Table 1. Clinical Impact of Postoperative Grade ≥2 Atelectasis on Clinical Outcomes in Earlier Cohort

Factors	Postoperative grade ≥2 atelectasis: No (n = 61)	Postoperative grade ≥2 atelectasis: Yes (n = 49)	p Value
Pneumonia, n (%)	1 (1.6)	9 (18.4)	0.002
PaO_2/FiO_2 ratio POD 1	381 ± 11	361 ± 12	0.235
PaO_2/FiO_2 ratio POD 3	343 ± 14	332 ± 13	0.576
PaO_2/FiO_2 ratio POD 5	329 ± 28	331 ± 19	0.956
Length of mechanically ventilation, d	2.0 ± 0.9	4.7 ± 0.9	0.028
Length of oxygen support, d	5.3 ± 0.8	9.6 ± 0.9	<0.001
ICU stay, d	4.0 ± 0.8	7.3 ± 0.9	0.011
Postoperative hospital stay, d	28 ± 3	39 ± 3	0.005
Tracheotomy, n (%)	0 (0)	2 (4.1)	0.111
Reintubation, n (%)	1 (1.6)	4 (8.2)	0.103

Unless stated otherwise, data are reported as mean ± SD.
POD, postoperative day.

Infectious pneumonia was diagnosed by the combinations of radiologic findings showing new or increasing infiltrates, clinical symptoms such as fever or dyspnea, and positive cultures. Three main radiologic patterns were considered indicative of pneumonia: focal pulmonary consolidation; nodules or rapidly growing masses, with or without central cavitation; and diffuse pulmonary infiltrates with an interstitial or alveolar pattern.

Statistical analysis

All statistical analyses were performed using SAS software (JMP 11.0.1; SAS Institute Inc). Continuous variables were expressed as means ± SD and compared using Mann-Whitney U-tests. Categorical variables were compared using chi-square tests. A receiver operating characteristic (ROC) curve analysis and Youden's index were used to identify ideal cutoff values in multivariate analysis.

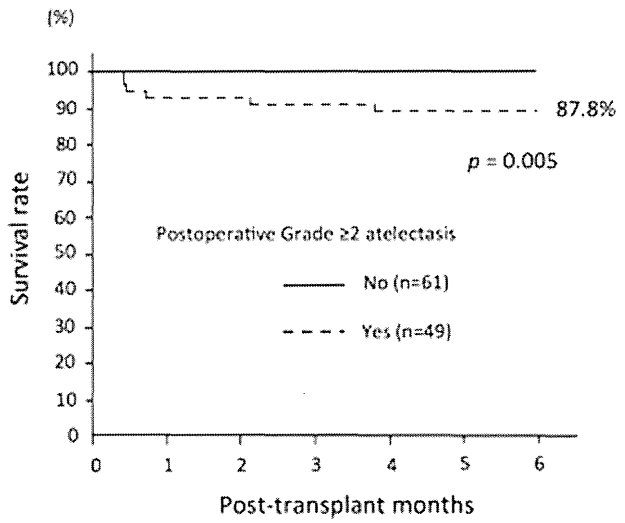

Figure 3. Six-month graft survival rates of the earlier cohort with and without postoperative grade ≥2 atelectasis.

RESULTS

Characteristics of the recipients, donors, and grafts

Demographic and clinical characteristics of all LDLT donors and recipients and the characteristics of the grafts are shown in Supplementary Table 1, online only.

Clinical sequelae of grade ≥2 postoperative atelectasis in the earlier cohort

The incidence of pneumonia was significantly higher in recipients with than without grade ≥2 postoperative atelectasis (18.4% vs 1.6%, p = 0.002). The durations of mechanical ventilation (4.7 ± 0.9 days vs 2.0 ± 0.9 days, p = 0.028), oxygen support after extubation (9.6 ± 0.9 days vs 5.3 ± 0.9 days, p < 0.001), ICU stay (7.3 ± 0.9 days vs 4.0 ± 0.8 days, p = 0.011), and postoperative hospital stay (39 ± 3 days vs 28 ± 3 days, p = 0.005) were significantly longer in patients with than without atelectasis (Table 1). The 6-month survival rate was significantly lower in recipients with than without postoperative atelectasis grade ≥2 (87.8% vs 100%, p = 0.005) (Fig. 3). The causes for mortality in the 6 recipients lost within 6 month after LDLT included respiratory failure (n = 3), retroperitoneal hemorrhage (n = 3), graft vs host disease (n = 1), and small for size syndrome (n = 1). Four of them were lost within 1 month after LDLT. There were no significant differences in the PaO_2 to FiO_2 ratio on PODs 1, 3, and 5, or in the percentages of recipients who underwent tracheotomy and reintubation.

Factors associated with grade ≥2 postoperative atelectasis in earlier cohort

Univariate analysis showed that Child-Pugh scores (10.5 ± 0.2 vs 9.5 ± 0.2, p = 0.003), Model for End-stage Liver Disease (MELD) scores (17.7 ± 0.8 vs 14.1 ± 0.8, p = 0.002) and body mass index (24.5 ± 0.4 kg/m^2 vs 23.0 ± 0.4 kg/m^2, p = 0.014) were significantly higher in patients with than without grade ≥2 postoperative atelectasis. The percentages of patients with vital capacity <80% (14.3% vs 5.0%, p = 0.034) and performance status ≥3 (46.9% vs 13.1%, p < 0.001) were significantly higher in patients with than without atelectasis, as were the amounts of transfused red cells (15.8 ± 1.9 units vs 10.6 ± 1.7 units, p = 0.044) and platelet (23.6 ± 2.3 units vs 16.3 ± 2.1 units,

Table 2. Univariate Analysis of Risk Factors for Postoperative Grade ≥2 Atelectasis

Factors	Postoperative grade ≥2 atelectasis: No (n = 61)	Yes (n = 49)	p Value
Recipient factors			
Sex, male, n (%)	30 (49.2)	17 (34.7)	0.125
Age, y	55 ± 1	55 ± 1	0.924
Primary diagnosis			0.091
Liver cirrhosis, n (%)	45 (73.8)	37 (75.5)	
Cholestatic disease, n (%)	13 (21.3)	5 (10.2)	
Others, n (%)	3 (4.9)	7 (14.3)	
Child-Pugh score, n	9.5 ± 0.2	10.5 ± 0.2	0.003
MELD score, n	14.1 ± 0.8	17.7 ± 0.8	0.002
Body mass index, kg/m^2	23.0 ± 0.4	24.5 ± 0.4	0.014
Diabetes, n (%)	9 (14.8)	7 (14.3)	0.945
Smoking, n (%)	13 (21.3)	12 (25.0)	0.650
FEV1.0% ≤ 70, n (%)	10 (16.7)	4 (8.9)	0.246
VC < 80, n (%)	3 (5.0)	7 (14.3)	0.034
Performance status ≥3, n (%)	8 (13.1)	23 (46.9)	<0.001
Donor factors			
Sex, male, n (%)	35 (57.4)	34 (69.4)	0.270
Age, y	36 ± 1	35 ± 1	0.769
ABO incompatibility, n (%)	8 (13.1)	6 (12.2)	0.892
Left lobe graft, n (%)	36 (59.0)	26 (53.0)	0.766
GV/SLV, (%)	39.2 ± 1.0	41.9 ± 1.1	0.086
GRWR	0.76 ± 0.09	0.94 ± 0.10	0.202
Recipient operation			
Operative time, min	782 ± 22	805 ± 25	0.489
Blood loss, L	3.6 ± 0.7	5.6 ± 0.8	0.054
RCC, U	10.6 ± 1.7	15.8 ± 1.9	0.044
FFP, U	17.6 ± 2.1	22.7 ± 2.4	0.113
PC, U	16.3 ± 2.1	23.6 ± 2.3	0.021
Ascites, mL	390 ± 280	1820 ± 320	0.001
Splenectomy, n (%)	52 (85.3)	36 (75.0)	0.178
Porto-systemic shunt ≥1 cm, n (%)	18 (29.5)	15 (31.3)	0.844

Unless stated otherwise, data are reported as mean ± SD.
FEV, forced expiratory volume; FFP, fresh frozen plasma; GRWR, graft/recipient weight ratio; GV, graft volume; MELD, Model for End-stage Liver Disease; PC, platelet concentrates; RCC, red cell concentrates; SLV, standard liver volume; VC, vital capacity.

p = 0.021) concentrates and the amount of ascites (1,820 ± 320 mL vs 390 ± 280 mL, p = 0.001) (Table 2).

Optimal cut-off values for atelectasis, as determined by receiver operating characteristic (ROC) curve analysis, were body mass index 27 kg/m^2 (area under the curve [AUC] = 0.62, sensitivity 51%, specificity 77%); MELD score 23 (AUC = 0.65, sensitivity 31%, specificity 93%); Child-Pugh score 11 (AUC = 0.65, sensitivity 49%, specificity 72%); ascites 500 mL (AUC = 0.64, sensitivity 44%, specificity 82%); red blood cell concentrates 6 units (AUC = 0.63, sensitivity 83%, specificity 36%); platelet concentrates 40 units (AUC = 0.60, sensitivity 21%, specificity 95%).

Multivariate analysis showed that body mass index ≥27 kg/m^2 (odds ratio [OR] 15.1, 95% CI 4.4 to 60.0, p < 0.001), performance status ≥3 (OR 7.1, 95% CI 2.0 to 28.0, p = 0.003) and MELD score ≥23 (OR 17.1, 95% CI 2.2 to 371.7, p = 0.005) were independent risk factors for postoperative atelectasis (Table 3).

Noninfectious pulmonary complications in the earlier cohort

Of the 120 patients, 103 (93.6%) experienced noninflammatory pulmonary changes during the early postoperative period, the most common being pleural effusion in 101 patients (91.8%) (Supplementary Table 2, online only). Atelectasis grade ≥2 occurred in 46 patients (41.8%), including 14 patients with atelectasis on both sides, 26 with atelectasis on the right side, and 6 with atelectasis on the left side. Of the 46 patients with atelectasis grade ≥2, 44 (95.7%) also had pleural effusions.

Demographic and clinical characteristics of the 2 cohorts

Recipient sex distribution, age, distribution of disease, MELD score, and body mass index were similar in the 2 cohorts (Supplementary Table 3, online only). The percentage of patients with performance status ≥3 was significantly higher in the later than in the earlier cohort (46.0% vs 29.2%, p = 0.025), although the percentages of patients with risk factors for postoperative atelectasis were similar in the 2 cohorts (p = 0.218).

Donors in earlier cohort were significantly younger than those in the later cohort (36 ± 1 years vs 39 ± 1 years, p = 0.020). However, there were no significant differences between groups in graft-to-standard liver volume ratio and graft-to-recipient weight ratio.

Operation times were similar in the 2 groups. Mean blood loss per patient was significantly greater in the later than in the earlier cohort (7.8 ± 1.1 L vs 4.7 ± 0.8 L, p = 0.027).

Clinical outcomes in the 2 cohorts

The percentages of patients with atelectasis (21.1% vs 42.5%, p = 0.005) and pneumonia (1.8% vs 10.0%, p = 0.049) were significantly lower in the later than in the earlier cohort (Table 4). Moreover, the mean length of ICU stay (3.6 ± 0.2 days vs 5.7 ± 0.6 days, p = 0.038) and the period with oxygen support (5.1 ± 0.8

Table 3. Multivariate Analysis of Risk Factors for Postoperative Grade ≥2 Atelectasis

Variables	Odds ratio	95% CI	p Value
Body mass index ≥27 kg/m^2	15.1	4.4–60.0	<0.001
Performance status ≥3	7.1	2.0–28.0	0.003
MELD score ≥23	17.1	2.2–371.7	0.005
PC > 40 U	6.3	0.9–58.3	0.064
RCC > 6 U	1.6	0.5–5.3	0.426
Child–Pugh score ≥11	1.5	0.5–4.7	0.526
Ascites > 500 mL	1.4	0.4–5.1	0.627
%VC < 80, %	4.3	0.6–39.5	0.141

MELD, Model for End-stage Liver Disease; PC, platelet concentrates; RCC, red cell concentrates; VC, vital capacity.

days vs 7.1 ± 0.5 days, p = 0.037) were significantly shorter in the later cohort. However, the mean length of postoperative hospital stay was similar in the 2 groups. The PaO_2 to FiO_2 ratio on POD 1 was significantly higher in the later cohort (418 ± 14 vs 372 ± 9, p = 0.005), but there were no differences between groups on PODs 3 and 5. Complications associated with intraoperative thoracic drainage did not differ significantly between the 2 cohorts. The recurrence of thoracic fluid correction with a positive culture occurred in 1 patient in the earlier cohort, and pneumothorax after drain removal occurred in 2 patients in the later cohort. The fluid correction with a positive culture was not accompanied by clinical symptoms and was successfully treated by exchanging the chest drain and administering of antibiotics; pneumothorax in both patients was successfully treated with reinsertion of a chest drain.

Subgroup analysis of clinical outcomes

The patients in each group were divided into 3 subgroups. Of the 120 patients in the earlier group, 10 (8.3%) had preoperative pleural effusion, while 56 (46.7%) had risk factors for postoperative atelectasis, and 54 (45.0%) did not. Of the 57 patients in the later group, 8 (14.0%) had preoperative pleural effusions; 28 (49.1%) had risk factors for postoperative atelectasis and 21 (36.8%) did not.

When the incidence of postoperative pulmonary complications was compared in each pair of subgroups, we observed significant differences in patients with risk factors for atelectasis. The percentages of patients with atelectasis (21.4% vs 71.4%, p < 0.001) and pneumonia (0% vs 16.1%, p = 0.025) were significantly lower in the later than in the earlier cohort. Additionally, the PaO_2 to FiO_2 ratio on POD 1 was significantly greater (421 ± 19 vs 364 ± 13, p = 0.014), and the mean length of oxygen support was significantly shorter (5.2 ± 0.9 days vs 7.7 ± 0.7 days, p = 0.029) in the later cohort (Table 5), but there was no difference in mean length of ICU stay. No differences were observed in the subgroups with preoperative pleural effusion and those without risk factors for postoperative atelectasis.

DISCUSSION

Postoperative pulmonary complications have been associated with early morbidity and mortality in liver transplant recipients.[1-7] These postoperative pulmonary complications may have serious clinical impacts due to poor patient condition, end-stage liver disease, pre-existing pulmonary abnormalities, high comorbidity rates, and immunosuppressive status.[1,2,6] Therefore, special attention should be paid to preventing pulmonary complications. This study demonstrated that preemptive thoracic drainage in LDLT recipients effectively reduced the rates of postoperative atelectasis and pneumonia and shortened the lengths of ICU stay and oxygen support.

We found that postoperative grade ≥2 atelectasis after LDLT was associated with prolonged respiratory recovery and a high mortality rate, and was an important target of

Table 4. Comparison of Clinical Outcomes in the 2 Recipient Cohorts

Factors	Earlier cohort (n = 120)	Later cohort (n = 57)	p Value
Postoperative grade ≥ 2 atelectasis, n (%)	51 (42.5)	12 (21.1)	0.005
Pneumonia, n (%)	12 (10.0)	1 (1.8)	0.049
PaO_2/FiO_2 ratio POD 1	372 ± 9	418 ± 14	0.005
PaO_2/FiO_2 ratio POD 3	340 ± 9	332 ± 15	0.615
PaO_2/FiO_2 ratio POD 5	332 ± 14	363 ± 24	0.275
Length of mechanically ventilation, d	3.2 ± 0.5	2.1 ± 0.7	0.196
Length of oxygen support, d	7.1 ± 0.5	5.1 ± 0.8	0.037
ICU stay, d	5.7 ± 0.6	3.6 ± 0.2	0.038
Postoperative hospital stay, d	33 ± 2	30 ± 3	0.373
Complications associated with intraoperative thoracic drainage, n (%)	1 (10)	2 (5.6)	0.615

Unless stated otherwise, data are reported as mean ± SD.
POD, postoperative day.

Table 5. Subgroup Analysis of Clinical Outcomes in the 2 Recipient Cohorts

Factors	Group	Earlier cohort	Later cohort	p Value
Postoperative atelectasis, grade ≥2, n (%)	P	2 (20.0)	1 (12.5)	0.671
	R (−)	9 (16.7)	5 (23.8)	0.476
	R (+)	40 (71.4)	6 (21.4)	<0.001
Pneumonia, n (%)	P	2 (20.0)	1 (12.5)	0.671
	R (−)	1 (1.9)	0 (0.0)	0.530
	R (+)	9 (16.1)	0 (0.0)	0.025
PaO_2/FiO_2 ratio on POD 1, n	P	365 ± 34	362 ± 40	0.952
	R (−)	382 ± 14	433 ± 22	0.056
	R (+)	364 ± 13	421 ± 19	0.014
PaO_2/FiO_2 ratio on POD 3, n	P	362 ± 30	382 ± 48	0.747
	R (−)	343 ± 12	304 ± 21	0.126
	R (+)	333 ± 14	340 ± 21	0.804
PaO_2/FiO_2 ratio on POD 5, n	P	357 ± 17	350 ± 20	0.791
	R (−)	311 ± 30	292 ± 52	0.766
	R (+)	339 ± 17	398 ± 30	0.101
Length of mechanical ventilation, d	P	3.9 ± 1.2	2.7 ± 1.3	0.534
	R (−)	2.1 ± 0.2	1.7 ± 0.4	0.323
	R (+)	4.2 ± 1.0	2.3 ± 1.4	0.258
Length of oxygen support, d	P	6.6 ± 2.5	7.0 ± 2.9	0.909
	R (−)	6.6 ± 0.9	4.4 ± 1.4	0.172
	R (+)	7.7 ± 0.7	5.2 ± 0.9	0.029
ICU stay, d	P	5.7 ± 1.7	5.0 ± 2.0	0.797
	R (−)	4.1 ± 0.3	3.2 ± 0.5	0.101
	R (+)	6.8 ± 1.0	4.0 ± 1.4	0.115
Postoperative hospital stay, d	P	31 ± 7	27 ± 10	0.788
	R (−)	29 ± 3	30 ± 4	0.867
	R (+)	36 ± 2	30 ± 4	0.171

Unless stated otherwise, data are reported as mean ± SD.
POD, postoperative day; P, patients with preoperative pleural effusions; R (−), patients without risk factors for postoperative atelectasis; R (+), patients with risk factors for postoperative atelectasis.

patient management. Atelectasis can be particularly problematic because it appears to be one of the primary mechanisms underlying acute lung injury[20] and impaired systemic oxygenation,[10,12] as well as being associated with prolonged ICU and hospital stay.[20] Moreover, atelectasis is thought to predispose to pneumonia,[8-10] which is also associated with a high early mortality rate[6] and prolonged mechanical ventilation and ICU stay after LDLT.[3] This study found that the incidence of pneumonia was significantly lower in the later than in the earlier cohort (1.8% vs 10.0%). All recipients with early mortality in the earlier cohort had post-transplant atelectasis. As many as 50% of those recipients (3 of 6) were lost due to respiratory failure. These results were comparable with past findings, and suggested that the prevention of atelectasis may reduce the rates of pneumonia, morbidity, and mortality.

Multivariate regression analysis showed that independent risk factors for postoperative grade ≥2 atelectasis were body mass index ≥27 kg/m^2, performance status ≥3, and MELD score ≥23. Other reports have also assessed risk factors for post-transplantation pulmonary complications.[1-7] Obesity was found to markedly reduce functional residual capacity, promoting airway closure to a greater extent than in normal weight recipients.[21] The weight of the torso and abdomen make diaphragmatic excursions difficult, especially when patients are in the supine position.[10] Owing to similar mechanisms, severe ascites may also contribute to the loss of aeration in caudal and dependent lung segments, leading to atelectasis and airway closure.[22] Liver transplant recipients with high MELD scores often have a greater incidence of pleural effusion, a need for more perioperative blood transfusions, a greater risk of fluid retention, more severely restrictive pulmonary patterns, and a greater incidence of muscle atrophy related to poor nutritional status.[2] A MELD score ≥25 was reported to be an

independent predictor of postoperative pulmonary complications.[23] To our knowledge, no studies have shown that performance status was a risk factor for postoperative pulmonary complications. A performance status ≥ 3 indicates that a patient is $\geq 50\%$ bedridden during the daytime.[15] Immobilized patients suffer profound and persistent impairments in physical function, typically with slow and incomplete recovery.[24] These patients often lost their muscle bulk, predominantly in proximal muscle, leading to sarcopenia.[24] We have reported that sarcopenia was a prognostic factor after LDLT.[25] Although recipient age, smoking history, diabetes, and cirrhotic encephalopathy have also been identified as risk factors for postoperative pulmonary complications,[2,4,6,20] they were not found to be risk factors in this study. These risk factors for atelectasis in this study implied that the patients with atelectasis had more severe preoperative systemic status than those without it. Atelectasis in these patients might cause vital systemic damages, resulting in higher mortality.[5]

Postoperative pulmonary atelectasis after orthotopic liver transplantation is accompanied in most patients by pleural effusion.[1] Similarly, we found that 40.8% of the earlier cohort had postoperative grade ≥ 2 atelectasis, with 95.7% of them having pleural effusion. Under the combination of general anesthesia and prolonged placement in a supine position, intrathoracic fluid retention contributes to a decrease in functional residual capacity and compression of lung tissue, causing compressive atelectasis.[26,27] Because atelectasis has several causes, various approaches have been used to prevent this condition, according to its mechanism and cause.[12] Lung mechanics and breathing patterns are often changed postoperatively, resulting in coughing and removal of particulate matter, both of which are particular to pulmonary defense mechanisms.[10] Treatment modalities targeting these defense mechanisms include pain control, chest physiotherapy, bronchodilators, fiberoptic bronchoscopy, and DNase treatment.[10,12] We have actively used these strategies in perioperative management of patients in both groups. Positive end-expiratory pressure has also been used to prevent and reverse atelectasis.[20] However, despite these efforts, 74.5% of recipients in earlier cohort had atelectasis. Those suggested that there was a strong relationship between postoperative atelectasis and intrathoraic fluid retention after LDLT. Therefore, preemptive thoracic drainage of transudative effusions was theoretically reasonable for the prevention of postoperative atelectasis.

The rates of postoperative pleural effusion and atelectasis we observed were higher than in previous studies. Rates of pleural effusion and atelectasis in the earlier cohort were 91.8% and 74.5%, respectively, rates higher than reported incidences in other groups of orthotopic liver transplant recipients, eg, 32% to 47% and 5% to 29%, respectively,[2] and 40.9% and 29.5%, respectively.[4] These differences may be due to the greater susceptibility of LDLT than deceased donor liver transplant (DDLT) recipients to postoperative noninfectious pulmonary complications. The incidence of pulmonary infections was found to be higher in LDLT than in deceased donor liver transplant recipients, perhaps due to the smaller liver volume in the former.[8] Indeed, slower recovery of liver function, prolonged cholestasis, and persistent ascites in LDLT recipients may also be due to smaller liver volume,[28,29] suggesting that the high incidence of postoperative pulmonary complications after LDLT may be associated with small liver volumes.

Thoracic drainage under mini-thoracotomy using an electronic scalpel was extremely safe and was not associated with any serious adverse events. Thoracentesis under ultrasound guidance is associated with many risks in liver transplant recipients. Recipients' collateral veins continued to develop owing to end-stage liver disease, even after liver transplantation.[30,31] The incidence of hemothorax after tube thoracostomy was reported to be 1.8% after orthotopic liver transplantation.[31] We also previously described 2 patients with hemothorax after thoracentesis under ultrasound guidance, emphasizing the importance of proper chest tube placement. In this study, chest tube placement was a safe technique because it was performed under direct vision and hemostasis was adequate. This procedure requires adequate sterile facilities, suggesting that it be performed at the same time as LDLT.

One important limitation of this study was that it was not a concurrent controlled study. Therefore, the impact of thoracic drainage could not be compared precisely. Although a randomized controlled study is required, our subgroup analysis may be adequate. This analysis showed that preemptive thoracic drainage of LDLT recipients with at least 1 risk factor for atelectasis contributed to improvements in the later cohort. Another limitation was our inability to determine whether our preemptive strategy improved mortality. Longer-term observation is therefore required.

CONCLUSIONS

In conclusion, preemptive thoracic drainage of LDLT recipients at high risk of pulmonary complications may reduce the rates of atelectasis and pneumonia. Chest tube placement could be performed safely under mini-thoracotomy using an electronic scalpel. However, it is

yet unclear whether this strategy improves patient mortality. Further observation and experience are therefore required.

Author Contributions

Study conception and design: Imai, Ikegami, Toshima, Yoshizumi, Yamashita, Ninomiya, Harimoto, Itoh, Uchiyama, Shirabe, Maehara

Acquisition of data: Imai, Ikegami, Toshima, Yoshizumi, Yamashita, Ninomiya, Harimoto, Itoh, Uchiyama, Shirabe, Maehara

Analysis and interpretation of data: Imai, Ikegami, Toshima, Yoshizumi, Yamashita, Ninomiya, Harimoto, Itoh, Uchiyama, Shirabe, Maehara

Drafting of manuscript: Imai, Ikegami, Toshima, Yoshizumi, Yamashita, Ninomiya, Harimoto, Itoh, Uchiyama, Shirabe, Maehara

Critical revision: Imai, Ikegami, Shirabe, Maehara

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Supplementary Table 1. Demographic and Clinical Characteristics of All Recipients, Donors and Grafts

Factors	Total cases (n = 177)
Recipient factors	
Sex, male, n (%)	75 (42.3)
Age, mean, y	54.9
Primary diagnosis	
Liver cirrhosis, n (%)	130 (73.4)
HBV, n	18
HCV, n	76
Alcoholic, n	16
NASH, n	12
Cryptogenic, n	8
Cholestatic disease, n (%)	30 (16.9)
PBC, n	22
PSC, n	8
Others, n (%)	17 (9.7)
Child-Pugh score, mean	10.2
MELD score, mean	16.7
Body mass index, mean, kg/m^2	23.6
Diabetes, n (%)	25 (14.1)
Smoking, n (%)	40 (22.6)
Performance status ≥3 (%)	62 (35.0)
Donor factors	
Sex, male, n (%)	110 (62.1)
Age, mean, y	36.6
ABO incompatibility, n (%)	17 (0.1)
Left lobe graft, n (%)	99 (55.9)
GV/SLV, %	40.7
GRWR	0.82

GRWR, graft/recipient weight ratio; GV, graft volume; HBV, hepatitis B virus; HCV, hepatitis C virus; MELD, Model for End-stage Liver Disease; NASH, nonalcoholic steatohepatitis; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; SLV, standard liver volume.

Supplementary Table 2. Noninfectious Pulmonary Complications in the Earlier Cohort (n = 120), %

Factors	Total	Right	Left
Pleural effusions			
None	8.2	13.6	20.9
Grade 1	42.7	42.7	57.3
Grade 2	44.6	39.2	20.9
Grade 3	4.5	4.5	0.9
Atelectasis			
None	25.5	31.8	48.2
Grade 1	32.7	31.8	33.6
Grade 2	40.9	35.5	18.2
Grade 3	0.9	0.9	0

Supplementary Table 3. Demographic and Clinical Characteristics of the 2 Recipient Cohorts, Donors, and Grafts

Factors	Earlier cohort (n = 120)	Later cohort (n = 57)	p Value
Recipient factors			
Gender, male, n (%)	55 (45.8)	20 (35.1)	0.176
Age, mean, y	55 ± 1	54 ± 1	0.590
Primary diagnosis, n (%)			0.134
Liver cirrhosis	90 (75.0)	40 (70.2)	
Cholestatic disease	19 (15.8)	15 (26.3)	
Others	11 (9.2)	2 (3.5)	
Child-Pugh score	10.1 ± 0.2	10.2 ± 0.2	0.763
MELD score	16.2 ± 0.6	17.4 ± 0.8	0.223
Body mass index, kg/m^2	23.6 ± 0.3	23.6 ± 0.5	0.969
Diabetes, n (%)	16 (14.6)	5 (8.8)	0.159
Smoking, n (%)	28 (23.5)	12 (21.1)	0.714
FEV1.0% ≤ 70, n (%)	17 (14.9)	4 (7.4)	0.135
%VC < 80, n (%)	15 (13.2)	12 (22.2)	0.170
Performance status ≥3, n (%)	33 (29.2)	29 (46.0)	0.025
Risk factors for post-transplant atelectasis, n (%)	64 (53.3)	36 (63.2)	0.218
Donor factors			
Sex, male, n (%)	76 (63.3)	33 (58.9)	0.649
Age, y	36 ± 1	39 ± 1	0.020
ABO incompatibility, n (%)	14 (11.7)	3 (5.3)	0.177
Left lobe graft, n (%)	67 (55.8)	32 (56.1)	0.978
GV/SLV, %	40.4 ± 0.7	41.2 ± 1.1	0.546
GRWR	0.83 ± 0.05	0.79 ± 0.08	0.645
Recipient surgery			
Operative time, min	796 ± 15	823 ± 22	0.301
Blood loss, L	4.7 ± 0.8	7.8 ± 1.1	0.027
Ascites, mL	2,200 ± 940	1,990 ± 1,330	0.894

Unless stated otherwise, data are reported as mean ± SD.
FEV, forced expiratory volume; GRWR, graft/recipient weight ratio; GV, graft volume; MELD, Model for End-stage Liver Disease; SLV, standard liver volume; VC, vital capacity.

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LETTER FROM THE FRONTLINE

Different Histological Sequelae of Immune-Mediated Graft Dysfunction After Interferon Treatment in Transplanted Dual Grafts From Living Donors

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TO THE EDITORS:

End-stage liver disease secondary to hepatitis C virus (HCV) infection is the leading indication for liver transplantation (LT) in Japan as well as Western countries. Reinfection with HCV is common after LT, and with the ensuing accelerated progress to cirrhosis, it is an important cause of impaired long-term survival after LT. Pegylated interferon (PEG-IFN)/ribavirin (RBV) therapy has been shown to be effective in eliminating HCV even after LT. Meanwhile, with respect to PEG-IFN/RBV's immunomodulatory properties, immune-mediated graft dysfunction (IGD), which is characterized pathologically by plasma cell hepatitis, has been reported in LT patients after PEG-IFN/RBV therapy.[1,2] IGD is considered to include acute cellular rejection, chronic rejection, and plasma cell hepatitis or a variant form of acute cellular rejection developing in post-LT patients receiving IFN treatment for recurrent HCV. Because IGD has been shown to be associated with high morbidity and mortality, its recognition has been increasing in recent years. Although some characteristics of IGD have been reported, in large part, its pathogenesis remains to be resolved. Here we present a rare case of an LT recipient who received dual grafts from 2 donors with different interleukin 28B (IL28B) genetic variants and showed a quite different severity of IGD in each graft after PEG-IFN/RBV therapy for recurrent HCV.

A 51-year-old Japanese male with uncompensated liver cirrhosis secondary to HCV had received LT with dual grafts from 2 donors. The left lobe was from his 21-year-old son, and the right lobe was from his 42-year-old wife, as previously described.[3] The clinical course after LT progressed without major surgical complications. Immunosuppression was maintained with tacrolimus (Prograf, Astellas, Tokyo, Japan), the dosages of which were adjusted to trough concentrations of 5 to 10 ng/mL. He started PEG-IFNα2b/RBV therapy for HCV reinfection 4 months after LT and achieved a virological response 16 months after LT. However, 6 months later, HCV RNA was detected again, and PEG-IFNα2a/RBV therapy was commenced. Twelve weeks later, however, an increase in liver function test results was observed, and antiviral therapy was terminated. As previously reported, the 2 liver grafts from different donors had different IL28B genetic variants: the right lobe from his wife was a minor genotype (TG), and the left lobe from his son was a major genotype (TT). Findings on liver biopsy conducted 4 years after LT were also different for the 2 grafts. The left lobe with the major genotype showed mild hepatitis and no fibrosis (A1F0), whereas the right lobe with the minor genotype exhibited moderate inflammation and bridging fibrosis (A2F2). At this time, HCV RNA was detected only in the right lobe graft by quantitative reverse transcriptase–polymerase chain reaction with total RNA extracted from each specimen. After the termination of PEG-IFNα2a/RBV therapy, liver function tests still showed mild elevations (alanine transaminase level = 47-85 U/L) with only liver-supportive therapy. Seven years after LT, when telaprevir was introduced into clinical use in Japan, antiviral therapy was reintroduced with telaprevir and PEG-IFNα2b/RBV because the atrophy of the right lobe graft was progressive. At this time, tacrolimus was converted to cyclosporin A (Neoral, Novartis Pharma, Tokyo, Japan), and its trough levels were maintained at 100 to 150 ng/mL during the treatment. Although HCV RNA was cleared at 12 weeks, liver function test results increased (alanine transaminase level = 102 U/L, alkaline phosphatase

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Potential conflict of interest: Nothing to report.

Address reprint requests to Mizuki Ninomiya, M.D., Ph.D., Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan. Telephone: 81-92-642-5463; FAX: 81-92-642-5482; E-mail: nino-m@surg2.med.kyushu-u.ac.jp

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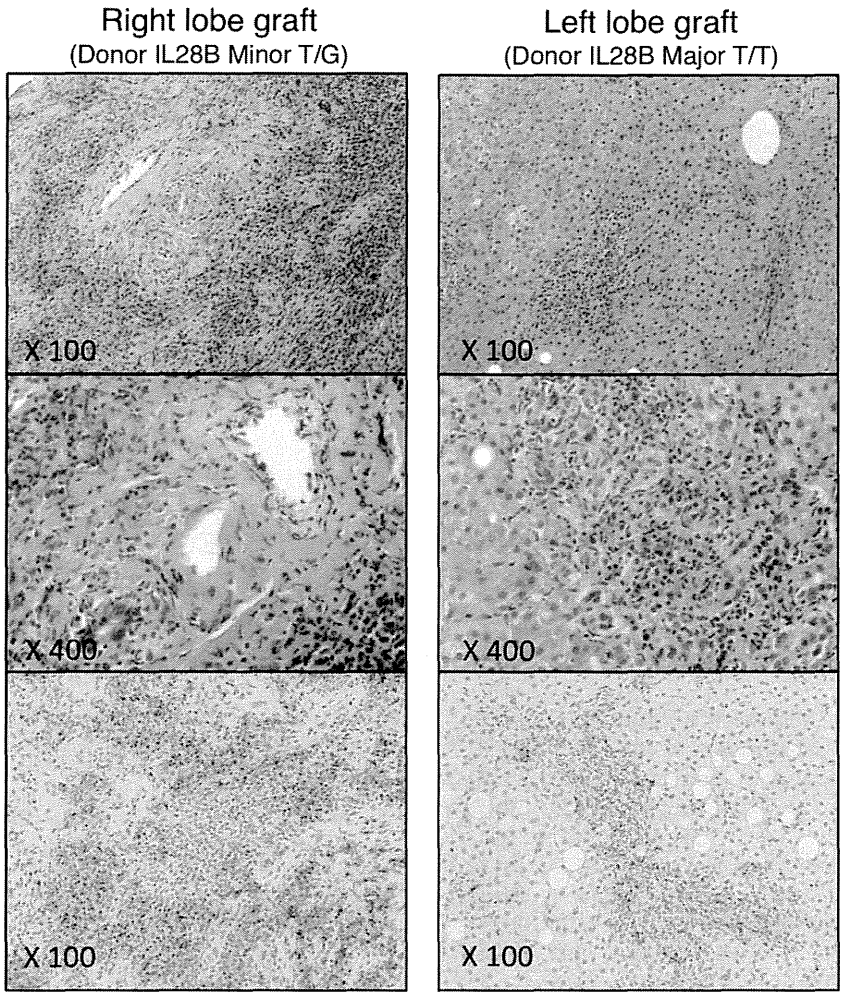

Figure. 1. Liver biopsies from each graft after telaprevir and PEG-IFNα2b/RBV treatment with HCV RNA clearance 7 years after LT. The right lobe showed an intense aggregation of plasma cells with some lymphocytes throughout the entire lobule and with prominent loss of liver parenchyma. Bile duct damage was not clear, but severe endotheliitis was recognized. Meanwhile, the left lobe graft showed mild to focally severe interface hepatitis with lymphoplasma cell, neutrophil, and eosinophil infiltration. An immunohistochemical examination for CD38 indicated that many of the infiltrating cells in the right lobe were plasma cells.

level = 858 U/L) in comparison with the pretreatment levels. Liver biopsy was performed on each graft with a laparoscopic approach because there was no percutaneous puncture route for the right lobe graft as a result of its atrophy. The histological findings for the left lobe graft showed mild to focally severe interface hepatitis with lymphoplasma cell, neutrophil, and eosinophil infiltration. Meanwhile, the histology of the right lobe showed an intense aggregation of plasma cells with some lymphocytes throughout the entire lobule and with prominent loss of liver parenchyma (Fig. 1). Bile duct damage was not clear, but severe endotheliitis was also recognized. Fibrotic changes were also present. An immunohistochemical examination for CD38 indicated that many of the infiltrating cells in the right lobe were plasma cells.

The patient was treated with a steroid pulse and mycophenolate mofetil (2000 mg/day), and the cyclosporine A trough level increased to 150 to 250 ng/mL. The liver enzymes gradually decreased and stabilized within the normal range within 1 month after the start of treatment. The HCV RNA levels remained negative during treatment.

There were quite different severities of plasma cell hepatitis between these 2 grafts in a single recipient. One of the possible mechanisms of IGD previously raised is that increased host immunoreactivity after PEG-IFN/RBV therapy with or without suboptimal immunosuppressive levels or lowering of the immunosuppression during the process of HCV clearance could lead to autoimmune-type hepatitis.[2] However, our current case clearly indicates that the extent of IGD is defined not only by the reactivity of the recipient's immune system but rather mainly by factors associated with the graft. Another hypothesis is that because most IGD is known to occur when HCV RNA has been cleared after antiviral therapy, a vigorous immune response promoting viral clearance favors tissue damage, with subsequent cryptic antigen release in a context of interferon-induced major histocompatibility

complex up-regulation.[2] Our group recently reported that telaprevir-based triple therapy led to greater occurrence of IGD in comparison with standard PEG-IFN/RBV therapy.[4] In this case, HCV RNA was already cleared after the first PEG-IFN/RBV treatment in the left lobe graft but not in the right lobe graft, probably because of different IL28B genetic variants. A potent viral clearance activity of the triple therapy to the right lobe graft might have led to more antigen release after the second antiviral triple therapy and subsequent IGD. The extreme histological sequelae of IGD in the right lobe graft could happen because the recipient had received dual grafts. Remaining viable liver parenchyma in the right lobe graft was quite scarce, so it would have been impossible to maintain minimal life-supporting function if the patient had not received dual grafts. Its extreme histology would be a manifestation of the characteristics of IGD associated with a very poor prognosis. Although the exact pathogenesis of IGD is yet to be clarified, the current case indicates that the severity of IGD is not determined primarily by host immunoreactivity but can be altered by factors associated with liver grafts.

Mizuki Ninomiya, M.D., Ph.D.[1]
Shinichi Aishima, M.D., Ph.D.[3]
Tomoharu Yoshizumi, M.D., Ph.D.[1]
Toru Ikegami, M.D., Ph.D.[1]
Huanlin Wang, M.D.[2]
Norifumi Harimoto, M.D., Ph.D.[1]
Shinji Ito, M.D., Ph.D.[1]
Hideaki Uchiyama, M.D., Ph.D.[4]
Yuji Soejima, M.D., Ph.D.[5]
Hirofumi Kawanaka, M.D., Ph.D.[1]
Ken Shirabe, M.D., Ph.D.[1]

Yoshihiko Maehara, M.D., Ph.D.[1]
Departments of [1]Surgery and Science and [2]Anatomic Pathology
Graduate School of Medical Sciences
Kyushu University
Fukuoka, Japan
[3]Department of Pathology and Microbiology
Saga University
Saga, Japan
[4]Department of Surgery
Fukuoka City Hospital Organization
Fukuoka City Hospital
Fukuoka, Japan
[5]Department of Surgery
Matsuyama Red Cross Hospital
Ehime, Japan

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Impact of Rituximab Desensitization on Blood-Type-Incompatible Adult Living Donor Liver Transplantation: A Japanese Multicenter Study

H. Egawa[1,*], S. Teramukai[2], H. Haga[3], M. Tanabe[4], A. Mori[5], T. Ikegami[6], N. Kawagishi[7], H. Ohdan[8], M. Kasahara[9] and K. Umeshita[10]

[1]*Department of Surgery, Institute of Gastroenterology, Tokyo Women's Medical University, Tokyo, Japan*
[2]*Innovative Clinical Research Center, Kanazawa University, Kanazawa, Japan*
[3]*Department of Diagnostic Pathology, Kyoto University, Kyoto, Japan*
[4]*Department of Surgery, Graduate School of Medicine, Keio University, Tokyo, Japan*
[5]*Department of Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan*
[6]*Department of Surgery, Graduate School of Medicine, Kyushu University, Fukuoka, Japan*
[7]*Department of Surgery, Graduate School of Medicine, Tohoku University, Miyagi, Japan*
[8]*Department of Surgery, Graduate School of Medicine, Hiroshima University, Hiroshima, Japan*
[9]*Department of Transplantation, National Center for Child Health and Development, Osaka, Japan*
[10]*Department of Surgery, Graduate School of Medicine, Osaka University, Osaka, Japan*
**Corresponding author: Hiroto Egawa, egawa@ige.twmu.ac.jp*

We evaluated the effects of rituximab prophylaxis on outcomes of ABO-blood-type-incompatible living donor liver transplantation (ABO-I LDLT) in 381 adult patients in the Japanese registry of ABO-I LDLT. Patients underwent dual or triple immunosuppression with or without B cell desensitization therapies such as plasmapheresis, splenectomy, local infusion, intravenous immunoglobulin and rituximab. Era before 2005, intensive care unit-bound status, high Model for End-Stage Liver Disease score and absence of rituximab prophylaxis were significant risk factors for overall survival and antibody-mediated rejection (AMR) in the univariate analysis. After adjustment for era effects in the multivariate analysis, only absence of rituximab prophylaxis was a significant risk factor for AMR, and there were no significant risk factors for survival. Rituximab prophylaxis significantly decreased the incidence of AMR, especially hepatic necrosis ($p < 0.001$). In the rituximab group, other B cell desensitization therapies had no add-on effects. Multiple or large rituximab doses significantly increased the incidence of infection, and early administration had no advantage. In conclusion, outcomes in adult ABO-I LDLT have significantly improved in the latest era coincident with the introduction of rituximab.

Keywords: Antibody-mediated rejection, blood-type incompatible, desensitization, living donor liver transplantation, rituximab

Abbreviations: ABO-I, ABO-blood-type incompatible; ACR, acute cellular rejection; AIH, autoimmune hepatitis; AMR, antibody-mediated rejection; AUC, area under the curve; CMV, cytomegalovirus; DSA, donor-specific antibody; FHF, fulminant hepatic failure; ICU, intensive care unit; IHBC, intrahepatic biliary complication; IVIG, intravenous immunoglobulin; LDLT, living donor liver transplantation; MELD, Model for End-Stage Liver Disease; RBC, red blood cell; ROC, receiver operating characteristic

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Introduction

Advances in ABO-blood-type-incompatible living donor liver transplantation (ABO-I LDLT) through innovations in B cell desensitization aimed at preventing antibody-mediated rejection (AMR) have expanded the donor pool in Japan. Local infusion through the portal vein or hepatic artery to decrease inflammatory reaction at the epithelium was introduced in 2000, and rituximab prophylaxis was introduced widely in 2004 in Japan (1). Although there have been several single-center reports of rituximab prophylaxis in ABO-I LDLT, all describe small numbers of patients (2–4). There is no information about how much, how many times or when rituximab should be administered, and there have been no comparisons of patient outcomes with and without rituximab in a large cohort.

Age is an important prognostic factor for AMR and patient and graft survival (5). Demand for an effective desensitization method is especially strong in adult ABO-I LDLT. This study aimed to assess the effects of rituximab prophylaxis in ABO-I LDLT and to determine an effective and safe rituximab regimen.

Patients and Methods

Data collection

The Japan Study Group for ABO-Blood-Type-Incompatible Transplantation and a national registry for liver transplantation were established in 2001 by transplant centers performing ABO-I LDLT in Japan. The study group meets yearly to report experiences and has established a consensus for AMR diagnosis, treatment strategies and quality control of antibody titer measurements. Questionnaires are updated yearly and were sent in 2012 to registered surgeons and hepatologists in transplant centers, inquiring about patient characteristics, treatments and clinical courses. Information assayed included age, sex, disease, blood types of the recipient and donor, preoperative status, Model for End-Stage Liver Disease (MELD) score, relation of donor to recipient, peak titer of anti-donor-blood-type antibodies before transplantation and anti-donor antibody titer at the time of operation. Each center was classified as a large (≥10 ABO-I cases) or small (<10 ABO-I cases) volume center. Patients who required hospitalization in an intensive care unit (ICU) or a ward before surgery were classified as "in-ICU" or "in-hospital," respectively. Patients who required medical care other than in an ICU or ward were classified as "at home" at the time of transplantation. Treatment data included graft type, splenectomy, immunosuppression, local infusion, plasmapheresis, intravenous immunoglobulin (IVIG) and rituximab. Data concerning dose, frequency and timing of rituximab treatment and its adverse effects were collected in 2012. Clinical course data included peak titer of anti-donor-blood-type antibodies after transplantation, as well as rejection, bacterial infection, fungal infection, cytomegalovirus (CMV) disease requiring treatments and patient survival. Data on mortality and cause of death were also collected.

Measurement of anti-A/B antibody levels

Titers of anti-donor-blood-type antibodies were measured at each institution and a quality control survey was performed yearly by The Japan Study Group for ABO-Blood-Type-Incompatible Transplantation (6). The standard protocol for the test tube agglutination test is described briefly below (6,7). For both IgM and IgG assays, red blood cells (RBCs) were combined with the patient's serum sample at a ratio of 1:2 and centrifuged for 15 s. For the IgM assay, serum samples were first serially diluted with saline, and then incubated with RBCs at room temperature for 15 min. For the IgG assay using anti-human globulin, serum samples were preincubated with 0.01 M dithiothreitol at 37°C for 30 min, and then serially diluted and incubated with RBCs at 37°C for 30 min. The final dilution at which the agglutination reactivity was positive (1+), not equivocal (+/−), was determined as the antibody titer.

Definitions

Clinical AMR was diagnosed on the basis of radiological findings and clinical course, as described previously (1,5). The clinical manifestations of AMR were hepatic necrosis and intrahepatic biliary complication (IHBC). Hepatic necrosis was diagnosed when hepatic enzyme levels increased markedly in laboratory studies and liver necrosis was observed by computed tomography, usually 1 week after transplantation. IHBC was diagnosed when refractory cholangitis had developed and sclerosing change of the hepatic duct was observed by cholangiography. Diagnosis of acute cellular rejection (ACR) and chronic rejection was based on Banff criteria (8). Infectious diseases were defined as infections requiring treatment.

Statistical analysis

Survival curves were constructed with the Kaplan–Meier method (1). In univariate and multivariate analyses, Cox regression and logistic regression were used to evaluate the association between patient characteristics and overall survival and AMR, respectively. In the multivariate analyses, all potential confounders ($p < 0.05$ in the univariate analysis), including the era of operation, were included, and all patient data, including those for which values were missing, were used to minimize confounding and biases. The incidences of clinical complications were compared by using the chi-squared test.

Receiver operating characteristic (ROC) curves were plotted and areas under the curve were calculated to assess the optimum cut-off values for independent predictors of AMR. In analyses of prognostic factors for AMR and patient survival, the antibody cut-off titers that we calculated previously (1) were used. In the subgroup analysis of patients treated with rituximab, the cut-off titers for antibodies were newly calculated. SAS version 9.3 (SAS Institute, Inc., Cary, NC) was used for statistical analysis, and JMP version 10.0 (SAS Institute, Inc.) was used for the ROC curve analysis.

This study was performed in accordance with the provisions of the Declaration of Helsinki (as revised in Seoul, Korea, October 2008).

Results

Patients

By December 2011, clinical and laboratory data on 663 patients who underwent ABO-I LDLT in 37 institutions were available in the Japanese registry of ABO-I LDLT; of these patients, 381 who were aged 16 years or older were included as adults in the study. All 136 adult patients enrolled in our previous study (1) were included in the current study. The annual number of adults undergoing ABO-I LDLT was higher in 2001 and 2004 than in the previous years (Figure 1).

Demographic data on the 381 patients are listed in Table 1. Recipient age ranged from 16 to 70 years (median, 52 years). MELD scores ranged from 17 to 66 (median, 18), and donor age ranged from 18 to 66 (median, 45). Graft type was left-side liver in 146 patients, right-side liver in 231 patients and unknown in 4 patients. The original diseases were hepatocellular carcinoma in 104 patients, hepatitis C cirrhosis in 58 patients, hepatitis B cirrhosis in 22 patients, alcoholic cirrhosis in 14 patients, primary biliary cirrhosis in 57 patients, primary sclerosing cholangitis in 10 patients, cirrhosis secondary to autoimmune hepatitis (AIH) in 5 patients, cirrhosis after Kasai operation for biliary atresia in 24 patients, fulminant hepatic failure (FHF) in 22 patients (including 2 cases of FHF due to AIH), Wilson's disease in 8 patients, cirrhosis secondary to nonalcoholic steatohepatitis in 6 patients, cryptogenic cirrhosis in 5 patients, idiopathic portal hypertension in 5 patients, re-transplantation in 16 patients and other diseases in 25 patients. In an analysis of the impact of the original disease, 7 patients with AIH (5 cases of cirrhosis and 2 of FHF), 57 patients with primary biliary cirrhosis and 10 patients with primary sclerosing cholangitis were classified as having autoimmune disease.

Immunosuppression

All patients underwent double (calcineurin inhibitor and steroids; n = 36) or triple (calcineurin inhibitor, steroids and antimetabolites; n = 345) immunosuppression. The

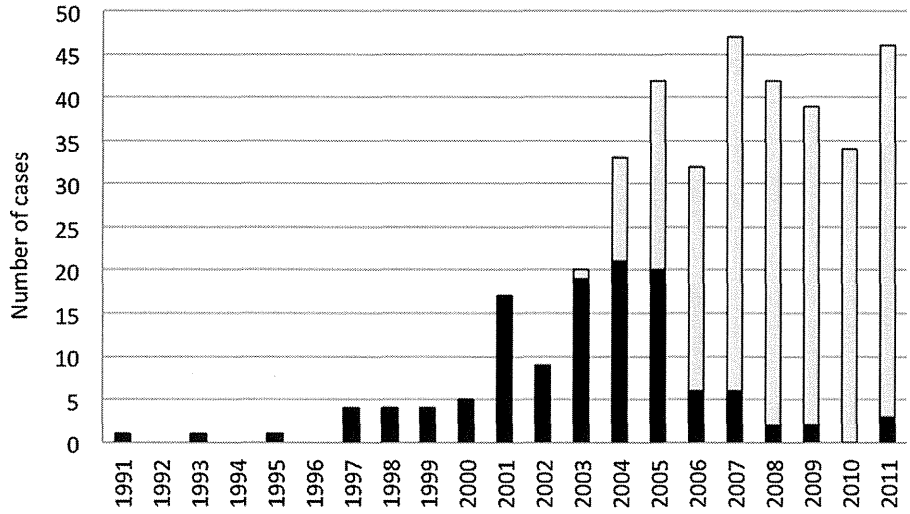

Figure 1: Annual numbers of adults undergoing ABO-I LDLT or rituximab prophylaxis at 37 institutions in Japan. ABO-blood-type-incompatible living donor liver transplantation (ABO-I LDLT) without rituximab prophylaxis (black bars); with rituximab prophylaxis (gray bars).

calcineurin inhibitor tacrolimus was administered in 364 cases, cyclosporine in 13 cases and an unknown drug in 4 cases. Regarding antimetabolites, cyclophosphamide was administered in 137 cases, mycophenolate mofetil in 286 cases, azathioprine in 18 cases, mizoribine in 20 cases and data were missing in 4 cases. Cyclophosphamide was switched to another antimetabolite in 105 cases. Antibody induction was performed by anti-lymphocytic antibody in 36 cases, anti-lymphocyte globulin in 15 cases, anti-IL-2 receptor antibody in 18 cases, muromonab-CD3 (OKT-3) in 2 cases and an unknown antibody in 1 case.

B cell desensitization

Plasmapheresis (n = 320), local infusion (n = 312), rituximab (n = 259), splenectomy (n = 241) and IVIG (n = 56) were performed. Local infusion, IVIG and rituximab were first used in 2000, 2003 and 2004, respectively. The number of times plasmapheresis was used before transplantation ranged from 0 to 11 (median, 2). Prophylactic IVIG was performed in seven institutions as center-specific policy, and it was performed in 6 patients before transplantation and 56 patients after transplantation. Here, we analyzed the effects of only posttransplantation IVIG. The dose ranged from 0.5 to 0.8 g/kg/injection, and the number of doses in regimens ranged from 2 to 5. There was no significant difference in titers between patients treated, or not treated, with IVIG (data not shown).

In the subgroup analysis of the rituximab group, regimens were classified into the following four groups: rituximab only without splenectomy or local infusion (R; n = 10); rituximab with splenectomy but without infusion (RS; n = 30); rituximab with infusion but without splenectomy (RI; n = 80); and rituximab with both infusion and splenectomy (RIS; n = 137).

Rituximab administration

Doses of rituximab were 500 mg/body in 113 cases, 300 mg/body in 60 cases and 375 mg/m^2 in 49 cases. The number of doses administered was 1 in 222 cases, 2 in 22 cases and 3 in 12 cases. The timing of initial administration ranged from preoperative days 0 to 66 and was ≤6 days before transplantation in 22 cases (Figure 2).

Analysis for prognostic factors

In univariate Cox regression analyses, prognostic factors that were significantly and favorably associated with patient survival were era (2005 onward), preoperative status (at home), low MELD score (<23), rituximab prophylaxis, low peak IgM and IgG donor-specific antibody (DSA) titers posttransplantation (<64), absence of bacterial and fungal infection and absence of AMR (Table 1). There was no significant factor among pretransplant characteristics and types of desensitization therapy in the multivariate analysis after adjustment for the era effect (Table 2).

In univariate analyses, significant risk factors for AMR were era (up to 2000 or 2001–2004), autoimmune disease, preoperative status (in-ICU), high peak IgG DSA titer before transplantation (≥64), high IgG DSA titer at transplantation (≥16), high MELD score (≥23), absence of rituximab prophylaxis, high peak IgM and IgG DSA titers posttransplantation (both ≥64) and presence of fungal infection (Table 1). Among pretransplant characteristics and types of desensitization therapy, only the absence of rituximab prophylaxis was a significant indicator of risk of AMR in the multivariate analysis after adjustment for the era effect (Table 3).

AMR was a significant risk for overall survival in the univariate analysis ($p < 0.001$; Figure 3).

Table 1: Prognostic factors for overall survival and antibody-mediated rejection: univariate analysis (n = 381)

			Overall survival				Antibody-mediated rejection			
			Hazard ratio	95% CI	p-Value	p-Value (global association without unknown)	Odds ratio	95% CI	p-Value	p-Value (global association without unknown)
Characteristics	Category	N	Cox regression analysis				Logistic regression analysis			
Characteristics before transplantation										
Sex	Male	169	1.000	–	–	–	1.000	–	–	–
	Female	212	1.062	0.762–1.479	0.723		1.455	0.759–2.789	0.259	
Center size	Less than 10 cases	49	1.000	–	–	–	1.000	–	–	–
	10 cases or more	332	1.102	0.684–1.845	0.705		1.171	0.438–3.132	0.749	
Era	Up to 2000	20	1.000	–	–	0.002*	1.000	–	–	<0.001*
	2001–2004	79	0.628	0.335–1.178	0.147		0.640	0.214–1.915	0.425	
	2005 onward	282	0.391	0.217–0.708	0.002*		0.188	0.065–0.539	0.002	
Autoimmune disease	No	304	1.000	–	–	–	1.000	–	–	–
	Yes	74	1.032	0.685–1.553	0.882		2.411	1.217–4.777	0.012*	
	Unknown	3	2.612	0.642–10.62	0.180		0.000	N/A	N/A	
Preoprative status	At home	143	1.000	–	–	0.013*	1.000	–	–	0.022*
	In-hospital	178	1.222	0.837–1.786	0.299		1.460	0.692–3.080	0.320	
	In-ICU	40	2.153	1.289–3.596	0.003*		3.639	1.438–9.208	0.006*	
	Unknown	20	1.489	0.727–3.048	0.277		0.575	0.071–4.673	0.605	
Recipient's blood type	A	91	1.000	–	–	0.860	1.000	–	–	0.116
	B	87	0.896	0.548–1.464	0.660		1.050	0.353–3.128	0.930	
	O	203	1.004	0.671–1.502	0.984		2.081	0.878–4.932	0.096	
Donor's blood type	A	183	1.000	–	–	0.654	1.000	–	–	0.654
	B	117	0.949	0.643–1.400	0.793		0.757	0.363–1.580	0.458	
	AB	81	1.166	0.772–1.762	0.465		0.726	0.311–1.693	0.459	
Antigen blood type	A	217	1.000	–	–	0.528	1.000	–	–	0.965
	B	153	0.992	0.705–1.396	0.962		1.024	0.537–1.951	0.943	
	AB	11	1.597	0.696–3.662	0.269		0.768	0.094–6.256	0.805	
Donor relative	No	188	1.000	–	–	–	1.000	–	–	–
	Yes	185	0.777	0.558–1.083	0.136		1.018	0.543–1.911	0.955	
	Unknown	8	0.350	0.049–2.523	0.298		0.000	N/A	N/A	
IgM (peak before transplantation)	Low (<256)	273	1.000	–	–	–	1.000	–	–	–
	High (≥256)	62	1.180	0.767–1.817	0.451		0.683	0.275–1.699	0.413	
	Unknown	46	0.908	0.528–1.563	0.729		0.142	0.019–1.060	0.057	
IgG (peak before transplantation)	Low (<64)	155	1.000	–	–	–	1.000	–	–	–
	High (>64)	182	1.229	0.863–1.749	0.253		2.352	1.159–4.771	0.018*	
	Unknown	44	1.112	0.627–1.973	0.717		0.568	0.122–2.637	0.470	
IgM (at transplantation)	Low (<16)	245	1.000	–	–	–	1.000	–	–	–
	High (≥16)	82	1.231	0.828–1.828	0.304		1.183	0.577–2.429	0.646	
	Unknown	54	1.007	0.613–1.653	0.979		0.130	0.017–0.976	0.047	
IgG (at transplantation)	Low (<16)	191	1.000	–	–	–	1.000	–	–	–
	High (≥16)	124	1.172	0.809–1.699	0.401		2.672	1.334–5.354	0.006*	
	Unknown	66	1.336	0.855–2.089	0.204		1.173	0.436–3.161	0.752	
MELD	Low (<23)	240	1.000	–	–	–	1.000	–	–	–
	High (≥23)	88	1.619	1.095–2.393	0.016*		3.172	1.565–6.428	0.001*	
	Unknown	53	2.039	1.325–3.138	0.001		2.193	0.898–5.352	0.085	
Desensitization therapies										
Local infusion	No	65	1.000	–	–	–	1.000	–	–	–
	Yes	312	0.904	0.582–1.405	0.655		0.929	0.410–2.105	0.861	
	Unknown	4	1.368	0.323–5.795	0.671		0.000	N/A	N/A	

(*Continued*)

Table 1: Continued

			Overall survival				Antibody-mediated rejection			
			Hazard ratio	95% CI	p-Value	p-Value (global association without unknown)	Odds ratio	95% CI	p-Value	p-Value (global association without unknown)
Characteristics	Category	N	Cox regression analysis				Logistic regression analysis			
Splenectomy	No	135	1.000	–	–	–	1.000	–	–	–
	Yes	241	0.841	0.599–1.181	0.317		1.094	0.564–2.122	0.0790	
	Unknown	5	0.874	0.213–3.587	0.852		0.000	N/A	N/A	
Rituximab prophylaxis	No	119	1.000	–	–	–	1.000	–	–	–
	Yes	259	0.501	0.358–0.702	<0.001*		0.214	0.111–0.414	<0.001*	
	Unknown	3	1.554	0.380–6.358	0.540		0.000	N/A	N/A	
Prophylactic IVIG after transplantation	No	325	1.000	–	–	–	1.000	–	–	–
	Yes	56	0.859	0.523–1.409	0.547		0.392	0.117–1.313	0.129	
Anti-lymphocyte antibodies	No	345	1.000	–	–	–	1.000	–	–	–
	Yes	36	1.232	0.732–2.073	0.432		0.953	0.320–2.836	0.931	
Plasmapheresis	No	47	1.000	–	–	–	1.000	–	–	–
	Yes	320	0.723	0.454–1.152	0.172		1.132	0.422–3.038	0.806	
	Unknown	14	0.913	0.368–2.263	0.844		0.646	0.069–6.041	0.702	
Plasmapheresis (times)	0	47	1.000	–	–	0.240	1.000	–	–	0.247
	1	68	0.639	0.353–1.155	0.138		0.813	0.233–2.837	0.745	
	2	89	0.865	0.505–1.483	0.277		1.185	0.386–3.637	0.767	
	3	93	0.622	0.355–1.091	0.098		0.684	0.205–2.283	0.537	
	4	28	1.159	0.597–2.249	0.664		2.801	0.793–9.888	0.110	
	≥5	28	0.659	0.302–1.439	0.295		1.008	0.222–4.584	0.992	
	Unknown	28	0.616	0.282–1.346	0.224		1.826	0.478–6.973	0.378	
Short-term outcomes										
IgM (peak posttransplantation)	Low (<64)	251	1.000	–	–	–	1.000	–	–	–
	High (≥64)	94	1.689	1.180–2.418	0.004*		7.935	3.973–15.85	<0.001*	
	Unknown	36	1.046	0.571–1.916	0.884		0.000	N/A	N/A	
IgG (peak posttransplantation)	Low (<64)	205	1.000	–	–	–	1.000	–	–	–
	High (≥64)	126	1.484	1.043–2.110	0.028*		10.453	4.467–24.46	<0.001*	
	Unknown	50	1.142	0.671–1.945	0.624		1.805	0.450–7.244	0.405	
Acute rejection	No	296	1.000	–	–	–	1.000	–	–	–
	Yes	78	0.964	0.640–1.453	0.862		1.133	0.533–2.408	0.745	
	Unknown	7	2.023	0.746–5.487	0.166		0.000	N/A	N/A	
Chronic rejection	No	349	1.000	–	–	–	1.000	–	–	–
	Yes	5	1.905	0.703–5.158	0.205		1.827	0.199–16.74	0.594	
	Unknown	27	1.750	1.006–3.044	0.048		0.281	0.037–2.126	0.219	
Bacterial infection	No	254	1.000	–	–	–	1.000	–	–	–
	Yes	124	4.160	2.965–5.835	<0.001*		1.843	0.975–3.485	0.060	
	Unknown	3	3.650	0.890–14.97	0.072		0.000	N/A	N/A	
Fungal infection	No	342	1.000	–	–	–	1.000	–	–	–
	Yes	34	5.718	3.772–8.667	<0.001*		3.776	1.666–8.558	0.002*	
	Unknown	5	1.394	0.344–5.648	0.641		0.000	N/A	N/A	
CMV disease	No	199	1.000	–	–	–	1.000	–	–	–
	Yes	180	0.784	0.562–1.095	0.153		0.911	0.485–1.713	0.773	
	Unknown	2	1.233	0.171–8.870	0.835		0.000	N/A	N/A	
Antibody-mediated rejection	No	337	1.000	–	–	–	–	–	–	–
	Yes	44	2.493	1.654–3.759	<0.001*		–	–	–	

CMV, cytomegalovirus; IVIG, intravenous immunoglobulin; MELD, Model for End-Stage Liver Disease.
*$p < 0.05$.

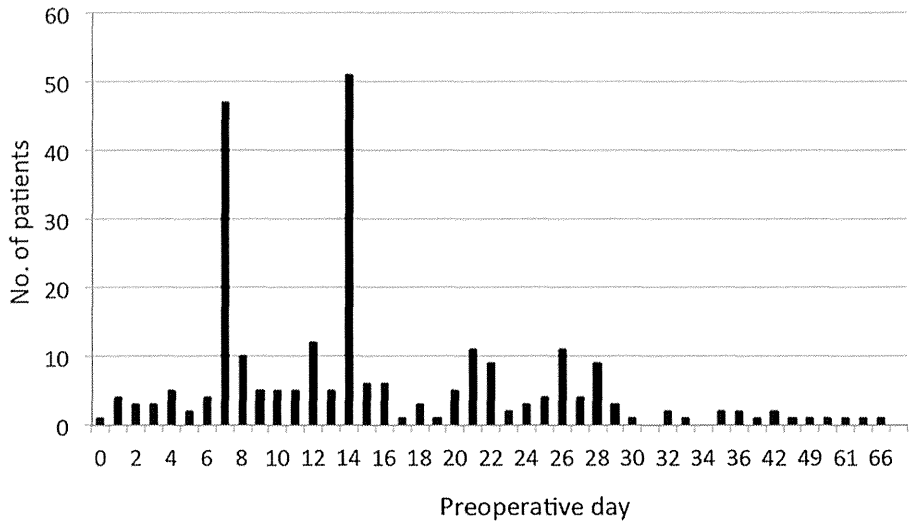

Figure 2: The timing of initial administration of rituximab ranged from preoperative days 0 to 66 and was within 6 days before transplantation in 22 cases.

Impact of rituximab on clinical outcomes

The AMR incidence was significantly lower in the rituximab group (6%) than in the nonrituximab group (23%) ($p < 0.001$; Figure 4, top); a significant difference was also observed for the subset of patients with hepatic necrosis-type AMR ($p < 0.001$; Figure 4, top). There were no significant differences between the incidences of ACR (Figure 4, top), bacterial infection or CMV disease (Figure 4, bottom) between the rituximab and nonrituximab groups. The rate of fungal infection was significantly lower in the rituximab group (4%) than in the nonrituximab group (19%) ($p < 0.001$; Figure 4, bottom).

Adverse effects of rituximab (kidney dysfunction, sepsis, neutropenia or lung edema) were observed in four patients, whose ages ranged from 56 to 62 years. Neutropenia occurred after a single dose of 300 mg/body, and the other complications manifested after the second or third dose of 500 mg/body. The patient with renal dysfunction died from a massive thrombus of the superior mesenteric artery on postoperative day 63, and the patient with sepsis died on postoperative day 202 from sepsis with an unknown focus. The other two patients are doing well.

Subgroup analysis of rituximab group

Because most ABO-I LDLT patients are currently administered rituximab, we analyzed the effects of additional desensitization therapies and the manner of rituximab administration to elucidate a better regimen. In a subgroup analysis of the rituximab group, local infusion, splenectomy, anti-lymphocyte antibodies and IVIG had no significant impact on overall survival or AMR incidence (Table 4).

Patients who were administered multiple doses of rituximab, or a regular dose of 500 mg/body or 375 mg/m^2, tended toward a lower incidence of AMR, but this was not

Table 2: Prognostic factors for overall survival: multivariate analysis ($n = 381$)

Characteristics	Category	N	5-Year survival (%)	Hazard ratio	95% CI	p-Value
Era	Up to 2000	20	40.0	1.000	–	–
	2001–2004	79	50.6	0.766	0.378–1.551	0.459
	2005 onwards	282	67.5	0.742	0.346–1.591	0.443
Preoperative status	At home	143	65.8	1.000	–	–
	In-hospital	178	63.6	1.087	0.735–1.606	0.676
	In-ICU	40	44.3	1.355	0.765–2.398	0.297
	Unknown	20	60.0	0.883	0.395–1.974	0.762
MELD	Low (<23)	240	66.9	1.000	–	–
	High (≥23)	88	57.2	1.364	0.894–2.080	0.149
	Unknown	53	48.8	1.420	0.827–2.437	0.203
Rituximab prophylaxis	No	119	48.4	1.000	–	–
	Yes	259	69.6	0.629	0.377–1.051	0.077
	Unknown	3	33.3	1.875	0.445–7.900	0.391

MELD, Model for End-Stage Liver Disease.

Table 3: Prognostic factors for antibody-mediated rejection: multivariate analysis (n = 381)

Characteristics	Category	N	AMR (%)	Odds ratio	95% CI	p-Value
Era	Up to 2000	20	30.0	1.000	–	–
	2001–2004	79	21.5	0.656	0.170–2.534	0.541
	2005 onwards	282	7.5	0.625	0.143–2.742	0.534
Autoimmune disease	No	304	9.5	1.000	–	–
	Yes	74	20.3	2.023	0.940–4.356	0.072
	Unknown	3	0.0	0.000	N/A	N/A
Preoperative status	At home	143	8.4	1.000	–	–
	In-hospital	178	11.8	0.929	0.404–2.134	0.862
	In-ICU	40	25.0	1.430	0.473–4.320	0.526
	Unknown	20	5.0	0.322	0.030–3.443	0.349
IgG (preoperative)	Low (<64)	155	7.7	1.000	–	–
	High (≥64)	182	16.5	1.805	0.724–4.505	0.205
	Unknown	44	4.6	0.744	0.100–5.555	0.773
IgG (at operation)	Low (<16)	191	7.9	1.000	–	–
	High (≥16)	124	18.6	1.933	0.790–4.731	0.149
	Unknown	66	9.1	1.066	0.269–4.234	0.927
MELD	Low (<23)	240	7.5	1.000	–	–
	High (≥23)	88	20.5	2.026	0.878–4.675	0.098
	Unknown	53	15.1	0.936	0.278–3.154	0.915
Rituximab prophylaxis	No	119	23.5	1.000	–	–
	Yes	259	6.2	0.248	0.089–0.690	0.008*
	Unknown	3	0.0	0.000	N/A	N/A

AMR, antibody-mediated rejection; MELD, Model for End-Stage Liver Disease.
*$p < 0.05$.

statistically significant (Table 4). In contrast, patients given multiple doses had significantly greater incidences of fungal infection and CMV disease than those given a single dose, and patients given the regular dose had a greater incidence of CMV disease than those given a small dose of 300 mg/body or less (Table 5). Patients subjected to local infusion together with rituximab prophylaxis (RI and RIS) had greater incidences of CMV disease than patients without local infusion or splenectomy (R) (Table 5). Finally, there were no significant differences among rituximab regimens in terms of AMR incidence or patient survival (Table 4; Figure 5).

Early administration of rituximab had no significant impact on AMR incidence or patient survival (Table 4). Twenty-two FHF patients underwent LDLT, and six of them were given

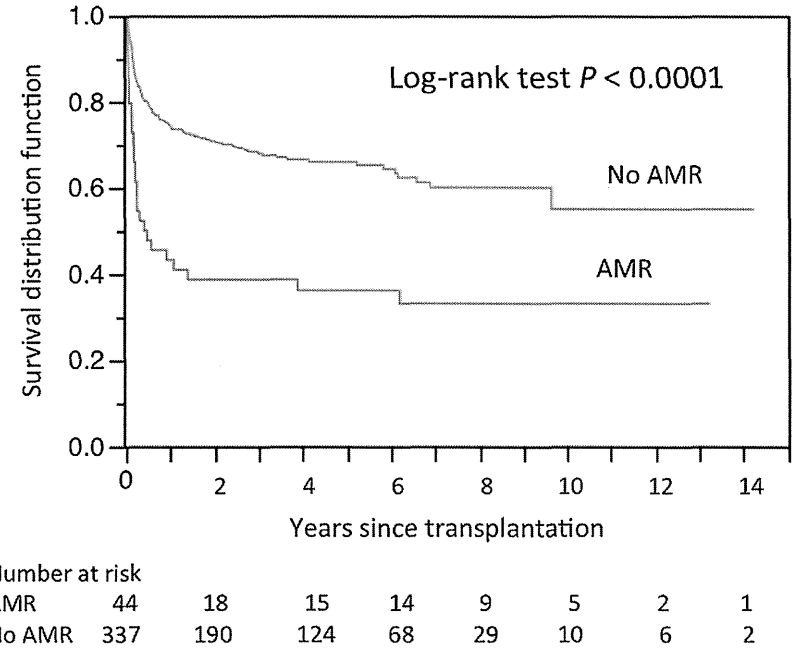

Figure 3: Comparison of overall survival between patients with and without antibody-mediated rejection. Patients with antibody-mediated rejection (AMR) had a significantly higher overall survival risk than those without AMR, $p < 0.001$.

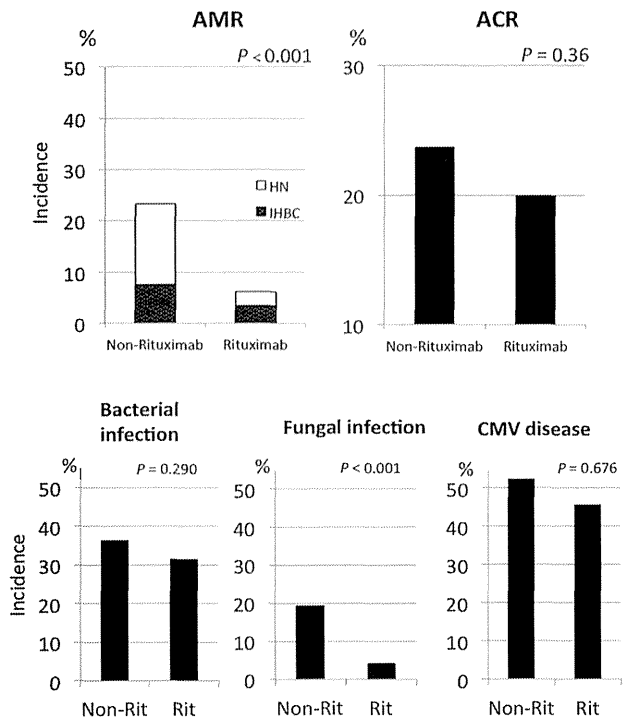

Figure 4: Comparison of incidences of complications between rituximab and nonrituximab groups. The incidences of antibody-mediated rejection (AMR) and acute cellular rejection (ACR) are shown (top); rates of intrahepatic biliary complication (IHBC) and hepatic necrosis (HN) type AMR were lower in the rituximab group than in the nonrituximab group (chi-squared test, $p < 0.0001$). The incidences of bacterial infection, fungal infection and cytomegalovirus (CMV) disease are shown (bottom); rates of bacterial infection and CMV disease were similar between the two groups (chi-squared test, $p = 0.36$), but the rate of fungal infection was significantly lower in the rituximab group (chi-squared test, $p < 0.0001$).

rituximab immediately before or during transplantation (three treated with RIS, two with RI and one with RS). All 6 patients survived transplantation without AMR, whereas AMR occurred in 7 patients and 1-year survival was 44% in the other 16 patients who were not given rituximab.

Peak IgG DSA titer before transplantation, IgG DSA titer at transplantation and peak IgG and IgM DSA titers post-transplantation showed a significant positive association with AMR incidence in the total cohort of adult ABO-I LDLT patients in the univariate analysis (Table 1). In the rituximab group, peak IgG and IgM DSA titers posttransplantation were significantly greater in patients with AMR than in those without AMR (Table 6). When the AMR incidence in the rituximab group was compared between high and low titers according to optimum cut-off values calculated from ROC curves, there were significant differences in peak IgG titers before transplantation (10% [10/104] vs. 3% [4/125] titer $\geq$128 vs. <128, $p = 0.042$), peak IgM titers post-transplantation (22% [10/45] vs. 3% [6/194], titer $\geq$64 vs. <64, $p < 0.001$) and peak IgG titers posttransplantation (19% [10/54] vs. 2% [3/171], titer $\geq$128 vs. <128, $p < 0.001$).

Discussion

Worldwide, the first case report of rituximab prophylaxis in kidney transplantation was published in Japan in 2002 (9); many rituximab protocols for kidney transplantation have been reported since. Monteiro et al (10) reported the first case of ABO-I liver transplantation using rituximab in 2003, and Usuda et al (3) reported the first case of rituximab prophylaxis in ABO-I LDLT in 2005. In the Japanese registry, the first adult case of rituximab prophylaxis was reported in November 2003. In our previous multicenter study (1) of 291 patients who underwent ABO-I LDLT up to and including March 2006, 44 adult patients were administered rituximab. The current study includes 259 adult patients who underwent rituximab prophylaxis up to and including December 2011.

After 2000, the evolution of innovation in the treatment of small-for-size syndrome in adult LDLT and desensitization for DSA was achieved (11–13). The era effect on overall survival is significant. In the total cohort of 381 adult patients, after adjustment for era effects in the multivariate analysis, only rituximab prophylaxis was a significant prognostic factor for AMR, but it was not a prognostic factor for overall survival. A prospective study is required to elucidate the effect of rituximab on patient survival; however, it would be difficult to remove rituximab prophylaxis when the current results are so much improved in the most recent era and when this may be attributable to rituximab.

To find the best regimen for rituximab, the impact of additional desensitization therapies and times and doses of rituximab were addressed. Splenectomy used to be considered an essential component of a successful ABO-I desensitization regimen for renal transplantation (14); however, it has been reported that rituximab can be used in place of splenectomy with similar outcomes (15,16). The Kyoto group suggested that splenectomy should be avoided in 2007 (2,17). In LDLT, however, splenectomy is performed not only for desensitization but also for portal flow adjustment in patients with small-for-size syndrome and for future anti-viral treatment using interferon in hepatitis C patients. An assessment of the effects of preserving the spleen is required in patients without small-for-size syndrome or hepatitis C infection in future.

Plasma exchange is a standard procedure to reduce DSA titers, but the titer required to prevent AMR is not defined. If titers increase again after plasmapheresis, another plasmapheresis is often performed. When peak titer before transplantation is very low, plasmapheresis is not performed. In other words, the more times the plasmapheresis

Table 4: Prognostic factors for antibody-mediated rejection and overall postsurgical survival: univariate analysis of 259 patients given rituximab prophylaxis

			Overall survival				Antibody-mediated rejection			
			Hazard ratio	95% CI	p-Value	p-Value (global association)	Odds ratio	95% CI	p-Value	p-Value (global association)
Characteristics	Category	N	Cox regression analysis				Logistic regression analysis			
Local infusion	No	40	1.000	–	–	–	1.000	–	–	–
	Yes	218	1.329	0.635–2.779	0.451	–	2.882	0.370–22.450	0.312	–
	Unknown	1	–	–	–	–	–	–	–	–
Splenectomy	No	90	1.000	–	–	–	1.000	–	–	–
	Yes	169	0.985	0.614–1.579	0.948	–	0.881	0.309–2.506	0.812	–
Anti-lymphocyte antibodies	No	244	1.000	–	–	–	1.000	–	–	–
	Yes	15	0.838	0.306–2.298	0.731	–	0.447	0.023–8.547	0.593	–
Prophylactic IVIG after transplantation	No	214	1.000	–	–	–	1.000	–	–	–
	Yes	45	0.984	0.529–1.830	0.960	–	0.664	0.146–3.031	0.598	–
Timing of rituximab administration before transplantation	≤6 days	22	1.000	–	–	–	1.000	–	–	–
	>7 days	236	1.241	0.535–2.883	0.615	–	1.425	0.179–11.330	0.738	–
	Unknown	1	–	–	–	–	–	–	–	–
Number of doses of rituximab	1	225	1.000	–	–	0.443	1.000	–	–	0.922
	2	22	1.504	0.747–3.031	0.253	–	0.947	0.161–5.560	0.730	–
	3	12	1.377	0.550–3.448	0.494	–	0.543	0.027–10.77	0.689	–
Dose of rituximab	Regular	162	1.000	–	–	–	1.000	–	–	–
	Small	66	1.282	0.745–2.207	0.370	–	2.655	0.952–7.404	0.062	–
	Unknown	31	–	–	–	–	–	–	–	–
Dose and number of doses of rituximab	Regular × 1	134	1.000	–	–	0.461	1.000	–	–	0.409
	Regular × 2	16	1.408	0.589–3.366	0.442	–	0.451	0.023–8.902	0.601	–
	Regular × 3	12	1.506	0.580–3.910	0.400	–	0.595	0.029–12.240	0.737	–
	Small × 1	60	1.264	0.694–2.310	0.444	–	2.086	0.738–5.897	0.165	–
	Small × 2	6	2.755	0.844–8.993	0.093	–	4.058	0.512–32.19	0.185	–
	Unknown	31	–	–	–	–	–	–	–	–
Regimen	RS	30	1.000	–	–	0.700	1.000	–	–	0.938
	R	10	2.053	0.490–8.597	0.325	–	0.937	0.031–28.37	0.970	–
	RI	81	1.568	0.596–4.128	0.362	–	1.693	0.266–10.790	0.577	–
	RIS	137	1.691	0.667–4.285	0.268	–	1.454	0.242–8.743	0.683	–
	Unknown	1	–	–	–	–	–	–	–	–

IVIG, intravenous immunoglobulin; R, only rituximab; regular dose, 500 mg/body or 375 mg/m^2; RI, rituximab and infusion; RIS, rituximab and infusion and splenectomy; RS, rituximab and splenectomy; small dose, 300 mg/body or less.