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ORIGINAL ARTICLE

Pretransplantation splenomegaly frequently persists after liver transplantation and can manifest as hypersplenism and graft fibrosis - a retrospective study

Nguyen Hai Nam, Kojiro Taura, Siyuan Yao, Toshimi Kaido, Yusuke Uemoto, Yusuke Kimura, Takayuki Anazawa, Ken Fukumitsu, Takashi Ito, Shintaro Yagi, Naoko Kamo, Koichiro Hata & Shinji Uemoto

Division of Hepato Biliary Pancreatic Surgery and Transplantation, Department of Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Correspondence
Kojiro Taura, MD, PhD, Division of Hepato Biliary Pancreatic Surgery and Transplantation, Department of Surgery, Graduate School of Medicine, Kyoto University, Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan.
Tel.: +81-75-751-3242;
fax: +81-75-751-4263;
e-mail: ktaura@kuhp.kyoto-u.ac.jp

ABSTRACT

The risk factors and clinical impact of post-transplantation splenomegaly (SM) are poorly understood. We investigated the predictors and impacts of post-transplantation SM in 415 LT patients at Kyoto University Hospital from April 2006 to December 2015. First, the predictors and clinical consequences of SM three years post-transplantation were analyzed among spleen-preserved recipients. Second, the clinical data of surviving recipients three years post-transplantation were compared between splenectomized and spleen-preserved recipients. There was no difference in indication for liver transplantation between these two groups. Third, survival outcomes were compared between splenectomized and spleen-preserved recipients. SM was determined as a SV/body surface area (BSA) higher than 152 ml/m^2. In the first analysis, preoperative SM occurred in 79.9% recipients and SM persisted three years post-transplantation in 72.6% recipients among them. Preoperative SV/BSA was the only independent predictor of three year post-transplantation SM, which was associated with lower platelet (PLT), white blood cell (WBC) counts and significant graft fibrosis (21.4% vs. 2.8%). In the second analysis, spleen-preservation was related to lower PLT, WBC counts and a higher proportion of significant graft fibrosis (26.7% vs. 7.1%) three years post-transplantation. In the third analysis, spleen-preserved recipients showed worse survival than splenectomized recipients. In conclusion, preoperative SM frequently persists more than three years post-transplantation and is associated with subclinical hypersplenism, graft fibrosis, graft loss, and even death.

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Key words
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Introduction

Splenomegaly (SM) is frequently observed during liver transplantation (LT) due to liver cirrhosis and subsequent portal hypertension. Extreme enlargement of spleen has been demonstrated to imply adverse impacts, including hypersplenism, collateral portosystemic shunt, and splenic artery steal syndrome [1,2]. Previously, splenectomy was recommended to improve the tolerance in recipients who use interferon (IFN) therapy for hepatitis C viral (HCV) infection and to avoid humoral rejection in ABO-incompatible LT [3–5]. In addition, splenectomy was widely encouraged to maintain inflow modulation in cases of suspected small-for-size graft syndrome [6]. Nonetheless, preoperative rituximab with plasmapheresis and the success of newer generation direct-acting antiviral (DAA) agents have limited the indications for splenectomy in ABO-incompatible and HCV-positive recipients, respectively [7,8]. Furthermore, simultaneous splenectomy (SS) in the context of cirrhosis was reported to negatively affect complications such as prolonged operative time, increased blood loss, portal vein thrombosis, and post-splenectomy sepsis syndrome [9–11].

On the other hand, extensive research has focused on the progress and clinical impacts of SM in the short term, frequently at six months [12–14] and one year [15] after LT. However, from a longer-term point of view, the consequences of preserving an enlarged spleen are hardly understood. To elucidate this information, we conducted a retrospective study to assess the predictive factors and clinical impacts of SM three years after LT.

Materials and methods

Study population

A single-center retrospective analysis of 538 patients who underwent LT at Kyoto University Hospital, Japan, between April 2006 and December 2015 was performed. Among them, we identified 415 patients, including 236 patients in the spleen-preserved group and 179 patients in the splenectomized group after applying the following common exclusion criterion: (1) retransplantation, (2) lost to follow-up, (3) splenectomy within three years after LT, (4) previous splenectomy, and (5) unavailable preoperative clinical data or SV. Details of the study population are presented in the flowchart of our approach (Fig. 1). Splenectomy was indicated for the following reasons: 1) HCV infection that required treatment with interferon after LT (until 2014); 2) ABO-incompatible LT (until 2006); 3) splenic arterial aneurysm; and 4) portal vein pressure> 15 mmHg after reperfusion [16]. As a result, splenectomy was performed in 43 patients (reason 1), 3 patients (reason 2), 13 patients (reason 3), 114 patients (reason 4) and additionally in 3 cases of accidental hemorrhage, 2 cases of unmentioned reasons, and 1 case of Hassab procedure.

In the first cohort study, 149 spleen-preserved recipients with available postoperative CT approximately three years after LT (median 1182 days, range 965 to 1377 days) were selected for analysis. They were classified into two groups according to their spleen volume/body surface area (SV/BSA) value three years after LT: post-transplantation SM and post-transplantation non-SM group. In the second cohort study, among 179 splenectomized and 236 spleen-preserved patients, 147 splenectomized and 185 spleen-preserved patients with available laboratory data approximately three years after LT were selected. Propensity score matching (PSM) subsequently identified 78 well-matched patients in each group. In the third cohort study, among 179 splenectomized and 236 spleen-preserved patients, PSM identified 116 patients in each group who were enrolled in the survival analysis.

This study was approved by the Ethics Committee of Kyoto University Graduate School and Faculty of Medicine (approval code: R1473-2), and all procedures were conducted in accordance with the 2013 Declaration of Helsinki.

Spleen volumetry evaluation

Spleen volumetry analyses were retrospectively performed with the preoperative and approximately three years postoperative CT with The Volume Analyzer Synapse Vincent (Fujifilm Medical, Tokyo, Japan), which calculated the volume of the reconstructed three-dimensional spleen images based on the multidetector computed tomography (MDCT) results. Namely, the circumscribed areas were automatically multiplied by each MDCT section thickness to yield an approximate value for the total SV in milliliters. Postoperative CT was proposed by the physician according to the clinical necessity. Since healthy human SV is correlated with individual weight and height, both in pediatric [17,18] and adult [19,20] patients, volumetric calculations of the spleen were normalized by BSA to eliminate the impact of patient size on SV. BSA was estimated according to the equation of Du Bois et al. [21] as BSA (m^2) = body weight(kg)$^{0.425}$ x body height(cm)$^{0.725}$ x

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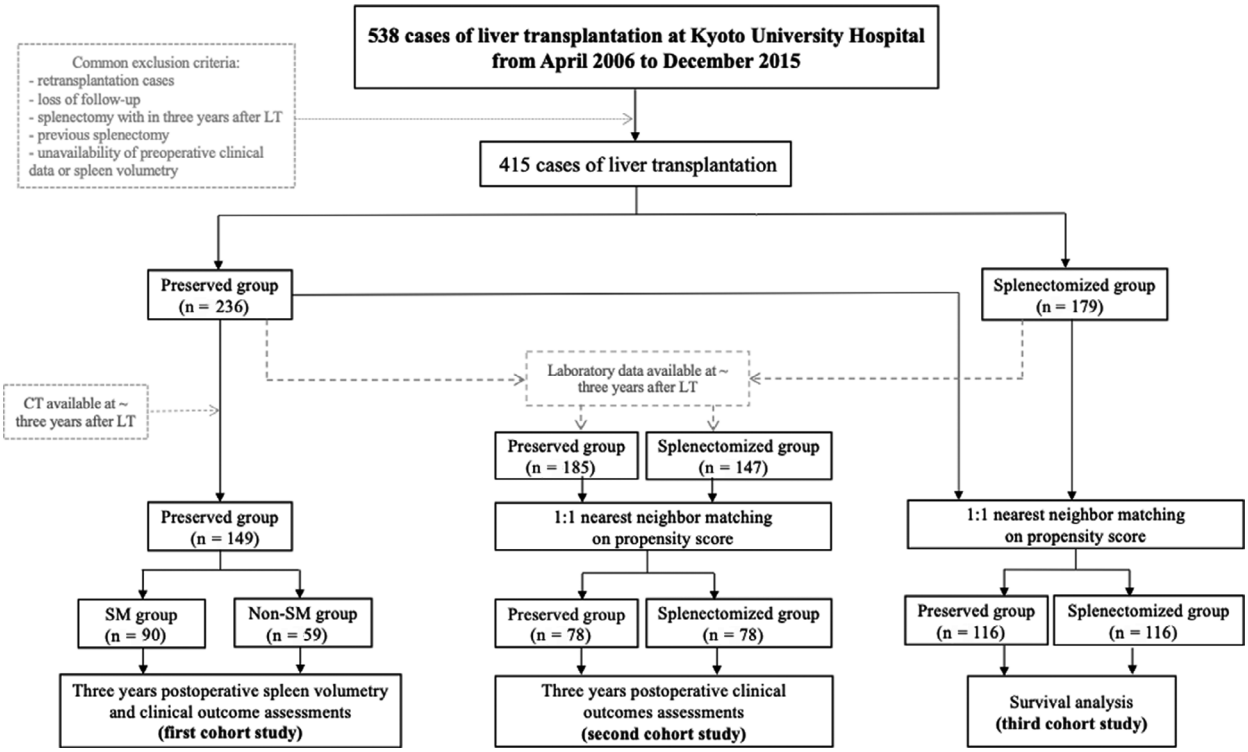

Figure 1 Flowchart of the study. LT, liver transplantation. SM, splenomegaly.

0.007184, and the SV/BSA value was used as a parameter to evaluate the SV of patients.

Determination of cutoff SM values based on the donor population

Currently, there is no consensus on the volumetry-based definition of SM. Obviously, SV identifying SM is outliers from the mean SV in the healthy population. In our study, a representative population of normal spleens was selected from healthy Japanese people who were liver donors for the 149 spleen-preserved patients in the first cohort study. Namely, these healthy subjects represented a random sample of the overall healthy Japanese population who do not suffer from any illnesses that affect SV. SM was defined as a SV/BSA 2.5 standard deviation (SD) above the mean, based on the approach used to define osteoporosis in the published literature [22,23].

Other clinical outcomes

The recovery of the PLT count was assessed at each threshold (>150.000/μl, >100.000/μl, >50.000/μl). The F-index of the METAVIR score was recorded as F0 (no fibrosis), F1 (mild portal fibrosis with no septa), F2 (portal fibrosis with few septa), F3 (portal fibrosis with numerous septa without cirrhosis), and F4 (cirrhosis) [24]. Indications for liver biopsy were determined according to the clinical needs.

Statistical analysis

Continuous variables are expressed as the median (interquartile range) and were compared using Wilcoxon rank sum test. Categorical variables are expressed as numbers and percentages, and were compared using chi-square test (or Fisher's exact test as appropriate).

To adjust for potential confounding effects according to the differences in baseline characteristics between splenectomized and spleen-preserved patients, the PSM method was applied. The PSM was calculated by using a logistic regression model that included variables considered to be directly associated with either post-transplantation SM or post-transplantation non-SM. After PSM, patients of these two groups underwent 1:1 nearest available matching of the logit of the PSM with a caliper width of 0.05 of the score's SD.

The recovery of PLT count at different thresholds and three-year survival rate was estimated using the Kaplan–Meier methods and compared with the log-rank test. Cox proportional hazards regression was used to calculate the hazard ratio (HR) and 95% confidence interval

(CI) for univariate and multivariate analyses. Factors with $P < 0.05$ in the univariate analysis were considered candidates for multivariate analysis. The receiver operating characteristic (ROC) curve was analyzed to identify the cutoff point at which the preoperative SV/BSA showed the highest accuracy in predicting post-transplantation SM and post-transplantation PLT < 100.000/μl. A P value < 0.05 (2-tailed) was considered statistically significant. All statistical analyses were performed using JMP Pro 14.0. (SAS Institute Inc. Cary, NC, USA).

Results

Proposed SV/BSA cutoff value to define SM

Among the 149 patients in the first study cohort, living donor LT was performed on 140 patients, and their donors' SVs were evaluated to determine the cutoff SV/BSA value for SM. The mean (SD) value of SV/BSA was 81 (28.4) ml/m^2. The threshold for SM was then determined as an SV/BSA 2.5 SD above the mean, which is 152 ml/m^2.

First cohort study: three year postoperative SV and clinical outcome assessments in the spleen-preserved population

Overview of persistent SM status three years after LT

The mean ΔSV/BSA was −37.9%, and there was a weak correlation between preoperative SV/BSA and post-transplantation SV/BSA ($R^2 = 0.386$, $P < 0.001$), indicating that SV decreased after LT to some extent but did not completely normalized. Indeed, among 119 patients with preoperative SM, only 33 patients (27.4%) had a decrease in SV that did not meet the definition of SM, whereas only 13.3% of the patients without SM before LT developed SM three years after LT.

Predictive factors for post-transplantation SM

Based on the postoperative SV/BSA value, patients were divided into two groups: post-transplantation SM ($n = 90$, 60.4%) and post-transplantation non-SM ($n = 59$, 39.6%). The patient demographics and clinicopathological characteristics of each group are outlined in Table 1. All donor factors were found to be approximately identical between these two groups. However, among the recipients, there were statistically significant differences in age, sex, etiology of liver disease, type of LT, SV/BSA, white blood cell (WBC) count, and INR. Namely, recipients in the post-transplantation SM group were younger than those in the post-transplantation non-SM group (13(2–51) vs. 45(5–56)), and the sex ratio (male:female) was not equivalent (48:42 vs. 19:40). Concerning the etiology of liver disease, the proportion of patients who suffered from biliary atresia (BA) was higher in the post-transplantation SM group than in the post-transplantation non-SM group (51.2% vs. 22%). Furthermore, pediatric LT was predominant in the post-transplantation SM group (58.9%), whereas adult LT was frequent in the post-transplantation non-SM group (67.8%). Additionally, the INR was significantly greater in the post-transplantation SM group than in the post-transplantation non-SM group (1.8 (1.3–2.9) vs. 1.4 (1.2–2.4), whereas the WBC count was found to be lower in the post-transplantation SM group (4.1 (2.8–6.5) vs. 5 (3.6–7.6)).

Univariate logistic analysis determined age, sex, type of living donor LT, BA, preoperative SV/BSA, and preoperative INR as predictive factors for post-transplantation SM three years after LT. These factors were then subsequently entered into multivariate regression analysis, which demonstrated that the preoperative SV/BSA was the sole significant predictor of persistent SM at three years postoperative (OR, 1.006; 95% CI, 1.003–1.010, $P < 0.001$) (Table 2). The odds of post-transplantation SM increased by 0.6% for each unit increase in preoperative SV/BSA.

Long-term consequences of post-transplantation SM

A comparison of the clinical outcomes three years after LT between post-transplantation SM and post-transplantation non-SM patients is shown in Table 3. The percent reduction in SV/BSA was obviously higher in the post-transplantation non-SM group than in the post-transplantation SM group (41% (28%-64%) vs. 36% (−17%-51%). The median interval between LT and postoperative laboratory tests was 1091 days with a range of 1029 to 1325 days. Patients who suffered from post-transplantation SM had significantly higher INR, AST and ALT (1.1 (1–1.2) vs. 1 (1–1.1); 31 (22–44) vs. 24 (16–35) and 23 (14–45) vs. 14(11–23), respectively) and markedly lower PLT and WBC (143 (89–189) vs. 203 (170–256); (5.2 (4–6.7) vs. 6.3 (5.1–7.7)) than the non-SM group.

Post-transplantation SM is associated with more advanced graft fibrosis three years after LT

An intra-operative graft biopsy at the time of LT was routinely performed and showed no evidence of fibrosis.

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Table 1. Comparison of the perioperative characteristics between splenomegaly and nonsplenomegaly patients three years after LT

Variables	Post-transplantation SM (n = 90) (60.4%)	Post-transplantation non-SM (n = 59) (39.6%)	P value
Age (year)	13 (2–51)	46 (5–56)	**0.013**
Sex (male:female)	48:42	19:40	**0.010**
Etiology of liver disease			**0.023**
AIH/PBC/PSC	11 (12.2%)	11 (18.6%)	
Alcohol	6 (6.7%)	4 (6.8%)	
BA	46 (51.2%)	13 (22%)	
Cryptogenic LC	1 (1.1%)	1 (1.7%)	
FLF	5 (5.6%)	10 (16.9%)	
HBV	2 (2.2%)	2 (3.4%)	
HCV	1 (1.1%)	2 (3.4%)	
HCC	8 (8.9%)	6 (10.2%)	
Hepatoblastoma	1 (1.1%)	3 (5.1%)	
Others	9 (10%)	7 (11.9%)	
Type of LT			**0.001**
Adult LT (%)	37 (41.1%)	40 (67.8%)	
Pediatric LT (%)	53 (58.9%)	19 (32.2%)	
BSA (m^2)	1.3 (0.5–1.6)	1.5 (0.8–1.7)	0.190
SV/BSA (ml/m^2)	430.7 (293.4–585.8)	186.1 (120.2–320)	**<0.001**
WBC count ($\times 10^3/\mu l$)	4.1 (2.8–6.5)	5 (3.6–7.6)	**0.026**
Hemoglobin (g/dl)	9.5 (8.3–11.3)	9.5 (8.7–11)	0.970
PLT count ($\times 10^3/\mu l$)	83 (52–146)	104 (59–139)	0.372
INR	1.8 (1.3–2.9)	1.4 (1.2–2.4)	**0.003**
AST (U/l)	96 (39–174)	73 (46–110)	0.193
ALT (U/l)	52 (24–91)	41 (28–72)	0.355
Total bilirubin (mg/dl)	6 (1.9–16.6)	8.5 (3.2–18)	0.221
Albumin (g/dl)	3.1 (2.7–3.4)	3.1 (2.6–3.6)	0.697

Other diseases: Wilson's disease, NASH, Budd–Chiari syndrome, polystic liver & kidney, neonatal cholestasis unknown origin (suspect of 5-β-reductase deficiency), Alagille syndrome, congenital portosystemic venovenous malformation, ornithine transcarbamylase deficiency, primary hyperoxaluria type 1, congenital biliary dilatation. ALT, alanine transaminase; AST, aspartate transaminase; AIH, autoimmune hepatitis; BA, biliary atresia; BSA, body surface area; FLF, fulminant liver failure; GRWR, graft-to-recipient weight ratio; HBV, hepatits B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; INR, international normalized ratio; LC, liver cirrhosis; LT, liver transplantation; NASH, nonalcoholic steatohepatitis; PBS, primary biliary sclerosis; PLT, platelet; PSC, primary sclerosing cholangitis; SM, splenomegaly; SV, spleen volume; WBC, white blood cell.

Bold indicates statistically significant P values.

The median interval between LT and postoperative liver biopsy was 1121 days with a range of 989 to 1140 days. At three years post-transplantation, there were 70 patients in post-transplantation SM group and 36 patients in post-transplantation non-SM group who underwent liver biopsy. The results revealed a significantly higher proportion of patients with a METAVIR score of F2 or higher in the post-transplantation SM group than in the post-transplantation non-SM group (21.4% vs. 2.8%, P = 0.01).

Preoperative SM delays recovery of PLT count

Univariate logistic analysis showed that sex, preoperative SV/BSA, preoperative PLT < 100.000/μl, and preoperative WBC < 5.000/μl were risk factors for nonrecovery of PLT> 100.000/μl three years after LT. Subsequent multivariate analysis determined that preoperative SV/BSA was the sole independent risk factor for nonrecovery of PLT count level (HR, 0.9978; 95% CI, 0.9967–0.9988, P < 0.001). As a result, the higher the preoperative SV/BSA determined in recipients, the less their PLT count recovered to normal levels three years after LT. The Kaplan–Meier curve revealed that the recipients who did not suffer from preoperative SM presented a better and faster capacity to recover a PLT> 50.000/μl (100% vs. 94.1%, P = 0.005), PLT> 100.000/μl (100% vs. 77.3%, P < 0.001), and PLT> 150.000/μl (76.7% vs. 45.4%, P < 0.001). Notably, 100% of patients in the non-SM group achieved a PLT> 50.000/μl within three quarters of the first year after LT, and these patients also entirely recovered to PLT> 100.000/μl within three years after LT.

Table 2. Multivariate analysis for predictors of splenomegaly three years after liver transplantation

	Univariate analysis		Multivariate analysis	
Variables	OR (95%CI)	*P* value	OR (95%CI)	*P* value
Age	0.98 (0.96–0.99)	**0.001**	0.99 (0.95–1.03)	0.598
Sex				
Female	1 (reference)			
Male	0.42 (0.21–0.82)	**0.010**	0.56 (0.17–0.24)	0.171
Type of LDLT				
pLDLT	1 (reference)			
aLDLT	0.33 (0.17–0.66)	**0.001**	0.99 (0.11–8.75)	0.995
Etiology of liver disease				
AIH/PBC/PSC	1 (reference)			
Alcohol	1.5 (0.33–6.83)	0.600		
BA	3.54 (1.25–9.99)	**0.017**		
Cryptogenic LC	1 (0.06–18.08)	1.000		
FLF	0.5 (0.13–1.95)	0.318		
HBV	1 (0.12–8.42)	1.000		
HCV	0.5 (0.04–6.35)	0.593		
HCC	1.33 (0.35–5.14)	0.675		
Hepatoblastoma	0.33 (0.03–3.72)	0.372		
Others	1.29 (0.35–4.69)	0.703		
Preoperative SV/BSA (ml/m^2)	1.007 (1.004–1.009)	**<0.001**	1.006 (1.003–1.010)	**<0.001**
Preoperative WBC count (×10^3/μl)	0.92 (0.84–1.01)	0.096		
Preoperative INR	2.11 (0.28–0.81)	**0.002**	0.82 (0.44–1.54)	0.527

AIH, autoimmune hepatitis; BA, biliary atresia; BSA, body surface area; FLF, fulminant liver failure; HBV, hepatits B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; INR, international normalized ratio; LC, liver cirrhosis; LT, liver transplantation; PBS, primary biliary sclerosis; PLT, platelet; PSC, primary sclerosing cholangitis; SM, splenomegaly; SV, spleen volume; WBC, white blood cell.

Bold indicates statistically significant *P* values.

Proposed value of SV/BSA suggesting post-transplantation SM and post-transplantation PLT < 100.000/μl

The area under the curve (AUC) determined that the preoperative SV/BSA value for indicating post-transplantation SM was 312 ml/m^2 (AUC, 0.8168; 95% CI, 0.7400–0.8818, $P < 0.001$) (Fig. 2), which yields a sensitivity of 73.3% and a specificity of 76.3%. Based on the same data, the optimum cutoff value for detecting post-transplantation PLT < 100.000/μl three years after LT was 475 ml/m^2 (AUC, 0.8233; 95% CI, 0.7010–0.9166, $P < 0.001$), which provides a sensitivity of 80.8% and a specificity of 84.5%. In combination, a preoperative SV/BSA value higher than 312 ml/m^2 was suggested to predict both post-transplantation SM and post-transplantation PLT < 100.000/μl.

Second cohort study: potential long-term impact of preserved-spleen on clinical parameters

After PSM, all differences in confounding variables used in PSM, such as age, graft-to-recipient weight ratio (GRWR), SV, BSA, SV/BSA, proportion of SM, WBC, and PLT counts, were well balanced. The preoperative baseline characteristics of the unmatched and matched cohorts are presented in Table 4.

Spleen preservation is associated with lower PLT and WBC counts

The laboratory findings three years after LT were compared between splenectomized patients and spleen-preserved patients (Table 5). Although no difference was observed in terms of Hb, INR, AST, ALT, or albumin levels, the PLT count and WBC count were lower in the spleen-preserved group than in the splenectomized group (163 (120–230) vs. 275 (226–347); 5.0 (4.1–6.4) vs. 6.8 (5.6–8.9)).

Spleen preservation delays recovery of the PLT count

The results from the Kaplan–Meier analysis emphasized that splenectomy promotes the recuperation of PLT> 100.000/μl ($P < 0.001$) and PLT> 150.000/μl ($P < 0.001$). All patients who underwent splenectomy at the time of LT

Table 3. Impact of splenomegaly three years after liver transplantation on laboratory data

Variables	Post-transplantation SM (n = 90) (60.4%)	Post-transplantation non-SM (n = 59) (39.6%)	P value
BSA (m^2)	1.4 (0.9–1.6)	1.5 (1.1–1.7)	0.346
SV/BSA (ml/m^2)	251.3 (199.1–377.3)	114.9 (87–131.4)	**<0.001**
Percent reduction of SV/BSA after LT	36% (−17%–51%)	41% (28%–64%)	**0.031**
Liver volume (ml)	917 (559–1172)	894 (591–1130)	0.992
WBC count ($\times 10^3/\mu l$)	5.2 (4–6.7)	6.3 (5.1–7.7)	**0.003**
Hemoglobin (g/dl)	12.7 (10.6–13.9)	12.5 (11.6–13.6)	0.707
PLT count ($\times 10^3/\mu l$)	143 (89–189)	203 (170–256)	**<0.001**
PLT count < 150.000/μl (%)	51 (56.7%)	9 (15.3%)	**<0.001**
PLT count < 100.000/μl (%)	26 (28.9%)	0 (0%)	**<0.001**
PLT count < 50.000/μl (%)	4 (4.4%)	0 (0%)	0.152
INR	1.1 (1–1.2)	1 (1–1.1)	**0.007**
AST (U/l)	31 (22–44)	24 (16–35)	**0.001**
ALT (U/l)	23 (14–45)	14(11–23)	**<0.001**
Total bilirubin (mg/dl)	0.7 (0.5–1.1)	0.7 (0.5–0.9)	0.797
Albumin (g/dl)	4 (3.7–4.3)	4 (3.7–4.3)	0.902
METAVIR score: ≥F2	15/70 (21.4%)	1/36 (2.8%)	**0.010**

ALT, alanine transaminase; AST, aspartate transaminase; BSA, body surface area; INR, international normalized ratio; LT, liver transplantation; PLT, platelet; SM, splenomegaly; SV, spleen volume; WBC, white blood cell.

Bold indicates statistically significant P values.

*Liver biopsy was available in 70 and 36 in the post-transplantation SM and non-SM groups, respectively.

†Fisher exact test.

achieved PLT> 100.000/μl and PLT> 150.000/μl three years after LT, whereas only 85.9% and 51.3% of spleen-preserved patients recovered PLT levels above those thresholds, respectively. All splenectomized patients reached PLT> 100.000/μl within approximately three months (116 days) and PLT> 150.000/μl within approximately 15 months (447 days) after LT, while spleen preservation could not guarantee that the whole population would recover these PLT counts, even three years after LT.

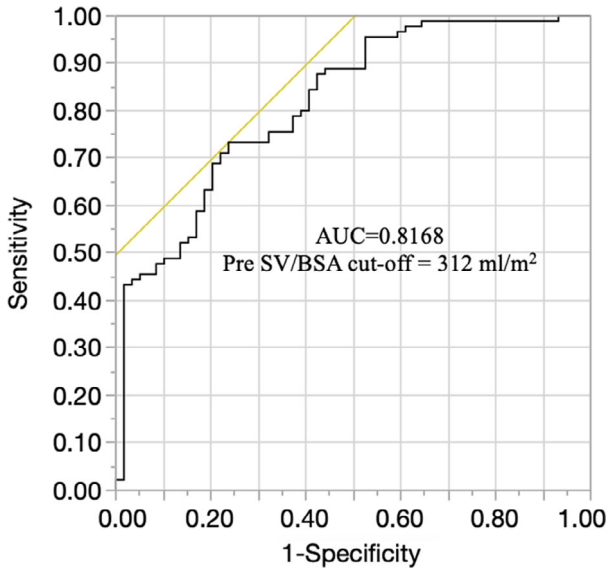

Figure 2 Receiver operating characteristic analysis for the cutoff value of preoperative SV/BSA for postoperative SM. AUC, area under the curve; BSA, body surface area; LT, liver transplantation; SM, splenomegaly; SV, spleen volume.

Preservation of the spleen is associated with more advanced graft fibrosis three years after LT

Among 78 splenectomized patients and 78 spleen-preserved patients in the previous analysis, liver biopsy findings were available for 42 splenectomized patients and 45 spleen-preserved patients. Record data determined all intra-operative graft biopsy at the time of LT showed no evidence of fibrosis. The median interval between LT and postoperative liver biopsy was 1108 days with a range of 1009 to 1184 days. Our results showed that the proportion of patients with METAVIR scores of F2 or higher was significantly higher in spleen-preserved patients than in splenectomized patients (26.7% vs. 7.1%, P = 0.022).

Third cohort study: Spleen preservation is associated with a reduced patient and graft survival rate

The differences in patient characteristics such as age, GRWR, SV, BSA, proportion of SM, hemoglobin, WBC,

Table 4. Baseline characteristics of the splenectomized and preserved patients in three years post-transplantation outcome evaluation using propensity score matching

	Unmatched population			Matched population		
Variable	Splenectomized group (n = 147)	Preserved group (n = 185)	P value	Splenectomized group (n = 78)	Preserved group (n = 78)	P value
Age (year)	55 (49–60)	39 (7–55)	**<0.001**	54 (46–59)	54 (46.5–61)	0.992
Sex (male:female)	81:66	82:103	0.051	38:40	40:38	0.748
GRWR (%)	0.87 (0.77–1.04)	1.11 (0.87–1.95)	**<0.001**	0.95 (0.79–1.1)	0.92 (0.76–1.1)	0.590
SV	536 (386–820)	356 (178.5–655.5)	**<0.001**	526 (384–801)	584 (365–858)	0.628
BSA (m^2)	1.7 (1.5–1.8)	1.5 (0.8–1.7)	**<0.001**	1.6 (1.5–1.8)	1.7 (1.6–1.8)	0.303
SV/BSA (ml/m^2)	311 (227–492)	325 (175.5–478)	0.331	308 (228.5–477)	354.4 (231–516)	0.647
SM (%)	133 (90.5%)	147 (79.5%)	**0.006**	70 (89.7%)	69 (88.5%)	0.797
WBC count ($\times 10^3/\mu l$)	3.6 (2.3–4.8)	4.4 (3.1–6.75)	**<0.001**	3.75 (2.36–5.2)	3.55 (2.6–5.2)	0.928
Hemoglobin (g/dl)	10.1 (8.9–11.7)	9.6 (8.6–11.5)	0.209	9.7 (8.5–11.7)	9.5 (8.7–11.6)	0.956
PLT count ($\times 10^3/\mu l$)	52 (35–77)	79 (51–128)	**<0.001**	55.5 (38–88)	60.5 (43–92)	0.547
INR	1.7 (1.4–2)	1.6 (1.3–2.1)	0.059	1.7 (1.4–2)	1.7 (1.4–2.3)	0.507
AST (U/l)	60 (39–92)	75 (38.5–125)	**0.023**	65 (41–98)	52 (35–90)	0.252
ALT (U/l)	33 (24–48)	41 (24–81)	**0.002**	35 (24–60)	33 (21–52)	0.368
Total bilirubin (mg/dl)	3.5 (2.1–9.4)	6.5 (2.2–16.3)	**0.034**	3.9 (2.2–12.5)	5.2 (2.1–14.3)	0.674
Albumin (g/dl)	3 (2.6–3.4)	(2.6–3.5)	0.400	3.15 (2.6–3.5)	2.9 (2.5–3.3)	0.079

Bold indicates statistically significant P values.

Table 5. Three years post-transplantation outcomes of splenectomized and spleen-preserved patients matched using propensity score matching

Variables	Matched population		
	Splenectomized group (n = 78)	Preserved group (n = 78)	P value
WBC count ($\times 10^3/\mu l$)	6.8 (5.6–8.9)	5 (4.1–6.4)	**<0.001**
Hemoglobin (g/dl)	12.7 (11.3–14.0)	13.1 (11.7–14.6)	0.287
PLT count ($\times 10^3/\mu l$)	275 (226–347)	163 (120–230)	**<0.001**
INR	1.0 (0.9–1.1)	1.0 (1.0–1.1)	0.758
AST (U/l)	24 (17–31)	23 (17–35)	0.857
ALT (U/l)	16 (11–27)	18 (13–38)	0.132
Total bilirubin (mg/dl)	0.7 (0.6–0.9)	0.8 (0.6–1.1)	0.055
Albumin (g/dl)	4.1 (3.8–4.2)	4.1 (3.8–4.3)	0.247
PLT count < 150.000/μl (%)	2 (2.63%)	33 (42.3%)	**<0.001**
PLT count < 100.000/μl (%)	1 (1.32%)	13 (16.7%)	**<0.001**
PLT count < 50.000/μl (%)	1 (1.32%)	1 (1.28%)	0.985
WBC count < 5.000/μl (%)	9 (11.8%)	39 (50%)	**<0.001**
METAVIR score: ≥F2	3/42 (7.1%)	12/45 (26.7%)	**0.022**

ALT, alanine transaminase; AST, aspartate transaminase; BSA, body surface area; INR, international normalized ratio; LT, liver transplantation; PLT, platelet; PSM, propensity score matching; WBC, white blood cell.

Bold indicates statistically significant P values.

*Liver biopsy was available in 42 and 45 in the splenectomized and preserved groups, respectively.

†Fisher exact test.

PLT, AST, ALT, and total bilirubin level between these two groups in the original cohort analysis disappeared after matching (Table 6). Regarding rejection rate, there was no significant difference between splenectomized and spleen-preserved groups (36.2% vs. 38.8%, $P = 0.786$). However, the proportion of patients with graft loss post-LT in splenectomized group was lower than that in spleen-preserved group (16.4% vs. 28.5%, $P = 0.040$). The Kaplan–Meier analysis demonstrated that the spleen-preserved group showed a worse patient survival rate and graft survival rate than the splenectomized group (Fig. 3). Even though the splenectomized patients suffered significantly increased operative time (842 minutes vs. 786 minutes, $P = 0.024$), there was no difference regarding blood loss (7465 gram vs. 6195 gram, $P = 0.082$), hemorrhage-related reoperation (6.9% vs. 4.3%, $P = 0.414$), post-transplantation bacteremia (38.8% vs. 48.3%, $P = 0.185$), and post-transplantation CMV infection (31.9% vs. 20.7%, $P = 0.073$). Particularly, splenectomy significantly alleviated post-transplantation small-for-size graft syndrome (5.2% vs. 16.4%, $P = 0.010$).

Discussion

In the last two decades, intense research with regard to ameliorating long-term outcomes of LT has been conducted. Among them, persistent SM after LT and indication of splenectomy in modulating the portal flow in the context of LDLT with small graft has been attracting great clinical interest. Thus, we evaluated the potential predictors and long-term outcomes of persistent SM in conjunction with comparisons of the clinical factors between splenectomy and spleen-preservation patients who underwent LT at Kyoto University Hospital.

One expectation from transplant surgeons after LT is the improvement of SM in recipients. However, 72.6% of patients who suffered from preoperative SM failed to recover from SM even after a long period. Previous studies showed that persistent SM varied from 27% [25] of the patients to 89% [26] one year after LT to 56% at two years after LT [27]. This dissimilarity might be due to the semiquantitative assessments of SV by conventional imaging diagnostic methods [15] and different definitions of SM, which were arbitrarily and inappropriately given. Namely, Sutedja defined SM by either physical examination or enlarged SV on MDCT without a specific threshold [25], while Chikamori determined SM as an SV two SD greater above the normal value in the recipient population, instead of using a normal healthy population such as the donors [26]. Regarding the impacts of SM, multivariate analysis

Table 6. Baseline characteristics of the splenectomized and preserved patients in a three-year survival analysis using propensity score matching

	Unmatched population			Matched population		
Variable	Splenectomized group (n = 179)	Preserved group#(n = 236)	P value	Splenectomized group (n = 116)	Preserved group#(n = 116)	P value
Age (year)	56 (49–61)	42.3 (12–56)	**<0.001**	54 (42–60)	54 (45–61)	1.000
Sex (male:female)	91:81	105:118	0.265	59–57	62–54	0.693
GRWR (%)	0.9 (0.8–1)	1.1 (0.8–1.6)	**<0.001**	0.9 (0.8–1.1)	0.9 (0.8–1.1)	0.540
SV (ml)	540 (387–817)	449 (213–745)	**<0.001**	523 (358–805)	576 (368–893)	0.407
BSA (m^2)	1.7 (1.5–1.8)	1.5 (1.2–1.7)	**<0.001**	1.67 (1.48–1.79)	1.67 (1.51–1.79)	0.841
SV/BSA (ml/m^2)	313 (228–490)	344 (205–502)	0.914	301 (222–500)	355 (226–557)	0.354
SM (%)	157 (91.3%)	189 (85%)	0.064	100 (86%)	98 (85%)	0.710
WBC count ($\times 10^3/\mu l$)	3.6 (2.3–4.8)	4.1 (2.8–6.3)	**<0.001**	3.7 (2.5–5.2)	3.5 (2.5–5.5)	0.890
Hemoglobin (g/dl)	10 (8.8–11.7)	9.4 (8.3–11.2)	0.053	9.5 (8.4–11.2)	9.2 (8.3–11.3)	0.704
PLT count ($\times 10^3/\mu l$)	51 (35–76)	73 (46–119)	**<0.001**	52 (36–87)	59 (42–80)	0.317
INR	1.6 (1.4–2.0)	1.5 (1.3–2.0)	**0.015**	1.7 (1.4–2.1)	1.6 (1.3–2.1)	0.598
AST (U/l)	57 (39–90)	70 (38–118)	**0.015**	60 (38–97)	56 (35–88)	0.587
ALT (U/l)	32 (23–47)	40 (24–74)	**0.002**	33 (22–50)	35 (23–51)	0.817
Total bilirubin (mg/dl)	3.4 (2–9.6)	6 (1.8–16.4)	0.087	3.7 (2–14.5)	4.7 (1.8–13.7)	0.723
Albumin (g/dl)	3 (2.6–3.4)	3 (2.6–3.4)	0.950	3 (2.5–3.4)	2.9 (2.6–3.2)	0.234

ALT, alanine transaminase; AST, aspartate transaminase; BSA, body surface area; GRWR, graft-to-recipient weight ratio; INR, international normalized ratio; LT, liver transplantation; PLT, platelet; PSM, propensity score matching; SM, splenomegaly; SV, spleen volume; WBC, white blood cell.

Bold indicates statistically significant P values.

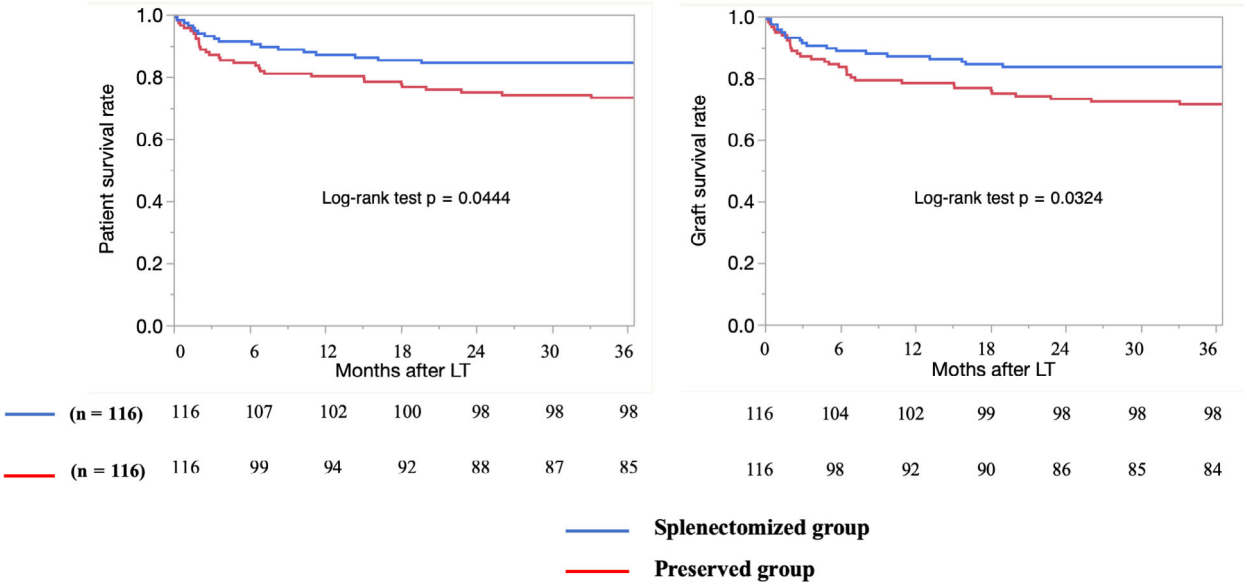

Figure 3 Kaplan–Meier curve for the patient survival rate and graft survival rates of splenectomized and spleen-preserved patients three years after LT using propensity score matching. LT, liver transplantation; PSM, propensity score matching.

determined that the preoperative SV/BSA was a significant predictor of persistent SM and was negatively associated with delayed PLT recovery. Yao et al. found that preserved spleen with an extremely low graft-to-spleen volume ratio was associated with short-term persistent hypersplenism one month after LT [28]. Similarly, Eyraud et al reported that the greater the SV increase was, the more important thrombocytopenia was one year after LT [15]. Hence, despite of restricted sample sizes and short follow-up periods, these findings contribute to the emphasis of our results about the correlation between SM and the risk for low PLT levels.

Subsequently, we demonstrated that recovery to PLT> 100.000/μl and to PLT> 150.000/μl was not achieved in 14.1% and 48.7% of spleen-preserved recipients, respectively, whereas 100% of the splenectomized patients successfully recovered to these thresholds, which again emphasizes the persistent influence of SM even long after LT. Furthermore, spleen preservation was also associated with a reduced patient and graft survival rate. Ito et al did not recommend splenectomy because of postoperative hemorrhage and lethal infectious complications [9]. However, in the aforementioned study, the patients did not routinely receive vaccinations against pneumococci and other bacteria, during either the preoperative or postoperative period and the proportion of lethal infections might be reduced by completing a prophylactic vaccination schedule prior to LT [29–31]. Regarding postoperative bleeding, splenectomy can be safe with a bloodless approach with a stapling device, and a careful surgical approach [32]. On the other hand, our results are in line with other studies suggesting that SS is required in some specific situations. Yoshizumi et al reported that SS was useful in preventing small-for-size syndrome [33], while graft size had an association with post-transplantation thrombocytopenia; splenectomy was required to maintain PLT> 100.000/μL for patients with a small graft [34]. Similarly, Cescon et al. approved splenectomy as a feasible option to correct thrombocytopenia [35].

Another important clinical implication of our study is the association between persistent SM and graft fibrosis. Current literature reporting on the long-term correlation between graft histology and SM is limited. Scheenstra et al. noted that enlarged spleens were mostly observed in patients with severe fibrosis rather than in those with no or mild fibrosis (56% vs. 34%) [36]. However, this difference was not statistically significant. The lack of a standard definition for SM, as well as the determination of SV by abdominal ultrasound, might affect the accuracy of these findings. In our study, we found that the proportion of patients with a METAVIR score of F2 or higher was significantly greater in two cohorts: patients suffering post-transplantation SM and patients with spleen preservation. It is a matter of interest whether significant graft fibrosis is a cause or a consequence of post-transplantation SM. Generally speaking, graft fibrosis can induce portal hypertension and can be a cause of SM. However, this

scenario is very unlikely in this situation for the following reasons. First, if this were the case, post-transplantation SM should have developed irrespective of the presence of preoperative SM. This contradicts our result that post-transplantation SM correlated strongly with the presence of preoperative SM. Second, the period of three years is too short for a healthy graft at the time of LT to become so fibrotic that it causes portal hypertension and SM. Therefore, it is highly plausible that graft fibrosis is not a cause of but a consequence of post-transplantation SM, implying the potential role of splenectomy in alleviating graft fibrosis.

Several experimental studies support this idea; the splenic contribution to hepatic fibrogenesis via spleen-derived transforming growth factor ß1 (TGF-ß1), which facilitates tissue fibrosis, has been reported [37–40]. Tanabe et al demonstrated that the migration of Th2-dominant splenic lymphocytes induces liver fibrosis by shifting the cytokine balance toward Th2 dominance [41]. Plenty of human studies also showed potential and significant effect of splenectomy in respect to the reversal of cirrhosis [42–44]. At one-year follow-up after splenectomy, Yamamoto at al. identified a significant improvement of total bilirubin level, prothrombin time, and Child-Pugh score in Child-Pugh class B cirrhosis patients [45]. In long-term follow-up, Ogata et al. also realized that an enhancement regarding total bilirubin, prothrombin time, platelet count, Child-Pugh score for 3 years, and albumin for 2 years after splenectomy [46]. Therefore, our study suggests that maintaining PLT levels and alleviating graft fibrosis by splenectomy could potentially enhance the prognosis of patients with high-risk SM (defined as preoperative SV/BSA> 312 ml/m^2). Nevertheless, another aspect that we should consider is the possibility of SM resulting from graft fibrosis. Elevated resistance to blood flow due to graft fibrosis leads to portal hypertension and could result in the manifestation of SM. The precise cause–effect relationship between graft fibrosis and SM remains unclear, and further research is needed to elucidate this mechanism.

In light of our study, the SV is not entirely restored after LT and approximately more than half of recipients that had preoperative SM continued to suffer SM after liver transplantation. Subsequently, post-transplantation SM was also proved as having association with lower PLT and WBC count and more advanced graft fibrosis three years after LT. Thus, splenectomy at the time of LT should be considered in patients with splenomegaly, especially in those with "high risk." In respect to the post-transplantation SM and post-transplantation PLT < 100.000/µl, the two adverse results which constitute the definition of "high risk," our analysis showed that a preoperative SV/BSA value greater than 312 ml/m^2 was implied to foresee these unfavorable long-term outcomes. Therefore, we would propose splenectomy at the time of LT, after cautiously consider its contraindication, in patients who suffer a preoperative SV/BSA value higher than the aforementioned value.

Despite cautious preparation, we acknowledge that our study has several limitations. The incomplete clinical records and the unavailability of liver biopsy at three years post-LT in a proportion of patients resulted in a limited sample size. This drawback might restrictively prevent us from establishing a convincing conclusion, especially to determine the anticipation of graft fibrosis in non-splenectomized patients and post-transplantation SM patients. There are also weaknesses inherent to retrospective analyses at a single institution, which inevitably results in heterogeneity and selection bias. Patients with fulminant liver failure and autoimmune hepatitis would be anticipated to have a higher risk of rejection and accumulate graft fibrosis more rapidly. However, these patients just occupy a small proportion and should not have so much impact on the result. Another shortcoming is the lack of comparison between splenectomy and splenic artery ligation (SAL) in modulation of portal vein pressure (PVP). We previously applied SAL for a few cases. Since it was not sufficient to decrease the PVP after reperfusion in some cases and splenectomy offers better control of PVP with acceptable rate of adverse effects, SAL was abandoned in our institution and splenectomy become the standard procedure for PVP modulation. Besides, with a large number of therapeutic splenectomies have been performed, we had the opportunity to observed effects not often seen in Western experience where LDLT is rare. The impact of portal pressure and portal flow on the graft is very different in a whole graft procured from a deceased donor as compared to a small graft procured from a living donor. Additionally, SM was investigated in both adult and pediatric patients, which also implicates a confounding bias. Nonetheless, by normalizing SV by the patient's BSA, we restricted the bias introduced by the patient's weight and height. Furthermore, the mentioned limitations were effectively addressed using PSM to overcome the potential confounding effects.

In summary, we found that SM persists more than three years after LT in a significant proportion of recipients and can be associated with not only reduced WBC and PLT counts but also more advanced graft fibrosis, graft loss, and even death. Our findings enabled us to

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identify high-risk patients and assisted us in generating an appropriate surgical strategy for the LT population. Further prospective and multicenter studies with larger samples are highly recommended to confirm our results.

Authorship

NHN: participated in research design, data collection, data analysis, and writing of the paper. KT, SY, TK, YU, YK, TA, KF, TI, SY, NK, KH, and SU: contributed to research design, data analysis, and review of the paper.

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Conflict of interest

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