blood loss, operative time, graft ischemic time, initial immunosuppressive agent, overexposure to CNI, and combined use of MMF. All 16 variables were entered into the multivariate analysis, even if deemed insignificant on univariate analysis, because of the potential importance of each variable [33]. All statistical analyses were performed using JMP software (release 6.0.3; SAS Institute Japan, Tokyo, Japan). Values of $P < 0.05$ were regarded as significant.

Results

Pre- and postoperative renal function and postoperative course

During the 28 days of postoperative follow-up, ARI, as determined by the RIFLE criteria, occurred in 121 (60.5%) of the study patients. The numbers of patients with ARI in the R-class, I-class, and F-class were 47 (38.8%), 42 (34.7%), and 32 (26.4%), respectively. The 1- and 5-year survival rates were 97.5% and 90.6% in the N-class, 95.7% and 89.2% in the R-class, 85.7% and 81.8% in the I-class, and 50.0% and 46.7% in the F-class, respectively (Fig. 1). Fatal outcomes in early post-transplant phase were seen in two cases in the N-class, two cases in the R-class, two cases in the I-class, and 10 cases in the F-class. Overall survival rates in the R-class were comparable to the rates in the N-class, and the survival rates in these groups were superior to those in the other classes. We therefore defined the combination of the N- and R-classes as the normal kidney function or mild ARI group (Group A, $n = 126$) and the combination of the I- and F-classes as the severe ARI group (Group B, $n = 74$). The 30 patients (15%) who required postoperative RRT in the acute postoperative phase comprised four Group A patients and 26 Group B patients. Every patient recovered from ARI, and no recipient required permanent RRT at 1-year follow-up. However, the rates of development to stage 3/4 CKD were 0.8% (1 of 126 patients) in Group A and 19% (14 of 74 patients) in Group B, respectively.

The in-hospital mortality rate was significantly lower for Group A (3.2%) than for Group B (15.8%; $P = 0.0015$). All cases of hospital mortality resulted from postoperative sepsis and/or graft perfusion obstruction, which were followed by graft failure. The 1-, 5- and 10-year survival rates were 96.7%, 90.6%, and 88.1% for Group A and 71.1%, 65.9%, and 59.3% for Group B, respectively. Group A showed more favorable post-transplant outcomes than Group B ($P < 0.0001$; Fig. 1). Late-phase mortality after follow-up for 1 year following LDLT was seen in nine patients (7%) in Group A and 14 patients (22%) in Group B as a result of HCV relapse, HCC recurrence, heart failure, *de novo* cancer, and chronic rejection. Forty-three percent of recipients with stage 3/4 CKD (6 of 14 patients) in Group B showed fatal outcomes in the chronic-phase, compared with uniformly satisfactory prognosis in Group A (Fig. 1). Unfortunately, each of these patients would have limited options for treatment modalities because of poor renal function, although the patients with chronic-phase deaths in Group A had a similar situation.

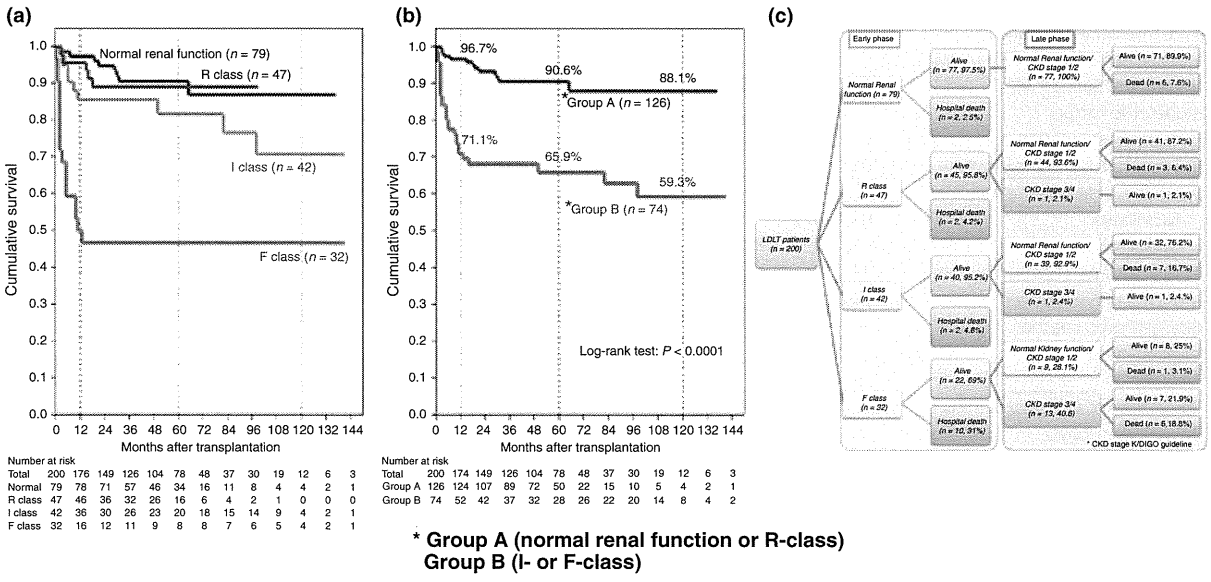

Figure 1 Overall survival curves and diagram of post-transplant prognosis. (a) Comparison of cumulative overall survival curves stratified by RIFLE criteria. (b) The patients were divided into two groups: Group A (normal renal function or R-class); and Group B (I- or F-class). Comparison of cumulative overall survival curves between Group A and Group B. (c) Diagram of prognosis for all patients after LDLT. LDLT, living donor liver transplantation.

Risk factors for severe ARI after LDLT

The background data for patients relevant to the RIFLE criteria are shown in Table 1. The results of univariate analysis of the studied variables for Groups A and B are summarized in Table 2. The patients in Group B had significantly higher MELD scores and higher frequency of insulin-controlled diabetes mellitus, but no other preopera-

Table 1. Demographic characteristics of patients according to RIFLE criteria.

	Normal renal function (n = 79)	R-class (n = 47)	I-class (n = 42)	F-class (n = 32)
Preoperative factors				
Age (years)	49.6 ± 1.3	51.0 ± 1.7	48 ± 1.6	47.9 ± 2.01
Sex				
Male/female (%)	54 (68)/25 (32)	24 (51)/23 (49)	21 (50)/21 (50)	16 (50)/16 (50)
Background disease				
Postnecrotic liver cirrhosis	50 (63%)	32 (68%)	26 (62%)	18 (56%)
HCV	22	16	13	11
HBV	22	4	7	2
Alcohol or non-HBV/HCV	6	12	6	5
Cholestatic disease	16 (20%)	8 (17%)	6 (14%)	9 (28%)
Acute liver failure	7 (9%)	6 (13%)	6 (14%)	5 (16%)
Metabolic disease	6 (8%)	1 (2%)	4 (10%)	0
MELD score	15.2 ± 0.8	15.4 ± 0.8	17.1 ± 0.9	18.2 ± 1.2
HCC (%)	25 (32)	13 (28)	15 (36)	8 (25)
Serum creatinine level (mg/dl)	0.85 ± 0.05	0.71 ± 0.05	0.71 ± 0.04	0.89 ± 0.13
GFR (ml/min)	75.9 ± 4.4	74.1.1 ± 4.5	70.5 ± 4.3	70.9 ± 6.9
Serum albumin level (g/dl)	3.0 ± 0.07	2.9 ± 0.07	2.8 ± 0.08	2.7 ± 0.11
Hypertension (%)	12 (15)	2 (4)	5 (12)	3 (9)
Diabetes mellitus (%)	4 (5)	7 (15)	9 (21)	3 (9)
Donor/graft factors				
Age (years)	38.3 ± 1.5	39.7 ± 1.8	39.2 ± 1.8	43.0 ± 2.3
Right/left lobe graft (%)	57 (72)/22 (28)	24 (51)/23 (49)	23 (55)/19 (45)	20 (62)/12 (38)
GW/RBW (%)	0.98 ± 0.03	0.87 ± 0.03	0.95 ± 0.05	0.91 ± 0.04
Operative factors				
Operative time (min)	567 ± 12.5	571 ± 13.6	674 ± 24.3	712 ± 79.1
Blood loss (ml/kg)	97.0 ± 18.2	91.0 ± 12.4	164.7 ± 22.8	130 ± 31.2
Cold ischemic time (min)	61.9 ± 4.2	60.2 ± 6.5	71.6 ± 10.2	82 ± 9.1
Warm ischemic time (min)	42.3 ± 1.6	44.2 ± 2.5	43.6 ± 2.2	43.1 ± 2.8
Transplant period				
Early/late period (%)*	42(53)/37(47)	17(36)/30(64)	23(55)/19(45)	18(56)/14(44)
Postoperative factors				
Initial induction of CNI				
Tacrolimus/cyclosporine (%)	61 (77)/18 (23)	33 (70)/14 (30)	32 (76)/10 (24)	27 (84)/5 (16)
Average CNI trough (ng/ml)				
Tacrolimus	9.6 ± 0.2	9.7 ± 0.46	10.5 ± 0.49	10.8 ± 0.59
Cyclosporine	188.6 ± 10.9	179.2 ± 11.0	177.0 ± 37.4	157.5 ± 16.4
Overexposure to CNI†	29 (36%)	18 (38%)	25 (59%)	18 (56%)
MMF use (%)	54 (68)	42 (89)	21(50)	19 (59)
Biopsy-proven rejection (%)	26 (13)	12 (6)	9 (4.5)	9 (4.5)
Clinical outcomes				
RRT (%)	2 (2.5)	2 (4.2)	6 (14)	20 (63)
Progression to I/E class	0	0	0	2 (6%)
Hospital stay (days)	56 ± 4.2	63 ± 6.0	76 ± 7.8	80 ± 10.5
Hospital mortality (%)	2 (2.5)	2 (4.2)	2 (4.8)	10 (31)
Progression to CKD (%)‡	0	1 (2)	1 (3)	13 (59)
Late-phase mortality (%)	6 (8)	3 (7)	7 (18)	7 (32)

CNI, calcineurin inhibitor; GW/RBW, graft weight-to-recipient body weight ratio; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; MMF, mycophenolate mofetil; RRT, renal replacement therapy.

*The first and second half of 200 cases.

†Averaged concentration: tacrolimus trough >10 ng/ml or cyclosporine trough >200 ng/ml within the first month.

‡Chronic kidney disease (KDIGO stage 3/4).

Table 2. Univariate analysis of variables between Group A and Group B.

	Group A (n = 126)	Group B (n = 74)	P-value
Preoperative factors			
Age (years)	49.6 ± 12.72	48.5 ± 10.8	0.530
Sex			
Male/female (%)	78 (62)/48 (38)	37 (50)/37 (50)	0.100
Body mass index (kg/m^2)	23.8 ± 3.3	24.0 ± 4.2	0.750
Background disease			
Postnecrotic liver cirrhosis	82 (65%)	44 (59%)	0.711
Cholestastic disease	24 (19%)	15 (20%)	
Acute liver failure	13 (10%)	11 (15%)	
Metabolic disease	7 (5%)	4 (5%)	
MELD score	15.1 ± 7.6	19.1 ± 0.8	<0.001
HCC (%)	38 (30)	23 (32)	0.843
Serum creatinine level (mg/dl)	0.79 ± 0.4	0.78 ± 0.5	0.870
GFR (ml/min)	75.1 ± 3.1	73.7 ± 3.9	0.388
Serum albumin level (g/dl)	2.98 ± 0.6	2.82 ± 0.6	0.078
Hypertension (%)	14 (11)	8 (11)	0.948
Diabetes mellitus (%)	10 (8)	13 (18)	0.039
Donor/graft factors			
Age (years)	38.5 ± 12.8	41.1 ± 12.5	0.156
Right/left lobe graft (%)	81 (64)/45 (36)	43 (58)/31 (42)	0.385
GW/RBW (%)	0.94 ± 0.27	0.92 ± 0.26	0.727
Operative factors			
Operative time (min)	565.3 ± 105.7	662.9 ± 156.6	<0.001
Blood loss (ml/kg)	95.5 ± 136.2	147.2 ± 153.9	0.017
Cold ischemic time (min)	63.5 ± 38.3	78.8 ± 55.6	0.039
Warm ischemic time (min)	42.2 ± 15.2	44.4 ± 14.7	0.465
Transplant period			
Early/late period (%)*	59 (47)/67 (53)	41 (55)/33 (44)	0.241
Postoperative factors			
Initial induction of CNI			
Tacrolimus/Cyclosporine (%)	94 (75)/32 (25)	59 (80)/15 (20)	0.409
Average CNI trough (ng/ml)			
Tacrolimus	9.4 ± 0.2	10.6 ± 0.3	0.008
Cyclosporine	182.0 ± 6.9	171.4 ± 8.9	0.315
Overexposure to CNI†	47 (37%)	43 (58%)	0.004
MMF use (%)	96 (76)	40 (54)	0.001
Biopsy-proven rejection (%)	37 (18.5)	19.(9.5)	0.574
Biliary fistula (%)	17 (13.5)	6 (8.1)	0.249
Major vascular complication (%)‡	11 (8.7)	10 (13.5)	0.287
Clinical outcomes			
RRT (%)	4 (3)	26 (35)	<0.001
Hospital stay (days)	69.7 ± 48.5	101.5 ± 68.8	<0.001
Hospital mortality (%)	4 (3)	12 (16)	0.001
Progression to CKD (%)§	1 (1)	14 (19)	<0.001
Late-phase mortality (%)	9 (7)	14 (22)	0.004

CNI, calcineurin inhibitor; GW/RBW, graft weight-to-recipient body weight ratio; HCC, hepatocellular carcinoma; MMF, mycophenolate mofetil; RRT, renal replacement therapy.

*The first and second half of 200 cases.

†Averaged concentration: tacrolimus trough >10 ng/ml or cyclosporine trough >200 ng/ml within the first month.

‡Hepatic artery, portal and hepatic vein stenosis needed surgical or radiological intervention.

§Chronic kidney disease (KDIGO stage 3/4).

tive factors appeared significant. Despite higher MELD score in Group B, preoperative serum creatinine (sCr) and GFR did not differ between the two groups. Among donor/graft and operative factors, operative time, blood loss, graft cold ischemic time, and use of MMF seemed to be significant factors related to severe ARI in univariate analysis. In

Table 3. Multivariate logistic regression analysis of variables associated with severe ARI.

	Number	Odds ratio	95% CI	*P*-value
Recipient age (years)				
<50	80	1	–	
≥50	120	0.58	0.22–1.45	0.247
Sex				
Male	115	1	–	
Female	85	1.91	0.79–4.71	0.149
Background disease				
Postnecrotic liver cirrhosis	126	1	–	–
Cholestatic disease	39	0.66	0.20–2.03	0.475
Acute liver failure	24	2.65	0.72–10.2	0.138
Metabolic disease	11	0.30	0.41–1.77	0.475
MELD score				
<20	158	1	–	
≥20	42	2.96	1.19–7.63	0.019
Hypertension				
No	178	1	–	
Yes	22	1.01	0.27–3.58	0.993
Diabetes mellitus				
No	177	1	–	
Yes	23	3.23	1.02–10.7	0.044
Donor age (years)				
<50	142	1	–	
≥50	58	0.91	0.38–2.12	0.839
Graft				
Right lobe graft	124	1	–	
Left lobe graft	76	1.56	0.64–3.81	0.321
Graft volume (GW/RBW, %)				
≥0.7	164	1		
<0.7	36	3.10	1.04–9.79	0.042
Operative time (h)				
<10	105	1	–	
≥10	95	1.13	0.47–2.69	0.776
Blood loss/body weight (ml/kg)				
<55	82	1	–	
≥55	118	3.70	1.53–9.53	0.003
Cold ischemic time (min)				
<80	149	1	–	
≥80	51	2.32	0.96–5.72	0.058
Warm ischemic time (min)				
<50	152	1	–	
≥50	48	1.00	0.39–2.47	0.995
Immunosuppressive induction of CNI				
Cyclosporine	47	1	–	
Tacrolimus	153	1.35	0.47–3.94	0.570
Overexposure to CNI*				
No	110	1	–	
Yes	90	2.59	1.14–6.11	0.022
Combined use of mycophenolate mofetil				
Yes	136	1	–	
No	64	2.50	0.957–6.67	0.061

ARI, acute renal injury; GW/RBW, graft weight-to-recipient body weight ratio; CNI, calcineurin inhibitor.
*Averaged concentration: tacrolimus trough >10 ng/ml or cyclosporine trough >200 ng/ml within the first month.

immunosuppressive therapy, the proportions of CNI were divided equally for two groups. The rate of overexposure to CNI was significantly higher in Group B. Furthermore, Group B also showed the higher average trough level for tacrolimus prior to develop renal dysfunction. As regards MMF-use, MMF was administered to 136 of all 146 patients after the introduction of MMF into our immunosuppression protocol, and in the other 10 patients MMF was stopped because of persistent afebrile diarrhea and bone marrow suppression. However, from another point of view, the average trough levels of tacrolimus in the MMF group were significantly lower than the levels in the non-MMF group (9.02 ± 0.2 ng/ml vs. 10.4 ± 0.26 ng/ml, $P < 0.0001$). And MMF showed the same efficacy in cyclosporine (173.0 ± 5.4 ng/ml vs. 212.7 ± 20 ng/ml, $P = 0.063$). Concerning clinical events, there were no differences between the two groups in biopsy-proven rejection episodes requiring rescue therapy, major biliary and vascular complications, and the transplant period; the first and second half of 200 cases. As a result, the patients in Group B were inferior in rates of requiring RRT, hospital stay and mortality, progression rates of CKD, and late-phase mortality.

On multivariate logistic regression analysis, independent risk factors associated with severe ARI were MELD ≥20 [odds ratio (OR), 2.96; $P = 0.019$], small-for-size graft [graft weight-to-recipient body weight ratio (GW/RBW) <0.7%; OR, 3.10; $P = 0.042$], blood loss/body weight >55 ml/kg (OR, 3.70; $P = 0.042$), overexposure to CNI (OR, 2.59; $P = 0.022$), and preoperative diabetes mellitus (OR, 3.23; $P = 0.044$). Graft size did not appear to be a significant factor in univariate analysis, but was identified as a significant factor after categorization with cutoff value of 0.7% for GW/RBW and consideration of confounding factors in multivariate analysis (Table 3).

A simple scoring system for all patients was then developed, with 1 point assigned to each significant patient-background factor: MELD ≥20; GW/RBW <0.7%; blood loss/body weight >55 ml/kg; overexposure to CNI; and preoperative diabetes mellitus, using a similar odds ratio to that used in multivariate analysis. The patients were divided into four groups according to the number of risk factors (R): R0 ($n = 22$); R1 ($n = 80$); R2 ($n = 61$); R3 ($n = 35$); R4 ($n = 2$); and R5 ($n = 0$). According to this risk classification scoring system, in which R4 was combined with R3, the proportion of postoperative ARI grade in each group was well categorized (Fig. 2).

Discussion

Acute renal injury is a common and important complication of orthotopic liver transplantation, representing a major cause of morbidity and mortality in the postopera-

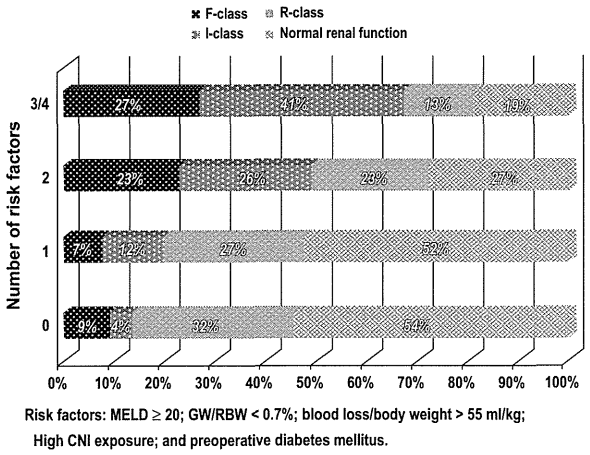

Figure 2 Proportion of acute renal injury after LDLT according to the risk-scoring system. A simple scoring system was developed with one point assigned to each significant risk factor: MELD ≥20; GW/RBW <0.7%; blood loss/body weight >55 ml/kg; high trough concentrations of CNI; and preoperative diabetes mellitus. It categorizes the proportion of ARI after LDLT. LDLT, living donor liver transplantation; MELD, Model for End-stage Liver Disease; GW/RBW, graft weight-to-recipient body weight ratio; CNI, calcineurin inhibitor; ARI, acute renal injury.

tive period [1–3,34]. ARI has been associated with an eight-fold increase in mortality risk [34], prolonged stay in the intensive care unit, and higher hospital costs [35]. Although mortality rates with ARI after OLT have been reported as high (45.1–67%), patients with ARI can have a good prognosis, with a recovery rate of 97% [7,36]. Previous studies have demonstrated preoperative renal injury [2,5,6,8], recipient age, male sex, HCV, preoperative hypertension, diabetes [37], red blood cell transfusion [15], use of vasopressors, overexposure to CNI [30,31,38], and hypoalbuminemia as risk factors for postoperative ARI [16]. However, early postoperative renal function after LDLT has rarely been investigated. This study therefore focused on the relationships between ARI after LDLT and prognosis, as well as on risk factors predicting this serious complication.

Using the RIFLE criteria, ARI after LDLT could be categorized into the R-, I-, or F-class. In our study, the incidence of ARI was 60%, which is a relatively high rate compared with previous reports. However, depending on the definition used for ARI, the incidence of ARI would have different rates. The occurrence of postliver transplant ARI has been reported as 51.5% using the definition of sCr >1.5 mg/dl [5], and as 39.2% using the definition of sCr >2 mg/dl [39]. In the RIFLE criteria, the R-class is defined as a 1.5-fold increase in the sCr and/or >25% decrease in the GFR. This comprehensive definition used in our study accounts for the high incidence of ARI that we observed. Using the definition of doubling in creatinine postliver transplant, the incidence of ARI rises to 37%, which is similar to values previously reported. We also divided the patients into two groups: Group A (normal renal function or R-class); and Group B (I- or F-class). The reason for this grouping related to the comparability and differences in post-transplant prognosis: the overall survival rate in the R-class was comparable to that in the normal renal function group, with survival in both the R-class and the normal renal function group significantly superior to that in the other classes, and with almost all patients in the R-class recovering renal function in the chronic phase. In other words, ARI in the R-class could be within the permissible range. On the other hand, ARI beyond the I-class led to higher hospital mortality rates and poor prognosis in the late phase. The 1- and 5-year overall survival rates were 95.7% and 89.0% in the R-class and 85.7% and 81.8% in the I-class, respectively. It is possible to speculate that ARI in the I-class could affect the lower survival rate in the late phase. We also focused on obvious perioperative ARI impact and simple risk analysis to derive and construct treatment strategies. Therefore, we decided to divide the study patients between the R- and I-class. ARI in Group B tended to progress to CKD and subsequent poor prognosis in the late phase. CKD after liver transplantation has been reported as an independent risk factor of lower patient survival in the late phase [40,41]. Our patients with stage 3/4 CKD had worse prognosis, which could have resulted from infectious episodes and poor tolerance of other treatment modalities for the adverse pathological episodes compared with Group A. The RIFLE criteria were also useful as a prognostic tool for ARI in LDLT. We emphasize that progression beyond the I-class could be a particularly hazardous sign, and may indicate irreversible renal injury after LDLT.

Multivariate analysis revealed that risk factors for severe ARI included preoperative diabetes mellitus, MELD ≥20, small-for size graft (GW/RBW <0.7%), blood loss/body weight >55 ml/kg, and overexposure to CNI. With regard to preoperative factors, diabetes mellitus was reported in 12.5% of pretransplant recipients, and 19.2% developed new-onset diabetes within 1 year after liver transplantation [42], along with increased risk of vascular disease, infection and CKD [43,44]. Some studies have identified pretransplant diabetes as a risk factor for the occurrence of ARI [42,45]. In our study, patients who had insulin-controlled diabetes prior to LDLT showed a significant increase in the incidence of severe ARI. Preoperative creatinine level, which can be used to indicate renal function, is a key component of the MELD calculation. An association between a higher MELD score and post-transplant ARI has been reported [46–48]. Our results support these previous findings that pretransplant renal impairment could have a negative influence on post-transplant renal function. Concerning operative factors, our study indicated that

surgical blood loss, which exerts a major effect on systemic hemodynamics, is a risk factor for severe ARI. Intraoperative hemodynamic instability resulting from blood loss is a well-recognized phenomenon during liver transplantation [49,50]. Vasopressors are known to constrict the renal vasculature, resulting in reductions in renal blood flow. Blood loss and hemodynamic instability are related to a certain extent, but could affect postoperative renal function through different mechanisms. This theory is supported by the fact that blood loss has been identified as an independent risk factor for severe ARI.

Compared to deceased donor liver transplantation, partial liver grafts sometimes cause serious complications. Particularly in adult LDLT, graft size mismatching with partial liver transplantation can cause various problems that may affect the prognosis when the graft cannot sustain excessive portal blood perfusion. This is defined as small-for-size syndrome (SFSS), characterized clinically by large-volume ascites, hyperbilirubinemia, coagulopathy, and ARI [17,51,52]. Some studies have found a significant relationship between small-for-size grafts (GW/RBW <0.8) and ARI after LDLT [52–54]. This condition affects the balance between vasoconstriction and vasodilatory factors and leads to renal dysfunction. ARI after adult LDLT may thus occur because of persistent portal hypertension and a hyperdynamic state in patients with a small-for-size graft [5]. Recent treatment strategies for SFSS, such as portosystemic shunt, splenectomy, and splenic artery ligation or embolization, could improve prognosis [20,55–60]. Furthermore, the lower limit of GW/RBW 0.8% could be reduced to <0.8% through these treatments [58,61]. In our institution, after the introduction of splenic artery ligation and preoperative embolization as portal modulation techniques, a risk cutoff value of 0.7% was set for the risk of SFSS and ARI. Multivariate analysis shows that use of this value has had a significant impact on the occurrence of severe ARI.

Nephrotoxicity resulting from use of a CNI has been well established as a cause of renal dysfunction, resulting from an imbalance in vasoactive substance release [62–64]. The direct toxic effects represent acute microvascular disease with a pattern of thrombotic microangiopathy resembling hemolytic uremic syndrome/thrombotic thrombocytopenic purpura [65]. A toxic concentration of CNI is a noticeable problem. The cutoff value of 10.4 ng/ml for tacrolimus trough and 198 ng/ml for cyclosporine trough for ARI after LDLT were calculated in ROC analysis. These data are in agreement with previous reports [30,38]. Recent studies in liver transplantation have shown that the use of MMF in combination with low CNI levels improves renal function while maintaining adequate immunosuppression [13,38,66]. In this analysis, MMF was less introduced for the patients in Group B, than Group A. As a result, the average trough level of tacrolimus in Group B was significantly higher than Group A. And CNI trough levels in immunosuppressive protocol with MMF were lower than those without MMF in all cases. So we speculated that the factor of MMF could be indicated as significant by an actually lowered CNI level and contribute to prevention of severe ARI. Thus, a reduced CNI exposure by adding MMF is beneficial in terms of renal impairment after LDLT and should be preferred to conventional dosage. Modification in nephrotoxic immunosuppressive regimens with MMF to avoid postoperative ARI could lead to favorable renal outcomes.

Concerning the treatment strategies for prophylaxis of severe ARI after LDLT, our scoring system that focuses on significant risk factors could offer a useful tool. For example, a recipient with a high MELD and insulin-controlled preoperative diabetes mellitus initially has a substantial risk of progressing to severe ARI. A systematic plan for perioperative and postoperative care should thus be considered, comprising a donor liver with sufficient graft volume, use of MMF in combination with reduced CNI use, transfusion in the perioperative phase, and early introduction of RRT to arrest progression toward severe ARI.

Severe ARI after LDLT is a risk factor for poor prognosis, which is associated with increased hospital mortality and which predicts the development of advanced CKD. We conclude that the RIFLE classification offers a simple and useful tool for stratifying the severity of ARI after LDLT. Discretionary choices in transplant surgery and the subsequent medical care are very restricted. So in these complicated situations, RIFLE is a very simple and useful predictive tool after LDLT and could contribute toward improved transplant prognosis in terms of medical care. However, the determination of RIFLE criteria after transplantation might be useful only with respect to the laboratory results and prediction made at that particular time in the patient's postoperative course. The essential point is the benefit of constructing suitable preventive and treatment strategies for ARI after LDLT. Such strategies should be based on the patient's etiology and risk factors for ARI. Our results suggest five risk factors for ARI after LDLT: MELD ≥20; GW/RBW <0.7%; blood loss/body weight >55 ml/kg; overexposure to CNI; and preoperative diabetes mellitus. Furthermore, the scoring system for these risk factors could categorize the grade of ARI severity after LDLT according to the RIFLE criteria. These risk factors could be mitigated through intentional care management: (i) strict therapeutic drug monitoring for CNI and (ii) accepting only donor livers with sufficient graft volume (i.e., GW/RBW more than 0.7% in high-risk recipients with MELD more than 20 and/or diabetes mellitus). The immunosuppressive regimen should be modified by MMF and any other agent for the sake of lowering CNI dose, especially in tacrolimus [38,67]. Perioperative treatment strategies should be designed and balanced based on the

risk factors for the further improvement of transplant prognosis.

Authorship

MU: participated in data analysis and writing of the paper. YU: participated in research design and writing of the paper. TY and TF: participated in research design. HS, TN, HM, AT, SS, RY, DS, DN and TF: participated in data analysis.

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Hepatology Research

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Case Report

First successful case of simultaneous liver and kidney transplantation for patients with chronic liver and renal failure in Japan

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Establishment of a preferential liver allocation rule for simultaneous liver and kidney transplantation (SLK) and revisions of laws regarding organ transplants from deceased donors have paved the way for SLK in Japan. Very few cases of SLK have been attempted in Japan, and no such recipients have survived for longer than 40 days. The present report describes a case of a 50-year-old woman who had undergone living donor liver transplantation at the age of 38 years for management of post-partum liver failure. After the first transplant surgery, she developed hepatic vein stenosis and severe hypersplenism requiring splenectomy. She was then initiated on hemodialysis (HD) due to the deterioration of renal function after insertion of a hepatic vein stent. She was listed as a candidate for SLK in 2011 because she required frequent plasma exchange for hepatic coma. When her Model for End-stage Liver Disease score reached 46, the new liver was donated 46 days after registration. The reduced trisegment liver and the kidney grafts were simultaneously transplanted under veno-venous bypass and intraoperative HD. The hepatic artery was reconstructed prior to portal reconstruction in order to shorten anhepatic time. Although she developed subcapsular bleeding caused by hepatic contusion on the next day, subsequent hemostasis was obtained by transcatheter embolization. Thereafter, her recovery was uneventful, except for mild rejection and renal tubular acidosis of the kidney graft. This case highlights the need to establish Japanese criteria for SLK.

Key words: deceased donor, kidney transplantation, liver transplantation, living donor, retransplantation, simultaneous

INTRODUCTION

WITH THE INDUCTION of the Model for End-Stage Liver Disease (MELD) score into the liver allocation system in 2002, the proportion of simultaneous liver and kidney transplantation (SLK) among those undergoing deceased donor liver transplantation (DDLT) has steadily increased in the USA. In fact, 7.1% ($n = 444$) of DDLT in the United Network of Organ Sharing database in 2007 were comprised of SLK.[1,2]

In Japan, liver transplantation was initiated as living donor liver transplantation (LDLT), and SLK had not been utilized to address concomitant hepatic and renal failure, mainly due to medical and ethical problems concerning multi-organ donation from a living donor. However, establishment of a preferential liver allocation rule for the SLK candidate in 2006 and revision of laws regarding organ transplants from deceased donors in 2010 have paved the way for SLK in Japan. The present report describes the first successful case of SLK in Japan that was performed for a critically ill patient that had previously undergone LDLT.

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CASE REPORT

A 50-YEAR-OLD WOMAN had been transplanted with a left lobe graft from her father at the age of 38 years in 2000 due to post-partum liver necrosis.[3] After surgery, she required repeated balloon dilatation

procedures of the hepatic vein (HV) in order to revise HV stenosis. She also underwent splenectomy in 2009 due to the development of pancytopenia related to hypersplenism. After splenectomy, she experienced recurrent monthly episodes of hepatic encephalopathy. In August 2010, her liver and kidney function declined, requiring placement of an 8-mm self-expandable stent within the HV and initiation of hemodialysis (HD). Retroperitoneal dissection with ligations of the porto-caval communicator was performed in December 2011, but hyperammonemia and encephalopathy persisted. Therefore, in July 2011, she was placed on a national waiting list as a candidate for SLK at another transplantation center. At initial registration, the severity of her hepatic failure was associated with a MELD score of 15 and a Child–Pugh score of 10.

Her serum bilirubin level increased to more than 30 mg/dL, and she developed portal thrombus, massive ascites, functional ileus and recurrent episodes of hepatic coma, which prompted repeated use of plasma exchange beginning in May 2012. Upon reevaluation at the original facility, she was deemed too ill to undergo SLK. In July 2012, she was referred to our institution with a MELD score of 46 and a Child–Pugh score of 13, grade C. A set of liver and kidney grafts became available 46 days after the patient established care at our facility, and she was subsequently airlifted to our institution.

On admission, the patient's serum total bilirubin level, prothrombin international normalized ratio and serum creatinine level after HD was 26, 2.72 and 6.16 mg/dL, respectively. She underwent HD twice a week but still produced over 400 mL of daily urine. Her serum erythropoietin level was 78.7 mIU/mL. Persistent functional ileus resulted in relative malnutrition, and daily output from a nasogastric tube was 2–3 L. Preoperative abdominal computed tomography (CT) revealed progression of Yerdel's grade 3 portal thrombus,[4] massive ascites and severe edema of the alimentary tract (Fig. 1).

Transplant surgery

The protocol of simultaneous liver and kidney transplantation was approved by the Ethical Committee of Okayama University Hospital and conformed to the provisions of the Declaration of Helsinki. Both the left axillary and the right saphenous vein were exposed for veno-venous bypass during the anhepatic phase.[5] The left femoral artery was also kept as a blood pumping route for cardiopulmonary support in case of unexpected cardiac arrest. After detachment of the previous jejunal loop at the hepatic hilum, the left lobe graft was dissected from the surrounding adhesion, which was composed of stony hard fibrous tissue containing network-like varices. During the dissection procedure, suppurative fluid was expressed from the surrounding tissue around the former hepatic vein anastomosis. The inflammatory fibrous ring squeezed the upper cava in a manner similar to that seen in Budd–Chiari syndrome. Then, veno-venous bypass was started to decrease the venous pressure of the inferior cava. After cross-clamp at the supra- and infrahepatic vena cava, the cirrhotic graft was explanted, and the anterior wall of the upper cava including the venous stent was resected. The new graft weighing 1360 g was mismatched to the small-built recipient (height, 150 cm; bodyweight, 45.3 kg) with severe edema in the intraperitoneal space. The graft was reduced to 1060 g by lateral segmentectomy, and kept 2.34% graft-to-recipient weight ratio. The reduced tri-

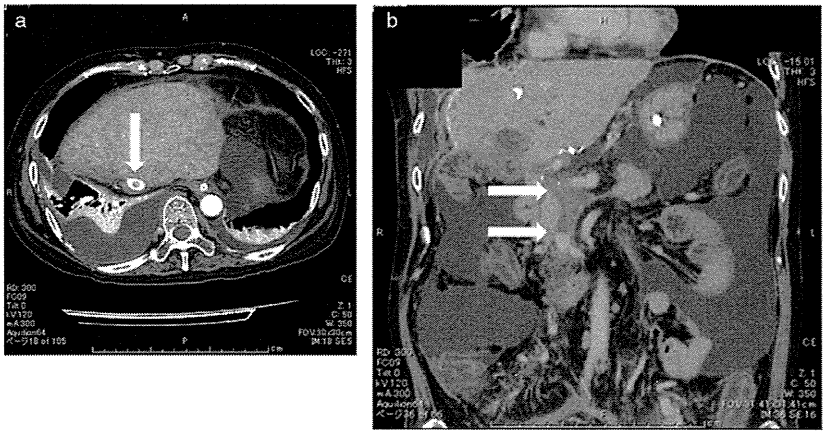

Figure 1 Computed tomography images before liver and kidney transplantation. (a) Perfusion in the hepatic parenchyma of the transplanted left lobe graft is poor. The self-expanding stent is seen in the orifice of the middle hepatic vein (arrow). (b) Portal thrombus extends from the intrahepatic portal branches to the infra-pancreatic superior mesenteric vein (arrows, Yerdel's grade 3). Massive ascites and severe edema of the alimentary tract are present.

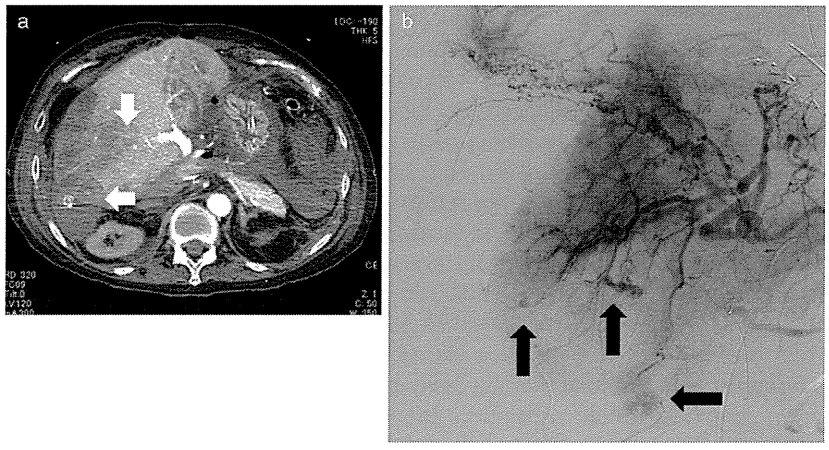

Figure 2 Computed tomography and celiac angiography images of intra-abdominal bleeding on postoperative day 1. (a) Subcapsular bleeding and retention of oozing blood around the retransplanted trisegment graft are seen (arrows). (b) Parenchymal contusion and extravasation from the arteries of segment 5 and 6 are shown (arrows).

segment graft with a 4.5-cm^2 caval patch was anastomosed to the defect of the anterior wall of the native vena cava. Next, the common hepatic artery of graft was anastomosed to the native hepatic artery in an end-to-end manner. The new graft was reperfused prior to the portal anastomosis, and the veno-venous bypass was removed after securing the hepatic circulation. Although either a reno-portal hemi-transposition or a jump graft technique was planned for management of portal thrombus,[6,7] intensive thrombectomy was chosen because the risk of uncontrollable bleeding made expansion of the dissecting area too dangerous. Reconstruction of the thrombectomized portal trunk provided 10 cm/s of hepatopetal mean flow, and choledochojejunostomy was subsequently performed. The celiac-mesenteric angiography on the next day revealed that most of the portal thrombus was removed by retrograde extirpation. Since there was little collateral circulation as shown in the preoperative CT, additional devascularization for collateral vessels was not carried out. After closure of the abdominal wound, the kidney allograft was transplanted to the iliac artery and vein in the right iliac fossa by the extraperitoneal approach. Initial urination was obtained at 10 min after reperfusion of the kidney graft. Operation time, cold ischemic time of the liver and the kidney were 16 h 35 min, 9 h 40 min and 16 h 27 min, respectively. Intraoperative blood loss was 22 970 mL, and 64 units of packed red blood cell were infused.

Postoperative course

The histological findings of the explanted liver showed the whole liver necrosis with severe portal thrombosis. Inflammatory cell infiltration into the Glisson's area, cholangitis and endotheliitis were not seen. Immunosuppression was induced with basiliximab and maintained by tacrolimus, methylprednisolone and mycophenolate mofetil. On postoperative day 1, progression of anemia and fresh bleeding from the intra-abdominal drains were observed. CT scan revealed massive subcapsular hematoma and parenchymal contusion of segments 5 and 6 in the new liver (Fig. 2a), and hemostasis was subsequently achieved by emergent transcatheter embolization (Fig. 2b). The prominent liver traumas were not detected in preoperative CT images of the donor and manipulation during organ procurement. Because the cause of brain death of the donor was a fall injury of the skull, the impact of the accident might have created the latent cracking wounds in the liver. The recipient experienced episodes of mild acute rejection of the kidney and an episode of type IV renal tubular acidosis, but these complications were treated with steroid pulses and the reduction of the trough level of tacrolimus, respectively (Fig. 3). The recipient was discharged in an ambulatory fashion on postoperative day 68.

DISCUSSION

THE HIGH MELD score, HD, history of poly-surgery, inserted HV stent and intestinal edema all indicated the likely complexity of transplant surgery in the present case. The patient continued to produce urine and have a high serum erythropoietin level at 2 years after initiation of HD induction; therefore, it was unlikely that she had hepatorenal syndrome alone. Rapid progression of renal and hepatic failure had been observed after placement of the HV stent, and thus the caval stricture may have

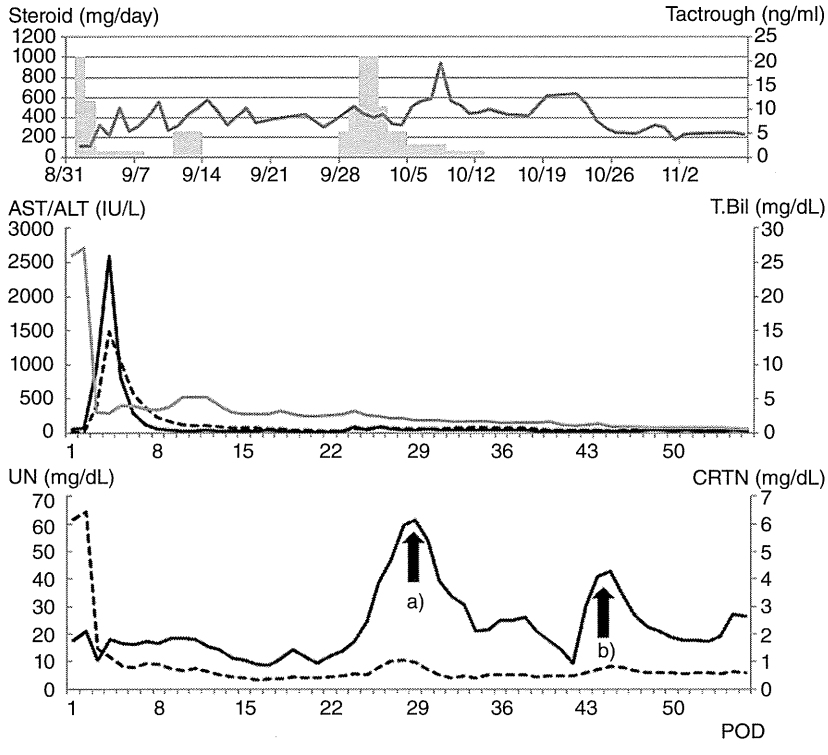

Figure 3 Clinical course of the recipient. The upper, middle and lower lines indicate the daily dose of steroid, the trough level of tacrolimus and the graft function of the liver and kidney, respectively. Acute cellular rejection of kidney alone (a) was treated with steroid pulse therapy. An episode of renal tubular acidosis resolved after reducing the dose of tacrolimus (b). ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRTN and Cr, creatinine; POD, postoperative day; T.Bil, total bilirubin; Tac, tacrolimus; UN, urea nitrogen. ▒, steroids (mg/day); ——, tacrolimus (ng/mL); ——, AST; - - -, ALT; ——, T.Bil; ——, UN; - - -, CRTN.

arisen secondary to the HV stent. We hypothesized that an additional decrease in lower caval flow led to congestive renal failure and persistent edema in the lower half of the body. The long-standing hypovolemic condition of the upper half of the body might have increased the risk of hypovolemic shock or cardiac arrest in the context of bleeding during transplant surgery. Therefore, we planned the following strategy: (i) the veno-venous bypass should be started from the early phase of surgery to correct blood imbalance between the upper and lower portions of the body; (ii) intraoperative HD should be simultaneously started to prevent hyperkalemia and acidosis that may otherwise occur in the context of massive transfusion; and (iii) the presence of portal thrombus lengthens the time required for the portal reconstruction, so reconstruction of the hepatic artery should be performed first to shorten the anhepatic time.

Simultaneous liver and kidney transplantation is not necessarily associated with a higher risk of postoperative mortality than liver transplantation alone (LTA).[1] Outcomes for HD patients undergoing LTA are worse than those for patients undergoing SLK.[8] As reported by Kamada *et al.*, liver graft has native immunomodulatory effects; therefore, multi-organ transplantation involving liver graft is associated with better outcomes than single organ transplantation before the popularization of tacrolimus.[9,10] Despite inclusion of kidney transplantation, SLK does not require a direct cross-match test between the ABO identical or compatible pair. When recipients require liver and kidney transplantation, SLK has an immunological advantage over serial transplantation (kidney transplantation after liver transplantationor liver transplantation after kidney transplantation) in terms of avoiding the risk of pre-sensitization.[11] Kamada also suggested that it may not be possible to rescue the kidney graft when the liver graft from the same donor is rejected. Therefore, prevention of acute rejection in the liver graft was the primary goal of immunosuppression. We planned for a target tacrolimus trough level of more than 10 ng/mL for 2 months, and thus despite an episode of minor rejection of the kidney graft, rejection of the liver graft did not occur (Fig. 3). However, maintenance of a high tacrolimus trough level led to renal tubular acidosis, a dose-dependent complication.[12,13]

Relatively heavy weighting of the serum creatinine in the equation of MELD score has resulted in greater priority for transplantation among patients with renal dys-

function than those without.[14,15] The increased use of SLK in the USA has raised the following questions related to organ allocation: (i) what are the indications for SLK for liver transplant recipients who have concomitant acute kidney injury (AKI) or chronic kidney disease (CKD)?; and (ii) how should kidney transplant recipients with mild liver failure be managed?

Ojo *et al.* reported that LTA recipients had an 18.1% rate of 5-year CKD morbidity requiring maintenance HD or renal transplantation. They also reported that increasing age, female sex, hepatitis C infection, hypertension, diabetes mellitus and postoperative AKI were risk factors for renal failure after LTA.[16] Northup *et al.* evaluated the correlation between preoperative duration of HD and postoperative native kidney function in LTA recipients. Recipients who had been HD-dependent less than 30 days, 30–60 days, 60–90 days and over 90 days had 70%, 56%, 23% and 11% recovery rates from HD, respectively.[17] Independent risk factors for mortality and graft loss ratio in patients undergoing SLK were as follows: recipient age of more than 65 years; male sex; black race; hepatitis C/diabetes mellitus status; donor age of more than 60 years; serum creatinine level of more than 2.0 mg/dL; cold ischemia time of more than 12 h; and warm ischemia time of more than 60 min.[1] The multidisciplinary American consensus conference suggested 6 weeks as a threshold for the preoperative HD period, after which SLK should be considered.[2] However, they also reported a significant gray zone between 6 and 12 weeks during which some AKI patients still recover renal function after LTA.[18] Renal biopsy is feasible in liver transplant candidates with AKI and provides reproducible histological information that does not relate to the pretransplant clinical data.[19] Therefore, intraoperative renal biopsy was planned for recipients in the gray zone as a new tool for clinical decision-making in patients undergoing SLK. However, intraoperative change in the treatment plan is not practical for use with organ procurement or within the organ allocation system. Nadim *et al.* recommended the newest SLK criteria, including preoperative kidney biopsy, in the "Simultaneous Liver–Kidney Transplantation Summit".[20] Indications of SLK are complicated and determined by nephrological aspects such as CKD or AKI, degree of proteinuria and presence of metabolic disease as follows:

1 Candidates with persistent AKI for 4 weeks or more with one of the following: (i) stage 3 AKI as defined by modified RIFLE (i.e. a threefold increase in serum creatinine [sCr] from baseline, sCr ≥4.0 mg/dL with an acute increase of ≥0.5 mg/dL or on renal replacement therapy); and/or (ii) estimated glomerular filtrating ratio (eGFR) of 35 mL/min or less or GFR of 25 mL/min or less (iothalamate clearance).
2 Candidates with CKD, as defined by the National Kidney Foundation, for 3 months with one of the following: (i) eGFR of 40 mL/min or less or GFR of 30 mL/min or less (iothalamate clearance); (ii) proteinuria of 2 g/day or more; (iii) kidney biopsy showing more than 30% global glomerulosclerosis or more than 30% interstitial fibrosis; or (iv) metabolic disease.

According to the Japanese Evaluation Committee of Indications (JECI) for DDLT, severity of hepatic dysfunction and urgency of liver transplantation is determined on the basis of MELD and Child–Pugh score. The major advantages of MELD scores (objectivity, simplicity and reproducibility) may be offset by concomitant use of the Child–Pugh score in our system. However, because the Child–Pugh score includes liver-originated factors such as albumin level, ascites and encephalopathy, the JECI system may help exclude kidney transplant recipients with mild liver failure from the waiting list for DDLT. Although our rule states that "the kidney graft should be preferentially allocated to patients with liver disease accompanied by irreversible kidney damage requiring liver transplantation", criteria for "irreversible" kidney damage have yet to be clearly defined, especially for patients with AKI. Debate regarding utilization of kidneys from deceased donors is required in Japan, especially due to dilemmas concerning the choice between SLK and LTA.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Which patients respond best to hepatitis B vaccination after a hepatitis B virus-related liver transplantation?

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Abstract
Background A combination of hepatitis B immunoglobulin and nucleos(t)ide analogues is the current standard of care for controlling hepatitis B recurrence after orthotopic liver transplantation (OLT). However, frequent immunoglobulin treatment is expensive and inconvenient. This study investigated the efficacy of hepatitis B virus (HBV) vaccination in preventing the recurrence of hepatitis B after living donor OLT.
Methods Twenty-seven patients who had undergone living donor OLT participated in the study; five had acute HBV infected liver failure (ALF-OLT) and 22 had HBV related liver cirrhosis (LC-OLT). Hepatitis B surface antigen (HBsAg)-containing vaccine was administered to them for at least 1 year after transplantation and continued once monthly for up to 36 months post-OLT. Patients who had anti-HBs antibody titers above 100 mIU/mL for a minimum of 6 months without immunoglobulin administration were defined as good responders; the others were defined as poor responders. Interferon-γ enzyme-linked immunospot assays against HBs and HBc antigens were used to assay cellular immune responses.
Results All five of the ALF-OLT patients had good responses after a median of four (range 2.5–5) vaccinations. Nine of the 22 LC-OLT patients had good responses after a median of 19 (range 11.5–30) vaccinations. Among the LC-OLT group, those with livers donated by relatively higher-aged, marital and high-titer anti-HBs antibody donors were good responders. LC-OLT patients classed as good responders showed interferon-γ responses comparable to those of the ALF-OLT patients.
Conclusions The ALF-OLT and LC-OLT patients who received livers from relatively higher-aged, marital, high-titer anti-HBs antibody donors were the best candidates for HBV vaccine administration. Boosting donors before transplantation may facilitate later vaccine response of the recipients.

Keywords Vaccination · Living donor liver transplantation · Hepatitis B immunoglobulin · Marital donor · Immune response

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Introduction

Prior to the introduction of effective post-transplantation antiviral prophylaxis, liver transplantation for hepatitis B virus (HBV)-related disease was usually followed by immediate HBV reinfection of the allograft, resulting in a fatal hepatitis B recurrence [1–3]. Recent studies have found that treatment with a combination of hepatitis B

immunoglobulin (HBIg) and nucleos(t)ide analogues decreases the risk of hepatitis B recurrence, and achieves a higher rate of graft survival [4–8]. However, long-term administration of HBIg is associated with several unresolved issues, including limited availability and extremely high cost, so several protocols for treatment with low-dose HBIg in combination with nucleos(t)ide analogue have been reported [9–12]. Previously, we reported that treatment with high-dose HBIg in the early period post-transplantation followed by low-dose HBIg with nucleos(t)ide analogues offers reliable, cost-effective control of hepatitis B recurrence [13]. However, even with such a simplified protocol, patients would still need to receive a drip infusion or intramuscular injection of hundreds to thousands of units of HBIg every 2–3 months.

Active immunization of post-orthotopic liver transplantation (OLT) recipients with HBV vaccine is a recently emerging approach. However, most studies report low response rates, even with double concentration of vaccines or prolonged vaccination regimens [14, 15]. Patients who had not been HBV carriers [e.g., acute liver failure (ALF) patients following sexual transmission of HBV as an adult; or non-chronic HBV carrier patients who received hepatitis B core antibody (HBcAb)-positive livers] are accepted as good candidates for vaccine administration [15, 16]. Vaccination in patients who have been HBV carriers or liver cirrhosis (LC) patients typically yields disappointing results [14, 15]. Understanding how different cohorts respond to HBV vaccination is critical to the design of safe, cost-saving, and custom-designed prophylaxis protocols.

It remains unclear to what extent cellular immune responses may contribute to protection from HBV reinfection. Since non-carrier patients respond well to the HBV vaccination, immune tolerance is expected to play a large role in this process. Yet only a few reports have mentioned T cell immune reaction after HBV-related OLT [14].

In this report, we assessed a monthly, long-term vaccination protocol starting 1 year after OLT, to investigate those characteristics that could discriminate between the vaccine-responsive and non-responsive patients. In addition to anti-hepatitis B surface (anti-HBs) antibody titer due to a humoral immune response, CD4 T cell immune responses to hepatitis B surface antigen (HBsAg) were used to assess the cellular immune response to vaccination in immunocompetent patients.

Methods

Patients

From October 1996 to June 2011, OLT was performed in 264 adults at Okayama University Hospital. Of these, ten patients had ALF due to acute HBV infection. Thirty-seven patients had end-stage LC due to chronic life-long HBV infection. Five-year survival rates were 88 and 87 % for HBV-related ALF patients and for HBV-related LC patients, respectively.

The HBV vaccine was administered to five ALF patients (ALF-OLT) and 22 LC patients (LC-OLT). The general characteristics of the patients included in this study are summarized in Table 1. All of them received living donor liver transplantation (LDLT). The numerical data are expressed as median and interquartile range values, and categorical data are presented as positive counts or percentages in all tables.

For analysis of the HBV-specific cellular immune response (Table 2), the study enrolled all five ALF-OLT patients, along with 15 of the 22 LC-OLT patients. Additionally, 11 healthy volunteers who had received the HBV vaccine and developed a successful anti-HBs antibody response (termed 'Healthy vaccine'), ten patients with chronic hepatitis B (termed 'Chronic hepatitis'), and five patients who recovered from acute hepatitis B (termed 'Self-limited') were enrolled as controls. The five patients who recovered from acute hepatitis B had a history of acute hepatitis B diagnosed with high-titer IgM-HBc antibody response, and presented as HBsAg negative, anti-HBs antibody positive, anti-HBc antibody positive at the time of

Table 1 Patient characteristics

N	ALF 5	LC 22
Recipient related factors		
Age at OLT	29 (27–46)	53 (47–56)
Age at start of vaccine	36 (30–51)	56 (49–59)
Sex (M)	1 (20 %)	19 (86 %)
HBsAg at OLT	0.7 (0–1)	2000 (100–2000)
HBV DNA at OLT (≥3.7)	0 (0 %)	8 (36 %)
MELD at OLT	21 [19–21]	15 [9–18]
HCC at OLT (+)	0 (0 %)	15 (68 %)
Donor related factors		
Age at OLT	32 (27–44)	46 (31–49)
Sex (M)	4 (80 %)	9 (40 %)
ABO (identical)	4 (80 %)	12 (54 %)
Blood relation (no)	0 (0 %)	8 (36 %)
Anti-HBs antibody (>100)	1 (20 %)	9 (40 %)
Anti-HBc antibody (+)	1 (20 %)	11 (50 %)
Anti-HBc(+)/anti-HBs(+)	1 (20 %)	10 (45 %)
Anti-HBc(+)/anti-HBs(−)	0 (0 %)	1 (4 %)
Anti-HBc(−)/anti-HBs(+)	0 (0 %)	0 (0 %)

ALF acute liver failure, *LC* liver cirrhosis, *OLT* orthotopic liver transplantation, *MELD* Model for End-stage Liver Disease, *HCC* hepatocellular carcinoma

Table 2 Characteristics of the cases for HBV antigen-specific T cell response

N	Healthy vaccine 11	Chronic hepatitis 10	Self-limited 5	ALF-OLT 4	LC-OLT-good 8	LC-OLT-poor 7
Age	29 (28–31)	53 (42.5–61)	67 (58.5–77)	41.5 (37.2–47.2)	60 (53–62)	55 (40–58)
Sex [M (%)]	10 (91)	7 (70)	2 (40)	0 (0)	8 (100)	7 (100)
HBs Ag (+)	0	10 [titer 2000 (1893–2000)]	0	0	0	0
HBs Ab (IU/l) (>100/≤100)	8/3	0/10	2/3	2/2	4/4	1/6

LC-OLT-poor patients received HBIG within 3 months

Age and HBsAg were shown as median (interquartile range)

ALF-OLT acute liver failure patients who received OLT, *LC-OLT-good* liver cirhosis patients who received OLT and had a good vaccine response, *LC-OLT-poor* liver cirrhosis patients who received OLT and had a poor vaccine response

the study. The chronic hepatitis B patients were followed for several years at our hospital and all were HBsAg positive with a median HBV-DNA titer of 2.5 (interquartile range 2.1–4.2) logcopies/mL. The healthy volunteers had no HBsAg and anti-HBc antibodies, and the median anti-HBs antibody level was 240 (interquartile range 100–797) mIU/mL.

Informed consent was obtained from each patient included in the study, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in the approval by the Ethics Committee at the Okayama University Hospital.

Antiviral prophylaxis

Our HBV prophylaxis protocol was as follows. We administered HBIg at 200 IU/kg intraoperatively. Recipients were administered another 2000 IU/week HBIg for an additional 1 week post-operatively. HBIg (2000 IU) was administered thereafter only when anti-HBs antibody titers fell below 100 mIU/mL. After 6 months, HBIg was administered only to maintain anti-HBs antibody titers at >10 mIU/mL. We measured levels of HBsAg and anti-HBs antibody and/or HBV-DNA every month for 6 months after LDLT, and every 2–3 months thereafter. Three of the ALF-OLT patients were anti-HBs antibody positive at the time of OLT, these patients were not administered nucleos(t)ide analogues. The remaining two ALF-OLT patients, and all of the LC patients were given nucleos(t)ide analogues. The two ALF-OLT patients were given lamivudine (LAM), and of the 22 LC-OLT patients, 14 received LAM, six were given LAM + adefovir dipivoxyl (ADV), and two received entecavir (ETV). Administration of nucleos(t)ide analogues was started a minimum of 1 month pre-operatively, when possible.

Post-OLT re-activation of HBV was defined as continuous positivity for serum HBsAg and/or serum HBV-DNA.

HBV vaccine protocol

HBV vaccine administration was initiated at least 1 year after OLT, and when patients showed no active infection or rejection episode in the preceding month. The vaccine consisted of recombinant purified HBsAg (Bimmugen; Kaketsuken, Kumamoto, Japan). Ten micrograms were administered every 1–2 months. Based on the effect of the vaccine, patients were classified as "good responders; LC-OLT good" or "poor responders; LC-OLT poor". Patients who showed anti-HBs antibody titers above 100 mIU/mL without HBIg for a minimum of 6 months were defined as good responders, since all of these patients did not need HBIg administration for an additional 2 years (median) of follow-up. All other patients were defined as poor responders. Patients who showed a good response within 36 months were given additional vaccinations when their anti-HBs antibody titer decreased, whereas vaccination was stopped in patients who showed no good response after 36 months.

Immune suppression

Patients were treated using a standard immunosuppressive regimen (tacrolimus or cyclosporine A with steroids and/or mycophenolate mofetil). One patient was free from calcineurin inhibitors at the time of vaccine administration.

Routine laboratory tests and serum HBV-DNA assay

Hepatitis B surface antigen, anti-HBs antibody, hepatitis Be antigen (HBeAg), and anti-HBe antibody (HBeAb) levels were measured routinely using a commercially available chemiluminescent enzyme immunoassay system (Lumipulse System; Fujirebio, Tokyo, Japan). HBV-DNA levels were measured using a transcription-mediated amplification assay (TMA) (SRL, Tokyo, Japan), a polymerase chain reaction (PCR) assay (Amplicor HBV

Monitor assay; Roche Diagnostics, Tokyo, Japan), or a real-time PCR assay (COBAS TaqMan HBV Test; Roche Diagnostics).

HBV recombinant proteins for cellular immune response analysis

Hepatitis B virus recombinant protein HBsAg was purchased from Advanced ImmunoChemical, Inc. (Long Beach, CA). Recombinant protein hepatitis B core antigen (HBcAg) was purchased from the Institute of Immunology (Tokyo, Japan). These proteins were used as stimulating antigens at 1 μg/mL for the enzyme-linked immunospot (ELISPOT) assay.

CD14-positive monocyte isolation and myeloid DC generation

Mononuclear cells were separated from peripheral blood by centrifugation on the Ficoll-Hypaque density gradient (Amersham Pharmacia, Uppsala, Sweden), as previously described. CD14-positive monocytes were purified using microbeads (Miltenyi Biotec, Auburn, CA) in accordance with the protocols of the manufacturer. Subsequently, CD4-positive T cells (T4) were positively sorted in the same way. T4 cells were frozen immediately. CD14-positive cells were cultured at 1×10^6/mL in RPMI containing 5 % heat-inactivated human AB serum (ICN Biomedicals; Aurora, OH) supplemented with 100 ng/mL of granulocyte macrophage colony-stimulating factor (kindly provided by Kirin Pharma, Tokyo, Japan) and 50 ng/mL of interleukin-4 (kindly provided by Ono Pharmaceuticals, Osaka, Japan) at 37 °C in 5 % CO_2 for 5 days. Cells were confirmed to be CD11c-positive myeloid immature dendritic cells (DC).

Interferon-γ (IFNγ) ELISPOT assay with myeloid DC and CD4-positive T-cells

The immature DC cultures were exposed to recombinant HBsAg and HBcAg (1 μg/mL each) for 1 day. To mature the DCs, 1 ng/mL of lipopolysaccharide (LPS) (Sigma, St. Louis, MO) was added to the culture 1 day after HBV protein addition. On the same day, mouse anti-human interferon-γ antibody (MABTECH, Sweden) was diluted to 5 μg/mL with ELISPOT buffer (0.159 % Na_2CO_3, 0.293 % $NaHCO_3$) and coated overnight at 4 °C onto 96-well filtration plates (Millipore, Billerica, MA) at 100 μL per well. The coated plate was washed with phosphate-buffered saline (PBS) and blocked with 10 % fetal calf serum in RPMI1640 medium for 1–2 h. Myeloid DCs were counted and seeded at 5×10^3/well. Cryopreserved T4 cells were thawed, counted, and seeded at 2×10^5/well. On the next day, the plate was washed six times with PBS. Wells were coated with rabbit anti-interferon-γ serum (diluted to 1/800 in PBS), and the plate was incubated at 37 °C for 2 h. The plate was washed six times with PBS and coated with goat anti-rabbit immunoglobulin G-alkaline phosphatase (IgG-AP; Southern Biotech, Birmingham, AL) diluted to 1/2000 with PBS. After a 1 h incubation at 37 °C, the plate was washed six times with water and spots were developed using 5-bromo-4-chloro-3-indolyl phosphate *p*-toluidine salt and nitroblue tetrazolium chloride (BCIP/NBT) as a substrate. Spot development was stopped after 10 min by washing with distilled water. The spots were viewed and counted under a microscope.

Statistical analysis

Statistical comparisons were performed using JMP version 9 (SAS Institute, Cary, NC, USA). The Wilcoxon rank-sum test was used to compare the continuous data and the Chi-square test was used to compare categorical data. For multivariate analysis, logistic regression analysis was used. The Steel–Dwass test was used for multiple group analysis. A p value of <0.05 was considered significant.

Results

The effects of HBV vaccination

None of the patients in the ALF-OLT group showed reactivation of the virus. One patient of the LC-OLT group showed transient positive responses for HBsAg and HBV DNA, however, these became negative again with frequent HBIg administration. At the final observation point, no patients showed HBsAg or HBV DNA-positive response. All five ALF-OLT patients had good responses to vaccination (Table 3). A median of four (range 2.5–5) vaccinations were sufficient to induce a good response. In contrast, LC-OLT patients were less responsive, with only nine of 22 displaying a good response. Additionally, these nine good responders required a median of 19 (range 11.5–30) vaccinations before these patients could be weaned from HBIg administration (Fig. 1).

Table 3 Results of HBV vaccination

N	ALF 5	LC 22
Response to vaccination (good/poor responders)	5/0	9/13
Number of vaccinations require before ceasing HBIg treatment	4 (2.5–5)	19 (11.5–30)

HBIg Hepatitis B immunoglobulin

Vaccine safety

None of the patients showed any adverse reactions as judged by their general condition, or by laboratory examination. One patient reported itchiness after injection of the eighth vaccination dose, although the symptom subsequently stopped.

The characteristics of vaccine responsiveness in LC-OLT patients

To determine the characteristics for defining a good response in LC-OLT patients, clinical data from recipients and donors were investigated (Table 4). The background data of the recipients, including HBV-DNA levels, HBeAg positive reactions, HBsAg levels at the time of OLT, and the anti-HBs antibody titer at the time of the initial vaccination did not differ between the good and poor responder groups (Table 5). However, the donor-related factors did differ. Notably, the good responders' donors were relatively high in age ($p = 0.019$) and not blood relatives of the recipients ($p < 0.001$). These donors (to good responders) showed high anti-HBs antibody titers at the time of OLT ($p = 0.038$). Since all of the patients in this study received LDLT, non-blood-related donors all corresponded to spouses of the OLT recipients. Multivariate logistic regression analysis was carried out with the following variables: donor age at OLT $\geq$47, non-blood-related donor, donor anti-HBs antibody titer >100 mIU/mL (Table 6). A status of non-blood-related donor was identified as a significant independent predictor of a good response to vaccination. Since the donor anti-HBs antibody was one of the factors associated with a good response, we asked whether the donors had received vaccination, and found that none of them had ever received an HBV vaccine. As shown in Table 4, none of the donors showed the anti-HBc antibody-negative, anti-HBs antibody-positive condition which indicates vaccine-induced seropositivity to the HBs antigen.

HBV antigen-specific immune responses

To determine the effectiveness of vaccine-induced cellular immune responses in post-OLT patients, we used the IFN-γ ELISPOT assay. First of all, we analyzed the clinical characteristics of those patients showing strong HBsAg-specific T cell immune responses when compared with those of non-transplanted patients, and vaccine-induced anti-HBs antibody-positive, healthy volunteers (Fig. 2). The patients with stronger HBsAg-specific CD4 T cell IFN-γ responses (equal or more than the median; 7 spots) showed lower levels of HBV DNA, lower HBsAg, higher anti-HBs antibody titer, and higher HBcAg-specific immune responses. The HBsAg and HBcAg-specific CD4 T cell immune response under different clinical conditions is shown (Fig. 3). Volunteer controls who were positive for anti-HBs antibodies (as a result of previous vaccine administration) showed numerous HBsAg-specific IFNγ spots. Spot numbers were reduced in control chronic hepatitis B patients, but remained high (against both HBsAg and HBcAg) in acute resolved hepatitis B patients. The ALF-OLT and LC-OLT good responders had relatively higher HBsAg-specific T-cell immune responses than LC-OLT poor responders. The LC-OLT patients with successful vaccine-induced humoral immune responses also showed higher cellular immune responses than control chronic hepatitis B patients. The LC-OLT patients with poor vaccine responses also had low cellular responses, similar to those seen in chronic hepatitis B patients.

Discussion

In this study we found that HBV vaccination was effective in OLT patients whose donors were relatively high in age, marital (non-blood-related), with high-titer anti-HBs antibodies. The multivariate analysis revealed that a marital (non-blood-related) donor was the only factor that associated strongly with a good response to vaccine. Among these OLT recipients, a good response to vaccination included effective responses in both the humoral and cellular arms of the immune system.

Controlling HBV reactivation after OLT is critical. In the absence of prophylaxis, hepatitis B recurs very frequently and results in early graft failure. The prophylaxis protocols have progressed from HBIg immunoprophylaxis in the early 1990s, to lamivudine in the late 1990s, to the more recent application of HBIg combined with nucleos(t)ide analogues. In 1991, Muller et al. [17] reported the first use of long-term HBIg immunoprophylaxis, reducing the HBV recurrence rate to 25 % after 6 months of OLT and 18 % after 12 months. A multicenter study revealed that the three-year risk of HBV recurrence was 75 ± 6 % without HBIg, 74 ± 5 % with short-term (2-month) HBIg, and 36 ± 4 % with long-term (>6-month) HBIg treatment [18]. Patients who were positive for HBeAg or HBV-DNA displayed the greatest risk of recurrence (83 %); patients with acute fulminant liver failure showed the lowest risk (16 %).

In 1996, Grellier et al. [19] reported a trial of LAM as a prophylactic treatment, achieving 18 % recurrence of HBV at 6 months after OLT. However, the long-term recurrence rate at 3 years after OLT progressed to 41 %, indicating that LAM monotherapy is not recommendable for post-transplantation prophylaxis.