), sepsis, EAD, ascites drainage (>1 l on POD 14), ICU/HDU, and hospital stay was significantly higher for patients with PLT $<30 \times 10^9$/l. Overall, the mortality rate after LDLT was 10%. The reasons for death in 12 patients were as follows: sepsis (9; 75%), graft dysfunction (2), and myocardial infarction (1). The mortality rate was higher for patients with PLT $<30 \times 10^9$/l (13.3% vs. 2.7%, OR = 5.50, 95% CI = 0.68–44.3) but this difference failed to reach statistical significance (Table 2).

On analysis of causes of EAD, only preoperative MELD, MELD sodium scores were predictive factors (P = 0.03 each, t-test for independent samples). EAD

Table 2. Comparison of postoperative outcomes in patients with low and high platelet group.

Variable	Patients with platelet counts $<30 \times 10^9$/l ($n = 83$)	Patients with platelet counts $\geq 30 \times 10^9$/l ($n = 37$)	Odds ratio (95% confidence interval)	*P* value
Grade III/IV complications, *n* (%)*	49 (59)	10 (27)	3.89 (1.66–9.08)	**0.001**
Mortality: grade V, *n* (%)*	11 (13.3)	1 (2.7)	5.50 (0.68–44.3)	0.10
Total intensive care unit/high dependency unit stay in days†	11 (8–15)	9 (6–12)	–	**0.01**
Hospital stay in days†	25 (21–31)	22 (18.5–25.5)	–	**0.04**
Early graft dysfunction, *n* (%)	27 (32.5)	4 (10.8)	3.97 (1.28–12.37)	**0.01**
Post-transplant sepsis, *n* (%)	34 (41)	4 (10.8)	5.72 (1.86–17.65)	**0.001**
Post-transplant ascites drainage (>1 l on postoperative day 14), *n* (%)	38 (45.8)	6 (16.2)	4.36 (1.65–11.57)	**0.002**

*According to Clavien–Dindo classification.

†The data are presented as medians and interquartile ranges. *P* values highlighted in bold are significant.

was not associated with recipient age, sex, preoperative bilirubin/platelet count, donor age, donor BMI, type of graft, or GRBWR. Patients with EAD had a significantly longer hospital, ICU stay ($P = 0.02$, 0.04, respectively, Mann–Whitney *U*-test), grade III/IV complications ($P = 0.02$, chi-square test), but no increase in mortality.

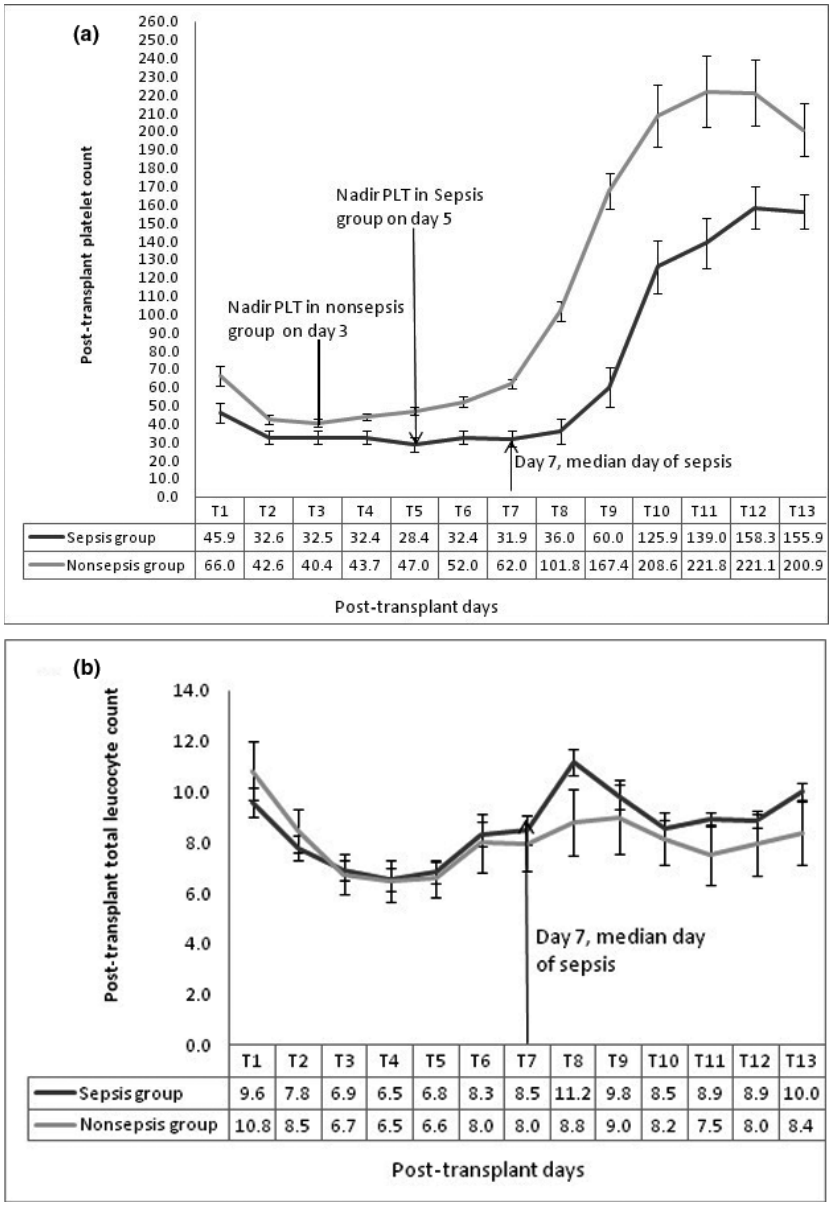
A significant correlation was documented between postoperative thrombocytopenia and sepsis within the first month after the transplant. The incidence of culture-positive bacterial infection was significantly higher for patients with PLT $<30 \times 10^9$/l versus the group with PLT $\geq 30 \times 10^9$/l (45 of 83 vs. 11 of 37, 54.2% vs. 29.7%, OR = 2.79, 95% CI = 1.23–6.39, $P = 0.01$). Overall, 41% (34 of 83) of the patients with PLT of $<30 \times 10^9$/l had a sepsis within 30 days of the transplant compared with 10.8% (4 of 37) of those with a PLT $\geq 30 \times 10^9$/l ($P = 0.001$) (Table 2). Based on this finding, patients were divided into two groups, sepsis group and nonsepsis group. Then, these two groups were compared with postoperative platelet recovery. The median day of sepsis in the sepsis group was post-transplant day 7. The median day of sepsis denotes the day where most patients had sepsis. Patients with sepsis had more protracted or persistent thrombocytopenia preceding the sepsis than those without sepsis; the lowest median PLT was reached significantly later in patients who developed sepsis (median 5 days vs. 3 days, $P = 0.02$) (Fig. 2a). Total leukocyte count in sepsis patients increased only after the sepsis was established, while PLT remained static or decreased prior to the development of sepsis (Fig. 2a and b).

Of the 120 patients in this study group, 11 (9.2%) were admitted in the week preceding transplantation with fever and suspected infection. They were treated with empirical third-generation cephalosporins. Two of the eleven were culture positive; one of them had coagulase-negative *Staphylococcus aureus*, which was thought to be a contaminant but treated with a course of teicoplanin, while the other had Klebsiella in the sputum for which he received antibiotics (Colistimethate sodium) as per culture sensitivity reports. All these patients were admitted to the ward and none of them required organ support. Nine of these 11 patients requiring hospital admission prior to transplant eventually belonged to the low platelet group. Five of these 11 patients (45.4%) developed severe sepsis as opposed to 27 of 109 (24.8%); $P = 0.16$. Five of these 11 patients died (45.4%) as opposed to 7 of 109 (6.4%); $P = 0.001$.

Comparison of different variables among complication (grade III/IV) versus noncomplication (grade I/II) group

The pretransplant PLT did not differ much between complication and noncomplication groups, but the postoperative PLT $<30 \times 10^9$/l was significantly different in two groups (Table 3). Compared with noncomplication group, other variables significantly predictive of complications within 3 months of transplantation were MELD score, intra-operative blood loss, intra-operative packed red cell transfusions, fresh frozen plasma transfusions and platelet transfusions (Table 3). On multivariate forward logistic regression analysis, PLT $<30 \times 10^9$/l was the only independent predictor for the development of grade III/IV complications [odds ratio (OR) = 3.47, 95% CI = 1.47–8.21, $P = 0.005$] after

Transplant International 2016; 29: 1126–1135



	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13
Sepsis group	45.9	32.6	32.5	32.4	28.4	32.4	31.9	36.0	60.0	125.9	139.0	158.3	155.9
Nonsepsis group	66.0	42.6	40.4	43.7	47.0	52.0	62.0	101.8	167.4	208.6	221.8	221.1	200.9

	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13
Sepsis group	9.6	7.8	6.9	6.5	6.8	8.3	8.5	11.2	9.8	8.5	8.9	8.9	10.0
Nonsepsis group	10.8	8.5	6.7	6.5	6.6	8.0	8.0	8.8	9.0	8.2	7.5	8.0	8.4

Figure 2 Dynamics of post transplant platelet counts and total leukocyte counts in patients with and without sepsis. (a) Postoperative platelet counts in patients with and without sepsis. (b) Postoperative total leukocyte counts in patients with and without sepsis.

LDLT, even after adjustment was made for intra-operative platelet transfusions (Table 3).

Impact of thrombocytopenia on graft and patient survival after LDLT

There was no graft loss or retransplantation. Patients with PLT $<30 \times 10^9$/l had statistically significant lower survival (1 year survival: 78.6% vs. 93.1%, $P = 0.04$) (Fig. 3). However, the median survival time was not reached as the numbers of events were very less. When survival analyses were repeated after excluding first 90 days mortality after LDLT ($N = 12$), the predictive value of PLT for survival was lost (1-year survival: 92.1% vs. 95.7%, $P = 0.37$). This result confirmed that low PLT after LDLT was associated with increased trend toward early mortality.

Discussion

Thrombocytopenia is common in LT recipients both pre- and postoperatively. While the risk of bleeding complications from thrombocytopenia in liver transplant recipients such as intracranial, alveolar, and

Table 3. Predictors of grade III/IV complications in patients undergoing living donor liver transplantation.

Variable	Complication group (grade III/IV) (n = 59)	Noncomplication group (grade I/II) (n = 61)	Odds ratio (95% confidence interval)	P value
Univariate logistic analysis				
Recipient age in years*	48 (39–55)	45 (39–54)	0.98 (0.97–1.02)	0.75
Recipient BMI*	23 (21.1–27.4)	25.2 (22.2–29.6)	1.06 (0.99–1.15)	0.11
MELD score*	20 (17–26)	18 (15–22)	1.07 (1.01–1.14)	**0.01**
Variceal bleeding n (%)	26 (44.1)	16 (26.2)	2.22 (1.03–4.78)	0.06
Hepatorenal syndrome n (%)	20 (33.9)	28 (45.9)	0.60 (0.29–1.26)	0.20
Hepatic encephalopathy n (%)	34 (57.6)	34 (55.7)	1.08 (0.52–2.22)	0.86
Spontaneous bacterial peritonitis n (%)	17 (28.8)	11 (18)	1.84 (0.78–4.36)	0.20
Pretransplant platelet count, $n \times 10^9$/l	61 (43–94)	75 (54–106)	0.99 (0.99–1.01)	0.31
Postoperative platelet count <30 $\times$ 10^9/l	49 (83.1)	34 (55.7)	3.89 (1.67–9.08)	**0.001**
Donor age in years*	34 (23–40)	26 (22.5–39)	1.03 (0.99–1.07)	0.21
Donor BMI*	24 (22.4–26.9)	24.6 (21.5–26.8)	0.98 (0.88–1.10)	0.75
Cold ischemia time in min*	94 (66–120)	96 (76–120)	1.00 (0.99–1.01)	0.71
Warm ischemia time in min*	40 (33–49)	42 (35–48)	1.00 (0.98–1.03)	0.70
Operative time in min*	960 (847–1080)	914 (797–1027)	1.00 (1.00–1.00)	0.08
GRBWR*	0.93 (0.80–1.20)	1.01 (0.89–1.10)	1.84 (0.40–8.54)	0.44
Intra-operative blood loss in ml*	3300 (2300–6550)	2200 (1350–3500)	1.00 (1.00–1.00)	**0.006**
Intra-operative blood products transfused (number of units)				
Packed red blood cells*	6 (4–12)	4 (2–9)	1.06 (1.00–1.12)	**0.02**
Fresh frozen plasma*	5 (3–7)	4 (2–4)	1.13 (1.03–1.24)	**0.04**
Platelets*	1 (0–2)	0 (0–1)	1.53 (1.06–2.21)	**0.01**

Variable	Odds ratio	95% confidence interval	P value
Multivariate forward logistic regression analysis			
Post-transplant platelet count <30 $\times$ 10^9/l	3.47	1.47–8.21	**0.005**

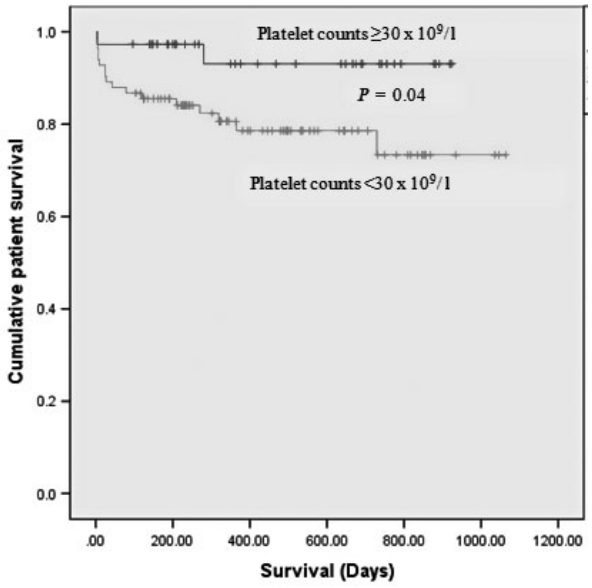
BMI, body mass index; MELD, model for end-stage liver disease; GRBWR, graft recipient body weight ratio.

*The data are presented as medians and interquartile ranges. P values highlighted in bold are significant.

intra-abdominal hemorrhage is well documented, its association with other important clinical outcomes, such as major postoperative complications, graft dysfunction, sepsis, ascites, and survival, is not well reported in the context of LDLT. The main aim of this study was to explore the value of postoperative thrombocytopenia in predicting graft- and patient-related outcomes in LDLT setting. Our study is prospective and included a homogenous group of patients with ESLD who underwent an LDLT and differs from other reports in literature, which studied DDLT [15] or LDLT [8]. Both of these were retrospective and included a heterogeneous group of patients including those with ALF, in whom the platelet dynamics is potentially different as patients with ALF usually do not have portal hypertension with hypersplenism. Portal hypertension-related splenic sequestration and decreased platelet function in the chronic liver disease to large extent contributes to preoperative thrombocytopenia [16].

The literature on the association of thrombocytopenia with outcome following LT has been described earlier in orthotopic liver transplantation [15,17–19]. Apart from the study by Li *et al.* [8], the relationship of platelets and outcome for LDLT has not been fully explored. From the study of serial PLT at different time points, we were able to plot the trajectory and timeline of platelet recovery in LDLT between low and high platelet groups, which is a unique contribution. In our study, the postoperative PLT reach a nadir on the 3rd postoperative day, and it gradually rises again to exceed pretransplant levels on about day 10. We found that the cutoff for predicting major complications was a platelet count of <30 $\times$ 10^9/l on POD 3. Lesurtel *et al.* [15]. found a PLT at day 5 of less than 60 $\times$ 10^9/l in the DDLT setting to correlate with major complications and mortality, while Li *et al.* [8]. found an immediate post-transplant PLT <68 $\times$ 10^9/l in the LDLT setting to predict early graft dysfunction. The difference may be

Transplant International 2016; 29: 1126–1135



Number at risk

Platelet counts <30 x 10^9/l (n = 83)	60	37	21	11	3
No. of Events in Group 1	12	4	0	1	0
Platelet counts ≥30 x 10^9/l (n = 37)	28	19	16	5	0
No. of Events in group 2	1	1	0	0	0

Figure 3 Kaplan–Meier curves depicting overall survival of patients with platelet counts <30 × 10^9/l (Group 1) and ≥30 × 10^9/l (Group 2) after liver transplantation (P = 0.04).

explained by the fact that in the study by Lesurtel *et al.* [15], day 5 PLTs were taken, by which time the PLT would have started recovering and in the study by Li *et al.* [8], the immediate post-transplant PLTs were used, which will always be high as our study showed that median nadir PLT was reached by day 3. The second reason for the higher platelet counts may be that both the studies included patients with acute liver failure who usually do not have portal hypertension with hypersplenism. A recent study by Akamastu *et al.* [20]. looked at perioperative variables affecting outcome in 445 LDLT recipients. PLTs on POD 3 and high body mass index were independent predictors of grade III, IV complications. High PT/INR on POD 5 was an independent predictor of 90-day mortality. In addition, they developed a scoring system giving a point each for PLT <50 × 10^9/l and INR > 1.6 within POD 5 and found that these were important predictors of severe complications and mortality, respectively. The findings are similar to the current paper in that PLTs on POD 3 predict grade III, IV complications.

The relationship between postoperative thrombocytopenia and the risk of morbidity has been observed both in DDLT [15,17–19] and LDLT settings [8]. In the present study too, a striking correlation between postoperative low platelet group and major complication rate and early graft dysfunction was observed. In addition, a significant correlation between sepsis and platelet count was found, for the first time in the LDLT setting. There is a growing body of evidence to suggest that platelets contribute significantly to antimicrobial host defense [21–23]. Chang *et al.* [21] showed that thrombocytopenia correlated significantly with a greater risk of early major infections after the DDLT. This held true in our patients too. There was a statistically significant difference in the incidence of culture-positive bacterial infections, and the incidence of sepsis was higher in patients with low platelet group. Sepsis, per se, can lead to thrombocytopenia; however, the nadir in PLT preceded the sepsis with a median lead time of 2 days. Furthermore, the patients with sepsis had significantly longer duration of thrombocytopenia preceding sepsis than those without sepsis. Thus, the direction of association suggests that thrombocytopenia was an antecedent to subsequent sepsis and not vice versa. Those with sepsis had a persistently low level of platelets in the first postoperative week as compared to those without sepsis who showed a rising trend after a nadir was reached on day 3. In the 34 (41%) of the 83 patients with low platelet counts who developed sepsis, cultures prior to day 3 were positive in 4 (11.7%), indicating that they already harbored infection at the time of thrombocytopenia. In the remaining 30 (88.3%), thrombocytopenia predated sepsis. This is an important observation as sepsis is the major determinant of outcome after LDLT and any marker that helps in predicting sepsis early before it becomes clinically obvious can change patient outcomes. While this study was not specifically designed to assess thrombocytopenia as a predictor for infective complications, we would think (i) carefully designed studies are needed in this context to make proper recommendations; (ii) to perhaps consider adding empirical antibiotics according to local sensitivity if the platelet counts continue to fall after day 3 and stop if day 3 cultures become negative and platelet counts improve; (iii) to escalate immunosuppression cautiously in this scenario taking into consideration the overall clinical status of the patient.

Ascites after liver transplant is relatively common in the immediate postoperative phase, and it generally resolves spontaneously within 7–14 days [14]. In the present study, the incidence of prolonged post-transplant ascites drainage (>1 l on POD 14) has been found to be significantly higher in patients with PLT

$<30 \times 10^9$/l. This finding suggests that a patient who had severe thrombocytopenia will need a longer duration for resolution of ascites drainage post-transplant, possibly because of greater severity of pretransplant portal hypertension and slow resolution of portal hypertension in partial liver grafts. EAD was significantly more common in patients with higher preoperative MELD, MELD sodium scores and these patients may present with a functional small-for-size syndrome (SFSS) despite acceptable GRBWR. Patients in this series had a median GRBWR of 1; this was not different between the low and high platelet groups. We used to measure intra-operative portal pressure after reperfusion earlier [24], but in our observation, none of the patients had a hepatic portal venous pressure gradient of more than 10 mm Hg; we have since then stopped routine intra-operative pressure measurement. Without pressure data, it is difficult to determine the exact incidence of SFSS in this cohort. With selection of grafts to provide a GRBWR of 0.8–1, SFSS is unlikely to develop, especially if the operation has been technically satisfactory with a triphasic outflow. Therefore, the authors did not consider inflow modulation in this series.

In several studies, preoperative thrombocytopenia has been found to be a risk factor for post-transplant thrombocytopenia [19,25]. In the present study, preoperative PLTs were significantly different in low and high PLT groups but this was not a predictive factor for negative outcome (grade III/IV complication) after LT for ESLD patients. We found that in addition to the pretransplant PLT, MELD score, intra-operative blood loss, intra-operative packed red blood cell transfusions, platelet transfusions were significantly different for the two groups (Table 1). These parameters appeared to be more a surrogate of the preoperative severity of the liver disease rather than a predictive factor of higher grade III/IV complication, as multivariate analysis showed that postoperative low platelet group was the only independent factor for higher morbidity after LDLT (Table 3).

Overall patient survival was significantly less in thrombocytopenia group. Also patients who were admitted in the week prior to transplant with fever/suspected infection had a higher mortality as compared to those who did not have these. The predictive value of platelet count on survival was lost after excluding first 90-day mortality. This signifies the fact that platelet count predicts short-term patient survival, once the platelet count has recovered, and its impact on survival nullifies. This is the first study of its kind in the LDLT setting to demonstrate this association, although a similar association has been observed in the DDLT setting [15].

In conclusion, in a homogenous population of patients undergoing LDLT for ESLD, postoperative PLT of $<30 \times 10^9$/l predicted major postoperative complications and is associated with high rate of early graft dysfunction, prolonged ascites drainage, and sepsis. The patients with low platelet count have higher short-term mortality.

Authorship

VP: contributed to study conception. SSM, NK and VK: contributed to data collection. SSM: contributed to manuscript drafting. VP, SK, KGSB, SVS and PKS: contributed to critical revision of the manuscript for important intellectual content.

Funding

The authors have declared no funding.

Conflict of interest

The authors have declared no conflicts of interest.

Acknowledgement

Authors wish to thank Guresh Kumar and Ajeet Singh Bhadoria for their support with statistical analysis.

REFERENCES

1. Klinger MH. Platelets and inflammation. *Anat Embryol (Berl)* 1997; **196**: 1.
2. Tang YQ, Yeaman MR, Selsted ME. Antimicrobial peptides from human platelets. *Infect Immun* 2002; **70**: 6524.
3. Sierko E, Wojtukiewicz MZ. Platelets and angiogenesis in malignancy. *Semin Thromb Hemost* 2004; **30**: 95.
4. Cywes R, Packham MA, Tietze L, *et al.* Role of platelets in hepatic allograft preservation injury in the rat. *Hepatology* 1993; **18**: 635.
5. Lesurtel M, Graf R, Aleil B, *et al.* Platelet-derived serotonin mediates liver regeneration. *Science* 2006; **312**: 104.
6. Nocito A, Georgiev P, Dahm F, Jochum W, Bader M, Clavien PA. Platelets and platelet-derived serotonin promote tissue repair after normothermic hepatic

ischemia in mice. *Hepatology* 2007; **45**: 369.
7. Pereboom IT, Lisman T, Porte RJ. Platelets in liver transplantation: friend or foe? *Liver Transpl* 2008; **14**: 923.
8. Li L, Wang H, Yang J, *et al.* Immediate postoperative low platelet counts after living donor liver transplantation predict early allograft dysfunction. *Medicine (Baltimore)* 2015; **94**: e1373.
9. Marubashi S, Dono K, Miyamoto A, *et al.* Impact of graft size on postoperative thrombocytopenia in living donor liver transplant. *Arch Surg* 2007; **142**: 1054.
10. 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.
11. Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Ann Surg* 2004; **240**: 205.
12. Garner JS, Jarvis WR, Emori TG, Horan TC, Hughes JM. CDC definitions for nosocomial infections. *Am J Infect Control.* 1988; **16**: 128.
13. Dellinger RP, Levy MM, Rhodes A, *et al.* Surviving Sepsis Campaign Guidelines Committee including The Pediatric Subgroup. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. *Intensive Care Med* 2013; **39**: 165.
14. Soejima Y, Taketomi A, Yoshizumi T, *et al.* Feasibility of left lobe living donor liver transplantation between adults: an 8-year, single-center experience of 107 cases. *Am J Transplant* 2006; **6**: 1004.
15. Lesurtel M, Raptis DA, Melloul E, *et al.* Low platelet counts after liver transplantation predict early post-transplant survival: the ''60-5 criterion''. *Liver Transpl* 2014; **20**: 147.
16. Afdhal N, McHutchison J, Brown R, *et al.* Thrombocytopenia associated with chronic liver disease. *J Hepatol* 2008; **48**: 1000.
17. Chin JL, Hisamuddin SH, O'Sullivan A, Chan G, McCormick PA. Thrombo cytopenia, platelet transfusion, and outcome following liver transplantation. *Clin Appl Thromb Hemost* 2016; **22**: 351.
18. de Boer MT, Christensen MC, Asmussen M, *et al.* The impact of intraoperative transfusion of platelets and red blood cells on survival after liver transplantation. *Anesth Analg* 2008; **106**: 32.
19. McCaughan GW, Herkes R, Powers B, *et al.* Thrombocytopenia post liver transplantation. Correlations with pre-operative platelet count, blood transfusion requirements, allograft function and outcome. *J Hepatol* 1992; **16**: 16.
20. Akamatsu N, Sugawara Y, Kanako J, *et al.* Low platelet counts and prolonged prothrombin time early after operation predict the 90 days morbidity and mortality in living-donor liver transplantation. *Ann Surg* 2016; [Epub ahead of print].
21. 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.
22. Kahn RA, Flinton LJ. The relationship between platelets and bacteria. *Blood* 1974; **44**: 715.
23. Miyata T, Yokoyama I, Todo S, Tzakis A, Selby R, Starzl TE. Endotoxaemia, pulmonary complications, and thrombocytopenia in liver transplantation. *Lancet* 1989; **2**: 189.
24. Sasturkar SV, Bharathy KG, Kumar S, Sarin SK, Chattopadhyay TK, Pamecha V. Right lobe adult living donor liver transplantation: does portal pressure predict outcome? [abstract]. In proceedings of the ILTS 20(th) Annual International Congress, June 3–7, 2014, London, United Kingdom. *Liver Transpl* 2014;**20**(Suppl. 1):S298. doi: 10.1002/lt.23901. Abstract # LB-39.
25. Chatzipetrou MA, Tsaroucha AK, Weppler D, *et al.* Thrombocytopenia after liver transplantation. *Transpla ntation* 1999; **67**: 702.