worse after LDLT than after DDLT [25, 26]. This issue is of critical importance in Japan and other countries in which almost all liver transplantations use living donor grafts. Therefore, this mini-review compares transplant outcomes for HCV-infected patients between LDLT and DDLT. Further, LDLT results for HCV-positive recipients in Japanese transplant centers are discussed, including the outcomes at Kyoto University.

Comparison between LDLT and DDLT in Western countries

Some published abstracts from US centers [11, 12] suggest that hepatitis recurs earlier and may be more aggressive and rates of graft loss may be greater following LDLT than following DDLT (Table 1). Although only small numbers of patients have been studied and the follow-up period was limited, this issue has attracted worldwide attention. Several full papers reported similarly poor graft outcomes following LDLT [13–16]. For example, Gaglio et al. [13] reported that 17 % (4/23) of LDLT recipients developed cholestatic HCV (defined as serum total bilirubin >10 mg/dL and histological features of portal expansion, ductular proliferation, and bile stasis with or without hepatocyte ballooning), yet none of the DDLT recipients ($n = 45$) developed this complication. A study from the United Network for Organ Sharing (UNOS) database by Thulubvath et al. [14] reported that HCV-positive LDLT recipients ($n = 207$) show significantly lower graft survival in comparison to a matched population that received DDLT ($n = 480$). Schiano et al. [15] compared the HCV kinetics immediately after transplantation between 11 LDLT and 15 DDLT patients and showed that HCV-RNA levels rise more rapidly between days 1 and 3 in LDLT recipients ($p = 0.0059$) and are significantly higher in this group on days 2–5, though the 2-year graft survival rates were not significantly different (73 % for LDLT and 80 % for DDLT recipients). Furthermore, a report from Spain demonstrated that severe recurrence of HCV, defined as the development of cirrhosis or clinically decompensated liver disease, is more frequent in LDLT recipients than in DDLT recipients [16]. Surprisingly, the 2-year probability of developing severe recurrence was 45 % after LDLT in comparison to 22 % after DDLT ($p = 0.019$). This report had a strong impact, because it was a well-designed, prospective study that used protocol liver biopsies [18].

In contrast, a second study by Russo et al. [19] using the UNOS database found no significant differences in the 2-year graft survival (72 vs. 75 %, $p = 0.11$) or 2-year patient survival (83 vs. 81 %, $p = 0.68$) between 279 LDLT and 3,955 DDLT recipients with a diagnosis of chronic HCV transplanted between 1999 and 2002. Schiffman et al. [20] prospectively evaluated the histological outcome of recurrent HCV using protocol liver biopsies in 23 LDLT and 53 DDLT recipients, and reported that there are no significant differences in the mean Knodell fibrosis stage (0.9 vs. 1.9) or in the percentage of patients with fibrosis (59 vs. 78 %) at 36 months. Several subsequent studies [21–26] reported that patients undergoing LDLT versus DDLT have comparable outcomes in terms of graft and patient survival or histological recurrence (Table 2). The most recent study by Jain et al. [26] retrospectively examined and compared survival outcomes and fibrosis progression between 35 LDLT and 65 DDLT recipients on long-term follow-up (mean 86.6 ± 6.8 months). They demonstrated that the 7-year patient and graft survivals are better for those undergoing LDLT in comparison to DDLT (77.1 vs. 51 %, $p = 0.026$, and 71.4 vs. 46.2 %, $p = 0.042$, respectively) and that the mean fibrosis scores according to the Ishak scoring system are lower for those undergoing LDLT than for those undergoing DDLT (1.5 ± 1.8 vs. 2.5 ± 1.9 at year 5, $p = 0.014$). However, the overall patient and graft survival

Table 1 Early reports of poor outcomes for LDLT

References	No. of LDLT patients	Follow-up (months)	Findings (LDLT vs. DDLT)
Gaglio et al. [11]	18	Median, 19	Recurrent hepatitis: 80 vs. 58 % ($p < 0.05$); severe hepatitis: 17 vs. 12 %
Ghobrial et al. [12]	9	6–12	Recurrent hepatitis: 86 vs. 30 %
Gaglio et al. [13]	23 (vs. 45 DDLT)	Mean, 25 (6–39)	Cholestatic hepatitis: 17 vs. 0 % ($p = 0.001$)
Thuluvath et al. [14]	207 (vs. 480 DDLT)	–	Lower graft survival ($p = 0.02$)
Schiano et al. [15]	11 (vs. 15 DDLT)	–	Rapid increase in HCV-RNA between days 1 and 3 ($p < 0.01$); significantly higher HCV-RNA level during 2–5 days
Garcia-Retortillo et al. [16]	22 (vs. 95 DDLT)	Median, 22 (2.6–44)	2-year probability of severe recurrence: 45 vs. 22 % ($p = 0.019$)

LDLT living donor liver transplantation, *DDLT* deceased donor liver transplantation

Table 2 Recent reports of comparable outcomes for LDLT and DDLT

References	No. of patients (LDLT vs. DDLT)	Findings (LDLT vs. DDLT)
Russo et al. [19]	279 vs. 3,955	2-year graft survival: 72 vs. 75 % ($p = 0.11$); 2-year patient survival: 83 vs. 81 % ($p = 0.68$)
Shiffman et al. [20]	23 vs. 53	3-year protocol biopsy (Knodell score): mean fibrosis stage 0.9 vs. 1.9 (n.s.) and percentage of patients with fibrosis 59 vs. 78 % (n.s.); 4-year graft survival: 82 vs. 75 % ($p = 0.46$)
Humar et al. [21]	12 vs. 32	1-year protocol biopsy (Batta and Ludwig score): mean grade of inflammation, 0.33 vs. 1.31 ($p = 0.002$) and mean fibrosis stage, 0.22 vs. 0.96 ($p = 0.07$)
Guo et al. [22]	15 vs. 52	No differences in histological recurrence, inflammation activity grade, or fibrosis stage up to 2 years
Terrault et al. [23]	181 vs. 94	3-year graft survival for LDLT case number <20, LDLT case number >20, and DDLT: 55 vs. 79 vs. 80 %, 3-year patient survival: 63 vs. 84, vs. 82 %, no differences between LDLT case number >20 and DDLT
Schmeding et al. [24]	20 vs. 269	3-year protocol biopsy (Scheuer score): mean fibrosis stage 1.59 vs. 1.80 ($p > 0.05$)
Gallegos-Orozco et al. [25]	32 vs. 168	1-year protocol biopsy (Scheuer score): mean fibrosis stage 1.3 vs. 0.8 ($p = 0.08$), advanced fibrosis/cirrhosis after LT: 13.8 vs. 14.3 % ($p = 0.9$), 5-year patient survival: 81 vs. 88 % ($p = 0.7$)
Jain et al. [26]	35 vs. 65	7-year graft survival: 71.4 vs. 46.2 % ($p = 0.042$), 7-year patient survival: 77.1 vs. 51 % ($p = 0.026$), 5-year protocol biopsy (Ishak score): mean fibrosis stage 1.5 vs. 2.5 ($p = 0.014$)

did not differ between those undergoing LDLT ($n = 32$) and those undergoing DDLT ($n = 32$), when donor age was less than 50 years and when the model for end-stage liver disease (MELD) score was less than 25.

There are several potential explanations for the discrepancies between prior and recent studies in terms of the transplant outcomes after LDLT in comparison to after DDLT. For example, there were a relatively small number of LDLT patients included in these studies. More importantly, recent data suggest that there is a learning curve effect associated with increased experience of LDLT. A report from the Adult-to-Adult Living Donor Liver Transplantation Cohort Study (A2ALL) by Terrault et al. [23] demonstrated lower graft and patient survival among the first 20 LDLT cases at each center (LDLT case number <20) in comparison to later cases (LDLT case number >20; $p = 0.002$ and $p = 0.002$, respectively). The cumulative graft survival for LDLT case number <20 ($n = 78$), LDLT case number >20 ($n = 103$), and DDLT ($n = 94$) at 3 years is 55, 79, and 80 %, respectively, and the cumulative patient survival at 3 years is 63, 84, and 82 %, respectively. The authors speculate that technical challenges in starting an LDLT program and the higher rate of graft loss early in the post-transplant period were related to vascular problems, biliary complications, and small-for-size syndrome might contribute to the worse outcomes seen in patients undergoing LDLT in those early reports, since the outcomes of HCV-infected patients are not significantly different when comparing LDLT and DDLT recipients once transplant centers have sufficient experience with LDLT. However, the relevance of such technical issues in relation to the higher rate and severe grade of HCV recurrence observed in the early series is not completely understood. Prospective comparative studies using a large sample size and uniform definitions for HCV recurrence between LDLT and DDLT are required to clarify the effects of the LDLT procedure on HCV recurrence.

Antiviral therapy for HCV-infected recipients in the Western countries

Antiviral therapy is also an important aspect affecting the transplant outcomes for HCV-positive recipients. Since the recommendation by International Liver Transplantation Society [2], post-transplant antiviral therapy in those with evidence of recurrent disease is the mainstay of management [27]. A combination of pegylated interferon and ribavirin is the treatment of choice, and a sustained virological response (SVR) rate is achieved with 48 weeks of treatment in approximately 30 % of treated patients [27, 28]. This SVR rate is far less than that reported for immunocompetent HCV-infected patients, which is attributed to poor tolerability and the frequent need for dose reduction and/or discontinuation. Alternative strategies have been studied including pre-transplant treatment of decompensated cirrhosis and pre-emptive antiviral therapy started within weeks of transplantation [2]. However, the safety and efficacy of these alternative approaches are limited so far. The majority of patients on the waiting list are not candidates for antiviral therapy, because tolerability is poor in those with advanced liver disease (Child class B and C). It has been also reported that pre-emptive treatment

in the early post-transplant period is hampered by poor tolerability of interferon and ribavirin therapy in most patients recently transplanted [29].

LDLT for HCV-positive recipients in Japan

A total of 5,653 LDLT were performed for 2,080 pediatric (<18 years old) and 3,573 adult patients as of the end of 2009, according to the registry of the Japanese Liver Transplantation Society [3]. These cases included 1,088 HCV-infected recipients, including 675 patients with hepatocellular carcinoma. The patient survival at 5 years after LDLT for HCV-infected recipients without HCC ($n = 413$) and for those with HCC ($n = 675$) was 68.2 and 65.7 %, respectively. These survival rates were nearly 10 % lower than that for HBV-infected recipients: 78.9 % for HBV-infected recipients without HCC ($n = 223$) and 73.4 % for those with HCC ($n = 357$).

Several Japanese studies have described outcomes for HCV-infected recipients after LDLT [30–34]. Tokyo University performed LDLT in 105 HCV patients between 1996 and 2008 [32]. The 5-year survival rate after LDLT did not differ significantly between HCV-negative ($n = 231$) and HCV-positive recipients (86 vs. 79 %, $p = 0.21$). The study utilized pre-emptive antiviral therapy with interferon (IFN) and ribavirin after LDLT, which yielded a 34 % SVR rate. Ikegami et al. [33] reported that 106 LDLT for HCV-related liver diseases were performed at Kyushu University between February 1999 and July 2008. Twenty-six patients that did not receive IFN treatment because of early mortality ($n = 16$), negative HCV-RNA ($n = 4$), or patient refusal ($n = 6$) were excluded, and 80 recipients were treated for recurrent HCV with IFN plus ribavirin therapy. These 80 patients were divided into four groups based on the treatment response: group I ($n = 18$), patients that achieved SVR; group II ($n = 25$), those with viral response (VR) but no SVR; group III ($n = 13$), those with biochemical response (BR) but no SVR; group IV ($n = 24$), those with no VR or no BR. The SVR rate was 23 %. The 5-year graft survival rate was 100, 91, 100, and 62 % for groups I, II, III, and IV, respectively ($p < 0.05$, group IV vs. groups I, II, and III).

These centers performed splenectomy concurrently with liver transplantation to alleviate blood cytopenia. The decrease of platelet and white blood cell (WBC) counts is one of the major causes for dose reduction or discontinuation of IFN-based treatment. The platelet count significantly increases soon after LDLT in recipients treated with simultaneous splenectomy and is maintained during the post-transplant IFN therapy [30]. Most Japanese transplant centers adopted this procedure to improve the tolerability of IFN treatment after LDLT.

LDLT for HCV-positive recipients at Kyoto University

Six hundred and thirty-eight adult patients underwent LDLT at Kyoto University from March 1999 to December 2008, including HCV-positive 180 patients (111 males, 69 females) [34]. One hundred and seven of these patients also had HCC. The overall patient survival for these HCV-positive patients was 71 % at 5 years after LDLT, with a median follow-up period of 41 months, which was similar to that for 458 non-HCV adult patients (70 %). The 5-year survival was also similar between those who had HCC ($n = 107$) and those that did not ($n = 73$; 71 % vs. 71 %, Fig. 1).

Recurrence of HCV after LDLT is diagnosed based on the histological evaluation. Follow-up protocol biopsies at 6 months or more were obtained from 137 patients. The fibrosis stage according to METAVIR score was F0 or F1 in 94 patients and was F2 or more in 43 patients at their last biopsy. The cumulative rate of progression to significant fibrosis, defined as F2 or more, was 51 % at 3 years and was 71 % at 5 years after LDLT. A univariate analysis shows that female gender for recipients, male gender for donors, and donor age of 50 years or more represented significant risk factors for progression to significant fibrosis (Figs. 2, 3). Furthermore, a multivariate analysis revealed that female gender for recipients and donor age ≥50 years are independent risk factors for significant fibrosis (hazard ratio, 2.331 and 1.980, 95 % confidential interval, 1.309–4.152 and 1.121–3.448, $p = 0.0003$ and $p = 0.009$, respectively).

The current treatment strategy for recurrent HCV after LDLT is (1) splenectomy during recipient transplant operation to increase the platelets and WBC counts suppressed by hypersplenism and to enhance the tolerability of

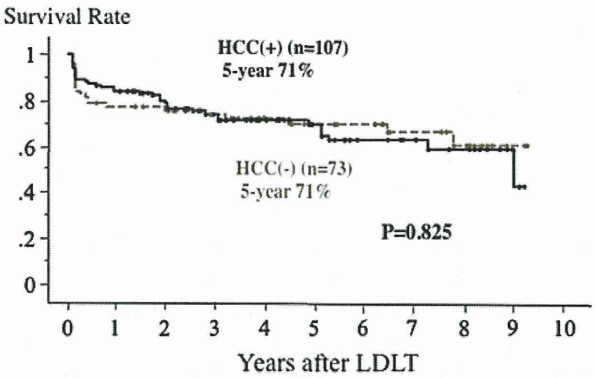

Fig. 1 Patient survival for HCV patients (with or without HCC) after LDLT. One hundred and eighty HCV-positive patients received LDLT at Kyoto University between March 1999 to December 2008, and the overall patient survival rate for patients with HCC ($n = 107$) was 71 % at 5 years, which was similar to that for patients without HCC (71 %, $n = 73$, $p = 0.825$)

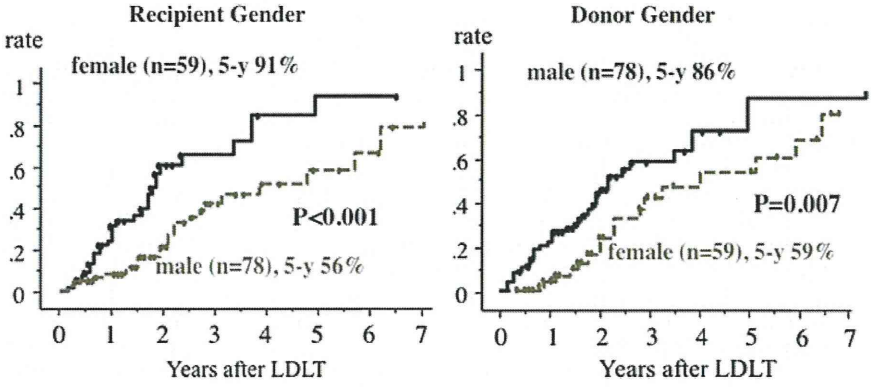

Fig. 2 The cumulative rate of progression to fibrosis, stage 2 or higher associated with recipient and donor gender. Results from the univariate analysis revealed that female gender of the recipients and male gender of the donors were significantly associated with progression to fibrosis of stage 2 or higher

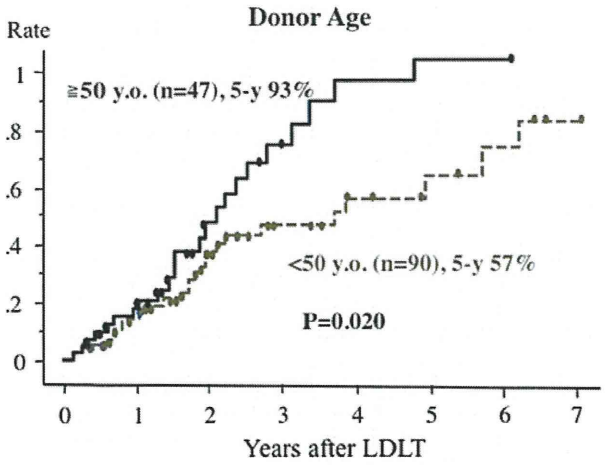

Fig. 3 The cumulative rate of progression to fibrosis, stage 2 or higher associated with the donor age. Results from the univariate analysis revealed that donor age ≥50 years was significantly associated with progression to fibrosis of stage 2 or higher

IFN treatment, (2) antiviral treatment with 1.5 μg/kg of peg-IFN α-2b once weekly plus ribavirin at an oral dose of 600–800 mg/day (full doses) when the recurrence of hepatitis is histologically confirmed, and (3) continue treatment for 12 months after serum HCV-RNA becomes negative [35]. Thirty-four recipients with genotype 1b were treated according to the protocol between February 2006 and February 2008 [35]. Serum HCV-RNA became undetectable at the median of 4.0 months (range, 1.2–9.9 months) after the initiation of treatment in 18 patients, for whom treatment was continued for an additional 12 months after serum HCV-RNA became undetectable. The results showed that 17 of these 18 patients achieved SVR, and the SVR rate was as high as 50 %. The efficacy of such extended treatment to prevent a relapse after a viral response has also been reported by other institutes [32, 36].

Conclusions

Recent reports from Western countries suggest that transplant outcomes for HCV-infected patients are similar between LDLT and DDLT. Although HCV recurrence could not be compared between LDLT and DDLT in Japan due to the small number of DDLT recipients, patient survival as well as the rate of progression to severe disease due to HCV recurrence seems similar when comparing LDLT recipients in Japan and DDLT recipients described in the literature. These findings suggest that LDLT is associated with acceptable outcomes for patients with HCV-related cirrhosis. However, patient survival for HCV-infected patients after either LDLD or DDLT was worse in comparison to HBV-infected patients treated with a prophylactic strategy against viral re-infection. Development of more effective modalities against post-transplant HCV recurrence is required to improve the outcomes in these patients.

Conflict of interest Yasutsugu Takada and Shinji Uemoto have no financial relationship with any organization that sponsored this research. We have full control of all primary data and agree to allow the journal to review our data if requested.

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Case Report

Two patients treated with pegylated interferon/ribavirin/telaprevir triple therapy for recurrent hepatitis C after living donor liver transplantation

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It is difficult to use protease inhibitors in patients with recurrent hepatitis C virus (HCV) infection after liver transplantation (LT) due to interaction with immunosuppressive drugs. We report our experience with two patients treated with telaprevir (TVR) combined with pegylated interferon/ribavirin (PEG IFN/RBV) for recurrent HCV genotype 1 infection after LT. The first was a 63-year-old man with HCV-related liver cirrhosis, who failed to respond to IFN-β plus RBV after LT. Treatment was switched to PEG IFN-α-2b plus RBV and TVR was started. The donor had TT genotype of interleukin (IL)-28 single nucleotide polymorphisms (SNP) (rs8099917). The recipient had TT genotype of IL-28 SNP (rs8099917). Completion of 12-week triple therapy was followed by PEG IFN-α-2b plus RBV for 36 weeks. Finally, he had sustained viral response. The second was a 70-year-old woman with HCV-related liver cirrhosis and hepatocellular carcinoma. She failed to respond to PEG IFN-α-2b plus RBV after LT, and was subsequently switched to PEG IFN-α-2b/RBV/TVR. Genotype analysis showed TG genotype of IL-28 SNP for the donor, and TT genotype of IL-28 SNP for the recipient. Serum HCV RNA titer decreased below the detection limit at 5 weeks. However, triple therapy was withdrawn at 11 weeks due to general fatigue, which resulted in HCV RNA rebound 4 weeks later. Both patients were treated with cyclosporin, starting with a small dose to avoid interactions with TVR. TVR is a potentially suitable agent for LT recipients who do not respond to PEG IFN-α-2b plus RBV after LT.

Key words: hepatitis C virus, liver transplantation, telaprevir

INTRODUCTION

THE HEPATITIS C virus (HCV) has infected 170 million people worldwide, which progresses in some patients to liver cirrhosis and/or hepatocellular carcinoma (HCC).[1] The current treatment for patients infected with HCV genotype 1 is the combination of pegylated interferon-α and ribavirin (PEG IFN/RBV) for 48 weeks.[2] However, this treatment produces sustained viral response (SVR) in only approximately 50% of patients with genotype 1 HCV infection. In 2011, the first direct-acting antiviral agent (DAA) for the treatment of HCV genotype 1, telaprevir (TVR), was approved and treatment with this agent improved SVR to approximately 70–80% of patients with genotype 1 HCV infection.[3,4]

Recurrence of HCV infection after liver transplantation (LT) is one of the major causes of morbidity and allograft loss after LT.[5,6] Because the outcome of post-LT therapy with the classic antiviral agents PEG IFN/RBV are at most moderate with respect to SVR, LT patients constitute one of the classic difficult-to-treat groups.[7–9] The newly introduced triple therapy of protease inhibitors (PEG IFN/RBV/TVR) offers promising perspectives

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for the management of LT patients, although TVR is not yet approved for use in LT patients.

Although there is urgent need for effective treatment of HCV recurrence after LT, significant concern has been expressed about the safety and efficacy of HCV protease inhibitors in this setting because of the side-effect profile and the potential for drug–drug interactions with immunosuppressive agents.[10] Both cyclosporin and tacrolimus are substrates of cytochrome P450 3A and P-glycoprotein. Thus, co-administration of TVR, a potent cytochrome P450 3A4 substrate and inhibitor with the potential to saturate or inhibit intestinal P-glycoprotein, substantially increases the blood levels of cyclosporin and tacrolimus.[11] Consequently, the blood concentration of tacrolimus increased 78-fold, and that of cyclosporin increased fourfold by interaction with TVR.[11] In their recent pilot study, Werner *et al.*[10] described the response to 12-week treatment with TVR plus tacrolimus, cyclosporin or sirolimus in nine patients. Pungpapong *et al.*[12] also reported the preliminary data of 35 patients treated with TVR plus cyclosporin and those of another group of 25 patients treated with boceprevir. Here, we report our preliminary data on protease inhibitors used in combination with PEG IFN/RBV for the treatment of recurrent HCV genotype 1 infection after LT.

CASE REPORT

Case 1

THIS PATIENT WAS a 63-year-old man with HCV-related liver cirrhosis. Living donor LT (LDLT) was performed after obtaining informed consent at May 2009. In August 2009, the patient was started on IFN-β (600 μg) plus RBV (200 mg) due to depression. Because serum HCV RNA titer never fell below the detection limit (1.2 log IU/mL) over the 48-month treatment period, tacrolimus was switched to cyclosporin. In April 2012, treatment was changed to PEG IFN-α-2b (100 μg) plus RBV (200 mg, due to anemia) and TVR (1500 mg) because of depression. At the start of triple therapy, the platelet count was 24.6×10^4/μL, alanine aminotransferase (ALT) was 45 IU/L, genotype was 1b and HCV RNA was 6.8 log IU/mL. Further analysis showed six amino acid (a.a.) substitutions in interferon sensitivity-determining region (ISDR), and mutant- and wild-type amino acids at a.a.70 and a.a.91 in the core region, respectively. The donor had TT genotype of IL-28 single nucleotide polymorphisms (SNP) (rs8099917) and TT/TT genotype of λ4 (ss469415590). The recipient had TT genotype of interleukin (IL)-28 SNP (rs8099917) and TT/TT genotype of λ4 (ss469415590) (Table 1, Fig. 1). Cyclosporin was started at 10 mg/day after triple

Table 1 Laboratory data of patient 1 at start of triple therapy after LT

CBC		LDH	219 IU/L	Tumor marker	
WBC	4630/μL	ALP	357 IU/L	AFP	4.8 ng/mL
RBC	4.01×10^6/μL	γ-GT	20 IU/L		
Hb	12.4 g/dL	TP	7.3 g/dL	HCV virus markers	
Ht	37.8%	Alb	4.0 g/dL	HCV RNA	6.8 KIU/mL
Plt	24.6×10^4/μL	TC	164 mg/dL	Genotype	1b
		TTT	12 U		
Blood coagulation test		ZTT	15 U		
PT	120%	BUN	24.6 mg/dl	a.a. substitution in ISDR	6
		Cr	1.07 mg/dl	a.a.70 in the core region	Mutant
Blood chemistry		CRP	0.10 mg/dl	a.a.91 in the core region	Wild
T-Bil	0.5 mg/dL	NH_3	32 μg/mL	*IL-28B* donor	TT genotype
AST	30 IU/L			*IL-28B* recipient	TT genotype
ALT	45 IU/L			ss469415590 donor	TT/TT genotype
FBS	98 mg/dL			ss469415590 recipient	TT/TT genotype
HbA1c	5.5%			AUC of telaprevir	103 μgh/mL

γ-GT, γ-glutamyltransferase; a.a. substitution in ISDR, amino acid substitutions in the interferon sensitivity-determining region; AFP, α-fetoprotein; Alb, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC, area under curve; BUN, blood urea nitrogen; CBC, complete blood count; Cr, creatinine; CRP, C-reactive protein; FBS, fasting blood sugar; Hb, hemoglobin; HbA1c, hemoglobin A1c; Ht, hematocrit; LDH, lactate dehydrogenase; LT, liver transplantation; RBC, red blood cells; Plt, platelets; PT, prothrombin time; T-Bil, total bilirubin; TC, total cholesterol; TP, total protein; TTT, thymol turbidity test; WBC, white blood cells; ZTT, zinc sulfate turbidity test.

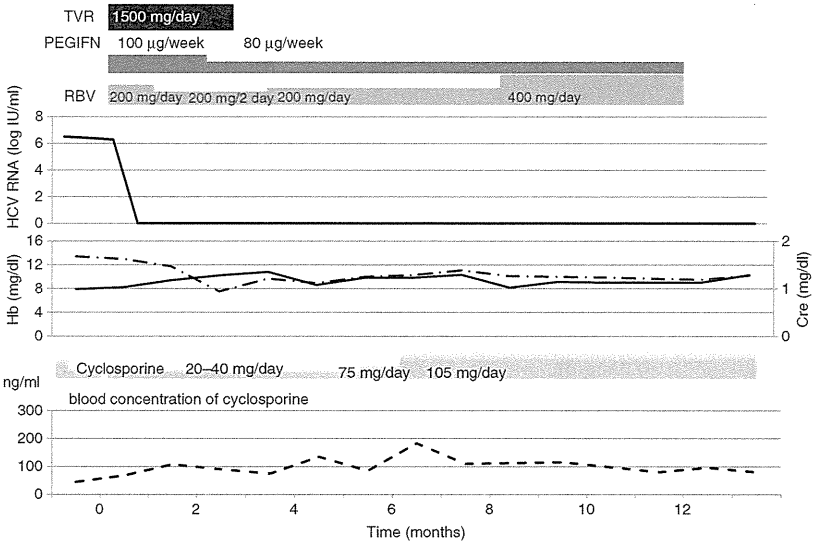

Figure 1 Clinical course of patient 1. Cre, creatinine; Hb, hemoglobin; HCV, hepatitis C virus; PEG IFN, pegylated interferon; RBV, ribavirin; TVR, telaprevir. ——, Cre; — ·, Hb.

therapy, but subsequently increased (based on measurement of its level in the peripheral blood during follow up) to 105 mg/day. The area under the curve (AUC) of TVR was 103 μgh/mL. Serum HCV RNA titer fell below the detection limit (1.2 log IU/mL) at 2 weeks after triple therapy. After 12-week triple therapy, PEG IFN-α-2b and RBV were continued for 36 weeks until April 2013. Finally, he achieved SVR.

Case 2

The patient was a 70-year-old woman with HCV-related liver cirrhosis and HCC. LDLT was performed in May 2006 after obtaining informed consent. Postoperatively, the patient was treated with PEG IFN-α-2b (80 μg) plus RBV (200 mg, due to anemia), which commenced in August 2006. Because serum HCV RNA titer never decreased below the detection limit (1.2 log IU/mL) in the subsequent 48 months, tacrolimus was changed to cyclosporin, and PEG IFN-α-2b plus RBV was changed to the combination of PEG IFN-α-2b (100 μg), RBV (200 mg, due to anemia) and TVR (1500 mg). At the start of triple therapy, platelet count was $19.8 \times 10^4/\mu L$, ALT was 15 IU/L, genotype was 1b, and HCV RNA was 6.2 log IU/mL. Further analysis showed no a.a. substitutions in the ISDR, but mutant- and wild-type a.a. at a.a.70 and a.a.91 in the core region, respectively were detected. The donor had TG genotype of IL-28 SNP (rs8099917) and TT/ΔG genotype of λ4 (ss469415590), while the recipient had TT genotype of IL-28 SNP (rs8099917) and TT/TT genotype of λ4 (ss469415590) (Table 2, Fig. 2). Cyclosporin was started at 10 mg/day, and based on measurement of its concentration in peripheral blood, the dose was increased gradually to 40 mg/day. Subsequent analysis showed a rise in serum creatinine and uric acid, but parameters improved following transfusion. Skin rashes of grade 2 appeared during the triple therapy, which was successfully treated with steroid cream. On the other hand, serum HCV RNA titer decreased below the detection limit (1.2 log IU/mL) at 5 weeks. However, triple therapy was stopped at 11 weeks due to general fatigue. HCV RNA rebounded 4 weeks later.

DISCUSSION

THE SVR RATE has improved since the introduction of PEG IFN/RBV for patients who undergo LT for HCV-related end-stage liver disease. The current estimated SVR rate for LT patients with history of HCV genotype 1 infection is 30–50%.[13–18] These results are much better than those reported in the 1990s and early 2000s, however, more than half of recipients still suffer from recurrent chronic hepatitis C.

It is often difficult to use protease inhibitors for HCV recipients after LT due to potential interaction with immunosuppressive drugs. We reported here our experience with two patients treated with protease inhibitors combined with PEG IFN/RBV for the treatment of recurrent post-LT hepatitis caused by genotype 1 HCV.

A recent study that examined the effect of TVR on the pharmacokinetics of cyclosporin and tacrolimus

Table 2 Laboratory data of Patient 2 at start of triple therapy after LT

CBC		LDH	241 IU/L	Tumor marker	
WBC	7530/μL	ALP	294 IU/L	AFP	5.6 ng/mL
RBC	4.23×10^6/μL	γ-GT	17 IU/L		
Hb	13.3 g/dL	TP	6.4 g/dL	HCV virus markers	
Ht	39.7%	Alb	3.5 g/dL	HCV RNA	6.2 log IU/mL
Plt	17.8×10^4/μL	TC	219 mg/dL	genotype	1b
		TTT	7 U		
Blood coagulation test		ZTT	12 U		
PT	121%	BUN	12.6 mg/dL	a.a. substitution in ISDR	0
		Cr	0.50 mg/dL	a.a.70 in the core region	Mutant
Blood chemistry		CRP	0.11 mg/dL	a.a.91 in the core region	Wild
T-Bil	0.7 mg/dL	FBS	106 mg/dL	*IL-28B* donor	TG genotype
AST	20 IU/L	HbA1c	6.9%	*IL-28B* recipient	TT genotype
ALT	15 IU/L	NH_3	57 μg/mL	ss469415590 door	TT/⊿G genotype
				ss469415590 recipient	TT/TT genotype

γ-GT, γ-glutamyltransferase; a.a. substitution in ISDR, amino acid substitutions in the interferon sensitivity-determining region; AFP, α-fetoprotein; Alb, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC, area under curve; BUN, blood urea nitrogen; CBC, complete blood count; Cr, creatinine; CRP, C-reactive protein; FBS, fasting blood sugar; Hb, hemoglobin; HbA1c, hemoglobin A1c; Ht, hematocrit; LDH, lactate dehydrogenase; LT, liver transplantation; RBC, red blood cells; Plt, platelets; PT, prothrombin time; T-Bil, total bilirubin; TC, total cholesterol; TP, total protein; TTT, thymol turbidity test; WBC, white blood cells; ZTT, zinc sulfate turbidity test.

reported a 78-fold increase in tacrolimus blood concentration and fourfold rise in cyclosporin blood concentration through interaction with TVR.[11] For this reason, we changed tacrolimus to cyclosporin before triple therapy. We also started cyclosporin using a small dose and checked the blood concentration of cyclosporin on a daily basis. Based on these measures, cyclosporin blood concentration remained at approximately 100 ng/mL. Considered collectively, it is important to change the dose of immunosuppressive drugs and frequently monitor cyclosporin blood concentrations.

It is noteworthy that the blood concentration of TVR also increased by interaction with cyclosporin. The AUC of TVR in patient 1 was 103 μgh/mL, while the AUC of TVR of 10 chronic hepatitis C patients treated with PEG IFN/RBV was 52 μgh/mL in our hospital (data not shown). These findings highlight the need for awareness of the potential side-effects of TVR. In fact, various side-

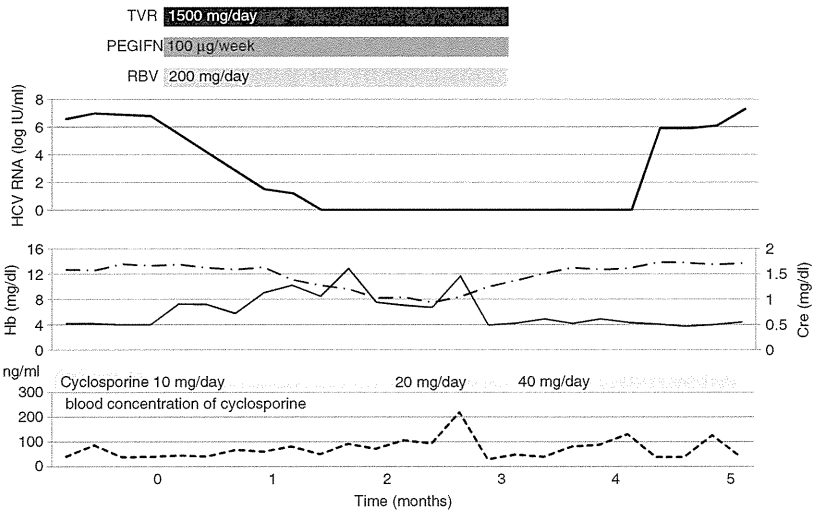

Figure 2 Clinical course of patient 2. Cre, creatinine; Hb, hemoglobin; HCV, hepatitis C virus; PEG IFN, pegylated interferon; RBV, ribavirin; TVR, telaprevir. ——, Cre; — ·, Hb.

effects were reported by patient 2, including anemia, renal dysfunction and skin rashes. Consequently, the triple therapy was discontinued at 11 weeks in this patient.

What are the indications for triple therapy? While there are no standardized rules for the initiation of this mode of treatment, we believe that triply therapy should be used under the following conditions: (i) laboratory tests should show normal hemoglobin and serum creatinine levels to avoid potential side-effects of TVR; and (ii) recipients who develop HCV RNA relapse while receiving PEG IFN/RBV dual therapy after LT. In naïve cases, we recommend PEG IFN/RBV therapy. There are some reports of triple therapy for recipients after LT.[19–21] However, there is no evidence in safety of triple therapy for recipients. Furthermore, Coilly *et al.* recommends PEG IFN/RBV dual therapy for naïve cases in review.[22]

Third, both the donor and recipient must have good SNP (IL28B or λ4). On the other hand, we recommend withholding triple therapy for patients who fail to respond to PEG IFN/RBV and those who have minor SNP (IL28B or λ4) of donor and recipient. In this regard, several groups have reported that *IL28B* of both recipients and donors influenced the SVR to PEG IFN/RBV in patients with recurrent hepatitis C after LT.[23–26]T.[19–22]

Another important question regarding treatment of recurrent post-LT HCV infection is the duration of IFN therapy. The answer to this question is difficult and currently there are no data on the ideal duration of triple therapy. However, we recommend long-term PEG IFN/RBV therapy following triple therapy from 12 to 36 weeks, with a total duration of treatment of 48 weeks. This is based on our previous finding that the majority of patients with genotype 1b in whom HCV RNA reached undetectable levels were able to achieve SVR (87.5%; 7/8).[23] Eradication of HCV by triple therapy should increase the SVR rate. In fact, Pungpapong *et al.* used 12-week triple therapy followed by 36-week PEG IFN/RBV therapy and reported an SVR rate associated with this regimen of 100% (7/7) for genotype 1b recipients.[12]

On the other hand, for such hard-to-treat patients after LT, DAA will become a standard therapy in the future. Because SVR rate and safety of DAA therapy is more higher than triple therapy.[27–29] However, there is a problem of mutation of HCV against DAA therapy.[30,31] In these instances, it may be necessary to recommence triple therapy. The experience of the present study provides a good reference for such an occurrence (e.g. dose of TVR and dose of immunosuppressive agents).

In conclusion, we reported our experience with two patients who developed recurrent HCV genotype 1 infection after LT and were treated with protease inhibitors combined with PEG IFN/RBV. The results point to possible achievement of SVR by triple therapy; however, more studies are needed to evaluate the clinical benefits and side-effects of triple therapy for recurrent post-LT HCV infection.

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

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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

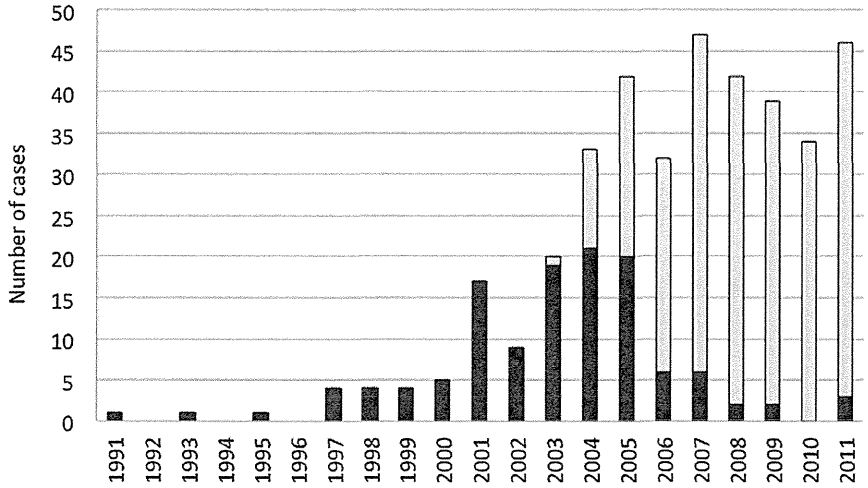

Figure 1: Annual numbers of adults undergoing ABO-I LDLT or rituximab prophylaxis at 37 institutions in Japan. ABO-blood-type-incompatible living donor liver transplantation (ABO-I LDLT) without rituximab prophylaxis (black bars); with rituximab prophylaxis (gray bars).

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 $\leq$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 ($\geq$64), high IgG DSA titer at transplantation ($\geq$16), high MELD score ($\geq$23), absence of rituximab prophylaxis, high peak IgM and IgG DSA titers posttransplantation (both $\geq$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.
*$p < 0.05$.

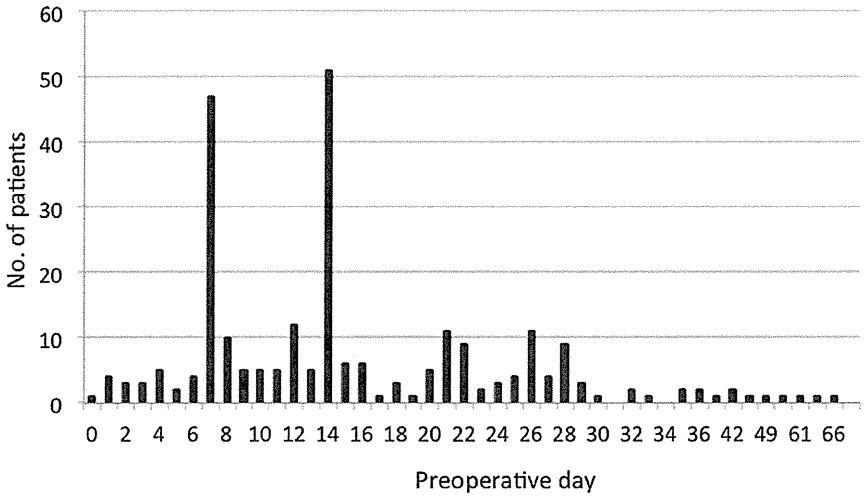

Figure 2: The timing of initial administration of rituximab ranged from preoperative days 0 to 66 and was within 6 days before transplantation in 22 cases.

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

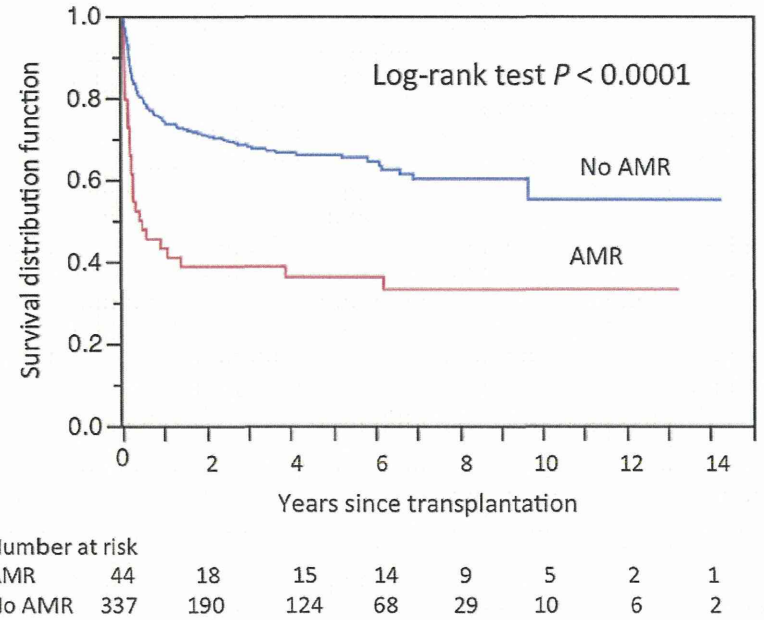

Figure 3: Comparison of overall survival between patients with and without antibody-mediated rejection. Patients with antibody-mediated rejection (AMR) had a significantly higher overall survival risk than those without AMR, $p < 0.001$.

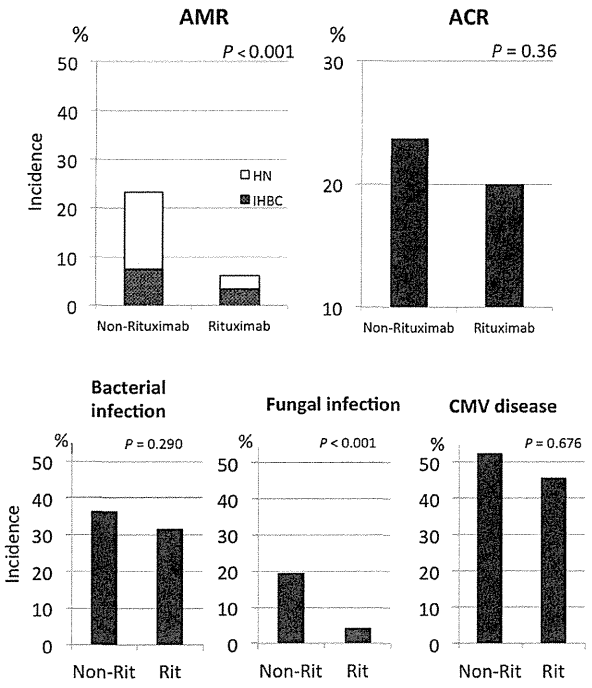

Figure 4: Comparison of incidences of complications between rituximab and nonrituximab groups. The incidences of antibody-mediated rejection (AMR) and acute cellular rejection (ACR) are shown (top); rates of intrahepatic biliary complication (IHBC) and hepatic necrosis (HN) type AMR were lower in the 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.