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REVIEW ARTICLE

Review of the surgical approach to prevent small-for-size syndrome in recipients after left lobe adult LDLT

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Abstract Left lobe liver grafts increase the donor safety in adult-to-adult living-donor liver transplantation (ALDLT). However, the left lobe graft provides about 30–50 % of the required liver volume to adult recipients, which is insufficient to sustain their metabolic demands, which can lead to small-for-size syndrome (SFSS). Transient portal hypertension and microcirculatory hemodynamic derangement, apart from outflow obstruction, during the first week after reperfusion are the critical events associated with small-for-size graft transplantation. The incidence of SFSS in left lobe ALDLT can be decreased by increasing the left lobe graft volume by effective utilization of the caudate lobe with preserved vascular supply, by modulating the portal pressure with splenectomy or a porto-systemic shunt or by hepatic venous outflow reconstruction to prevent the development of venous congestion. In this review, we discuss the pathophysiology of SFSS and the various surgical strategies that can be performed to prevent SFSS in an effort to enhance the donor safety during living-donor liver transplantation.

Keywords Portal flow modulation · Hemiportocaval shunt · Caudate lobe · Outflow reconstruction

Abbreviations

SFSS	Small-for-size syndrome
ALDLT	Adult living-donor liver transplantation
SFS	Small-for-size
HPCS	Hemiportocaval shunt
SAE	Splenic artery embolization
LHV	Left hepatic vein
MHV	Middle hepatic vein
GRWR	Graft–recipient weight ratio

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Introduction

Adult-to-adult living-donor liver transplantation (LDLT) (ALDLT) effectively decreases the donor shortage for liver transplantation being performed to treat end-stage liver disease [1–3]. Traditionally, the right lobe graft is the choice for ALDLT, because it helps to meet the metabolic demands of a recipient. However, the donor morbidity and mortality after right lobe donation have remained a formidable challenge over the last two decades, with 20–78 % of donors experiencing postoperative complications [4–9], with about 20 donor deaths [10]. Despite improvements in the donor evaluation process and more refined surgical techniques [5, 11], the donor complication rates following right lobe donation have remained unchanged over the years [5, 12]. Donor safety has the highest priority in living-donor liver transplantation (LDLT). The risk of donor mortality with the left lobe donation is lower (0.1 %) compared to right liver donation (0.5 %) [13]; moreover, the biliary and venous reconstruction is straightforward after left lobe donation [14]. In a survey of 1,508 donors from five major Asian centers, Lo et al. [15] reported a higher complication rate associated with right lobe donation (28 %) than left lateral segment (9.3 %) or left lobe (7.5 %) donation. Hashikura et al. [16] similarly reported higher donor complication rates after the right lobe donation from an analysis of 3,565 living liver donors from 38 Japanese centers.

Although using a left lobe liver graft improves the donor safety, left lobe graft ALDLT shifts the risk of complications to the recipients. The use of a left lobe graft provides only about 30–50 % of the required liver volume to an adult recipient [17], which is insufficient to sustain their metabolic demands, leading to small-for-size syndrome (SFSS). In this review, we discuss the pathophysiology and prevention of SFSS in an effort to decrease the recipient risks and enhance the safety of ALDLT.

Definition and clinical presentation

Liver grafts with a graft weight to standard liver volume ratio <40 % or a graft to recipient weight ratio (GRWR) <0.8 % have been considered to be SFS grafts. This results in functional impairment clinically characterized by cholestasis, prolonged coagulopathy, portal hypertension, ascites and encephalopathy at the end of the first week after transplantation, which has been described as SFSS [3, 18].

Pathogenesis

Understanding of pathogenesis of SFSS is crucial for predicting adverse clinical outcomes after left lobe liver transplantation. The transient portal hypertension and microcirculatory hemodynamic derangement in the initial first week after reperfusion are the critical events in the pathogenesis of small-for-size (SFS) graft [19]. These events significantly decrease the rate of liver regeneration. Chen et al. evaluated the regeneration of the left lobe liver graft in ALDLT by reviewing the pre- and post-operative images within 6 months after liver transplantation and compared these with images of right liver grafts. They revealed a lower regeneration rate among the patients with a left liver graft and GRWR <1. The regeneration ratio was proportional to the spleen volume and portal inflow [20].

Portal hyperperfusion

The increased portal blood flow through a reduced hepatic microvascular bed is a vital component in the pathogenesis of SFSS [21–23]. Partial liver grafts are exposed to much higher portal inflow compared to whole liver grafts in cadaveric transplantation. The hemodynamic changes after reperfusion of a left-lobe graft are more pronounced, as all portal flow is diverted to about 30–40 % of the mass of the native organ. In deceased donor liver transplantation with a whole liver graft, the mean portal blood is around 130 mL/min/100 g graft weight [24]. Shimamura et al. [21] concluded from their experience with LDLT that a portal flow exceeding 260 mL/min/100 g graft weight was associated with a significantly higher peak total bilirubin level, with a poor outcome. Troisi et al. [22] claimed that the portal flow should be reduced to <250 mL/min/100 g graft weight using graft inflow modification. Yagi et al. [25] reported that early elevation of the postoperative PVP ≥20 mmHg was associated with SFSS and poor outcomes. Portal hyperperfusion and insufficient venous outflow decrease the arterial perfusion, with a reduced capacity for regeneration, resulting in impaired liver function. The histological examination of grafts with SFSS shows sinusoidal congestion and disruption of the sinusoidal lining, with mitochondrial swelling, impaired bile secretion and severe cholestasis [26, 27]. Man et al. studied 40 LDLT cases, including 10 patients implanted with grafts that were <40 % of the standard liver weight. The transient portal hypertension in these patients was accompanied by intragraft upregulation of endothelin-1 expression and ultrastructural evidence of sinusoidal damage. The transient portal hypertension after reperfusion, subsequent endothelin-1 overexpression and plasma nitric oxide level reduction, together with the down-regulation of heme oxygenase-1 and heat shock protein 70, may account for the SFS graft injury [28]. The contribution of the arterial flow to the total liver blood flow was significantly lower after reperfusion and during the first week in patients with SFS grafts [25, 29].

Outflow obstruction

Apart from the increased inflow and hyperperfusion injury, hepatic outflow obstruction also significantly contributes to the development of SFSS. Recent experiments in a rat model with ligation of the right median hepatic vein, combined with 50 % hepatectomy, have demonstrated that a focal venous outflow obstruction in the remnant liver causes confluent centrilobular necrosis. In the early post-operative phase, the proliferative activity in the obstructed zone was markedly reduced compared with that in the normal zone due to the venous outflow occlusion. These findings indicate that the hepatic outflow obstruction may be crucial for the development of SFSS [30].

Finally, SFSS does not depend on just the volume of the graft, but on a combination of factors, such as the recipient's preoperative condition, the degree of portal hypertension, the donor age, the presence of parenchymal steatosis and the quality of the graft [31]. The preoperative Child's score often correlates with the portal venous pressure, including the portal hyperperfusion state, and this association influences the incidence of SFSS. Ben-Haim et al. [31] reported that the overall incidence of SFSS was 12.5 %; however, the incidence of SFSS in Child's class B or C was 83 %. Tanaka et al. [3] described that there is a combined effect of the graft size and length of pre-

transplantation intensive care unit stay on the incidence of SFSS and graft survival. Many reports have suggested that the donor age affects patient survival after liver transplantation [32]. There is concern that livers from older donors will have diminished regenerative capacity [33, 34]. Furthermore, livers from older donors have decreased blood flow and diminished function because of aging. Previous evidence suggests that the early graft function is better when the graft has been given by a young donor than an older donor, with an increase in the rate of graft failure as the donor age increases [35, 36].

Prognosis of SFSS

Soejima et al. [37] reported the initial results of adult LDLT using the left lobe in 36 recipients in 2003. The overall 1-year patient and graft survival rates were 85.7 and 82.9 %, respectively. SFSS occurred in seven of 16 patients (43.8 %) with cirrhosis and only one of 20 patients (5.0 %) without cirrhosis. Recipients who developed SFSS had inferior graft survival. At the same center, after 107 LL LDLTs were performed over 8 years, the reported the 1-, 3- and 5-year patient survival rates in LL-LDLT were 81.4, 76.9 and 74.7 %, respectively, with three losses directly attributable to SFSS [13]. Recent reports suggest that the incidence of SFSS is 19.5 % in LL LDLT, which is mainly due to the use of portal inflow modulation techniques with smaller grafts. The 1-, 5- and 10-year patient survival rates of LL LDLT were 85.6, 77.9 and 69.5 %, respectively, which are comparable to those of RL LDLT [38].

Strategy for preventing SFSS with left lobe grafts

The prevention of SFSS is based on controlling the factors that hamper the liver regeneration during the early postoperative period. The volume of the left lobe graft can be augmented by reconstructing the vascular supply to the caudate lobe and shifting the transection plane further to the right. Controlling the portal pressure by graft inflow modulation and adequate venous drainage to prevent early graft dysfunction and enhancing the regeneration are also helpful.

Increasing the graft volume

Effective utilization of the caudate lobe

The left lobe graft volume is usually <35 % of the total liver volume. The supplementation of the caudate lobe to a left liver graft increases the graft volume up to 9 % in most ALDLTs [39, 40] and can help to overcome a borderline graft–recipient size mismatch. A thick caudate lobe on preoperative imaging can effectively contribute to the graft volume if the portal and hepatic venous branches are preserved during donor operation. However, the concomitant resection of the caudate lobe is technically difficult, and many series have reported using the left lobe without the left caudate lobe [41, 42]. The biliary ducts of the left caudate lobe drain into the left ductal system [43]. Thus, parenchymal transection in the middle of the paracaval portion usually preserves the biliary drainage in the left caudate lobe.

Reconstruction of the short hepatic veins is controversial; even though it restores the outflow and enhances regeneration, it is technically difficult. Couinaud et al. [44, 45] reported that the caudate lobe is drained not only through the short hepatic veins, but also through the intraparenchymal communications with the left and middle hepatic veins. Ikegami et al. [46] reported the transplantation of a left lobe with caudate lobe graft without reconstruction of the short hepatic veins. The caudate lobes were increased in size and enhanced with contrast medium on postoperative CT scans, indicating a well-vascularized caudate lobe. However, the regeneration rate was significantly lower in the caudate lobe than segments II–IV (62 vs. 152 %). The most obvious cause of the lower regeneration is an insufficient venous drainage of the transplanted caudate lobe. Hashimoto et al. [47] described reconstruction of the short hepatic vein of the caudate lobe, which was resected along with the cuff of the vena cava, like a Carrel patch. After 1 month, the regeneration rate of the caudate lobe in 12 patients with reconstruction was comparable with that of the left liver (167 vs. 186 %). On the other hand, 13 patients without reconstruction of the short hepatic vein had a lower rate of regeneration of the caudate lobe (134 %). Hwang et al. [48] showed similar enhanced regeneration after reconstruction of short hepatic veins (Table 1).

The size, number and the distance of short hepatic veins from the MHV-LHV orifice are key determinants of the reconstruction of short hepatic veins. According to studies by Couinaud [44, 45], 69 % of caudate lobes have a single vein and 20 % have two. Most of the veins (91 %) enter directly into the vena cava. A single short hepatic vein with a size >3 mm is generally the dominant site of drainage of the caudate lobe, and reconstruction of such veins is strongly recommended.

The portal venous supply to the caudate lobe can be maintained by reconstruction of an isolated caudate branch of the portal vein, which is seen in 5.9 % of donors. Kokudo et al. [49] recommended reconstruction of an isolated caudate portal vein if its size was >0.5 mm. This technique increased the volume of the caudate lobe from 4 to 21 % in 1 month after transplantation.

Table 1 Effects of reconstruction of the short hepatic vein on the regeneration of the caudate lobe

	Ikegami et al. [46]	Hashimoto et al. [47]	Hwang et al. [48]
Reconstruction of the SHV	No	Yes	Yes
Preoperative volume of CL	24 ml	22 cm^3	25.6 ml
Postoperative volume	37 ml	35 cm^3	Not reported
Regeneration rate of the CL	62 %	167 %	142.6 %
Regeneration rate of the remaining liver	152 %	186 %	190.8 %

CL caudate lobe, *SHV* short hepatic vein

Extension of the transection plane to the right of demarcation line

The tributaries of the middle hepatic vein have significant crossover in the right and left hemi-liver. When the border of the left lobe graft is defined with inflow segmentation, a larger left lobe graft can be harvested than when it is defined with the conventional MHV border. The extra well-perfused part of the anterior segment of the liver can be added to the left lobe graft by extending the parenchymal transection plane 1 cm to the right of the demarcation line and left to the right anterior Glisson's pedicle. Imura et al. [50] added an extra 8–9 % liver volume to the left lobe graft using this technique.

Portal flow modulation

The regeneration of the liver can be enhanced by minimizing the graft damage by diverting the portal flow to the systemic circulation or by decreasing the portal inflow by controlling the splenic blood flow.

Hemiportocaval shunt (HPCS)

A HPCS diverts the portal blood flow into the systemic circulation and decreases the early graft damage induced by portal hyperperfusion [51]. Botha et al. [52] described 21 left lobe ALDLTs with construction of a HPCS in 16 patients with the median GRWR of 0.67. The portal flow was diverted to the systemic circulation by creating a HPCS between the right portal vein and inferior vena cava. The portocaval gradient was reduced from a median of 18 to 5 mmHg with reconstruction of a HPCS. SFSS developed in only 1 patient, who required re-transplantation. In a similar study, Yamada et al. [51] described LDLT with a HPCS based on a portal vein pressure >20 mmHg at the time of transplantation. Although the HPCS decreased the incidence of SFSS, systemic shunting of the portal flow can cause encephalopathy. Botha et al. reported encephalopathy in 10 patients, and two of them remained encephalopathic beyond 2 months, requiring occlusion of the HPCS. Apart from encephalopathy, graft atrophy was observed 6 months after the HPCS due to insufficient portal inflow. The graft volume decreased to 60 % of the initial volume, resulting in deterioration of the clinical condition and graft function. Although hyperperfusion of the graft causes SFSS during the early period after ALDLT, it is necessary to maintain a certain degree of portal inflow to trigger the liver regeneration cascade by shear stress [53]. Thus, permanent HPCS is not an appropriate choice for resolving SFSS, as it is impossible to regulate the caliber ratio of the portal vein to the portocaval shunt during the postoperative period. If the intrahepatic resistance increases during the post-LDLT period, as in acute cellular rejection or acute cholangitis, the portal vein flow may be reversed through the HPCS due to the pressure gradient, which could result in accelerated graft atrophy. To overcome these complications, Botha et al. [54] described the percutaneous endovascular closure of the shunt to restore the portal flow back to the liver. The HPCS also can be closed by leaving an endo-loop around the shunt at the time of transplantation and tightening the loop during the postoperative period. Sato et al. [55] interposed an obliterated ligamentum teres of the liver to create the portocaval shunt, which functioned as a memory graft, closing the portocaval shunt after regeneration of the liver graft.

Meso-renal shunt

Using a meso-renal shunt, which involves anastomosis of the inferior mesenteric vein to the left renal vein, has some advantages over the HPCS [56, 57]. For example, the inferior mesenteric vein is adjacent to the left renal vein, and the anastomosis can be performed easily. The shunt flow is modest due to the small size of the inferior mesenteric vein, and excessive porto-systemic shunting resulting in a portal flow steal or hyperammonemia can be avoided while still providing an effective decrease in the portal vein pressure (Fig. 1).

Delayed ligation of spontaneous porto-systemic shunts

Spontaneous porto-systemic shunts due to preoperative portal hypertension divert the portal blood flow to the systemic circulation. Delayed ligation of the spleno-renal shunt can alleviate the initial portal hypertension after graft implantation, and thus enhance liver regeneration. Sato et al. [58] described the delayed postoperative ligation of spontaneous spleno-renal shunts in five patients with an

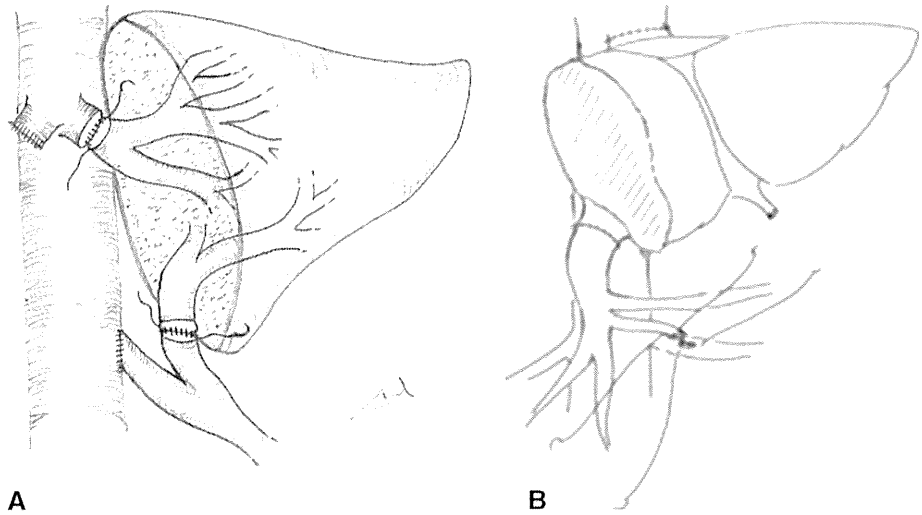

Fig. 1 **a** Using a hemi portocaval shunt (HPCS), it is difficult to control the flow across the HPCS between the liver and inferior vena cava. In the early phase, this can lead to encephalopathy, while during the late phase, it can be associated with the portal-flow steal phenomenon, kinking or torsion of the left portal vein because of massive graft regeneration, which can lead to late graft atrophy. **b** A meso-renal shunt is a more selective shunt that is not associated with complications such as hyperammonemia and portal flow steal (modified from references [50] and [54] with permission)

average GRWR of 0.68 ± 0.14. During the ALDLT, the left renal vein and the spleno-renal shunt were identified, and the left renal vein was encircled with prolene, which was passed through a Nelaton tube. The tube was then passed outside the body through the left abdominal side wall. The prolene was ligated on postoperative day 14, either by hand or using the Pringle method.

Splenectomy

The splenic circulation is an important contributor to the portal inflow, which can be modified by splenectomy or splenic artery ligation. The beneficial effects of splenectomy are not mediated only due to the decrease in portal flow, but also due to an increase in the hepatic arterial blood flow with an increased oxygen supply [59]. Furthermore, the hepatic oxygen delivery and hepatic oxygen consumption were positively correlated with the extent of liver regeneration after partial hepatectomy in rats [60]. Ogura et al. [61] introduced the intentional portal pressure control with splenectomy, and their selection of SFS grafts (GRWR <0.8) increased from 7.8 to 23.9 %, and the selection of left lobe grafts increased from 4.9 to 32.1 %. Despite the increase in the number of smaller grafts, the 1-year patient survival was significantly improved (76.2 vs. 87.9 %). However, patients with end-stage liver disease or splanchnic blood flow exhibited a hyper-dynamic state with collateral circulation around the splenic artery, such as a gastric coronary vein and spleno-renal shunt [62–64], and had an increased risk of hemorrhage. Splenectomy also increases the risks of infectious complications, the length of the operation and the blood product requirement [65]. In addition, cirrhotic patients are at high risk of developing portal vein thrombosis after splenectomy [66]. The current strategy at Kyoto University is to keep the portal pressure below 15 mmHg after reflow [61]. Splenectomy is the primary procedure performed to achieve this goal; if the portal pressure is >15 mmHg after splenectomy, an additional porto-systemic shunt (e.g., inferior mesenteric vein to left renal vein) is created.

Splenic artery embolization/ligation (SAE\SAL)

Preoperative SAE reduces the portal blood flow without increasing the risk of hemorrhage during the transplantation, and also shortens the length of the operation [67]. SAE effectively decreases the portal inflow without the risks associated with splenectomy, such as portal vein thrombosis or septic complications. SAE can also be used as a rescue treatment for post-transplantation SFSS [68].

The portal flow can also be reduced by ligation of the splenic artery, particularly if the portal venous pressure after transplantation does not exceed three to four times the portal vein pressure in the donor. However, most of the patients with end-stage liver disease have a portal venous pressure more than four times higher than the one measured in the donor, and other methods of portal inflow modulation are needed. In patients with an enlarged spleen due to portal hypertension, SAL can lead to the development of a splenic abscess due to a splenic infarction [69].

Umeda et al. [67] described 39 ALDLT recipients with a GRWR of <0.8. Twenty-one patients underwent splenic artery modulation (preoperative SAE in 15 patients and

intraoperative SAL in six patients). The excessive portal flow was significantly reduced in the SAL/SAE group, and the effect of SAE on portal decompression was equivalent to that of SAL. SFSS occurred in only one patient in the SAL/SAE group, compared to five in the without SAL/SAE group.

Even though the successful application of portal inflow modulation has led to renewed interest in the use of the left lobe, the optimal portal circulation for a liver graft is still unclear. Ishizaki et al. recently reported left lobe ALDLT without portal inflow modulation [70]. The actual GV/SLV ratio was <40 % in 24 of the 42 cases, and the GRWR was <0.8 % in 17 of the 42 recipients. The mean portal vein pressure was 21.5 ± 3.6 mmHg after graft implantation. None of the recipients developed SFSS, and the 1-, 3- and 5-year patient and graft survival rates were 100, 97 and 91 %, respectively.

Outflow modulation

Hepatic vein anastomosis is the most crucial step in the recipient surgery, as the optimum graft function depends on an adequate outflow. Hepatic outflow obstruction leads to congestion and eventual graft dysfunction after left lobe living-donor liver transplantation. The middle and left hepatic veins tend to distort and stretch during graft regeneration. These characteristics seem to be associated with outflow disturbances, resulting in graft failure. End-to-end anastomosis of the hepatic veins is the standard anastomosis in ALDLT. To ensure an adequate hepatic venous flow, it is necessary to obtain a wide ostium in the recipient and venoplasty of the graft hepatic vein for anastomosis. Emond et al. [71] advocated a triangulation method to create a wide outflow orifice. Tanaka et al. [72] described the reconstruction venoplasty of the middle and left hepatic veins with a right caudate extension on the inferior vena cava, while de Villa et al. [73] described the widening of the recipient orifice achieved by cutting across the intervening septa among the three recipient hepatic veins and trimming the irregular edges. Adequate hepatic venous flow was ensured by venoplasty of the hepatic veins of the graft and the recipient. For left liver transplantation, the left and middle hepatic vein (LHV and MHV) in the recipient is used for venoplasty. When the orifice of the combined LHV and MHV is smaller than the graft hepatic vein, a wide orifice is created by venoplasty of three hepatic veins.

Postoperative management

Early nutrition Delayed oral intake and the use of parenteral nutrition results in prolonged cholestasis and poor synthetic function, further aggravating the SFSS. A feeding jejunostomy hastens the liver functions, with an early recovery of the liver functions [74].

Conflict of interest None.

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Association of Anti-Human Leukocyte Antigen and Anti-Angiotensin II Type 1 Receptor Antibodies With Liver Allograft Fibrosis After Immunosuppression Withdrawal

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Background. Many pediatric patients who receive a living-donor liver transplant undergo withdrawal of immunosuppression (IS). For them, the high incidence of long-term progressive graft fibrosis is of particular concern.
Methods. We conducted a cross-sectional study including 81 pediatric patients who underwent IS withdrawal after living-donor liver transplant at Kyoto University Hospital and whose serum samples and pathological data could be obtained during the analysis period. We examined the association of donor-specific anti-human leukocyte antigen (HLA) antibody (DSA) and angiotensin II type 1 receptor antibody (anti-AT1R Ab) with posttransplant graft fibrosis. Normalized mean fluorescence intensity (MFI) 5,000 or higher and anti-AT1R Ab concentrations 17 U/mL or higher were both considered high level. The patients were classified into an advanced fibrosis group (AFG) (Ishak score≥3) and a control group (CG) (Ishak score≤2).
Results. Only one patient demonstrated DSA class I. Among those who demonstrated DSA class II, more AFG patients than CG patients demonstrated high-level mean fluorescence intensity, although the difference was not significant (64% vs. 39%; P=0.053). The incidence of high-level DSA-DRB1, however, was significantly higher in the AFG than that in the CG (40% vs. 4%; P<0.001), but there was no significant difference in DSA-DQB1 or DSA-DRB345. High-level anti-AT1R Ab was significantly more frequent in the AFG than in the CG (65% vs. 36%; P=0.02). All patients with both high-level DSA-DRB1 and high-level anti-AT1R Ab were found to have advanced fibrosis (P<0.001).
Conclusion. Anti-AT1R Ab and DSA-DRB1 may be candidates as biomarkers of graft fibrosis; both HLA and non-HLA immunity may be involved in graft fibrosis after IS withdrawal.

Keywords: DSA, Angiotensin II, Liver transplantation, Fibrosis, Tolerance.

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Operational tolerance (OT)—absence of graft rejection without immunosuppression (IS)—remains the goal of organ transplantation and remains generally elusive (*1*). However, since 1996, some 15% of our pediatric living-donor liver transplant (LDLT) patients maintained normal allograft function after IS withdrawal (*2*), consistent with other transplant centers' results (*3*).

Progressive fibrosis in liver allografts of long-term pediatric patients has been reported since 2006 (*4–10*), and its high incidence is of particular concern today. Such fibrosis may be caused by immune response, transplant-related factors, or both. Certainly, nonimmunologic causes of injury, such as prolonged cold ischemia time, young age when transplanted, high donor-recipient age ratio, and use of partial grafts, cannot be ignored, especially in pediatric transplants (*7*). However, we earlier reported that pediatric living-donor recipients, successfully weaned from IS, tended to have more fibrosis than those still on IS (*11*), and that resumption of minimal maintenance IS was effective for some patients who were found to have graft fibrosis (*11, 12*). Moreover, advanced fibrosis was accompanied by C4d deposition, which was

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attenuated or became undetectable after reintroduction of IS, along with improvement of fibrosis (*10, 12*). Altogether, we could reasonably hypothesize that fibrosis after IS withdrawal was at least partly associated with an antibody-mediated immune response.

Whatever its cause, fibrosis must be detected so measures can be taken against it. The Banff Working Group on Liver Allograft Pathology recently posted guidelines based on liver biopsy findings in IS management (*13*), strongly encouraging protocol follow-up biopsies 1, 3, 5, and 10 years after major decreases or total withdrawal of IS even for patients without symptoms or biochemical evidence of liver injury. Of course, fibrosis cannot be diagnosed without biopsies, but the attendant complications include serious hemorrhage, pneumothorax, biliary peritonitis, and possibly even death (*14*). So development of a way to predict the risk of fibrogenesis before initiating IS weaning would help clinicians select candidates for it. If that risk could also be monitored during weaning and after withdrawal, the need for biopsy might be reduced or at least postponed—or, conversely, the appearance of the demonstrated risk factors might trigger immediate biopsy, facilitating a more timely treatment of fibrosis.

Although some biomarkers for predicting OT have been investigated (*3, 15–17*), no reports on predictive biomarkers of graft fibrosis after IS withdrawal exist. However, some findings have been suggestive in light of our hypothesis about the role of immune response in fibrogenesis. Liver transplants have been considered exceptional with regard to antibody immune response. However, some reports have demonstrated the association of donor-specific anti-human leukocyte antigen (HLA) antibody (DSA) with long-term outcome in liver transplantation (*18–20*), which means that the humoral theory (*21, 22*) may explain graft fibrosis after IS withdrawal. Furthermore, non-HLA complement and non-complement-fixing antibodies, which may occur as alloantibodies or autoantibodies, have been reported to be responsible for a variety of allograft injuries (*23*). Dragun presented an overview of common molecular targets for non-HLA antibody responses: major histocompatibility complex class I chain-related genes A and B, vimentin, intercellular adhesion molecule-1, and angiotensin II type 1 receptor (AT1R) (*23*). Among these, AT1R is relatively well known for its important role in liver fibrogenesis (*24–27*). Moreover, in renal transplantation, angiotensin II type 1 receptor antibody (anti-AT1R Ab) may have a strong association with antibody-mediated rejection (*28, 29*).

Accordingly—as one of the first institutions attempting IS withdrawal—we conducted a cross-sectional study that included significant numbers of long-term patients and analyzed the association of DSA and anti-AT1R Ab with fibrosis in IS withdrawal after pediatric LDLT to identify biomarker candidates.

RESULTS

Twenty-six LDLT recipients who had stopped IS or were in weaning were found to have advanced fibrosis; 55 patients with no, or mild, fibrosis comprised the control group (CG). All advanced fibrosis group (AFG) patients stopped weaning or resumed IS because of rejection (n=7) or biopsy-proven fibrosis (n=19). Fifteen CG patients remained off IS, whereas 30 resumed IS because of rejection (n=16) or fibrosis (n=14); 10 patients continued weaning.

Baseline Characteristics

Table 1 details CG and AFG patient characteristic. At transplantation, AFG age was significantly lower than CG age (1.3 vs. 2.6 years; P=0.04) as was the percentage of female (46% vs. 71%; P=0.048). Advanced fibrosis group's percentage of HLA-A mismatches was significantly higher than CG's (88% vs. 61%, P=0.02); differences for HLA-B, HLA-DR, and HLA-DQ were not significant.

Advanced fibrosis group white blood cell and platelet counts were significantly lower than those in the CG (4.7 vs. 5.4; P=0.046 and 147 vs. 189; P=0.004) but with no significant differences in liver function tests.

DSA Analysis

Table 2 shows the frequency of high-level DSA and anti-AT1R Ab. We used 5,000 mean fluorescence intensity (MFI) as positive cutoff for DSA. One CG (2%) and zero AFG patients demonstrated positive DSA class I, whereas 21 CG (39%) and 16 AFG patients (64%) demonstrated positive DSA class II. The AFG tended toward a higher percentage of high-level MFI, although the difference did not reach significance (64% vs. 39%; P=0.053).

The AFG's percentage of high-level DSA-DRB1 was significantly higher than the CG's (40% vs. 4%; P<0.001), but with no significant difference in DSA-DQB1 or DSA-DRB345.

Anti-AT1R Antibody Analysis

The AFG's percentage with high-level anti-AT1R Ab was significantly higher than the CG's (65% vs. 36%; P=0.02) (Table 2). Sensitivity of high-level anti-AT1R Ab was 65%, its specificity 64%.

Hypertension is usually diagnosed and treated according to the World Health Organization guidelines (*30*) and is often found in patients with high-level anti-AT1R Ab. However, no patients in our study were diagnosed as hypertensive.

The Predictive Value of DSA-DRB1 and Anti-AT1R Ab

Note that *all* patients with both high-level DSA-DRB1 and high-level anti-AT1R Ab were found to have advanced fibrosis (P<0.001) (Table 3).

When the patients were categorized as double negative for the Abs, single positive and double positive, the percent of advanced fibrosis was 11% if double negative, 42% if single positive, and 100% if double positive. The proportion of patients with high Ishak scores was also significantly greater if double positive. (P=0.002) (Fig. 1).

Multivariate Analysis of AFG Versus CG Patients

Table 4 shows multivariate analysis results. High-level DSA-DRB1 was associated with fibrosis, odds ratio (95% confidence interval [CI]) of 30.4 (2.35–398) (P=0.009), so was Anti-AT1R Ab, odds ratio of 7.4 (1.4–40) (P=0.02). Although these levels are statistically significant, further analysis is needed to reach an appropriate odds ratio because of the large CI.

DISCUSSION

This cross-sectional study demonstrates the association of high-level DSA-DRB1 and high-level anti-AT1R Ab with advanced graft fibrosis during IS withdrawal after

TABLE 1. Baseline characteristics of study population

	Groups		
	Control (n=55)	Advanced fibrosis (n=26)	*P*
At the time of transplant			
Recipient age in years, mean (SD)[a]	2.6 (2.8)	1.3 (1.5)	0.04
Donor age in years, mean (SD)	32.1 (6.0)	32.3 (4.8)	0.88
Recipient female, n (%)[a]	39 (71%)	12 (46%)	0.048
Donor female, n (%)	29 (54%)	18 (69%)	0.23
Underlying disease, n (%)			0.77
Biliary atresia	43 (78%)	23 (88%)	
Fulminant hepatitis	2 (4%)	1 (4%)	
Metabolic liver disease	3 (5%)	0	
Other	7 (13%)	2 (8%)	
ABO blood types compatible, n (%)	47 (85%)	23 (88%)	1.00
Graft weight (GRBW%), mean (SD)[a]	2.8 (1.1)	3.2 (1.0)	0.08
Rejection episode within 1 mo post-LDLT, n (%)	14 (30%)	8 (31%)	1.00
Presence of HLA-A mismatch, n (%)[a]	31 (61%)	22 (88%)	0.02
Presence of HLA-B mismatch, n (%)	45 (88%)	24 (96%)	0.42
Presence of HLA-DR mismatch, n (%)	41 (80%)	21 (84%)	0.84
Presence of HLA-DQ mismatch, n (%)	25 (49%)	13 (52%)	0.87
Factors at the time of analysis			
Outcome of IS withdrawal, n (%)			<0.001
Operational tolerance	15 (27%)	0	
Rejection	16 (29%)	7 (27%)	
Fibrosis	14 (25%)	19 (73%)	
Under weaning process	10 (18%)	0	
Tacrolimus dose (mg/day), mean (SD)	0.9 (1.2)	2.0 (1.7)	0.002
Ishak score, n (%)			<0.001
0	12 (22%)	0	
1	23 (42%)	0	
2	20 (36%)	0	
3	0	21 (81%)	
4	0	5 (19%)	
Normal blood tests, mean (SD)			
WBC, $\times 10^3/\mu L$[a]	5.4 (1.5)	4.7 (1.8)	0.046
PLT, $\times 10^3/\mu L$[a]	189 (60)	147 (63)	0.004
CRP, mg/dL	0.04 (0.08)	0.03 (0.09)	0.79
Creatinine, mg/dL	0.7 (1.5)	0.6 (0.1)	0.60
Liver function tests			
AST, U/L	29.3 (17.6)	29.8 (12.7)	0.91
ALT, U/L	30.8 (44.4)	31.3 (30.7)	0.96
Albumin, mg/dL	4.3 (0.3)	4.3 (0.4)	0.89
Total bilirubin, mg/dL	0.8 (0.4)	0.9 (0.5)	0.26
PT (INR)	1.1 (0.2)	1.1 (0.1)	0.55
Time from transplantation to serum collection in years, mean (SD)	13.5 (4.0)	14.4 (3.5)	0.34
Time from the initiation of IS withdrawal to serum collectionin in years, mean (SD)[a]	10.1 (4.4)	11.6 (2.7)	0.11

[a] incorporated into a multivariable model.

GRBW, graft recipient to body weight ratio; WBC, white blood cell; PLT, platelet; CRP, C-reactive protein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; PT, prothrombin time; INR, international normalized ratio; HLA, human leukocyte antigen; IS, immunosuppression; LDLT, living-donor liver transplant.

TABLE 2. Frequency of high-level DSA and anti-AT1R Ab

	Groups		
	Control (n=55)	Advanced fibrosis (n=26)	*P*
DSA-class I	1 (2%)[a]	0	1.00
DSA-A	0	0	1.00
DSA-B	1 (2%)	0	1.00
DSA-class II	21 (39%)[a]	16 (64%)[a]	0.053
DSA-DQB1	18 (33%)[a]	11 (44%)[a]	0.45
DSA-DRB1	**2 (4%)[a]**	**10 (40%)[a]**	**<0.001**
DSA-DRB345	8 (15%)[a]	2 (8%)[a]	0.49
Anti-AT1R Ab	**20 (36%)**	**17 (65%)**	**0.02**

[a] Human leukocyte antigen (HLA)-typing was unknown in one patient.
Significant factors ($P<0.05$) are in bold.
DSA, donor-specific anti-HLA antibody.

pediatric LDLT. This may support our hypothesis that fibrosis after IS withdrawal is at least partly associated with an antibody-mediated immune response.

Donor-Specific Anti-HLA Antibody

High-level DSA-DRB1 was detected in 40% of the AFG but only 4% (two patients) of the CG (Table 2); its specificity was 96%, suggesting that high-level DSA-DRB1 could be a biomarker of advanced fibrosis after IS withdrawal.

The finding was different with other HLA class II specificities. Most of the CG with high-level DSA-DQB1 or DSA-DRB345 maintained good liver function without advanced fibrosis. With renal allografts, Kobayashi et al. found DSA-DRB more detrimental than DSA-DQB—more associated with chronic antibody-mediated rejection—although DSA-DQB was readily detectable (*31*); most of our study patients with DSA-DQB also demonstrated high-level MFI. Kobayashi suggested that expression of DQ is much lower than that of DRB in vascular endothelial cells. The same difference was also seen in liver tissue. Hubscher et al. (*32, 33*) reported that expression of HLA-DR antigen in bile ducts and vascular endothelium was significantly greater in patients who experienced rejection, though expression of HLA-DQ did not increase significantly.

In our group, five of the 15 patients who achieved OT also demonstrated high-level DSA-DQB1 or DSA-DRB345

TABLE 3. Predictive value of DSA-DRB1 and anti-AT1R Ab

		Groups		
DSA-DRB1	Anti-AT1R Ab	Control (n=54)	Advanced fibrosis (n=25)	Total
Negative	Negative	33 (89%)	4 (11%)	37
Negative	Positive	19 (63%)	11 (37%)	30
Positive	Negative	2 (33%)	4 (67%)	6
Positive	Positive	0	6 (100%)	6
			P<0.001	

DSA, donor-specific anti-HLA antibody.

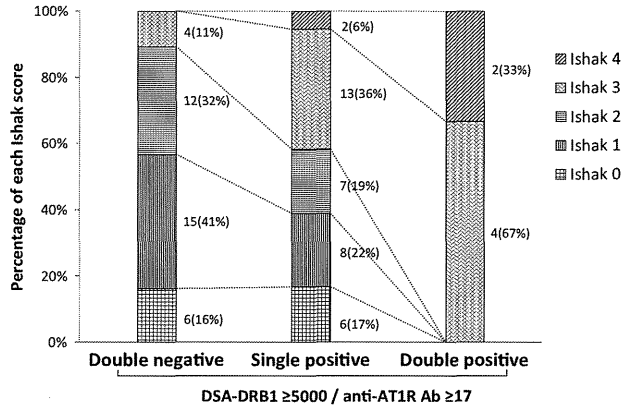

FIGURE 1. The proportion of Ishak score in each category ($P=0.002$).

without advanced fibrosis at the time of analysis. Three possibilities suggest themselves. First, high-level DSA in OT patients may be a warning of forthcoming fibrosis: Girnita et al. (*18*) reported that most OT liver recipients did not develop DSA. Second, the graft was not injured because expression of HLA-DQ and HLA-DRB345 was low. Finally, our previous studies demonstrated that OT after LDLT was likely maintained by some regulatory mechanisms, such as regulatory T cells and γδT cells (*34, 35*). Therefore, those mechanisms may counteract harmful immunoresponses against grafts despite the presence of alloreactive antibodies.

Anti-AT1R Antibody

Advanced fibrosis group patients demonstrated a significantly higher percentage of high-level anti-AT1R Ab than CG patients (Table 2). Although this may indicate that AT1R plays an important role in fibrogenesis after liver transplant (as well as in patients with hepatitis C), it only suggests the correlation between high-level anti-AT1R Ab and advanced graft fibrosis for projecting and implementing

TABLE 4. Logistic regression analysis of predictors for advanced fibrosis

Variables	Odds ratio	95% CI	*P*
Recipient age	0.70	0.34–1.43	0.33
Recipient female	0.43	0.07–2.5	0.35
Graft weight (GRBW%)	0.73	0.20–2.71	0.64
Presence of HLA-A mismatch	4.85	0.67–35.1	0.12
WBC, $\times 10^3/\mu L$	0.80	0.49–1.32	0.39
PLT, $\times 10^3/\mu L$	0.99	0.98–1.00	0.11
Time from the initiation of IS withdrawal to serum collection in years, mean (SD)	1.08	0.88–1.32	0.46
High-level DSA-DRB1 (MFI≥5,000)[a]	30.40	2.35–398.4	0.009
High-level anti-AT1R (≥17 U/mL)[a]	7.42	1.37–40	0.02

[a] Significant factors ($P<0.05$).
GRBW, graft recipient to body weight ratio; WBC, white blood cell; PLT, platelet; 95% CI, 95% confidence interval; HLA, human leukocyte antigen; DSA, donor-specific anti-HLA antibody; IS, immunosuppression.

IS withdrawal. Multivariate analysis did show both DSA-DRB1 and anti-AT1R Ab as significant factors for advanced fibrosis (Table 4), but their 95% CI was so wide, they were not reliable. *All* patients with both high-level DSA-DRB1 and high-level AT1R Ab were found to have advanced fibrosis. In contrast, 89% negative for both kinds of antibody was not found to have advanced fibrosis (Table 3). This means that assessing anti-AT1R Ab status along with DSA status will provide additional information about fibrosis.

Future Treatment for Graft Fibrosis

We usually used tacrolimus, prednisolone, and mycophenolate mofetil to treat our AFG's advanced fibrosis. Table 1 shows that their platelet and white blood cell counts were significantly lower than those of the CG, whereas no significant differences were detected in liver function tests. This may indicate that the current therapy is insufficient. Terui et al. reported that, with hepatitis C, AT1R antagonists may be beneficial in the early stage of hepatic fibrosis (*27*); perhaps they have a therapeutic effect after IS withdrawal. In renal transplant, Alachkar et al. (*36*) found depletion of anti-AT1R Ab with plasmapheresis and losartan therapy associated with resolution of antibody-mediated rejection in some patients.

Recipients being of female sex was associated with low Ishak score (P=0.048; Table 1)—consistent with the natural history of liver fibrosis progression in chronic hepatitis C (*37*). Yasuda et al. (*38*) demonstrated suppressive effects of estradiol on fibrosis of the liver in rats. Estradiol also may be effective with liver fibrosis.

Our study has some potential limitations. First, we included only pediatric LDLT patients with whom IS withdrawal was attempted, possibly minimizing the dispersion of baseline characteristics: for example, underlying disease and whether or not the graft was partial. Moreover, detectable antibodies might be merely a consequence of IS withdrawal. If antibodies are not detectable even in immunosuppressed recipients with advanced fibrosis, they are not useful for indicating biopsy. Second, because this study was cross-sectional, antibodies were not examined after advanced fibrosis impelled reintroduction of IS, so determining whether levels of circulating antibodies would correlate with a degree of C4d deposition that potentially can be altered after reintroduction of IS remains elusive. Third, for the same reason, the status of preformed or de novo antibodies is unknown because only the most recent post-transplant sera were available. Finally, of note, this study's results were not consistent with our previous findings that HLA-A matching and development of OT were positively correlated (*39*). Nonetheless, the role of DSA class I in rejection cannot be ignored. In this study, only OT recipients—with a normal liver test absent IS—were examined. Therefore, we cannot exclude the possibility that subjects exhibiting HLA class I were not basically included if DSA class I is mainly associated with clinically overt rejection. Clearly, prospective longitudinal studies including immunosuppressed recipients (reweaning and after reintroduction of IS) are necessary to elucidate whether antibody levels can be validated as biomarkers of fibrosis. However, the present study necessarily had to be cross-sectional to include a significant number of long-term patients, so our current study could be the first step in detecting biomarkers that contribute to the safety of IS management in long-term pediatric liver transplant patients.

In conclusion, we examined factors associated with liver fibrosis in IS withdrawal after pediatric LDLT. High-level DSA-DRB1 and high-level anti-AT1R Ab were associated with advanced graft fibrosis (Ishak≥3). It is suggested that HLA and non-HLA immunity may have important roles in fibrosis. Further large prospective studies are needed to substantiate the predictive value of these antibodies.

MATERIALS AND METHODS

Study Design and Population

Our cross-sectional study included all pediatric patients for whom IS withdrawal was attempted after LDLT at Kyoto University Hospital from June 1990 to December 2010 and whose serum samples and pathological data could be obtained during the analysis period, January 2011 to December 2012 (n=81). In that period, 719 pediatric patients underwent LDLT at our hospital, with 578 of them surviving (80%); IS withdrawal was attempted in 204 of these surviving patients. As noted, our study enrolled 81 (40%) whose serum samples were collected during the analysis period at the time of protocol or follow-up biopsy. The median time since transplantation for the latter group was 16.3 years (5.1–22.5).

Based on the guidelines for liver biopsies in which Ishak's modified score (≥3) was defined as advanced fibrosis (*13*), those patients in our study group who were found to have advanced fibrosis were classified as AFG and the others (with no, or mild, fibrosis—Ishak score≤2) were the CG.

No significant differences were seen between the groups in the interval from transplantation to serum collection (CG, 13.5 years; AFG, 14.4 years; P=0.34) or from initiation of IS withdrawal to serum collection (CG, 10.1 years; AFG, 11.6 years; P=0.11) (Table 1).

All patients met the criteria for IS withdrawal that we previously reported (*2, 39*): being more than 2 years after LDLT and having normal graft function, with no rejection episodes for more than 1 year. Serum samples were obtained from all at the time of protocol biopsy and were screened for DSA and anti-AT1R Ab. The institutional review board approved this study, and the ethics of the Declaration of Helsinki were followed (*40*).

Immunosuppression Protocol

When the first 14 cases in our study group were transplanted, cyclosporine A was administered with low-dose steroids. Then tacrolimus was substituted for cyclosporine A and was used for all subsequent patients. For the first 60 of these, tacrolimus was continuously given intravenously immediately after LDLT. Subsequently, it was given orally starting 1 day before transplantation, as it is today. The target trough level of tacrolimus is 10 to 12 ng/mL for the first 2 weeks and 5 to 10 ng/mL for the next 2 months. After discharge, the dose of tacrolimus is determined individually, depending on each patient's condition. Steroids are started during LDLT, then tapered gradually and stopped after 3 months.

In contrast to renal transplant, for which many new protocols have emerged (*41*), we have used mainly tacrolimus and steroid for two decades. There were no substantive differences in IS treatment between AFG and CG patients before IS weaning.

Protocol Biopsy and Fibrosis Scoring

Since January 2003, with pediatric LDLT, protocol biopsy of liver grafts has been performed at 1, 2, 5, 7, and 10 years after transplantation. If advanced fibrosis (bridging fibrosis, Ishak score≥3) was observed in our study group with a single biopsy—or progression of fibrosis with repeated biopsies—tapering was interrupted, and IS was increased or restarted, followed by annual biopsy for continual monitoring. The liver specimens were fixed in 10% buffered formalin, processed routinely, and sliced into 3-μm paraffin sections. The routine staining methods included hematoxylin-eosin, Masson trichrome, and cytokeratin 7 (CK-7, OV-TL 12/30, dilution 1:200; Dako, Glostrup, Denmark) (*10, 20*). The modified hepatic activity index (Ishak) system was used in this analysis. This is the system most widely used to

stage fibrosis, based on the degree of necroinflammatory lesions (*42*). The Banff Working Group on Liver Allograft Pathology recommended Ishak for staging fibrosis for uniformity and for finer gradation of fibrosis scoring (*13*).

HLA Typing

Haplotypes were serologically defined for HLA-A, HLA-B, HLA-DRB1, HLA-DRB345, and HLA-DQB1 loci in donors and recipients according to the standard methods of the National Institutes of Health at transplantation.

Detection of Anti-HLA Antibodies

Serum samples were stored and shipped to the Terasaki Foundation Laboratory in Los Angeles for evaluation of anti-HLA antibody and anti-AT1R antibody. The detection of HLA antibodies was performed using LABScreen Single Antigen class I (Lot 007) and class II (Lot 009) beads (One Lambda, Inc., Canoga Park, CA) for all the samples.

Trimmed mean values were obtained from the output file generated by the flow analyzer and normalized using the formula: (sample #N bead−sample negative control bead)−(negative control #N bead−negative control serum negative control bead).

Determination of Donor-Specific Anti-HLA Antibody Specificities

To identify DSA specificities, the donor-recipient mismatched HLA loci were compared to the antibody profile for each patient's sample. Mean fluorescence intensity was normalized in all cases, and MFI of 5,000 or greater was defined as high level.

Detection of Anti-AT1R Antibody

Anti-AT1R Ab was analyzed using enzyme-linked immunosorbent assay (CellTrend GmbH, Germany), following the manufacturer's instructions, and, based on previous studies (*29*), concentrations 17 U/mL or higher were considered high level. To determine the level of AT1R Abs, the standard curve (a four-parameter fit with five standard sera [2.5, 5, 10, 20, 40 U/mL] collected from patients positive for anti-AT1R) was plotted using AT1R software Version 1.0.0 (One Lambda). The enzyme-linked immunosorbent assay kits' positive controls (allosera from patients) and negative controls (sera from healthy individuals) were used to validate each run. The assay was validated if the positive control value was in the range of 15 to 25 U/mL and the negative control less than 10 U/mL.

Statistical Analysis

Patient characteristics of the AFG were compared with those of the CG using the *t* test or Wilcoxon rank sum tests for continuous variables. Categorical variables were summarized and compared with Fisher's exact test. Variables with a *P* value less than 0.20 in the univariate analysis were incorporated into a multivariable analysis using a logistic regression model.

Statistical significance was defined as a *P* less than 0.05. Stata version 11.2 (Stata, College Station, TX) was used for all statistical analysis.

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ORIGINAL ARTICLE

Prolonged thrombocytopenia after living donor liver transplantation is a strong prognostic predictor irrespective of history of splenectomy: the significance of ADAMTS13 and graft function

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Abstract The precise mechanism of prolonged thrombocytopenia following living donor liver transplantation (LDLT) remains unclear. To determine risk factors associated with prolonged thrombocytopenia following LDLT, with a focus on the activity of ADAMTS13 (a disintegrin-like and metalloproteinase with thrombospondin type-1 motifs member 13) and the influence of splenectomy. Adult LDLT patients were divided into two groups on the basis of platelet counts ($100 \times 10^3/\mu L$) on POD 14: high and low platelet (HP and LP) groups. Survival analysis was performed in the 100 patients, and ADAMTS13 activity and von Willebrand factor (VWF) levels in the plasma were measured in 65 adult recipients. The 6-month survival rate was significantly lower in the LP group ($n = 36$) than in the HP group ($n = 62$) (61.1 vs. 93.5 %). ADAMTS13 activity had been significantly lower in the LP group ($n = 23$) than in the HP group ($n = 42$). The VWF/ADAMTS13 ratio was significantly higher in the LP group than in the HP group. The independent risk factors for thrombocytopenia on POD14 were preoperative AT levels and ADAMTS13 activity on POD14. TPO levels on POD14 were significantly higher in the LP group than in the HP group, while those on POD28 in the LP group were significantly decreased, despite the low platelet levels. Irrespective of splenectomy history, platelet counts and ADAMTS13 activity in the LP group remained low until POD28, while VWF/ADAMTS13 ratio significantly increased until POD28. These results suggest that prolonged thrombocytopenia after LDLT was associated with not only a decrease in ADAMTS13 due to sinusoidal endothelial cell injury, but also low TPO production due to hepatocyte dysfunction, irrespective of history of splenectomy.

Keywords ADAMTS13 · Living donor liver transplantation · Thrombocytopenia · Splenectomy · von Willebrand factor

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Introduction

Although splenectomy is not usually performed for deceased donor liver transplantation (DDLT) patients, several transplant centers performing living donor liver transplantation (LDLT) have introduced simultaneous splenectomy for the purposes of controlling portal pressure in small-for-size graft recipients, preventing thrombocytopenia in HCV positive recipients, who is postoperatively planned to undergo interferon treatment, and undergoing ABO-incompatible LDLT [1–3]. After simultaneous splenectomy for LDLT, however, a few studies examined the changes in postoperative platelet counts. Marubashi et al. [2] revealed that seven patients, who underwent a simultaneous splenectomy showed a remarkable increase in their

platelet counts after LDLT on postoperative day (POD) 14 and the peak of the platelet count was seen on POD 28. In our previous report, we encountered several LDLT patients, who had suffered from prolonged thrombocytopenia even after splenectomy. Although this precise mechanism remained unclear, we suggested that the decreased activity of a disintegrin-like and metalloproteinase with thrombospondin type-1 motifs member 13 (ADAMTS13) after LDLT might be associated with prolonged thrombocytopenia [4].

ADAMTS13 is a metalloproteinase that specifically cleaves multimeric von Willebrand factor (VWF), which is mainly synthesized by vascular endothelial cells and mediates the adhesion of platelets to sites of vascular damage by binding to specific platelet membrane glycoproteins [5–8]. ADAMTS13 is almost entirely produced by stellate cells in the hepatic sinusoid. In vascular endothelial cells, if plasma ADAMTS13 activity decreases, the number of unusually large VWF multimers (UL-VWFM) significantly increases. Since UL-VWFM show a strong activity of platelet aggregation, an increase in UL-VWFM leads to platelet clumping and/or thrombus formation [9, 10]. It is reported that low levels of ADAMTS13 activity may result from low production or from increased consumption. In liver cirrhosis patients, production of ADAMTS13 in hepatic stellate cells decrease [11], and this enzyme is consumed through the continuing cleavage of VWF in disseminated intravascular coagulation (DIC) patients [12].

Transient thrombocytopenia is a common phenomenon after liver transplantation (LT), and the recovery of platelet counts is clinically significant. In 1992, McCaughan et al. [13] revealed that nadir platelet counts after LT predicted allograft dysfunction. Following this report, three additional studies [14–16] confirmed that severe thrombocytopenia after LT was associated with graft and patient survival. However, the precise mechanisms of post-transplant thrombocytopenia and its relationship with graft dysfunction still remain unclear. The mechanisms contributing to graft dysfunction are multifactorial and include ischemic reperfusion injury (IRI), sinusoidal endothelial cells (SEC) injury, platelet aggregation, immunological reactions, and inflammatory responses [17, 18]. Based on the previous evidence that the nadir platelet counts are related to graft dysfunction, we consider that SEC injury and platelet aggregation, which might be associated with decreased activity of ADAMTS13, play an important role in the pathogenesis of graft dysfunction.

There have been few studies on the correlation between thrombocytopenia and ADAMTS13 activity after LDLT; especially, paying attention to splenectomy. The aims of the present study were to evaluate how prolonged thrombocytopenia after LDLT can affect patient survival and to determine potential risk factors associated with persistent thrombocytopenia, focusing on a change of ADAMTS13 activity and on the influence of splenectomy.

Patients and methods

I. Comparison of postoperative platelet counts between LDLT and other operative procedures with or without splenectomy

Serial platelet counts were collected from the medical records in the adult patients with the following operative procedures (from March 2002 to July 2010): splenectomy with distal pancreatectomy for pancreatic tumors ($n = 20$), splenectomy in liver cirrhosis ($n = 42$), LDLT with splenectomy ($n = 35$), and LDLT without splenectomy ($n = 64$).

II. Factor analysis contributing to persistent thrombocytopenia after LDLT

We reviewed the LDLT database at Mie University Hospital, and consecutive 100 adult patients, who underwent LDLT at our institution between March 2002 and June 2011 were retrospectively analyzed to examine whether the changes in platelet counts after LDLT were associated with patient survival. Table 1 shows the patient characteristics and surgical parameters of the patients. The previous study [19] showed that the patients with low ($<100 \times 10^3/\mu L$) postoperative platelet counts after major hepatectomy had unfavorable postoperative liver function. Therefore, we defined the cutoff level of low platelet count as $<100 \times 10^3/\mu L$. The patients were divided into the low and high platelet count group (LP group and HP group) on the basis of the platelet counts on postoperative day (POD) 14.

ADAMTS13 activity and VWF antigen level in the plasma were measured from 65 consecutive adult recipients between 2002 and 2005 in our institution to determine the cause of thrombocytopenia after LDLT. We evaluated preoperative, intraoperative, and postoperative variables, including recipient and donor age, sex, Child-Pugh (C-P) score, model for end-stage liver disease (MELD) score, graft/recipient weight ratio (GRWR), platelet count, antithrombin (AT) level, ADAMTS13 activity and VWF antigen level, VWF/ADAMTS13 ratio, intraoperative portal venous pressure (PVP), splenectomy, cold ischemic time (CIT), warm ischemic time (WIT), blood loss, blood transfusion, total bilirubin (TB) level, prothrombin time international normalized ratio (PT-INR), and C-reactive protein (CRP) level, and analyzed the risk factors for prolonged thrombocytopenia. The primary regulator of platelet production is thrombopoietin (TPO) which is

Table 1 Background of the 100 adult LDLT recipients ($n = 100$)

Age (year old)	53.7 (20–70)
Gender (male female)	61/39
Etiology of liver disease	
Liver cirrhosis (HCC)	68 (39)
PBC	14
PSC	2
Fulminant hepatitis	11
Others	5
C-P score	9.6 (5–15)
MELD score	17.9 (6-45)
Donor age (year old)	38.0 (18–65)
Graft (right/left/post)	64/34/2
GRWR (%)	0.969 (0.441–1.571)
WIT (min)	48.7 (21–113)
CIT (rain)	108.9 (10–424)
Splenectomy	29 (29 %)
Blood loss (ml)	11115[a] (1426–74480)
Transfusion	
KBC (unit)	41[a] (4–213)
Platelet (unit)	40[a] (0–120)
FFP (unit)	40[a] (0–152)

HCC hepatocellular carcinoma, *PBC* primary biliary cirrhosis, *PSC* primary sclerosing cholangitis, *C-P* Child-Pugh, *MELD* modified end-stage liver disease, *GRWR* graft to recipient weight ratio, *WIT* warm ischemia time, *CIT* cold ischemia time, *RBC* red blood cells, *FFP* flesh frozen plasma

[a] Median

produced at a constant rate mainly in the liver (by hepatocytes). In the present study, therefore, the serum levels of TPO were additionally measured in 46 patients.

These recipients were divided into the following paired groups on the basis of the platelet counts on POD14 and splenectomy: the HP group with or without splenectomy and the LP group with or without splenectomy.

ADAMTS13 activity was measured using FRETS-VWF73, chemically synthesized by the Peptide Institute, Inc. (Osaka, Japan) according to the method described by Kokame et al. [20]. VWF antigen level was measured using an enzyme immunosorbent assay, IMUBIND VWF, according to the manufacturer's instructions (American Diagnostics Inc., CT, USA). Quantitative measurements of TPO levels were carried out by using the Thrombopoietin Human ELISA Kit (abcam, Cambridge Science Park, Cambridge, UK).

Statistical analysis

Statistical analyses were performed using SPSS version 19.0 (SPSS, Inc., Chicago, IL). The Kaplan–Meier estimator and log-rank test were used to evaluate the survival rate. The results for continuous variables are expressed as mean values and standard error. Categorical data were compared between the groups using a Chi-squared or Fisher's exact test. Student's t tests with the Bonferroni's correction were used to compare continuous variables between the two groups. Forward stepwise multiple regression analysis was used to identify independent risk factors for thrombocytopenia on POD14. A $p < 0.05$ was considered to be statistically significant.

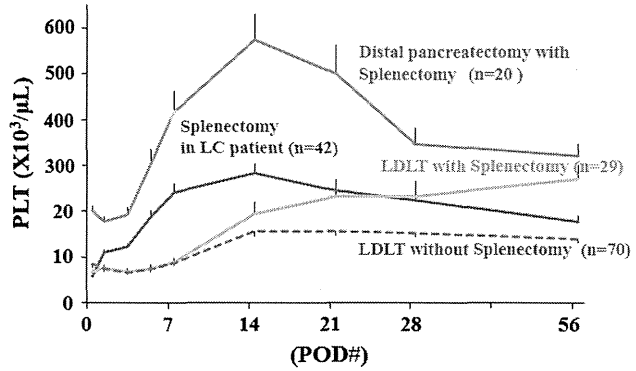

Fig. 1 Comparison of postoperative platelet counts between LDLT and other operative procedures with or without splenectomy (with standard *error bars*)

Results

I. Comparison of postoperative platelet counts after operation (distal pancreatectomy with splenectomy, splenectomy in LC patient, and LDLT with or without splenectomy)

The platelet counts in splenectomy with distal pancreatectomy and splenectomy in LC patients significantly and steadily increased until POD14 followed by gradual decrease (Fig. 1). On the other hand, the platelet counts in LDLT with or without splenectomy remained low until POD7 and increased on POD14. Thereafter, those in LDLT with splenectomy gradually and steadily increased until POD56, while those in LDLT without splenectomy remained the same levels as those on POD14. Based on these results, we focused on the platelet counts on POD14.

II. Factor analysis contributing to thrombocytopenia after LDLT

Overall survival after LDLT based on the platelet counts on POD14

The 100 patients were divided by platelet counts on POD14 into the HP group ($n = 62$), and the LP group

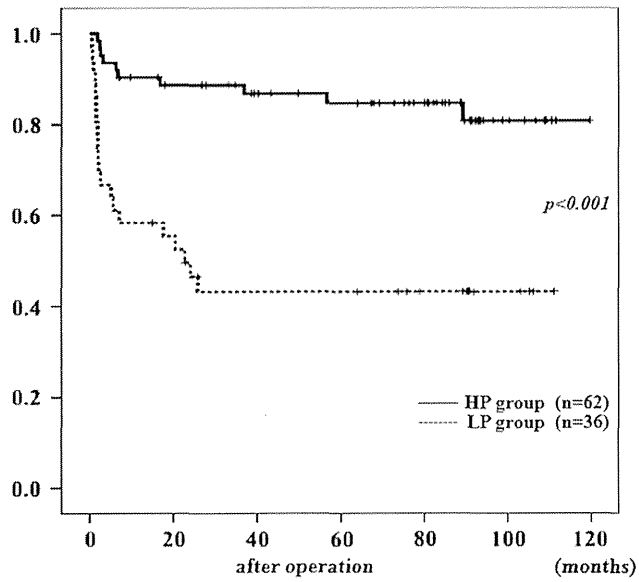

Fig. 2 Survival curves of the two groups classified according to platelet count on POD14

($n = 36$). Two patients died of pneumonia and hepatic infarction within POD14. The 6-, 12-, and 36-month survival rates in the LP group were significantly lower than those in the HP group (61.1, 58.3, and 43.2 % vs. 93.5, 90.3, and 88.6 %, respectively; $p < 0.001$) (Fig. 2). Causes of death within 6 months included sepsis ($n = 2$) in the HP group, and sepsis ($n = 8$), liver failure ($n = 2$), cerebral hemorrhage ($n = 2$), hepatic infarction ($n = 1$), gastrointestinal bleeding ($n = 1$), rupture of the splenic artery aneurysm ($n = 1$), and others ($n = 2$) in the LP group. Incidence of septic death was significantly higher in the LP group than in the HP group: 22.2 vs. 3.2 % ($p < 0.01$).

Postoperative complications in each group

Patients in the LP group suffered from complications more frequently than those in the HP group. Acute renal failure (ARF) occurred more frequently in the LP group ($n = 7$), comparing to those in the HP group ($n = 2$) ($p = 0.01$) (Table 2).

Patient characteristics of 65 patients in the two groups

In the patients LP group, CP and MELD score were higher, preoperative platelet counts and AT levels were lower, postoperative (measured on POD14) TB levels were higher, and postoperative PT-INR, AT levels and ADAMTS13 activity were lower than those in HP group (Table 3).

Table 2 Postoperative complications in LP and HP groups

	LP group ($n = 36$)	HP group ($n = 62$)	p value
Complication	15 (41.7 %)	12 (19.4 %)	**0.02**
Pneumonia	5	3	0.12
Sepsis	4	5	0.33
Biliary leakage	0	5	0.10
ARP	7	2	**0.01**
HAT	4	2	0.13

Bold values indicate statistically significant differences

ARF Acute renal failure (all recipients underwent renal replacement therapy), *HAT* Hepatic artery thrombosis

Changes in platelet count, TPO, ADAMTS13 activity, VWF antigen level, and VWF/ADAMTS13 ratio in LP and HP group

The platelet counts in the LP group were significantly lower than those in the HP group on POD 7, 14, and 28 ($p < 0.01$, $p < 0.01$, and $p < 0.01$, respectively). The platelet counts in the LP group remained lower than $100 \times 10^3/\mu L$ until POD28 (Fig. 3a). TPO levels were significantly higher in the LP group than in the HP group on preoperative day and POD 14 ($p < 0.01$ and $p < 0.01$, respectively). On POD28, however, TPO levels in the LP groups were significantly decreased, showing the levels similar to the HP group (Fig. 3b).

ADAMTS13 activity significantly decreased on POD1 in both groups compared with the preoperative levels. ADAMTS13 activity increased gradually in the HP group. In contrast, the activity in the LP group did not show any increase until POD28. ADAMTS13 activity in the LP group was significantly lower than those in the HP group on POD 14, and 28 ($p < 0.01$, and $p < 0.01$, respectively) (Fig. 4a).

VWF antigen level significantly decreased on POD1 in both groups as compared to the preoperative levels. Thereafter, those in both groups increased around the preoperative levels on POD7. There were no significant differences between two groups (Fig. 4b).

The VWF/ADAMTS13 ratio in the LP group was higher than that in the HP group, although there were no statistical differences between the two groups by t test with the Bonferroni's correction (rough p value on POD7, 14, and 28; $p = 0.04$, $p = 0.04$, $p = 0.03$, respectively) (Fig. 4c).

Risk factors for postoperative thrombocytopenia on POD 14

In univariate analysis, the risk factors for postoperative thrombocytopenia on POD14 were increased C-P and MELD scores, decreased preoperative platelet counts and

Table 3 Patient Characteristics (HP and LP group)

	AH ($n = 65$)	HP group ($n = 42$)	LP group ($n = 23$)	p
Preoperative factor		Mean ± S.D.		
Age (year)	53.09 ± 10.44	52.36 ± 11.71	54.39 ± 7.98	0.461
Gender (male)	39 (60 0 %)	27 (64.37.)	12 (52.2 %)	0.198
C-P score	**9.65 ± 2.58**	**8.69 + 2.32**	**11.17 ± 2.04**	**<0.001**
MELD score	**18.30 ± 9.57**	**15.60 ± 7.86**	**22.22 ± 10.00**	**0.009**
GRWR(K)	1.02 ± 0.19	1.04 ± 0.19	0.97 ± 0.18	0.168
Platelet	75.47 ± 61.13	82.69 ± 58.84	64.17 ± 65 34	0.248
AT (%)	**51.33 + 25.19**	**58.17 + 25.50**	**39.22 + 20.41**	**0.003**
ADAMTS13 (%)	69.09 ± 36.96	75.84 ± 37.47	59.24 ± 32.94	0.080
vWF (%)	285.35 ± 156.22	275.26 ± 150.77	309.30 ± 167.97	0.406
vWFJADAMTSO	6.77 ± 8.57	5 56 ± 5.001	872 ± 12.73	0.158
Intraoperative factor				
Splenectomy	14/65 (21.5 %)	9/42 (21.4 %)	5/23 (217 %)	0.606
OIT (mm)	110.34173.82	106 56 ± 54.88	120.17 ± 100.09	0.551
WIT (mm)	46.78 ± 16.31	48.76 ± 18.09	43.13 ± 12.53	0.191
Blood loss (mL)	16370 + 15363	17122 + 17822	15379 + 9987	0615
Transfusion				
RBO (unit)	43.02 ± 40.44	43.26 ± 45.42	43.30 ± 31.20	0.997
FFP (unit)	37.33 + 34.58	38.33 + 36.94	36.26 + 31.20	0.820
Platelet (unit)	33 56 + 27 04	32 26 + 29 64	3478 + 22.08	0722
PVP (mmHg)	18.92 + 5.05	18 62 + 4.72	19.57 + 570	0.481
Postoperative factor (POD14)				
TB (mg/dD)	**5.63 ± 6.93**	**3.38 + 4.92**	**9.65 ± 8.18**	**0.002**
PT-INR	**1.18 + 0.27**	**1.09 + 0.15**	**1.35 + 0.36**	**0.003**
CRP	4.40 ± 4.65	3.95 ± 4.38	5.61 ± 5.21	0.192
AT (%)	**81.49 ± 22.11**	**89.54 ± 14.48**	**67.49 ± 26.13**	**0.001**
ADAMTS13 (%)	**33.34 ± 21.03**	**39.94 ± 21.22**	**21.73 ± 15.09**	**0.001**
vWF (%)	285.35 ± 156.22	343.40 ± 100.96	336.03 ± 125.66	0.802
vWF/ADAMTS13	23.31 ± 38.47	15.99 ± 21.50	35.87 ± 55.44	0.129

Bold values indicate statistically significant differences

C-P Child-Pugh score, *MELD* modified stage liver disease, *GRWR* graft to recipient weight ratio, *AT* antithrombin, *ADAMTS13* a disintegrin and metalloproteinase with a thrombospondin type 1 motifs 13, *vWF* von Willebrand Factor, *CIT* cold ischemia time, *WIT* warm ischemia time, *RBC* red blood cell, *FFP* fresh-frozen plasma, *PVP* portal venous pressure, *TB* total bilirubin, *PT-INR* prothrombin time international normalized ratio, *CRP* C-reactive protein

AT levels, not undergoing splenectomy, increased (measured on POD14) TB levels and PT-INR, and decreased (measured on POD14) ADAMTS13 activity and AT levels (Table 4).

Stepwise multiple regression analysis revealed that decreased preoperative AT levels and decreased (measured on POD14) ADAMTS13 activity were independent risk factors for postoperative thrombocytopenia on POD14 (preoperative AT levels; regression coefficient 0.417; $p = 0.006$, ADAMTS13 activity on POD14; regression coefficient 0.331; $p = 0.011$).

Correlation among ADAMTS13, platelet counts, TPO, and liver function

In assessment of associations with TB levels and PT-INR as the graft function on POD14, platelet counts and ADAMTS13 activity showed a significant negative correlation to TB levels (Fig. 5a, b), but not to PT-INR (Fig. 5d, e). There were no significant correlations between TPO levels and TB levels or PT-INR (Fig. 5c, f).

Changes in platelet counts and TPO levels in LP and HP group with or without splenectomy

In the HP group, platelet counts were significantly increased after LDLT regardless of splenectomy, showing no significant differences between the patients with and without splenectomy within POD14, but showing significantly higher in those with splenectomy on POD28. TPO levels were not significantly increased after LDLT regardless of splenectomy, showing no significant differences between the patients with and without splenectomy. On the other hand, in the LP group, platelet counts were not increased after LDLT regardless of splenectomy, remaining low levels in both patients until POD28. Since TPO levels were measured in only few patients with splenectomy, we mention the data in those without splenectomy: