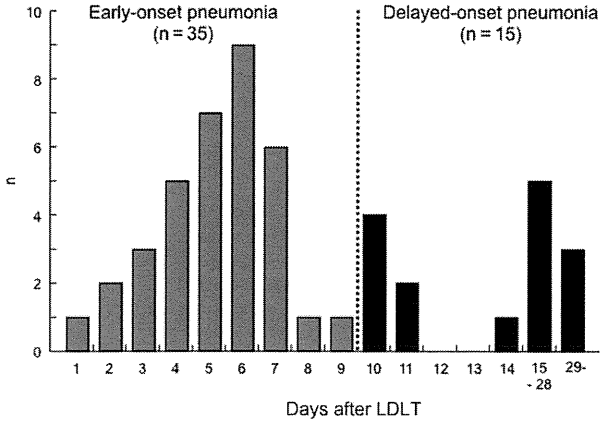

Figure 2. Time to the first episode of bacterial pneumonia. The pneumonia patients were divided into 2 groups: early-onset pneumonia (n = 35) and delayed-onset pneumonia (n = 15).

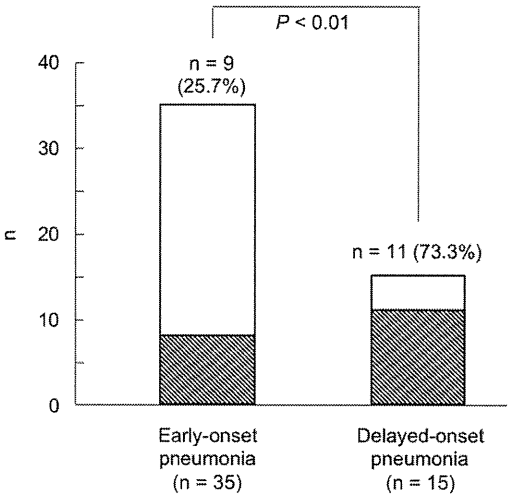

Figure 3. Early graft loss in patients with early-onset or delayed-onset bacterial pneumonia. The shaded portion of each bar shows the number of patients who experienced early graft loss.

the anterior segment of a right lobe graft (n = 1), portal vein thrombosis (n = 1), and prolonged mechanical ventilation due to pulmonary hypertension (n = 1).

Susceptibilities of Gram-Negative Bacteria Causing Pneumonia to Antibiotic Regimens

The susceptibility of gram-negative bacteria in patients with pneumonia (n = 42) is summarized in Fig. 4. The gram-negative bacteria had low susceptibility to cefazolin (CAZ; 5.3%) and ABPC/SBT (17.9%) and moderate susceptibility to cefepime (CFPM; 59.4%), piperacillin (PIPC)/tazobactam (TAZ; 61.5%), MEPM (66.7%), and GM (66.7%). The gram-negative bacteria were more susceptible to levofloxacin (LVFX; 74.4%) and combinations of broad-spectrum antibiotics with GM (CFPM and GM, 72.7%; PIPC/TAZ and GM, 74.4%; MEPM and GM, 74.4%; and LVFX and GM, 76.9%). *Stenotrophomonas maltophilia* (n = 10) accounted for 23.8% of the gram-negative bacteria responsible for pneumonia, and it showed high resistance to broad-spectrum antibiotics (except for minocycline hydrochloride). LVFX and combinations of broad-spectrum antibiotics with GM provided cover for almost all gram-negative bacteria except *S. maltophilia*.

DISCUSSION

In a 1996 study of patients who underwent DDLT, Singh et al.[6] reported that 14.8% of the patients experienced pneumonia with a mortality rate of 53% after transplantation. In contrast, Weiss et al.[3] reported that 15.5% of patients developed pneumonia within 6 days after transplantation, but the mortality rate was much lower: 21.7%. The mortality rate of patients with early-onset pneumonia was 25.7% in our study of patients after LDLT and was thus similar to the rate reported by Weiss et al. The mortality rate of patients with delayed-onset pneumonia, however, was quite high in our study: 73.3%. The precise impact of graft dysfunction on delayed-onset pneumonia is difficult to evaluate because both events could affect or cause each other.[10]

Only 1 study has investigated the prevalence of postoperative bacterial pneumonia in LDLT. Saner et al.[5] analyzed 55 LDLT recipients with a mean MELD score of 14.2 and found that 18.2% of the patients experienced pneumonia with a low 1-year survival rate of 42%. They concluded that the high mortality rate might be due to the longer warm ischemia time and the smaller graft size. Although GRWR was not described in their report, our series used small LDLT grafts (mean GRWR = 0.81%) and had the same warm ischemia time (39 minutes), but the mean MELD score was 17.4. The main difference between the Essen series[5] and our series is that the Essen series included more patients with alcoholic cirrhosis (21.8% versus 4%) and had fewer patients (55 versus 346); this suggests some learning curve effect.

Risk factors for developing pneumonia after LDLT included diabetes, pretransplant UNOS status 1 or 2A, and massive operative blood loss. A negative impact of diabetes on various infections has been reported within the context of abnormal neutrophil function (particularly impaired chemotaxis, phagocytosis, and bacterial killing).[16] Kornum et al.[17] performed a case-control study and showed that well controlled diabetes (hemoglobin A1c < 7%) and poorly controlled diabetes (hemoglobin A1c > 9%) were associated with increased risks of pneumonia (22% and 60%, respectively).

The deterioration of the patient's general status (ie, UNOS status 1 or 2A) is largely associated with poor short-term graft outcomes, as previously reported for DDLT.[18] Even for LDLT, it has been reported that both the disease severity and the general condition have a great impact on short-term graft survival,

TABLE 3. Characteristics of Patients With Early-Onset Pneumonia and Patients With Delayed-Onset Pneumonia

	Posttransplant Pneumonia		
	Early Onset (n = 35)	Delayed Onset (n = 15)	*P* Value
Recipient factors			
Male sex [n (%)]	16 (45.7)	7 (46.7)	0.95
Age (years)*	54.9 ± 10.6	49.1 ± 8.7	0.07
Child class C [n (%)]	7 (20.0)	9 (60.0)	<0.01
MELD score*	18.2 ± 7.6	18.3 ± 8.5	0.96
UNOS status 1 or 2A [n (%)]	12 (34.3)	14 (93.3)	<0.01
Acute liver failure [n (%)]	4 (11.4)	4 (26.7)	0.18
Major shunt vessels [n (%)]	19 (54.3)	7 (46.7)	0.62
Diabetes [n (%)]	14 (40.0)	4 (26.7)	0.37
Donor factors			
Male sex [n (%)]	26 (74.3)	8 (53.3)	0.15
Donor age (years)*	36.6 ± 11.8	42.3 ± 13.5	0.14
Left lobe graft [n (%)]	22 (62.9)	7 (46.7)	0.29
GV/SLV (%)*	42.1 ± 9.7	44.3 ± 7.0	0.42
GRWR (%)*	0.81 ±0.22	0.82 ± 0.17	0.93
Recipient surgery			
Cold ischemia time (minutes)*	78 ± 44	103 ± 47	0.07
Warm ischemia time (minutes)*	36 ± 9.4	45 ± 11	0.01
Hepatic artery flow (mL/minute)*	88 ± 57	125 ± 42	0.03
PVF/GV ratio (mL/100 g)*	378 ± 140	229 ± 80.5	<0.01
PVP at closure (mm Hg)*	16.1 ± 3.5	20.4 ± 6.9	<0.01
Splenectomy [n (%)]	18 (51.4)	7 (46.7)	0.76
Duct to duct [n (%)]	29 (82.9)	10 (66.7)	0.20
Operative time (minutes)*	803 ± 144	873 ± 210	0.18
Operative blood loss (L)*	6.6 ± 7.5	13.5 ± 12.8	0.02
Transfused red blood cells (U)*	14 ± 9	40 ± 30	<0.01
Transfused frozen plasma (U)*	20 ± 12	40 ± 28	<0.01
Postoperative factors			
Hepatic artery thrombosis [n (%)]	2 (5.7)	1 (6.7)	0.89
Portal vein thrombosis [n (%)]	0 (0.0)	1 (6.7)	0.12
Acute cellular rejection [n (%)]	4 (11.4)	1 (6.7)	0.61
Total bilirubin on day 14 (mg/dL)*	10.1 ± 8.3	17.9 ± 11.9	0.01
Ascites output on day 14 (L/day)*	0.67 ± 0.78	0.99 ± 1.2	0.27
Prothrombin time/international normalized ratio on day 14*	1.2 ± 0.2	1.6 ± 0.2	< 0.01

*The data are presented as means and standard deviations.

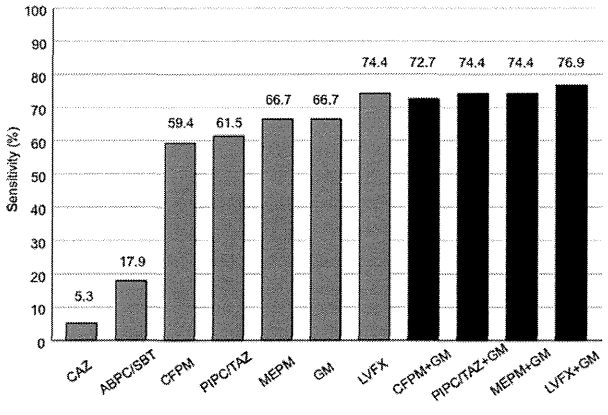

Figure 4. Susceptibility of gram-negative bacteria (n = 42). *Stenotrophomonas maltophilia* [n = 10 (23.8%)] was highly resistant to the commonly used broad-spectrum antibiotics.

although the impact of the MELD system on post-LDLT outcomes has been denied or is still under discussion.[19-21] In the current analysis, delayed-onset pneumonia was largely associated with poor graft dysfunction (whether the cause was primary or secondary). The prevalence of ventilator-associated nosocomial organisms, including *S. maltophilia* in the current series, could be attributed to the fact that secondary delayed-onset pneumonia was preceded by poorly functioning grafts. The duration of mechanical ventilation was significantly prolonged in patients with pneumonia caused by *S. maltophilia* versus patients with pneumonia caused by other types of bacteria.

Dysfunctional immunity caused by massive blood loss and resulting massive transfusions has also been reported.[22,23] Shorr et al.[22] reported a transfusion volume–dependent increase in ventilator-associated pneumonia in trauma patients. Meanwhile, Bernard

et al.[23] reported that packed red blood cell transfusions of 1, 2, or 10 U increased the risk of septic shock with odds ratios of 1.29, 1.53, and 2.29, respectively, in general surgery patients.

In our series of patients, gram-negative bacteria were the most common causative pathogens (84%), as compared with the earlier reports in DDLT. In patients with delayed-onset pneumonia, almost all of the bacteria responsible for pneumonia were gram-negative bacteria (93%). The most common bacteria isolated in this study were *P. aeruginosa* and *S. maltophilia*; the latter is an emerging and clinically significant causative pathogen for posttransplant infections. It is an aerobe gram-negative bacterium and is a relatively rare human pathogen.[24-26] However, this species is highly resistant to antibiotics because of its overproduction of β-lactamase and its high mutation rate.[24-26] It has been suggested that high-dose sulfamethoxazole/trimethoprim may be the only effective treatment for this species.[24-26] In the current series, the susceptibility of *S. maltophilia* to common antibiotics was as follows: 0% for CAZ, SBT/ABPC, CFPM, PIPC/TAZ, MEPM and sulfamethoxazole/trimethoprim; 10% for GM; 20% for LVFX; and 90% for minocycline hydrochloride.

Although it has been suggested that sulfamethoxazole/trimethoprim is the most effective treatment for *S. maltophilia*, our results do not support this. Instead, we found particularly high susceptibility to minocycline hydrochloride. Nevertheless, it is also true that a high sensitivity of *S. maltophilia* to minocycline hydrochloride has been demonstrated only in vitro and not in vivo.[24-26] Antimicrobial regimens such as LVFX, CFPM and GM, TAZ/PIPC and GM, and MEPM and GM are appropriate for posttransplant pneumonia because they cover most gram-negative bacteria, including *P. aeruginosa* (but not *S. maltophilia*). Further studies are necessary to better optimize the treatment of bacterial pneumonia after LDLT.

In conclusion, bacterial pneumonia (particularly delayed-onset pneumonia) is the most serious type of infection after LDLT. Risk factors for bacterial pneumonia include diabetes, the deterioration of the patient's general condition at the time of transplantation, and massive blood loss during surgery. Delayed-onset pneumonia and the emergence of *S. maltophilia* are major issues that need to be addressed.

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En Bloc Stapling Division of the Gastroesophageal Vessels Controlling Portal Hemodynamic Status in Living Donor Liver Transplantation

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Gastroesophageal shunts are commonly seen in patients with terminal liver disease requiring liver transplantation.[1] These shunts cause increased portal pressure in the gastroesophageal varices, increasing the risk of rupture and also allowing hepatofugal portal flow, which causes graft hypoperfusion and dysfunction after living donor liver transplantation (LDLT).[2,3] However, isolation and division of the vessels is difficult to achieve because of their anatomic properties. Moreover, obstruction of the shunt vessels may cause excessively high portal pressure, resulting in small-for-size graft dysfunction.[4] We describe a safe and rational technique for dividing the gastroesophageal hepatofugal shunts and left gastric arteries en bloc using end-stapling devices. Using this method, we can eradiate the shunts without increasing portal pressure.

METHODS

Indications for en bloc division of gastroesophageal vessels with major hepatofugal shunts include the larger caliber (>1 cm) vessels. In cases in which the left hepatic artery is replaced with a vessel originating from the left gastric artery, the technique is postponed until the graft arterialization is completed from other arterial sources in the recipient. Portal venous pressure is continuously monitored during LDLT surgery using a cannula (Medicut LCV-UK catheter 14G, Nippon Sherwood Inc) inserted into the superior mesenteric vein via a terminal jejunal vein.

We perform en bloc stapling division of the gastroesophageal vessels including huge hepatofugal shunts and the left gastric arterial systems as follows. After reperfusion of the graft, splenectomy is performed using a vessel-sealing system (LigaSure Atlas, Valleylab Inc) and endo-stapling devices (Echelon Flex Endopath Staplers 60–2.5, Ethicon Endo-Surgery Inc) to decrease the portal pressure, as previously described.[5] The gastrocolic and gastrosplenic ligaments are completely divided during splenectomy using the vessel-sealing system. Division of the left gastric ligament is started only when arterial reconstruction is complete because the gastric arterial system might be used for hepatic artery reconstruction.

The greater curvature of the stomach is manually lifted, the endo-stapling devices are applied to the base of the left gastric ligament including the left gastric artery, engorged coronary vein, and collateral vessels (Fig. 1A). The left gastric ligament is then divided en bloc using endo-stapling devices (Fig. 1B). Before the staples are fired, the esophagogastric junction, in which a nasogastric tube has been inserted, is manually palpated to prevent possibly injuring the esophagus. The tip of the endo-stapling device should be pointed vertically to the crus muscle for this purpose. Two sessions of this maneuver might be necessary to divide the ligaments, including the tortuous shunt vessels, therefore exposing the diaphragmatic crus. After division of the ligament, the stapled stump is mass-sutured using continuous 3–0 Prolene sutures with an SH needle (Ethicon Inc) to prevent postsurgical bleeding and occlude the retroperitoneal collateral veins. The stapled stump is also mass-sutured on the stomach side using the same sutures.

RESULTS

Between January 2011 and January 2012, 40 LDLTs were performed at Kyushu University Hospital. Among these cases, stapling division of the gastroesophageal vessels to control portal hemodynamic status was performed in 13 patients (32.5%) with simultaneous splenectomy. The mean Model for End-Stage Liver Disease (MELD) score of these patients was 15.5. ± 4.4. The endo-stapling devices were applied safely in all of the patients (Fig. 2A) without significant blood loss (Fig. 2B). The gastroesophageal shunt vessels (Fig. 2C) were successfully obstructed using

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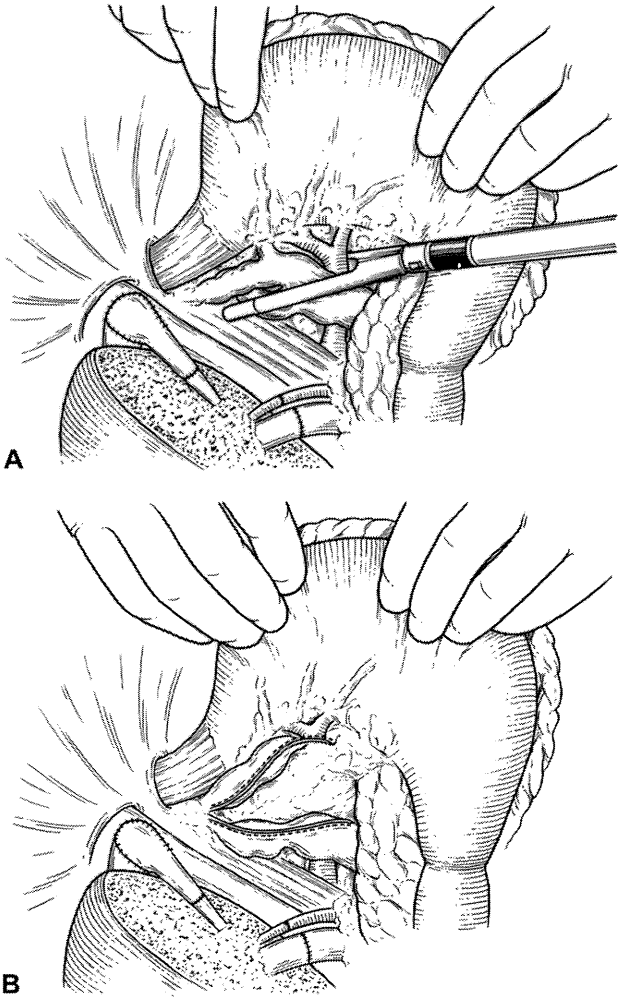

Figure 1. (A) The en bloc endo-stapling device was applied at the base of the left gastric ligament including the left gastric arterial system and the gastroesophageal shunts. (B) The left gastric ligament was divided en bloc.

this approach (Fig. 2D) in all of the patients. Although the left hepatic artery was replaced with a branch from the left gastric artery in 4 patients, our technique could be applied after graft arterial reconstruction using the right hepatic arteries.

The portal pressure decreased from 18.8 ± 5.6 mmHg to 17.4 ± 4.2 mmHg ($p = 0.02$, paired t-test) after stapling division of the gastroesophageal shunt vessels. There was no significant change in portal flow (1.65 ± 0.51 L/min vs 1.73 ± 0.60 L/min, $p = 0.79$, paired t-test) after division of the gastroesophageal shunt vessels. We did not perform pyloroplasty in this series because of the risk of postoperative complications including bleeding or leakage. However, no apparent gastroparesis or gastric stasis was observed.

DISCUSSION

Gastroesophageal shunt vessels are commonly seen in patients with terminal liver disease who undergo liver transplantation. However, surgical isolation and ligation of the shunts in LDLT is a technically difficult procedure often associated with massive bleeding. This en bloc procedure may also increase portal pressure, resulting in graft dysfunction.

Although gastroesophageal shunt vessels in terminal liver disease are derived from coronary or left gastric veins, their appearances differ greatly.[1] Gastroesophageal shunts are usually multiple in number, are coiled or tortuous in shape, engorged with a thin wall, and are buried in the retroperitoneum on the diaphragmatic crus. Therefore, manual isolation and ligation of such vessels is technically very difficult and may cause massive bleeding. In contrast, en bloc division of such vessels using endo-stapling devices is much safer, and does not require dissection or tying. In 1998, Hashizume and colleagues[6] first reported en bloc division of upper gastric vessels and splenectomy using endo-stapling devices under laparoscopy for patients with portal hypertension. Once the left gastric ligament is divided in two with the stapling devices, the retroperitoneal or gastric varices are easily mass-sutured for occlusion under a broad surgical field. Such mass-sutures are also useful for reinforcing the stapled stumps to prevent later bleeding or oozing. Moreover, stapling division followed by suturing could eliminate the multiple shunts; the isolation technique cannot.

Increases in portal pressure are also associated with isolated ligation of gastroesophageal shunt vessels.[4] It is well known that increased portal pressure is a significant cause of graft dysfunction in LDLT, and is characterized by prolonged cholestasis and intractable ascites. Therefore, to safely obstruct the portosystemic shunt vessels, the splanchnic or portal inflow should be controlled.[7] By simultaneous en bloc division of the left gastric arteries and the gastroesophageal shunts using endo-stapling devices, the portal pressure can be controlled. We previously reported splenic artery ligation in 2004[8] and splenectomy to control portal pressure in 2008.[9] The technique described here represents a third approach to controlling portal inflow in LDLT.

Although gastroesophageal varices or other portosystemic shunts might be improved during the years after LDLT, they might be causes of insufficient portal inflows into the transplanted grafts.[3] Therefore, we indicate the stapling division of the gastroesophageal vessels as major hepatofugal shunts including the larger caliber (>1 cm) vessels. Currently, we indicate the technique regardless of the portal pressure after reperfusion in LDLT. For other

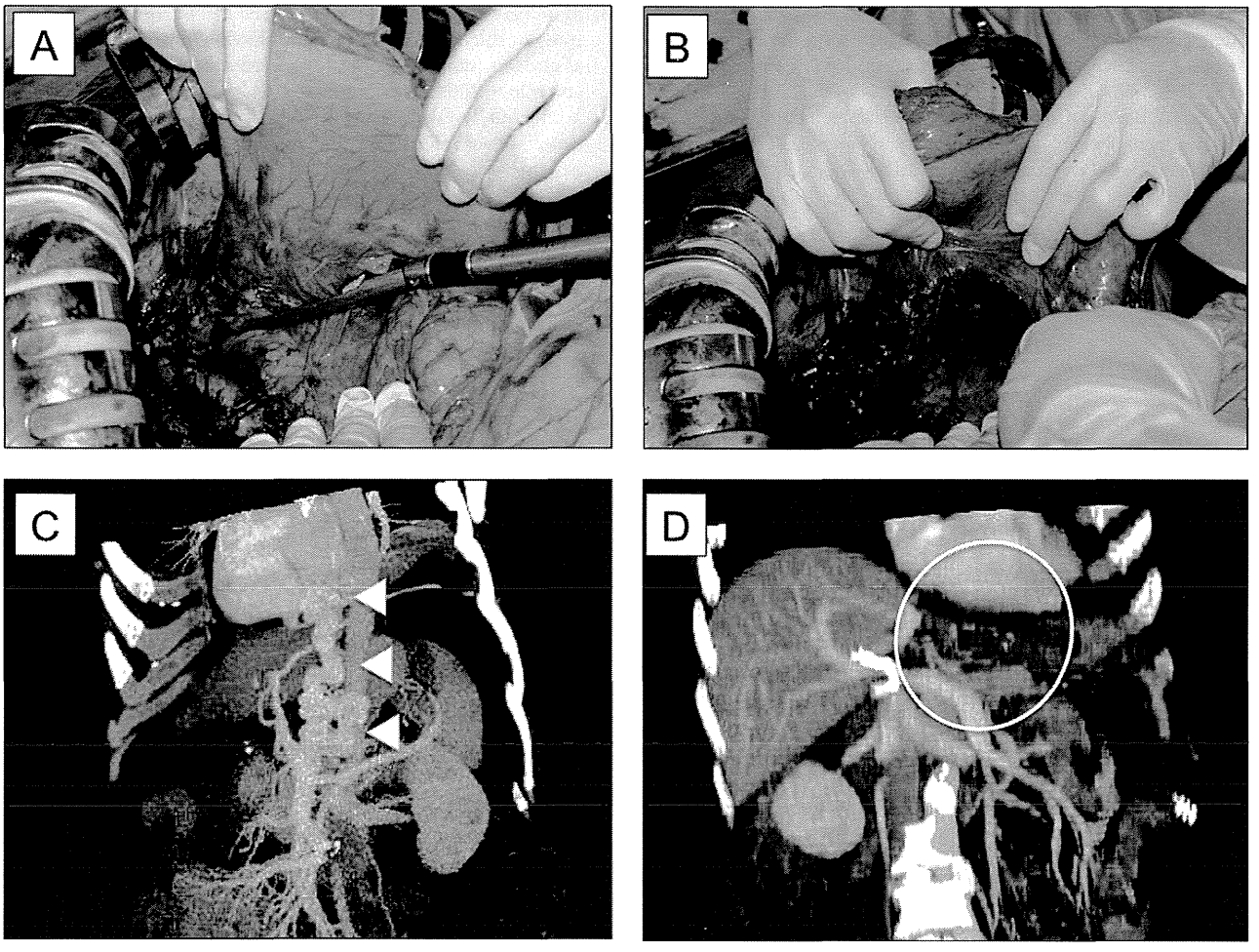

Figure 2. (A) Application and (B) firing of the endo-stapling devices. (C, white arrowheads) The major gastroesophageal shunt vessels (D, white circle) were eradicated, as confirmed by CT with 3-dimensional reconstruction.

types of major portosystemic shunts, including sprenorenal shunts and mesocaval shunts, we obstruct all such vessels during LDLT, simplifying the portal circulation system, because of the early experiences with portal steal phenomena, as Lee and associates[3] reported.

CONCLUSIONS

En bloc stapling division of the gastroesophageal vessels is a safe and rational technique in obstructing gastroesophageal hepatofugal shunts without increasing the portal pressure in LDLT.

Author Contributions

Study conception and design: Ikegami, Yoshizumi, Soejima
Acquisition of data: Yoshizumi, Yoshiya, Toshima, Motomura, Uchiyama
Analysis and interpretation of data: Ikegami, Yoshizumi, Soejima
Drafting of manuscript: Ikegami
Critical revision: Shirabe, Maehara

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Primary Graft Dysfunction After Living Donor Liver Transplantation Is Characterized by Delayed Functional Hyperbilirubinemia

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The purpose of this study is to propose a new concept of primary graft dysfunction (PGD) after living donor liver transplantation (LDLT), characterized by delayed functional hyperbilirubinemia (DFH) and a high early graft mortality rate. A total of 210 adult-to-adult LDLT grafts without anatomical, immunological or hepatitis-related issues were included. All of the grafts with early mortality (n = 13) caused by PGD in LDLT had maximum total bilirubin levels >20 mg/dL after postoperative day 7 ($p < 0.001$). No other factors, including prothrombin time, ammonia level or ascites output after surgery were associated with early mortality. Thus, DFH of >20 mg/dL for >seven consecutive days occurring after postoperative day 7 (DFH-20) was used to characterize PGD. DFH-20 showed high sensitivity (100%) and specificity (95.4%) for PGD with early mortality. Among the grafts with DFH-20 (n = 22), those with early mortality (n = 13) showed coagulopathy (PT-INR > 2), compared with those without mortality ($p = 0.002$). Pathological findings in the grafts with DFH-20 included hepatocyte ballooning and cholestasis, which were particularly prominent in the centrilobular zone. PGD after LDLT is associated with DFH-20 caused by graft, recipient and surgical factors, and increases the risk of early graft mortality.

Key words: Donor age, graft dysfunction, hyperbilirubinemia, living donor liver transplantation, small-for-size

Abbreviations: DDLT, deceased donor liver transplantation; DFH, delayed functional hyperbilirubinemia; GRWR, graft recipient weight ratio; GV, graft volume; GW, graft weight; LDLT, living donor liver transplantation; MELD, model for end-stage liver disease; PGD, primary graft dysfunction; PNF, primary graft nonfunction; POD, postoperative day; PT-INR, prothrombin time international normalized ratio; PVF, portal venous flow; PVP, portal venous pressure; ROC, receiver operating characteristic curve; SLV, standard liver volume; T.Bil, total bilirubin.

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Introduction

In deceased donor liver transplantation (DDLT), primary graft nonfunction (PNF) is one of the most serious and life-threatening conditions in the immediate postoperative period (1–3). PNF has been attributed to graft steatosis, prolonged cold ischemic time and advanced donor age (1,2). Unfortunately, PNF is usually irreversible, which means that early retransplantation is still the only treatment option (3).

In living donor liver transplantation (LDLT), the clinical characteristics of functional graft failure, hereafter referred to as primary graft dysfunction (PGD), are expected to be very different from those of PNF after DDLT because of differences in graft quality, size and preservation time (4,5). In LDLT, a qualified liver graft is usually obtained after exhaustive donor selection processes, and is transplanted after a very short cold ischemic time (4–7). However, LDLT grafts are always small-sized liver grafts, which may not be sufficient for the recipient's requirements (8,9). Therefore, in the mid-2000s, many institutes started to recommend the use of larger grafts to prevent "small-for-size syndrome" (SFSS), which might be a typical presentation of PGD after LDLT, and is characterized by prolonged cholestasis and increased ascites volume, with reduced graft survival (6–10).

Nevertheless, transplantation centers are also considering smaller grafts, with improved outcomes under refined and established surgical techniques (11–14). Indeed, recent studies have documented that small grafts do not necessarily cause or correspond to PGD, which is attributed to multiple factors including disease severity, portal pressure, graft regeneration and donor age (15). Therefore, the term SFSS is now unsuitable to refer to delayed PGD occurring after LDLT because its characteristics differ from those of PNF after DDLT.

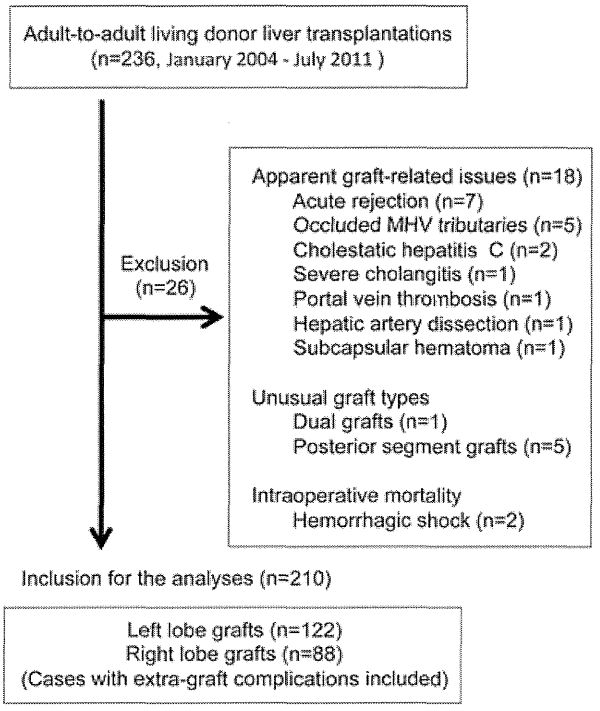

Figure 1: Twenty-six cases were excluded and 210 cases were included for the analyses. MHV = middle hepatic vein.

Therefore, the aim of this study was to characterize delayed PGD occurring after LDLT. We also sought to identify the factors associated with these disease processes and examine the pathological findings.

Materials and Methods

Patients

Between January 2004 and July 2011, a total of 236 adult-to-adult LDLTs were performed at Kyushu University Hospital, Fukuoka, Japan. Cases that underwent complex LDLT procedures using dual grafts (n = 1) or posterior segment grafts (n = 5), cases that died during surgery (n = 2) and cases with graft dysfunction caused by technical, immunological or recurrent hepatitis-related issues within 1 month of surgery (total, n = 18; acute rejection, n = 7; occlusion of the reconstructed middle hepatic vein tributaries, n = 5; cholestatic hepatitis C, n = 2; cholangitis, n = 1; portal vein thrombosis, n = 1; hepatic artery dissection, n = 1; and graft subcapsular hematoma because of percutaneous transhepatic cholangiogram, n = 1) were excluded from this study (Table S1). Cases with infectious complications (e.g. primary sepsis, pneumonia or spontaneous peritonitis) were not excluded in this analysis, because these complications overlapped with graft-oriented hepatic dysfunction in the same time in many cases, and it is difficult to delineate the cause and effect, as graft insufficiency could contribute to these infectious issues (15,16). Thus, 210 cases were included in this study (Figure 1). All of the LDLTs were performed after obtaining full informed consents from all patients and approval by the Liver Transplantation Committee of Kyushu University. The mean follow-up time was 3.4 ± 2.3 years.

Graft selection process

Grafts were selected as previously described (17). Left lobe grafts were considered to be the primary graft type if the desired graft volume (GV)/standard liver volume (SLV) is >35%. Right lobe grafts were considered if the simulated GV/SLV of the left lobe graft was <35% and the donor's remnant liver volume was >35%. Major middle hepatic vein tributaries >5 mm were maximally reconstructed to maintain uncongested GV/SLV >40% in right lobe grafts.

Surgical procedures

The donor parenchymal transection was performed using the Cavitron Ultrasonic Surgical Aspirator (CUSA™, Valleylab Inc., Boulder, CO, USA) and a saline-linked radio-frequency dissecting sealer (Tissuelink™, Tissuelink Medical Inc., Dover, DE, USA) with the hanging maneuver (18). After donor hepatectomy, the graft was perfused, weighted and stored in University of Wisconsin solution (Viaspan ™, DuPont Inc., Wilmington, DE, USA). For right lobe grafts, the middle hepatic vein tributaries were reconstructed on the back table using the explanted portal vein or other vessels procured from the recipient (19). After recipient hepatectomy, the grafts were transplanted in a piggyback fashion. The orifice of the recipient hepatic vein was enlarged with an incision on the vena cava for the venous anastomosis to provide sufficient outflow. The venous anastomoses were performed using continuous 5–0 PDS-II™ sutures (Ethicon Inc., Somerville, NJ, USA). Reconstruction of the portal vein with continuous 6–0 PDS-II™ sutures was followed by reperfusion. Arterial reconstruction was performed using interrupted 8–0 Prolene™ sutures (Ethicon Inc.), under microscope. Bile duct reconstruction was performed by duct-to-duct biliary anastomosis or by hepaticojejunostomy using interrupted 6–0 PDS-II™ sutures.

Measurement of arterial and portal hemodynamics properties

Portal venous pressure (PVP) was continuously monitored during LT surgery using a cannula (Medicut LCV-UK catheter 14G™, Nippon Sherwood Inc., Tokyo, Japan) placed in the superior mesenteric vein via a terminal jejunal vein by direct cut down. Intraoperative blood flow was measured in the recipients after reperfusion using an ultrasonic transit time flow meter (Transonic System™, Ithaca, NY, USA) in the recipients after reperfusion. Hepatic arterial flow is expressed as mL/min and portal venous flow is expressed as L/min.

Immunosuppression

The basic immunosuppression protocol consisted of tacrolimus or cyclosporine with mycophenolate mofetil and steroids. The target tacrolimus level was 10–15 ng/mL in the first month after LDLT month and was decreased to 5–10 ng/mL over the next few months. The target cyclosporine level was 200–250 ng/mL in the first month after LDLT and was decreased to 100 to 200 ng/mL over the next few months. One gram of methylprednisolone was given after reperfusion, and decreased from 200 mg to 20 mg daily over 1 week, then switched to oral prednisolone, tapered off at 3 months.

Clinical laboratory and ascites data

The serum total bilirubin (T.Bil) level, prothrombin time-international normalized ratio (PT-INR) and ammonia level were determined daily after LDLT and were analyzed in this study. The amount of ascites drained via the indwelling abdominal drains was also recorded. Prolonged coagulation profile (PT-INR > 1.8) was corrected by giving fresh frozen plasma. Fluid loss because of drainage of the ascites was corrected using intravenous 5% albumin solution to maintain circulatory stability and urinary output.

PGD and delayed functional hyperbilirubinemia (DFH)

PGD was defined as graft insufficiency with possible early graft loss, without technical, anatomical, immunological or hepatitis-related issues. DFH was

Table 1: Patient demographics

Variables	Total (n = 210)	Early graft loss		p-Value
		Yes (n = 13)	No (n = 197)	
Recipient age (years)	53.9 ± 10.5 (55, 18–73)	50.4 ± 10.9 (52, 21–64)	54.1 ± 10.5 (55, 18–73)	0.230
Recipient gender, male	102 (48.6)	5 (38.4)	97 (49.2)	0.451
Diseases				
Acute liver failure	16 (7.6)	1 (7.7)	15 (7.6)	0.748
Cholestatic cirrhosis	35 (16.7)	2 (15.4)	33 (16.8)	
Postnecrotic cirrhosis	154 (73.3)	9 (69.2)	145 (73.6)	
Others	4 (1.9)	0 (0.0)	4 (2.0)	
Hepatocellular carcinoma	112 (53.3)	3 (23.1)	109 (55.3)	0.024
Child-Pugh class				
A	10 (5.2)	0 (0.0)	10 (5.5)	0.380
B	56 (29.0)	2 (16.7)	54 (29.8)	
C	127 (65.8)	10 (83.3)	117 (64.7)	
MELD score	16.4 ± 6.4 15 (6–40)	20.7 ± 4.6 (20, 13–29)	16.2 ± 6.4 (15, 6–40)	0.019
Hospitalized status	82 (39.0)	10 (76.9)	72 (36.5)	0.004
Major shunt vessels	82 (39.0)	6 (46.1)	76 (38.6)	0.587
Donor gender, male	132 (62.9)	10 (76.9)	122 (61.9)	0.278
Donor age (years)	35.0 ± 10.6 (34, 19–62)	33.8 ± 12.9 (28, 21–62)	35.1 ± 12.9 (34, 19–62)	0.649
Incompatible blood type	10 (4.7)	0 (0.0)	10 (5.1)	0.461
Left lobe graft	122 (58.1)	8 (61.5)	114 (57.8)	0.795
GV (g)	476 ± 106 (464, 250–734)	486 ± 113 (480, 280–734)	475 ± 106 (460, 250–720)	0.725
GV/SLV ratio (%)	41.2 ± 8.4 (40.6, 23.7–72.5)	42.4 ± 8.1 (42.8, 29.7–58.7)	41.3 ± 8.4 (40.5, 23.7–72.5)	0.646
GRWR (%)	0.80 ± 0.18 (0.77, 0.45–1.78)	0.81 ± 0.14 (0.81, 0.61–1.06)	0.79 ± 0.18 (0.77, 0.45–1.78)	0.767
PVP at laparotomy (mmHg)	24.1 ± 6.1 (24, 7–40)	23.0 ± 5.1 (24.5, 15–30)	24.2 ± 6.2 (24, 7–40)	0.512
Splenectomy	136 (64.7)	7 (53.8)	129 (65.4)	0.380
Cold ischemic time (min)	98 ± 89 (72, 25–377)	100 ± 42 (96, 40–179)	98 ± 91 (71, 25–377)	0.951
Warm ischemic time (min)	39 ± 11 (37, 22–102)	42 ± 12 (40.5, 26–62)	39 ± 11 (36, 22–102)	0.352
Hepatic arterial flow (mL/min)	101 ± 64 (89, 15–580)	108 ± 53 (114, 30–192)	101 ± 65 (88.5, 15–580)	0.701
Portal venous flow (L/min)	1.76 ± 0.67 (1.64, 0.27–3.85)	1.73 ± 0.56 (1.53, 1.00–2.65)	1.77 ± 0.67 (1.65, 0.27–3.85)	0.855
PVP at the closure (mmHg)	16.3 ± 4.1 (16, 6–37)	17.9 ± 7.4 (16, 11–37)	16.2 ± 3.8 (16, 6–26)	0.165
Duct-to-duct biliary reconstruction	181 (86.2)	12 (96.2)	168 (85.7)	0.454
Operation time (min)	805 ± 180 (777, 437–1519)	861 ± 246 (818, 579–1315)	802 ± 176 (773, 437–1519)	0.293
Operative blood loss (L)	5.2 ± 6.4 (3.4, 0.2–50.4)	10.7 ± 12.3 (4.0, 0.75–35.4)	4.9 ± 5.8 (3.3, 0.2–50.4)	0.003
LDLT before 2008	112 (53.3)	9 (69.2)	103 (52.3)	0.223
Maximum values within POD 28				
Total bilirubin (mg/dL)	10.5 ± 8.2 (7.6, 1.4–46.7)	29.5 ± 6.3 (29.4, 9.2–46.7)	9.1 ± 6.3 (7.0, 1.4–32.1)	<0.001
Daily ascites output (L)	1.2 ± 1.4 (0.8, 0.2–11.3)	2.1 ± 1.4 (1.5, 0.7–1.8)	1.1 ± 1.4 (0.7, 0.2–11.3)	0.017
PT-INR	1.8 ± 0.4 (1.8, 1.2–3.8)	2.2 ± 0.6 (2.2, 1.8–3.8)	1.8 ± 0.3 (1.8, 1.2–3.6)	<0.001
Ammonia (μg/dL)	77 ± 40 (71, 14–353)	121 ± 77 (87, 48–353)	74 ± 34 (70, 14–286)	<0.001

Continued

Table 1: Continued.

Variables	Total (n = 210)	Early graft loss		p-Value
		Yes (n = 13)	No (n = 197)	
Values on POD 14				
Total bilirubin (mg/dL)	6.0 ± 7.1 (2.8, 0.4–41.3)	19.7 ± 5.6 (18.6, 2.9–41.3)	5.1 ± 5.6 (2.6, 0.4–29.3)	<0.001
Daily ascites output (L)	0.4 ± 0.9 (0, 0–8.9)	1.3 ± 1.6 (0, 0.7–5.7)	0.3 ± 0.8 (0, 0–8.9)	<0.001
PT-INR	1.3 ± 0.6 (1.1, 0.9–3.3)	1.5 ± 0.5 (1.3, 1.1–3.3)	1.2 ± 0.6 (1.1, 0.9–2.6)	0.032

MELD = model for end-stage liver disease; GV = graft volume; SLV = standard liver volume; GRWR = graft recipient weight ratio; PVP = portal venous pressure; LDLT = living donor liver transplantation; POD = postoperative day; PT-INR = prothrombin time international normalized ratio.

defined as hyperbilirubinemia (e.g. T.Bil > 20 mg/dL for >seven consecutive days occurring after postoperative day [POD] 7). Early graft loss was defined as graft loss occurring within 6 months after LDLT.

Liver biopsy

Graft biopsies early after LDLT were obtained percutaneously or under laparotomy. For left lobe grafts, percutaneous biopsy was performed because manual compression of the punctured liver is possible. For right lobe grafts, open biopsy for suturing the punctured site was performed if indicated. If PGD was highly suspected because of hyperbilirubinemia with stable transaminase levels, biopsy was postponed.

Statistical analysis

Values are expressed as the mean ± standard deviation (median, minimum–maximum). Variables were analyzed using the χ^2 tests for categorical values or the Mann–Whitney's test for continuous variables. Cumulative survival analyses were determined using the Kaplan–Meier method with the log-rank test. Sensitivity (%) was calculated as true positive (n)/[true positive (n) + false negative (n)]. Specificity was calculated as true negative (n)/[true negative (n) + false positive (n)]. Values of p value <0.05 were considered statistically significant. Receiver operating characteristic curve analysis was also performed.

Results

Characteristics of the recipients, donors and grafts

The mean age of the recipients was 53.9 ± 10.5 years (Table 1). Indications for LDLT included acute liver failure (n = 16, 7.6%), cholestatic cirrhosis (n = 35, 16.7%), post-necrotic viral or nonviral cirrhosis (n = 154, 73.3%) and others (n = 4, 1.9%). Approximately half of the patients had hepatocellular carcinomas (n = 112, 53.3%). The majority of the patients were Child-Pugh class C (n = 127, 65.8%). The mean MELD score was 16.4 ± 6.4. Overall, 39% of the patients (n = 82) had been hospitalized before LDLT and had major (>1.0 cm) shunt vessels (n = 82, 39.0%).

The mean age of the donors was 35.0 ± 10.6 years. Graft types included left lobe grafts (n = 122, 58.1%) and right lobe grafts (n = 88, 41.9%). Ten donors (4.7%) provided blood type incompatible donors. The mean GV was 476 ± 106 g, the mean GV/SLV was 41.2 ± 8.4 and the mean GRWR was 0.80 ± 0.18. Splenectomy was performed in 136 cases (64.7%) and duct-to-duct biliary reconstruction was performed in 181 cases (86.2%). The mean operative

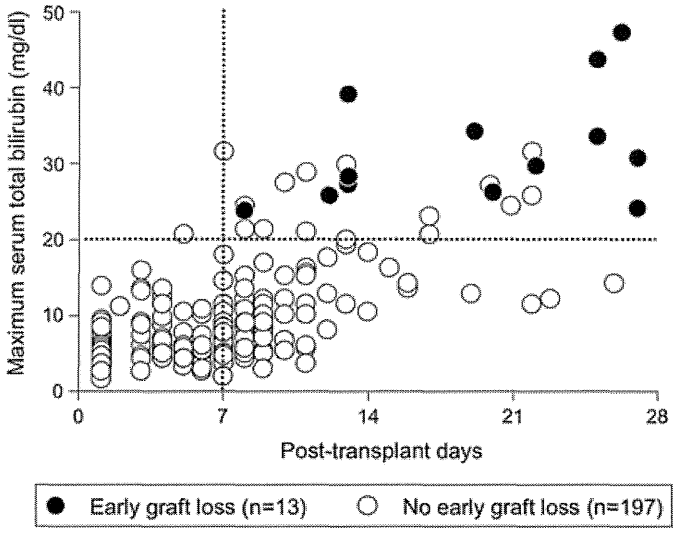

Figure 2: Maximum total bilirubin values within 28 days after transplantation plotted against the post-operative date (n = 210). All of the patients with early graft loss (black dots; n = 13) had maximum total bilirubin >20 mg/dL later than 1 week after transplantation (p < 0.001).

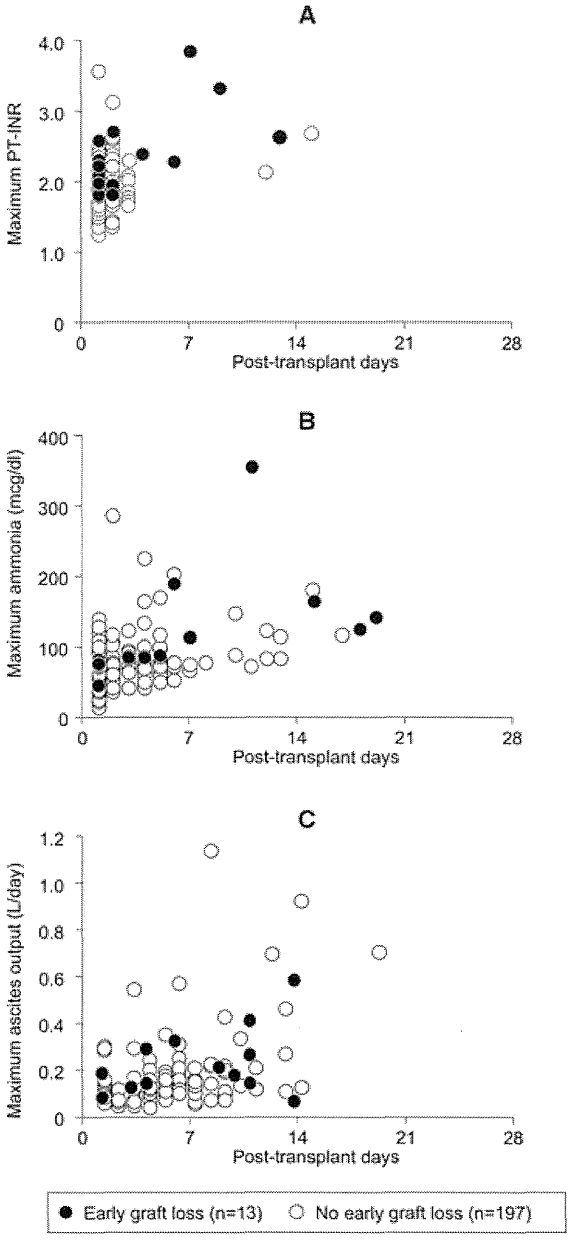

Figure 3: Maximum prothrombin international normalized ratio (PT-INR, A), ammonia levels (B) and ascites output (C) plotted against the postoperative date (n = 210). The black dots (n = 13) represent patients with early graft losses.

time was 805 ± 180 min and the mean blood loss was 5.2 ± 6.4 L.

We have performed 346 adult-to-adult LDLTs between May 1997, adult-to-adult LDLT program started, and July 2011. The cumulative 2-year graft survival rate since 2004 (87.0%, n = 228) was significantly better than before 2004 (70.8%, n = 118, p < 0.001). Therefore, to exclude possible technical or learning bias and to focus on PGD, only cases treated since 2004 were included in the current analysis.

PGD

Overall, 13 cases experienced early graft loss within 6 months after adult-to-adult LDLT; none of these cases were associated with technical, immunological or hepatitis-related issues (Table 1). The mean graft survival of these 13 cases was 1.7 ± 1.0 months. Early graft loss in all of the cases was caused by PGD. Prior hospitalization of the recipient (46.1% vs. 38.6%; p = 0.004), higher MELD score (20.7 ± 4.6 vs. 16.2 ± 6.4; p = 0.019), absence of hepatocellular carcinoma (23.1% vs. 55.3%; p = 0.024) and massive intraoperative blood loss (10.7 ± 12.3 L vs. 4.9 ± 5.8; p = 0.003) were significantly associated with early graft loss. Graft GV/SLV ratio and GRWR were not associated with early graft loss.

The maximum T.Bil values (29.5 ± 6.3 mg/dL vs. 9.1 ± 6.3 mg/dL; p < 0.001), daily ascites output (2.1 ± 1.4 L vs. 1.1 ± 1.4 L; p < 0.001), PT-INR (2.2 ± 0.6 vs. 1.8 ± 0.3, p < 0.001) and ammonia levels (121 ± 77 μg/dL vs. 74 ± 34 μg/dL; p < 0.001) measured by POD 28 were significantly greater in cases with early graft loss. The mean values of T.Bil (19.7 ± 5.6 mg/dL vs. 5.1 ± 5.6 mg/dL; p < 0.001), daily ascites output (1.3 L ± 1.6 vs. 0.3 ± 0.8 L; p < 0.001) and PT-INR (1.5 ± 0.5 vs. 1.2 ± 0.6; p = 0.032) on POD 14 were also significantly greater in cases with early graft loss. Grafts with early mortality had significantly worse hepatic parameters at both a fixed date (i.e. on POD 14) and within a fixed time (i.e. within POD 28). However, grafts may show worse hepatic parameters in the early postoperative period because of the deteriorated recipient's condition or may show delayed worsening as a result of PGD.

Therefore, maximum T.Bil (Figure 2) and other hepatic parameters (Figure 3), including maximal PT-INR, ammonia and ascites output, were plotted against their corresponding POD. All 13 cases with early functional graft loss (n = 13) had maximum T.Bil >20 mg/dL after POD 7 (p < 0.001; Figure 2). No definite relationship with early graft loss was observed between the other hepatic parameters including PT-INR, ammonia and ascites output (Figure 3).

The maximum T.Bil values within POD 28 after LDLT were also plotted against GV/SLV or GRWR (Figure 4). Grafts with maximum T.Bil >20 mg/dL were evenly distributed with GV/SLV and GRWR.

DFH

Because the maximum T.Bil >20 mg/dL detected after POD 7 was associated with PGD, which persisted for a number of consecutive days, we defined DFH as described in the methods. We calculated the sensitivity and specificity for early graft loss caused by functional graft failure

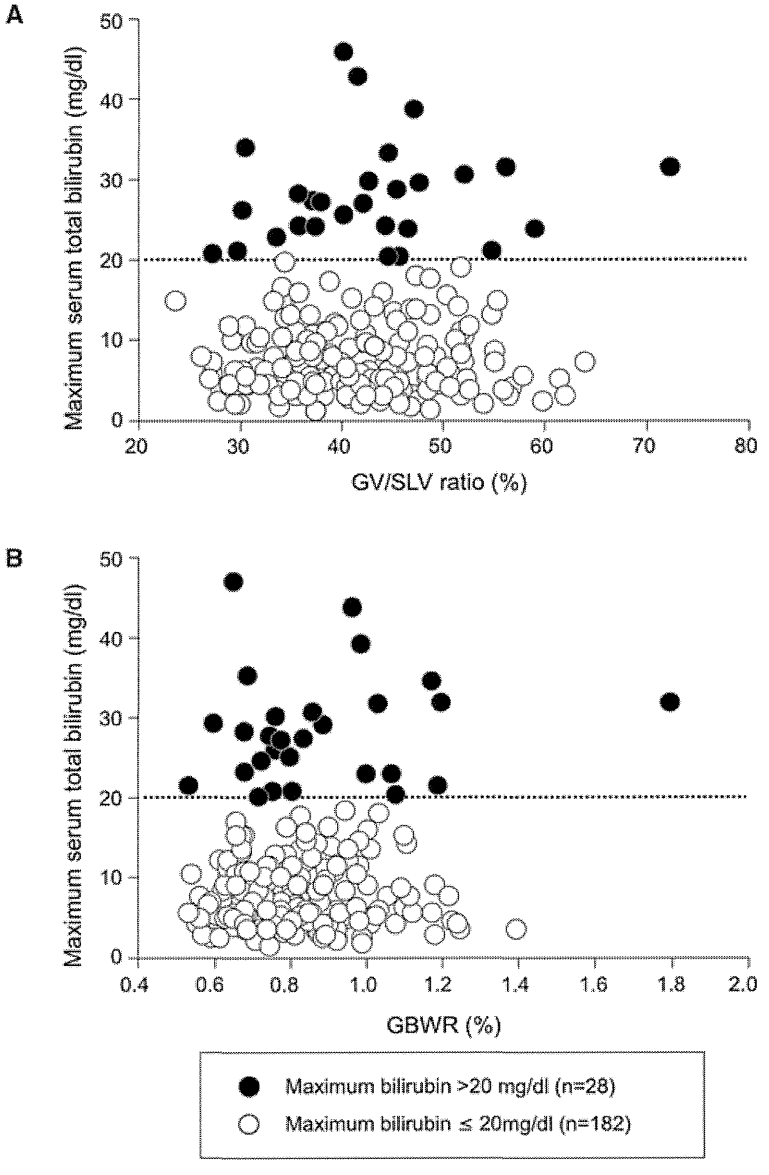

Figure 4: Maximum total bilirubin values within 28 days after transplantation plotted against GV/SLV (A) and GRWR (B) (n = 210). GRWR = graft recipient weight ratio; GV = graft volume; SLV = standard liver volume.

using several definitions (Table 2), including DFH with T.Bil >10, 15, 20 or 25 mg/dL for >seven consecutive days after POD 7 (DFH-10, DFH-15, DFH-20 and DFH-25, respectively), small-for-size graft dysfunction as defined by Dahm et al. (8), SFSS as defined at our institute in 2006 (15), and SFSS as defined by Hill, et al. (14). The sensitivities of the previous definitions of small-for-size graft dysfunction or syndrome for early loss caused by PGD were <50%. On the other hand, DFH-20 (i.e. T.Bil > 20 mg/dL for >seven consecutive days after POD 7) showed the highest sensitivity (100%) and the second highest (95.4%) specificity for detecting early graft loss caused by nontechnical, non-immunological and nonhepatitis-related PGD.

Characteristics of grafts with DFH-20

The effects of DFH-20 on cumulative graft survival are shown in Figure 5. The 1- and 2-year graft survival rates were 40.9% and 35.1% for grafts with DFH-20 (n = 22) versus 97.6% and 93.8% for grafts without DFH-20 (n = 188), respectively ($p < 0.001$).

Risk factors for DFH-20

Univariate analyses (Table 3) showed that recipient Child class C (yes; 14.1% vs. 5.1%; p = 0.047), MELD score >15 (yes; 15.5% vs. 4.3%; p = 0.008), hospitalized status (yes; 17.3% vs. 6.3%; vs. p = 0.012), the presence of major shunt vessels >1 cm in diameter (yes; 15.9% vs. 7.0%;

Table 2: Sensitivity and specificity for detecting early graft loss caused by primary graft dysfunction after LDLT

Definitions	Early graft loss because of primary graft dysfunction		
	Sensitivity (%)	Specificity (%)	Area under ROC
Delayed functional hyperbilirubinemia (DFH)			
with T.Bil >10 mg/dL (DFH-10)	100.0	71.6	0.857
with T.Bil >15 mg/dL (DFH-15)	100.0	89.3	0.946
with T.Bil >20 mg/dL (DFH-20)	100.0	95.4	0.977
with T.Bil >25 mg/dL (DFH-25)	53.8	97.4	0.756
*For >seven consecutive days after POD 7, excluding technical, immunological and hepatitis factors.			
Small-for-size graft dysfunction, Dahm et al. (8)	23.1	95.9	0.595
*GRWR <0.8 and the presence of two of the followings for three consecutive days during the first postoperative week: T.Bil >100 μmol/L, PT-INR >2 and encephalopathy grade 3 or 4, excluding technical, immunological and infectious factors.			
Small-for-size graft syndrome, Soejima, et al. (16)	30.7	87.3	0.590
*Prolonged cholestatis (T.Bil >10 mg/dL on POD14) and intractable ascites (ascites > 1 L on POD 14 or >0.5 L on POD 28).			
Small-for-size graft syndrome, Hill et al. (14)	46.2	95.9	0.687
*T.Bil >10 mg/dL (and continuing to increase) after POD 7, PT-INR >1.5 and ascites >2 L, excluding mechanical/technical problems.			

LDLT = 8 living donor liver transplantation; ROC = receiver operating characteristic curve; T.Bil = total bilirubin; DFH = delayed functional hyperbilirubinemia; POD = postoperative day; GRWR = graft recipient weight ratio; PT-INR = prothrombin time-international normalized ratio.

p = 0.042), donor age >45 years (yes; 21.3% vs. 7.4%; p = 0.006), PVP >20 mmHg at the end of the surgery (yes; 20.6% vs. 8.5%; p = 0.004) and intraoperative blood loss >1 0 L (yes; 38.1% vs. 7.4%; p < 0.001) were risk factors for DFH with T.Bil >20 mg/dL. On the other hand, graft type, GV/SLV and GRWR were not significant risk factors. Multivariate analysis was not included in the current report because of the smaller number of the patients with DFH-20.

We performed additional univariate analyses (Table 4) to compare cases with (n = 13) or without (n = 9) early graft loss among those with DFH-20 (n = 22). The maximum PT-INR was 2.4 ± 0.6 and 1.8 ± 0.3 for grafts with and without

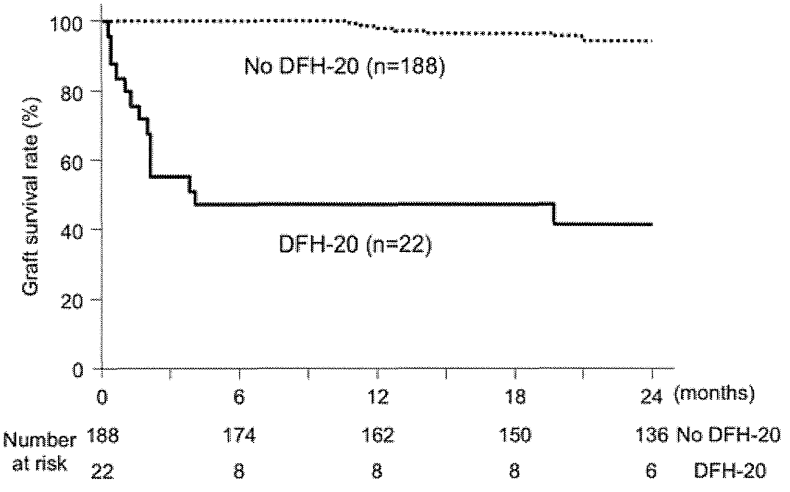

Figure 5: Cumulative graft survival of patients with (n = 22) or without (n = 188) DFH. The difference in survival was significantly different (p < 0.001). DFH = delayed functional hyperbilirubinemia: total bilirubin >20 mg/dL for >7 consecutive days after postoperative day 7, excluding technical, immunological and hepatitis factors.

early graft loss, respectively. Categorical analysis showed that early graft loss was more frequently associated with coagulopathy (PT-INR >2) compared with grafts without early graft loss (76.9% vs. 11.1%; p = 0.011).

Pathological findings of the grafts with DFH-20

Liver biopsy specimen was obtained in 8 of the 22 patients (21.3 ± 9.1 days after LDLT). Centrilobular hepatocyte ballooning and cholestasis were the most prominent and characteristic findings (Figure 6). Although ductular reaction was observed in six cases, other findings including necrosis, steatosis, nonspecific portal infiltration were not the consistent findings (Table 5).

Discussion

The primary endpoint of this study was to characterize PGD after LDLT, in which a smaller but qualified graft is usually transplanted with a short cold ischemic time is usually transplanted. We found that DFH-20 essentially encompassed PGD after LDLT with a high mortality rate. Other factors such as ammonia levels, PT-INR and ascites volume were not associated with PGD. The superior sensitivity and specificity of DFH-20 for detecting PGD after LDLT, compared with other definitions, is clinically relevant because it is important to know whether the graft is likely to recover, or fail and require retransplantation. Worryingly, the rate of graft loss was particularly high in cases with DFH-20 and coagulopathy (i.e. PT-INR >2). Therefore, these cases should be considered as candidates for retransplantation.

The significance of hyperbilirubinemia has been reported in both transplant and nontransplant settings in the literature. In LDLT, Marubashi et al. (20) reported that T.Bil >27 mg/dL is a significant indicator for early graft loss, regardless of the cause. Even in pediatric LDLT, Emond et al. (21) reported that cholestasis was more prominent and was prolonged in recipients with smaller LDLT grafts. Although studies evaluating prolonged hyperbilirubinemia in DDLT are limited, Fusai et al. (22) reported that functional hyperbilirubinemia >100 μmol/L sustained for at least 1 week after DDLT was associated with poor prognosis. After hepatic resection for tumors, Balzan et al. (23) showed that PT <50% and T.Bil >50 μmol/L on POD 5 is associated with a mortality rate exceeding 50% after hepatectomy.

PGD after LDLT and PNF after DDLT are quite different in terms of their pathogeneses and clinical manifestations. PNF after DDLT usually becomes evident during the immediate postoperative period with rapidly rising transaminase levels, absence of bile production, severe coagulopathy, acidosis and hemodynamic instability (1–3). These clinical characteristics of PNF after DDLT are attributed to massive hepatic cytolysis following reperfusion of a graft

Table 3: Univariate analysis of risk factors for DFH-20

	DFH with T.Bil >20 mg/dL		
Variables	Yes (n = 22)	No (n = 188)	p-Value
Recipient gender, male	10 (45.5)	92 (48.9)	0.721
Recipient age >60 years	4 (18.2)	52 (27.7)	0.341
Child-Pugh class C	18 (81.8)	109 (57.9)	0.066
MELD score >15	18 (81.8)	98 (52.2)	0.008
Total bilirubin >8 mg/dL	7 (31.8)	38 (20.2)	0.209
PT-INR > 1.8	10 (45.5)	34 (18.1)	0.003
Creatinine >1.0 mg/dL	4 (18.1)	23 (12.2)	0.430
Hospitalized status	14 (63.6)	68 (36.2)	0.012
Acute liver failure	1 (4.5)	16 (8.5)	0.518
Major shunt vessels	13 (59.1)	69 (36.7)	0.042
Donor gender, male	15 (68.2)	117 (62.2)	0.584
Donor age >45 years	10 (45.5)	37 (19.7)	0.006
Left lobe graft	12 (54.5)	110 (58.5)	0.721
GV/SLV ratio <30%	1 (4.5)	14 (7.4)	0.617
GV/SLV ratio <40%	8 (36.4)	92 (48.9)	0.264
GRWR < 0.6%	1 (4.5)	19 (10.1)	0.401
GRWR < 0.8%	12 (54.5)	104 (55.3)	0.944
PVP > 30 mmHg at laparotomy	2 (7.1)	26 (14.2)	0.963
No splenectomy	8 (36.4)	66 (35.1)	0.907
Cold ischemic time >120 min	8 (36.4)	43 (22.9)	0.167
Warm ischemic time >50 min	6 (27.3)	26 (13.9)	0.095
PVP > 20 mmHg at closure	7 (31.8)	27 (14.4)	0.036
Blood loss > 10 L	8 (36.4)	13 (6.9)	< 0.001

DFH-20 = delayed functional hyperbilirubinemia: total bilirubin >20 mg/dL for >7 consecutive days after postoperative day 7, excluding technical, immunological and hepatitis factors; MELD = model for end-stage liver disease; PT-INR = prothrombin time international normalized ratio; GV = graft volume; SLV = standard liver volume; GRWR = graft recipient weight ratio; PVP = portal venous pressure.

Table 4: Characteristics of cases with or without early graft loss under DFH-20

	Early graft loss		
Variables	Yes (n = 13)	No (n = 9)	p-Value
Recipient age (years)	50.9 ± 11.2 (52, 21–64)	48.2 ± 8.7 (46, 34–62)	0.552
MELD score	19.7 ± 4.0 (20, 13–27)	17.1 ± 3.8 (16, 12–26)	0.144
Donor age (years)	43.2±10.8 (46, 20–56)	36.4 ± 14.7 (36, 21–62)	0.249
GV (g)	483 ± 114 (480, 280–734)	524 ± 89 (540, 400–620)	0.385
GV/SLV ratio (%)	41.8 ± 8.3 (41.0, 29.7–58.7)	45.9 ± 11.8 (42.8, 33.7–72.5)	0.351
GRWR (%)	0.79 ± 0.16 0.79 (0.56–1.1)	0.91 ± 0.35 (0.74, 0.67–1.78)	0.289
PVP at laparotomy (mmHg)	23.5 ± 5.3 (25, 15–30)	25.4 ± 5.5 (27, 16–32)	0.459
Cold ischemic time (min)	141 ± 107 (90, 40–179)	100 ± 42 (162, 43–377)	0.951
Warm ischemic time (min)	41 ± 12 (40, 26–62)	43 ± 13 (40, 24–66)	0.799
Hepatic arterial flow (mL/min)	102 ± 55 (104, 29–192)	96 ± 66 (70.5, 21–224)	0.817
Portal venous flow (L/min)	1.67 ± 0.58 (1.46, 0.99–2.65)	1.61 ± 0.60 (1.40, 0.81–2.3)	0.842
PVP at the closure (mmHg)	18.4 ± 7.4 (17, 11–37)	17.0 ± 4.6 (17.5, 11–23)	0.640
Operation time (min)	854 ± 236 812 (579–1315)	942 ± 224 (978, 597–1360)	0.397
Operative blood loss (L)	13.1 ± 14.1 (5.9, 0.7–38.0)	111.5 ± 7.8 (10.0, 2.4–26.5)	0.774
Maximum values within POD 28			
Total bilirubin (mg/dL)	31.4 ± 8.1 (29.4, 21.8–46.7)	27.7 ± 3.1 (27.5, 23.1–32.1)	0.232
Daily ascites output (L)	1.8 ± 1.3 (1.5, 0.6–4.0)	1.6 ± 1.2 (1.6, 0.4–2.7)	0.820
PT-INR	2.4 ± 0.6 2.3 (1.9–3.8)	1.8 ± 0.3 1.8 (1.4–2.3)	0.011
PT-INR > 2	10 (76.9)	1 (11.1)	0.002
Ammonia (μg/dL)	125 ± 80 (90, 48–353)	115 ± 59 (89, 41–204)	0.767

DFH-20 = delayed functional hyperbilirubinemia: total bilirubin >20 mg/dL for >seven consecutive days after postoperative day 7, excluding technical, immunological and hepatitis factors; MELD = model for end-stage liver disease; GV = graft volume; SLV = standard liver volume; GRWR = graft recipient weight ratio; PVP = portal venous pressure; POD = postoperative day; PT-INR = prothrombin time international normalized ratio.

with steatosis, higher age or prolonged cold ischemic time (1,2,24). On the other hand, PGD after LDLT becomes evident in a gradual and delayed fashion, and is characterized by hyperbilirubinemia and sometimes massive ascites, without elevated of serum transaminase levels, representing functional intolerance during regeneration (13–16). Because these LDLT grafts show insufficient function rather than being nonfunctional, we termed than as PGD. Thus, PNF after DDLT corresponds to necrosis and PGD after LDLT represents functional intolerance.

The pathologic findings of DFH-20 were consistent with those of nonspecific preservation injuries as previously described, including prominent centrilobular hepatocyte ballooning, cholestasis, possibly accompanied by steatosis, necrosis, portal infiltration and ductular reaction (21,25,26). In electron-microscopic studies using postliver transplant specimens after DDLT, Ng et al. (27) showed that ballooned hepatocytes were characterized dilatation of the cisternae of the rough endoplasmic reticulum and mitochondria. In LDLT, Emond et al. (21) examined 25 patients and reported that the pathologic changes after LDLT are more prominent in smaller grafts. Thus, such centrilobular pathologic changes after LDLT might be attributed to overperfusion and ischemic stress.

In LDLT, the issue of graft size, namely SFSS, has been of significant concern since the first adult-to-adult LDLT

Table 5: Summary of the pathological findings in the grafts (n = 8/22) with DFH-20

	Case							
	#1	#2	#3	#4	#5	#6	#7	#8
Graft type	Left	Left	Left	Left	Left	Right	Left	Left
GV/SLV (%)	33.7	41.0	44.2	36.9	29.7	44.3	30.1	35.3
GRWR (%)	0.67	0.88	1.05	0.74	0.71	0.72	0.64	0.56
Maximum T.Bil (mg/dL)	24.1	43.3	33.3	24.9	25.9	24.5	34.2	28.1
Early graft loss	No	Yes	Yes	Yes	Yes	No	Yes	Yes
Pathological findings								
Cholestasis	++	++	++	++	+++	++	++	++
Ballooning	++	++	+++	+++	++	++	++	++
Necrosis	++	+	+	−	−	−	+	+
Steatosis	+	+	−	−	−	+	+	++
Portal infiltration	+	−	+	−	−	−	−	−
Ductular reaction	−	+	+	+	+	−	+	+

DFH = delayed functional hyperbilirubinemia: total bilirubin >20 mg/dL for >seven consecutive days after postoperative day 7, excluding technical, immunological and hepatitis factors; GRWR = graft recipient weight ratio; GV = graft volume; SLV = standard liver volume.

was performed in 1998 (28). We consider that SFSS after LDLT is included in the concept of PGD after LDLT, because functional graft dysfunction after LDLT is now thought to be caused by graft size as well as several other factors (11–15). In this analysis, such additional factors included Child class C, MELD score >15, prolonged pretransplant hospitalized status, the presence of major shunt vessels, donor age >45 years, PVP >20 mmHg at the end of the surgery and intraoperative blood loss >10 L. Although small-for-size graft dysfunction was originally defined in 2005 (8) and has been used in many reports published since then, the descriptions better resemble PNF in DDLT, namely severe coagulopathy (PT-INR >2) and advanced encephalopathy (Grade 3 or 4) with T.Bil >100 μmol/L during the first postoperative week. It seems that PGD after LDLT characterized by DFH-20 encompasses SFSS and represents functional graft intolerance caused by multiple factors after LDLT (Figure 7).

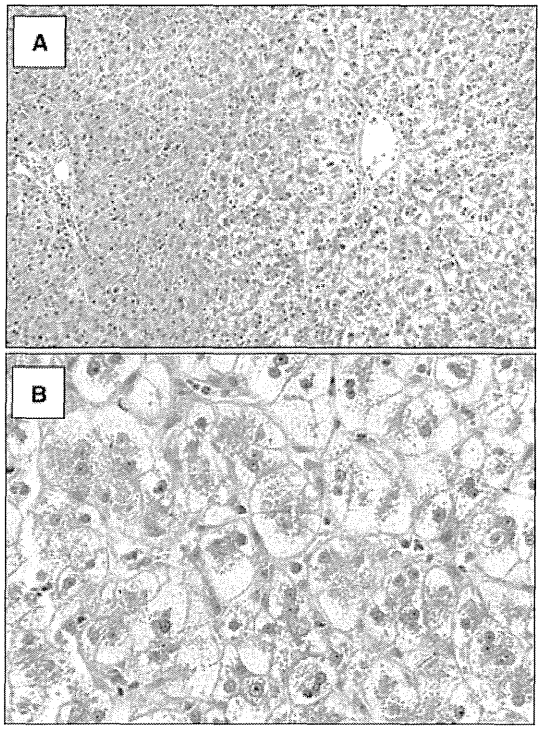

Figure 6: The representative microscopic findings of the graft with DFH-20 (Case #4). Centrilobular hepatocyte ballooning with cholestasis was prominent and the ballooned cells were well demarcated from the uninvolved cells (A, H.E. ×100). The ballooned hepatocytes showed intracellular cholestasis (B, H.E. ×400). DFH-20, delayed functional hyperbilirubinemia: total bilirubin >20 mg/dL for >seven consecutive days after postoperative day 7.

Nevertheless, it is also true that graft size is one of the main contributors to the clinical outcome (8,9). We started our adult-to-adult LDLT program in 1997 and exclusively used left lobe grafts with GV/SLV >30% (29) with an inferior 2-year graft survival rate (70.8%) before 2004. Since 2004, we have revised the graft selection criteria and now use left or right lobe grafts with GV/SLV >35% to provide an adequate safety margin (17). Therefore, all of the data in the current series were under obtained from our intent to maintain, keeping GV/SLV >35%. Besides intension of achieving a GV being an adequate safety margin, our transplant center has always considered the severity of recipient illness and potential surgical difficulties. We use a formula to score the overall risk of the procedure to maintain appropriate safety limits (30). Moreover, our surgical techniques to modulate portal and venous flows have been refined as previously reported (19,31–33). Based on these approaches, the 2-year graft survival rate at our center has reached 87.0% since 2004.

The main limitation of this study is that selection bias may confound our interpretations because GV was not correlated with mortality. Reports from other centers are also necessary to help generalize our current findings. Our results do not necessarily imply that small grafts without intentional selection or surgical refinements could yield satisfactory outcomes. The other limitation of this study is

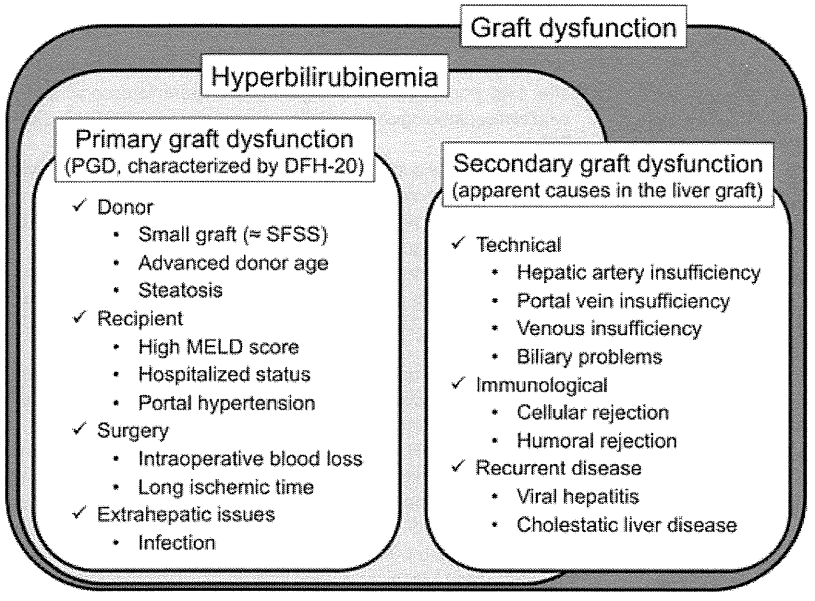

Figure 7: Associations between PGD, SFSS and secondary graft dysfunction. PGD, characterized by DFH-20, encompasses SFSS and other causes of functional and primary graft dysfunction. DFH-20: delayed functional hyperbilirubinemia (total bilirubin >20 mg/dL for >7 consecutive days after postoperative day 7, excluding technical, immunological and hepatitis factors); MELD, model for end-stage liver disease; PGD, primary graft dysfunction; SFSS, small-for-size syndrome.

that the immunological factors have not been completely ruled out because of insufficient histological evidence in the study population, no assessment of antibody and no thorough analysis of transfusion related immune events. Further prospective analysis is necessary to address such issues.

In conclusion, we have proposed a new concept for PGD after LDLT with a higher early graft mortality rate and characterized by DFH-20, which is caused by multiple factors.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1: The details of the cases excluded from the analysis

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