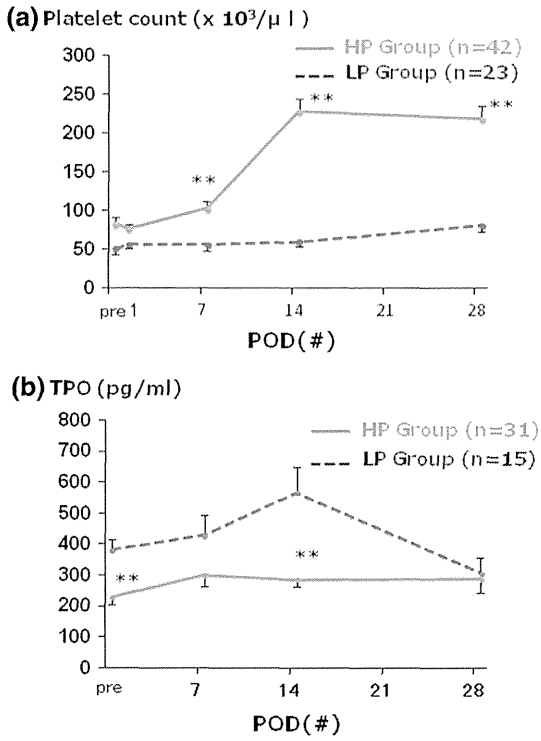


**Fig. 3** Comparison of serial platelet counts (**a**) and TPO levels (**b**) between the two groups classified on the basis of platelet count on POD14. ** vs. LP group $p < 0.01$

the levels, which on preoperative day were significantly higher than those in the HP group, were significantly ($p = 0.04$) increased on POD14 and decreased to the preoperative levels on POD28, showing the levels similar to those in the patients without splenectomy of the HP group (Fig. 6).

*Changes in ADAMTS13 activity, VWF antigen level and VWF/ADAMTS13 ratio in HP group with or without splenectomy and LP group with or without splenectomy*

In HP group, there were no significant differences between the patients with and without splenectomy on ADAMTS13 activity, VWF antigen level, and VWF/ADAMTS13 ratio within POD14 (Fig. 7). On POD28, ADAMTS13 activity in HP group with splenectomy gradually decreased and those in HP group without splenectomy gradually increased after LDLT. ADAMTS13 activity on POD28 in HP group with splenectomy was lower than without splenectomy, although there were no statistical differences between the two groups by $t$ test with the Bonferroni's correction (rough $p$ value; $p = 0.01$). VWF antigen level in HP group with splenectomy was significantly higher than those in HP group without splenectomy ($p = 0.02$). VWF/ADAMTS13 ratio increased after LDLT until POD14 and decreased on

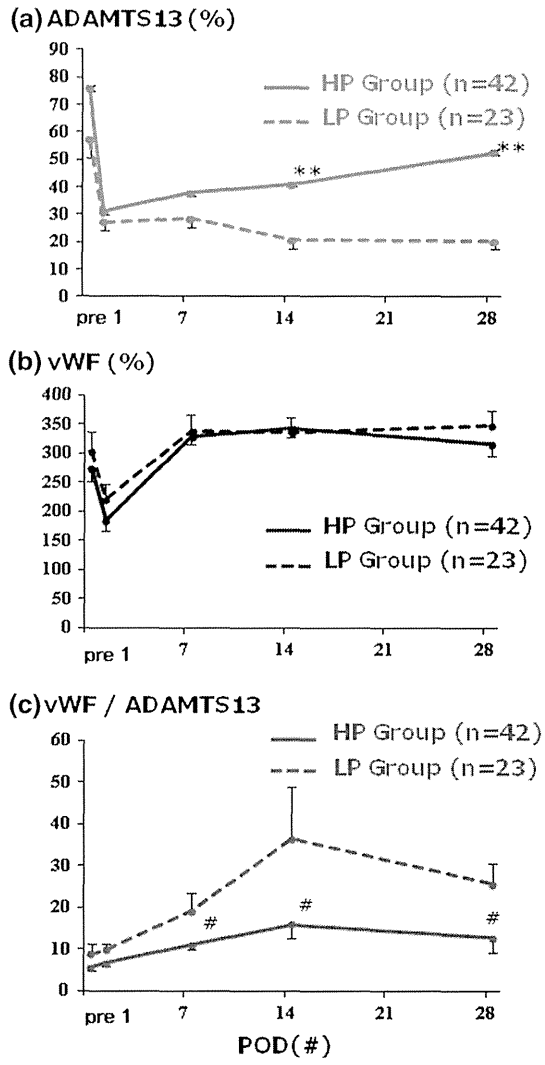


**Fig. 4** Comparison of serial ADAMTS13 activity (**a**), VWF antigen level (**b**), and VWF/ADAMTS13 ratio (**c**) between the two groups classified on the basis of platelet count on POD14. ** vs. LP group $p < 0.01$. VWF/ADAMTS13 ratio were lower in the HP group than in the LP group, although there were no statistical differences between the two groups by $t$ test with the Bonferroni's correction (rough $p < 0.01$ (#) on POD 7.14 and 28)

POD28 between each groups and there was no significant difference on VWF/ADAMTS13 ratio on POD28.

On the other hand, in LP group, there were no significant differences on ADAMTS13 activity, VWF antigen level, and VWF/ADAMTS13 ratio after LDLT between LP group with splenectomy and without splenectomy. ADAMTS13 activity decreased significantly after LDLT on the each groups.

## Discussion

Splenectomy increases platelet counts almost unexceptionally, because it removes the major site of platelet

**Table 4** Risk factors for postoperative thrombocytopenia on POD14

| | Univariate analysis ($n = 65$) | | Multivariate analysis | |
|---|---|---|---|---|
| | Correlation coefficients | $p$ | Regression coefficient | $p$ |
| Preoperative factor | | | | |
| C-P score | **−0.445** | **<0.001** | −0.088 | 0.632 |
| MELD score | **−0.309** | **0.012** | 0.053 | 0.296 |
| Platelet | 0.362 | **0.003** | 0.032 | 0.829 |
| AT (M) | **0.379** | **0.003** | **0.417** | **0.002** |
| Postoperative factor (POD 14) | | | | |
| TB (mg/dD) | **−0.430** | **<0.001** | −0.171 | 0.244 |
| PT-INE | **−0.324** | **0.010** | −0122 | 0.352 |
| AT (%) | **0.431** | **<0.001** | 0.212 | 0.137 |
| ADAMTS13 (%) | **0.416** | **0.001** | **0.331** | **0.011** |

Bold values indicate statistically significant differences

*C-P* Child-Pugh score, *MELD* modified end-stage liver disease, *AT* antithrombin, *TB* total bilirubin, *PT-INR* prothrombin time international normalized ratio, *ADAMTS13* a disintegrin and metalloproteinase with a thrombospondin type 1 motifs 13

destruction and reduces antibody production, resulting in prolonging the platelet survival times [21]. In addition, postsplenectomy transient thrombocytosis occurs and unusually reaches a maximum level on POD14. Although the precise mechanism of transient thrombocytosis remains unclear, Ichikawa et al. [22] pointed out the role of TPO which is catabolized by platelets within the spleen: serum TPO levels after splenectomy reached the maximum levels on POD 3–5 followed by significant reduction, which in turn caused transient thrombocytosis on POD14. On the other hand, it was reported that splenectomy after LDLT showed the peak platelet counts on POD28 [2, 3]. Our study also showed that the platelet counts after splenectomy in the operation other than LDLT significantly increased on POD7 and reached a maximum on POD14. In LDLT, however, the platelet counts remained low levels until POD7 and significantly increased on POD14 regardless of splenectomy, not showing peak level on POD14 even after splenectomy. Therefore, we focused on the mechanism of the delayed recovery of the platelet counts in LDLT patients regardless of splenectomy.

Thrombocytopenia during early period after LT is a common phenomenon and its mechanism has been thought to be the sequestration of platelets in the graft liver, which is due to local thrombin generation and platelet aggregation [23]. The grafted liver is injured before extraction, by cold preservation, by hypoxic rewarming, and/or upon reperfusion. Within the injured graft liver, especially SEC injury plays an important role of the platelet aggregation [24–27]. In our study, early thrombocytopenia after LDLT was gradually recovered within 14 days. However, the problem was the delayed recovery of the platelet counts, that is, prolonged thrombocytopenia.

The previous studies demonstrated the relationship between low platelet counts and patient survival after LT [13–16]. In 1992, McCaughan et al. were the first to report that thrombocytopenia on POD14 after LT was associated with patient survival: platelet counts in nonsurvivors were significantly lower than those in survivors ($88 \times 10^3$ vs. $174 \times 10^3/\mu l$; $p < 0.01$). Furthermore, they reported that graft liver dysfunction was the most independent predictor of the nadir platelet counts after LT, although the exact mechanisms of thrombocytopenia remained uncertain. In our present study, the platelet counts of $<100 \times 10^3/\mu l$ on POD14 was a strong predictor of patient survival after LDLT. Furthermore, we analyzed the risk factors associated with thrombocytopenia on POD14, focusing on a change of ADAMTS13 activity and VWF antigen level. In the univariate analysis, the risk factors were C-P and MELD scores, preoperative platelet count, preoperative AT levels, postoperative (POD14) TB levels, PT-INR, ADAMTS13 activity, and AT levels. The multivariate analysis revealed that preoperative AT levels and postoperative ADAMTS13 activity on POD14 were independent risk factors.

It is known that the patients with low ADAMTS13 activity and an increased VWF/ADAMTS13 ratio have a tendency towards platelet thrombus formation [28]. In sepsis-associated thrombocytopenia, VWF increases by releasing from endothelial cells and platelets with response to the systemic inflammation, and then ADAMTS13 activity decreases due to consumption by cleaving VWF multimur [29]. The dysbalance between ADAMTS13 activity and VWF antigen level is negatively correlated with platelet count and positively correlated with the severity of inflammation and the degree of organ failure.

As for LT, there have been few reports focusing on ADAMTS13 activity and VWF antigen level [4, 30–33]. We previously reported that ADAMTS13 activity significantly decreased after LDLT and that there was the association with thrombotic microangiopathy (TMA) like syndrome. However, our previous study did not investigate how the decreased platelet count could be related to ADAMTS13 activity, graft function, or patient survival. Pereboom et al. [31] revealed that the patients undergoing LT had persistently elevated levels of VWF antigen and concomitantly acquired a deficiency of ADAMTS13 by examining plasma levels of VWF and ADAMTS13 during and after DDLT in 20 patients: 30 min after induction of anesthesia, 30 min after the start of the anhepatic phase, 30 min after reperfusion, at the end of surgery and at days 1, 5, and 10 after surgery. ADAMTS13 activity steadily decreased during the transplantation, with nadir levels at the end of surgery, followed by immediate recovery within

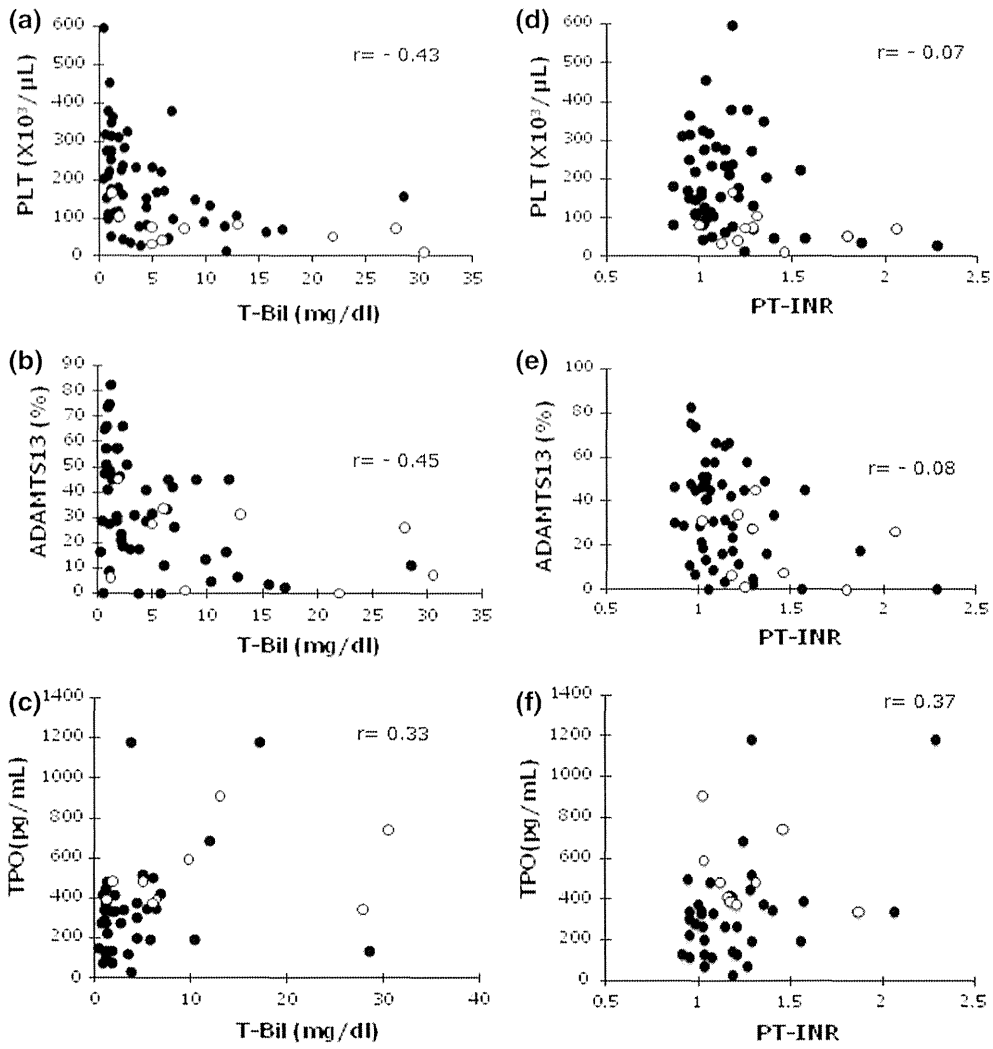


**Fig. 5** Correlation between PLT and TB/PT-INR, ADAMTS13 and TB/PT-INR, and TPO levels and TB/PT-INR on POD14. The Open circles designate patients who died within 6 months of LT

5 or 10 days. On the contrary, VWF antigen level decreased at the end of surgery, and persistently elevated levels of VWF antigen. Therefore, they speculated that VWF/ADAMTS13 dysbalance could easily flip toward a thrombogenic state and might contribute to the risk of perioperative thrombotic complications. However, they did not examine the VWF/ADAMTS13 ratio, platelet count, and graft function.

Our present study also examined serial changes of ADAMTS13 activity, VWF antigen level, and VWF/ADAMTS13 ratio in the 65 recipients, paying special attention to the platelet counts on POD14. In the HP group, similarly to Pereboom's report, ADAMTS13 activity significantly decreased on POD1 and thereafter increased gradually after LDLT. In the LP group, however, ADAMTS13 activity remained lower level and did not show any increase until POD28. The VWF/ADAMTS13 ratio in the LP group was higher than that in the HP group, although there were no statistical differences between the two groups by $t$ test with the Bonferroni's correction (rough $p$ value on POD7, 14, and 28; $p = 0.02, p = 0.03, p = 0.02$, respectively), suggesting that the patients in the LP group had a tendency toward a thrombogenic state. On the other hand, as of the liver function tests, TB level and PT-INR on POD14 were significantly higher in the LP group than in the HP group, and moreover TB levels on POD14 showed a significant negative correlation to ADAMTS13 activity. Taking these results into account, although the mechanisms of thrombocytopenia after LDLT may be multifactorial, we considered that the decreased production of ADAMTS13 in the graft, which seemed to reflect graft dysfunction, under the circumstances of persistently elevated VWF may be one of the causes of prolonged thrombocytopenia after LDLT as shown in the LP group. These conditions were similar to TMA like syndrome, although all of the patients in the LP group did not match the criteria of TMA like syndrome [34].

Because prolonged thrombocytopenia in cirrhotic patients is commonly associated with hypersplenism, we furthermore examined the data in the HP and LP groups with or without splenectomy. In the LP group, the platelet

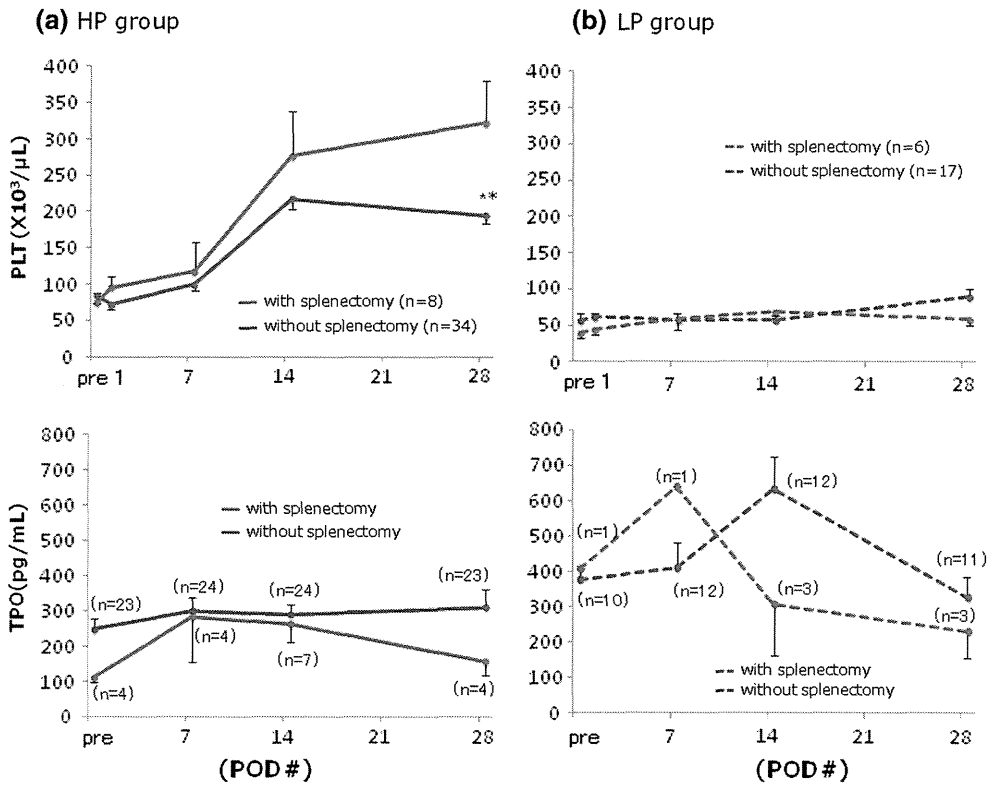


**Fig. 6** Comparison of serial platelet counts between the two groups after LDLT with or without splenectomy in the HP group (**a**) and LP group (**b**). ** vs. with splenectomy $p < 0.01$

counts and ADAMTS13 activity remained low until POD28 regardless of splenectomy, while VWF/ADAMTS13 ratio significantly increased until POD28 regardless of splenectomy. These results suggested that the main cause of prolonged thrombocytopenia was not sequestration of platelets in the spleen, but VWF/ADAMTS13 dysbalance. In the HP group, the platelet counts significantly increased especially after splenectomy, and ADAMTS13 activity in the patients without splenectomy gradually increased after LDLT, while that in those with splenectomy decreased gradually. VWF/ADAMTS13 ratio in the patients without splenectomy remained low, while that in those with splenectomy increased significantly compared to the preoperative data, showing no significant difference between the patients with or without splenectomy. Although the reason why ADAMTS13 activity in the HP group gradually decreased after splenectomy remained unclear, we speculated that ADAMTS13 produced in the graft had been gradually consumed in the peripheral blood to overcome significantly increased platelet counts.

TPO, which is produced at a constant rate mainly in the normal hepatocytes [35], has been known as the primary regulator of platelet [36, 37], and a steady-state amount of TPO is regulated by thrombopoietin receptor present on platelets [38]. TPO receptor agonists have recently been developed for treatment of idiopathic thrombocytopenic purpura [39]. After TPO is binding to platelets, the receptor–ligand complex undergoes internalization and the bound TPO is degraded [40, 41]. In the patients with normal liver function, when the platelet counts significantly decrease in the circumstances such as massive bleeding, TPO levels significantly increase, resulting in the production of platelet by megakaryocytes. In our study, TPO levels on POD14 were significantly higher in the LP group than in the HP group, while those on POD28 in the LP group were significantly decreased, showing the levels similar to the HP group, despite the low platelet levels. It was considered that a constant production of TPO in the hepatocyte was preserved in the LP group on POD14, while on POD28 its production was significantly impaired, suggesting graft dysfunction. Although the reason why preoperative TPO levels were significantly higher in LP group than in HP group remained unclear, preoperative low platelet counts in LP group may be associated with high TPO levels. Taking the results of TPO levels and VWF/ADAMTS13 ratio together, it was suggested that the cause of thrombocytopia was different between PODs 14 and 28: the former could be interpreted as a thrombotic tendency with the decrease in ADAMTS13 levels due to SEC injury, while the latter could be done as not only the decrease of ADAMTS13, but also low TPO production due to hepatocyte dysfunction.

In conclusion, low platelet count as $<100 \times 10^3/\mu L$ on POD14, which means prolonged thrombocytopenia after LDLT, was the strong predictor of survival regardless of splenectomy, and it was furthermore considered that prolonged thrombocytopenia after LDLT was associated with

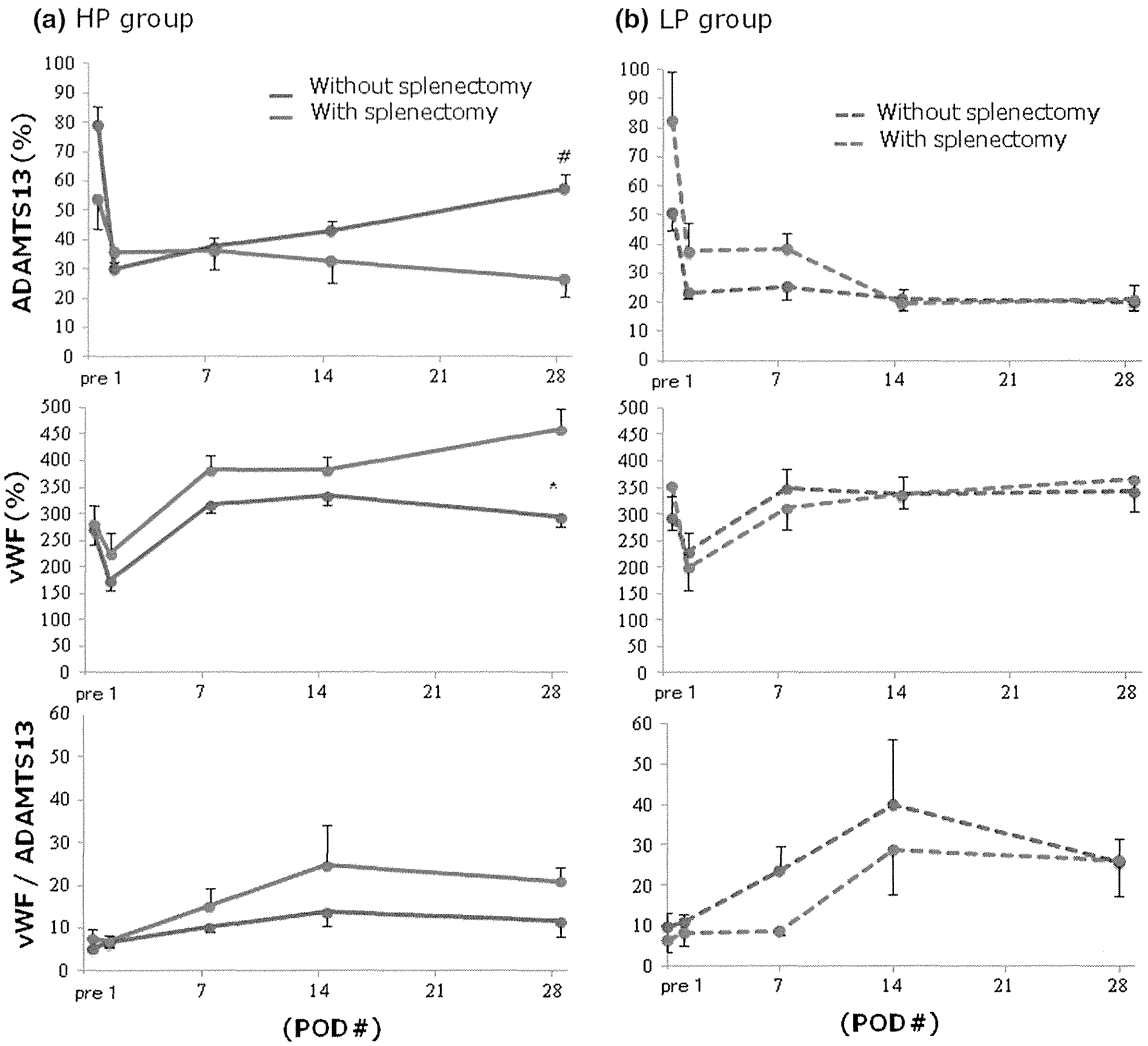


**Fig. 7** Comparison of serial ADAMTS13 activity, VWF antigen level, and VWF/ADAMTS13 ratio between the two groups after LDLT with or without splenectomy in the HP group (**a**) and LP group (**b**). * vs. with splenectomy $p < 0.05$, ADAMTS13 activity were higher in the HP group without splenectomy than with splenectomy, although there were no statistical differences between the two groups by $t$ test with the Bonferroni's correction [rough $p = 0.01$ (#)]

not only the decrease of ADAMTS13 due to sinusoidal endothelial cell injury, but also low TPO production due to hepatocyte dysfunction. In the present study, however, the frequency of septic death in the LP group was significantly higher, and; therefore, in these patients, the presence of sepsis might be additionally associated with the decrease in ADAMTS13 activity and prolonged thrombocytopenia. Consequently, the patients with prolonged thrombocytopenia are in greater need of organ support as well as aggressive infection control measures.

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LIVER TRANSPLANTATION 20:576–583, 2014

ORIGINAL ARTICLE

# Pregnancy Outcomes After Living Donor Liver Transplantation: Results From a Japanese Survey

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A national survey of pregnancy outcomes after living donor liver transplantation (LDLT) was performed in Japan. Thirty-eight pregnancies in 30 recipients resulted in 31 live births (25 recipients), 3 artificial abortions in the first trimester (3 recipients), 1 spontaneous abortion (1 recipient), and 3 fetal deaths (3 recipients). After the exclusion of the 3 artificial abortions, there were 35 pregnancies in 27 recipients: pregnancy-induced hypertension developed during 6 pregnancies (5 recipients), fetal growth restriction developed during 7 pregnancies (6 recipients), acute rejection developed during 2 pregnancies (2 recipients), and ileus developed during 1 pregnancy (1 recipient). Preterm delivery (<37 weeks) occurred for 10 pregnancies (10 recipients), and cesarean delivery was performed for 12 pregnancies (12 recipients). After delivery, acute rejection developed in 3 recipients. Twelve neonates were born with low birth weights (<2500 g), and 4 of these 12 neonates had extremely low birth weights (<1500 g). Two neonates had congenital malformations. The pregnancy outcomes after LDLT were similar to those reported for cadaveric liver transplantation (LT). The incidence of pregnancy-induced hypertension in recipients who were 33 years old or older at the diagnosis of pregnancy was significantly higher than the incidence in recipients who were less than 33 years old at the diagnosis of pregnancy. The incidences of fetal growth restriction, pregnancy-induced hypertension, and extremely low birth weight were significantly higher in the early group (<3 years after transplantation) versus the late group (≥3 years after transplantation). In conclusion, it is necessary to pay careful attention to complications during pregnancy in recipients who become pregnant within 3 years of LT, particularly if the age at the diagnosis of pregnancy is ≥33 years. ***Liver Transpl 20:576-583, 2014.*** **© 2014 AASLD.**

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The number of patients undergoing liver transplantation (LT) has increased; therefore, the number of women of reproductive age undergoing LT has also increased. In the United States, recipients who become pregnant after organ transplantation are registered, and their statistics are regularly reported.[1-5] Many studies concerning pregnancy after LT have been reported by the UK Transplant Pregnancy Registry and transplantation centers.[6-22] Recent case-control studies and meta-analyses have shown that LT recipients and their infants have an increased risk of obstetric complications, although most pregnancy outcomes are favorable.[23,24] Although the pregnancy outcomes for some recipients after living donor liver

**Abbreviations:** γ-GTP, gamma-glutamyl transpeptidase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC, area under the receiver operating characteristic curve; CI, confidence interval; LDLT, living donor liver transplantation; LT, liver transplantation; MMF, mycophenolate mofetil; ROC, receiver operating characteristic.

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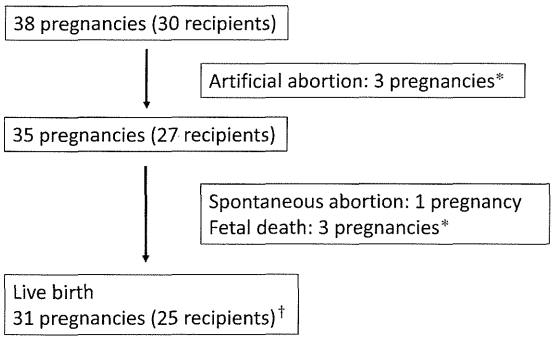


Figure 1. Subjects of this study. *In one recipient, artificial abortion was performed at the first pregnancy, and the second pregnancy was resulted in fetal death. †Six recipients had live births twice.

transplantation (LDLT) have been reported in 1 study,[4] most participants in previous studies have been cadaveric LT recipients. Here, the results of a national survey of pregnancy outcomes after LDLT in Japan are presented and discussed.

## PATIENTS AND METHODS

In Japan, data on LT, including LDLT and cadaveric LT, and the institutes (hospitals or medical centers) that perform LT are registered with the Japanese Liver Transplantation Society. By the end of 2011, 139 cadaveric LT procedures and 6503 LDLT procedures were registered with the society.[25] The Japanese Liver Transplantation Society performed a national survey of pregnancy outcomes after LDLT in Japan. The society sent questionnaires to the institutes and retrospectively assessed data on pregnancy outcomes after LT until May 2012. The questionnaires included information about LDLT, clinical courses of pregnancies and deliveries, and neonates.

Pregnancy-induced hypertension was defined as a systolic blood pressure $\geq$ 140 mm Hg or a diastolic blood pressure $\geq$ 90 mm Hg after 20 weeks of gestation in a woman with previously normal blood pressure.[26] Fetal growth restriction was defined as an estimated fetal weight $< -1.5$ standard deviations of the normal reference range. The fetal weight was estimated with formulas from ultrasound measurements based on neonatal specific gravities and volumes.[27] In 22 of the 23 recipients who received tacrolimus during pregnancy (25 of 29 pregnancies), consecutive serum trough levels of tacrolimus during pregnancy (at several times) were available, and the mean trough level was calculated. The pathological degree of acute rejection (the rejection activity index) was assessed according to the Banff classification.[28]

This study was approved by the ethics committee of the Osaka City University Graduate School of Medicine (no. 1856) and was conducted in accordance with the Declaration of Helsinki of 1996. Informed consent was obtained from the participants. No patient was excluded from the study because informed consent could not be obtained.

TABLE 1. Indications for LDLT

| Disease | Patients (n) |
|---|---|
| Congenital biliary atresia | 14 |
| Acute hepatic failure | 9 |
| Primary sclerosing cholangitis | 2 |
| Autoimmune hepatitis | 1 |
| Hepatitis B virus | 1 |
| Budd-Chiari syndrome | 1 |
| Familial amyloid polyneuropathy | 1 |
| Hepatocellular carcinoma | 1 |

### Statistics

To assess the relationships between complication rates during pregnancy and pregnancy outcomes and the age at pregnancy and interval from LDLT to pregnancy, receiver operating characteristic (ROC) curves were constructed. In addition, areas under the receiver operating characteristic curve (AUCs) with 95% confidence intervals (CIs) were calculated. The optimal age and interval cutoff values were determined with Youden's index (sensitivity + specificity − 1). Categorical variables were compared with the chi-square test or Fisher's exact test as appropriate. The Student $t$ test was used to analyze differences in ages. A $P$ value $< 0.05$ was considered significant. All statistical data were generated with JMP 9.0 (SAS Institute, Cary, NC).

## RESULTS

### Recipient Characteristics

The study participants were 30 LT recipients who had 38 pregnancies (Fig. 1). The recipients underwent LDLT at 11 institutions. The indications for LDLT included congenital biliary atresia (14 recipients), acute liver failure (9 recipients), primary sclerosing cholangitis (2 recipients), autoimmune hepatitis (1 recipient), liver cirrhosis caused by hepatitis B virus (1 recipient), Budd-Chiari syndrome (1 recipient), familial amyloid polyneuropathy (1 recipient), and hepatocellular carcinoma (1 recipient; Table 1). The age of the recipients at the time of LDLT ranged from 4 to 38 years. The age at which pregnancy was diagnosed ranged from 22 to 41 years (mean = 30.3 years). The time from LDLT to the diagnosis of pregnancy ranged from 356 to 6798 days (median = 1751 days).

At the diagnosis of pregnancy, tacrolimus was being administered to 23 recipients (27 pregnancies); cyclosporine was being administered to 2 recipients (2 pregnancies); a combination of tacrolimus and steroids was being administered to 2 recipients (2 pregnancies); a combination of cyclosporine and sirolimus was being administered to 1 recipient (1 pregnancy); and a combination of tacrolimus, steroids, and mycophenolate mofetil (MMF) was being administered to 1 recipient (1 pregnancy). The mean trough level of tacrolimus at the diagnosis of pregnancy was 4.5 ng/mL (range = 0.9-10.0 ng/mL), and the mean trough level during

TABLE 2. Interval From LDLT to Pregnancy and Delivery Outcomes

| Outcome | Total | Interval | | *P* Value |
|---|---|---|---|---|
| | | <3 Years | ≥3 Years | |
| Age at pregnancy (years)* | 27 (22-41) | 35 (24-41) | 29 (22-40) | 0.0014 |
| Indications for LT (n) | | | | 0.327 |
| Congenital biliary atresia | 16 | 3 | 13 | |
| Acute hepatic failure | 12 | 4 | 8 | |
| Primary sclerosing cholangitis | 1 | 1 | 0 | |
| Other | 6 | 2 | 4 | |
| Complications during pregnancy [n (%)]† | | | | |
| Spontaneous abortion | 1 (2.9) | 0 | 1 (4.0) | >0.999 |
| Fetal death | 3 (8.6) | 2 (20.0) | 1 (4.0) | 0.190 |
| Fetal growth restriction | 7 (20) | 5 (50.0) | 2 (8.0) | 0.0120 |
| Liver dysfunction | 4 (11.4) | 2 (20.0) | 2 (8.0) | 0.561 |
| Pregnancy-induced hypertension | 6 (17.1) | 5 (50.0) | 1 (4.0) | 0.0040 |
| Delivery outcomes [n (%)]‡ | | | | |
| Preterm delivery | 10 (32.3) | 4 (50.0) | 6 (26.1) | 0.381 |
| Cesarean delivery | 12 (38.7) | 4 (50.0) | 8 (34.8) | 0.676 |
| Low birth weight (<2500 g) | 12 (38.7) | 5 (62.5) | 7 (30.4) | 0.206 |
| Extremely low birth weight (<1500 g) | 4 (12.9) | 3 (37.5) | 1 (4.3) | 0.0432 |
| Birth defects | 2 (6.5) | 1 (12.5) | 1 (4.3) | 0.456 |

NOTE: There were 35 pregnancies in 27 recipients (3 pregnancies in 3 recipients ended by artificial abortions were excluded from the analysis).
*The data are reported as medians and ranges.
†There were 10 pregnancies in the <3-year group and 25 pregnancies in the ≥3-year group.
‡There were 8 pregnancies in the <3-year group and 23 pregnancies in the ≥3-year group (4 pregnancies in 4 recipients ending in a spontaneous abortion or fetal death were excluded from the analysis).

pregnancy was 4.5 ng/mL (range = 1.5-10.0 ng/mL). No immunosuppressive drugs were administered during 3 pregnancies at the time of the pregnancy diagnosis because of auxiliary partial orthotopic LT (1 pregnancy in 1 recipient) or the discontinuation of drugs after LDLT in childhood (2 pregnancies in 1 recipient). The serum creatinine levels at the diagnosis of pregnancy were available for 32 pregnancies (24 recipients), and they were within the reference range.

## Pregnancy Outcomes

Thirty-eight pregnancies in 30 recipients resulted in 31 live births (81.6%) for 25 recipients, 3 artificial abortions for 3 recipients, 1 spontaneous abortion for 1 recipient, and 3 fetal deaths for 3 recipients (Fig. 1). Artificial abortions were performed in the first trimester because of MMF use in 1 pregnancy (1 recipient), sirolimus use in 1 pregnancy (1 recipient), and a short time after LDLT (356 days) in 1 pregnancy (1 recipient).

## Obstetric Complications

After the exclusion of the 3 artificial abortions in 3 recipients, there were 35 pregnancies in 27 recipients: a spontaneous abortion occurred during 1 pregnancy (2.9%) in 1 recipient, and fetal death occurred during 3 pregnancies (8.6%) in 3 recipients as previously described (Table 2). Pregnancy-induced hypertension developed during 6 pregnancies (17.1%) in 5 recipients, fetal growth restriction developed during 7 pregnancies (20.0%) in 6 recipients, and ileus developed during 1 pregnancy in 1 recipient. Liver dysfunction [elevated serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and/or gamma-glutamyl transpeptidase (γ-GTP)] was detected during 4 pregnancies in 4 recipients. Acute rejection, diagnosed by liver biopsy (rejection activity index = 2) and laboratory test results, occurred in 2 of these 4 recipients; an increased dose of cyclosporine and steroid pulse therapy was given to 1 recipient, and an increased dose of tacrolimus was administered to 1 recipient. Other obstetric complications such as gestational diabetes, infections, placental abruption, and thromboembolic disorders did not occur in any recipient. Two recipients did not receive immunosuppressive drugs, and for the one who underwent auxiliary partial orthotopic LT, fetal death occurred because of umbilical cord coiling. In another patient (2 pregnancies), no complications developed during pregnancy.

In 1 of the 8 recipients who were pregnant twice, the second pregnancy resulted in a spontaneous abortion (at 7 weeks of gestation), although the first pregnancy was uneventful. Another recipient had pregnancy-induced hypertension in both the first and second pregnancies; fetal death ended the first pregnancy (at 25 weeks), and fetal growth restriction occurred during the second pregnancy.

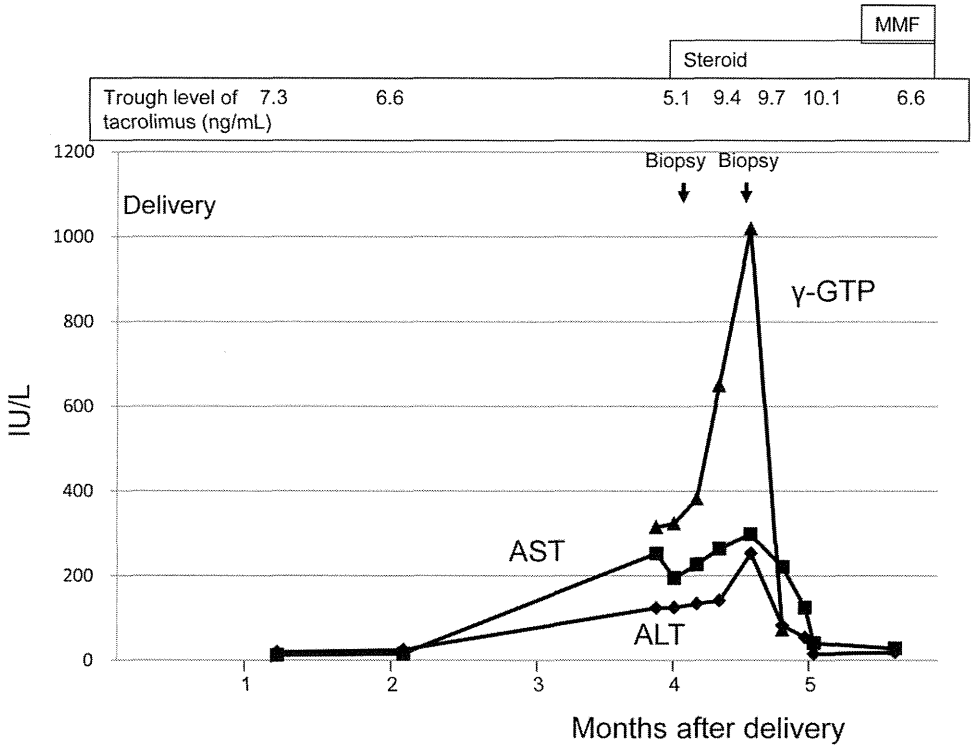


Figure 2. Clinical course of recipients suffering acute rejection after delivery. Acute rejection was diagnosed with a second liver biopsy (rejection activity index = 4).

## Delivery Outcomes

There were 31 pregnancies in 27 recipients, and preterm delivery (<37 weeks) occurred for 10 of these pregnancies (32.3%) in 10 recipients. Cesarean delivery was performed for 12 pregnancies (38.7%) in 12 recipients because of pregnancy-induced hypertension (6 pregnancies in 6 recipients), hypotonic contraction during labor (1 pregnancy in 1 recipient), transient bradycardia of the fetus (1 pregnancy in 1 recipient), ileus (1 pregnancy in 1 recipient), previous multiple abdominal operations (1 pregnancy in 1 recipient), previous cesarean delivery (1 pregnancy in 1 recipient), and the recipient's will (1 pregnancy in 1 recipient).

After delivery, liver dysfunction (elevated serum activities of AST, ALT, and/or γ-GTP) occurred during 4 pregnancies (4 recipients), and acute rejection, diagnosed by liver biopsy (rejection activity index = 2-4), occurred within 4 months of LDLT in 3 of these 4 recipients. For acute rejection, steroid pulse therapy was administered to 2 recipients, and a steroid and MMF were added to tacrolimus therapy for 1 recipient (Fig. 2). The recipients' liver function improved with these treatments. In 1 recipient, artificial respiration was necessary because of acute respiratory distress syndrome after delivery, and renal dysfunction persisted after recovery. Puerperal fever developed in 1 recipient. The pregnancy-induced hypertension improved after delivery in all recipients who had hypertension during pregnancy. In 1 recipient, retransplantation was performed because of the recurrence of primary sclerosing cholangitis 5 years after delivery.

There were 31 live births, and neonatal asphyxia occurred in 1 neonate. Twelve neonates were born with low birth weights (<2500 g), and 4 of the 12 low-birth-weight neonates were born with extremely low birth weights (<1500 g). Although intracranial bleeding developed after delivery in 1 neonate with an extremely low birth weight, the condition improved without complications.

One neonate had tetralogy of Fallot, and 1 neonate had hypospadias.

## Risk Factors for Obstetric Complications, Delivery Outcomes, and Birth Defects

Relationships between the mean trough level of tacrolimus and obstetric complications, delivery outcomes, and birth defects were not found.

Relationships between the age at the diagnosis of pregnancy and complications during pregnancy were studied with ROC curves. The AUC was 0.784 (95% CI = 0.613-0.905) for pregnancy-induced hypertension (Fig. 3A). The optimal cutoff value was 33 years (sensivitity = 83.3%, specificity = 69.0%). No significant relationship was found between the age at pregnancy and other complications such as spontaneous abortion, fetal death, fetal growth restriction, and liver dysfunction. The incidence of pregnancy-induced hypertension in recipients who were 33 years old or older at the diagnosis of pregnancy was significantly

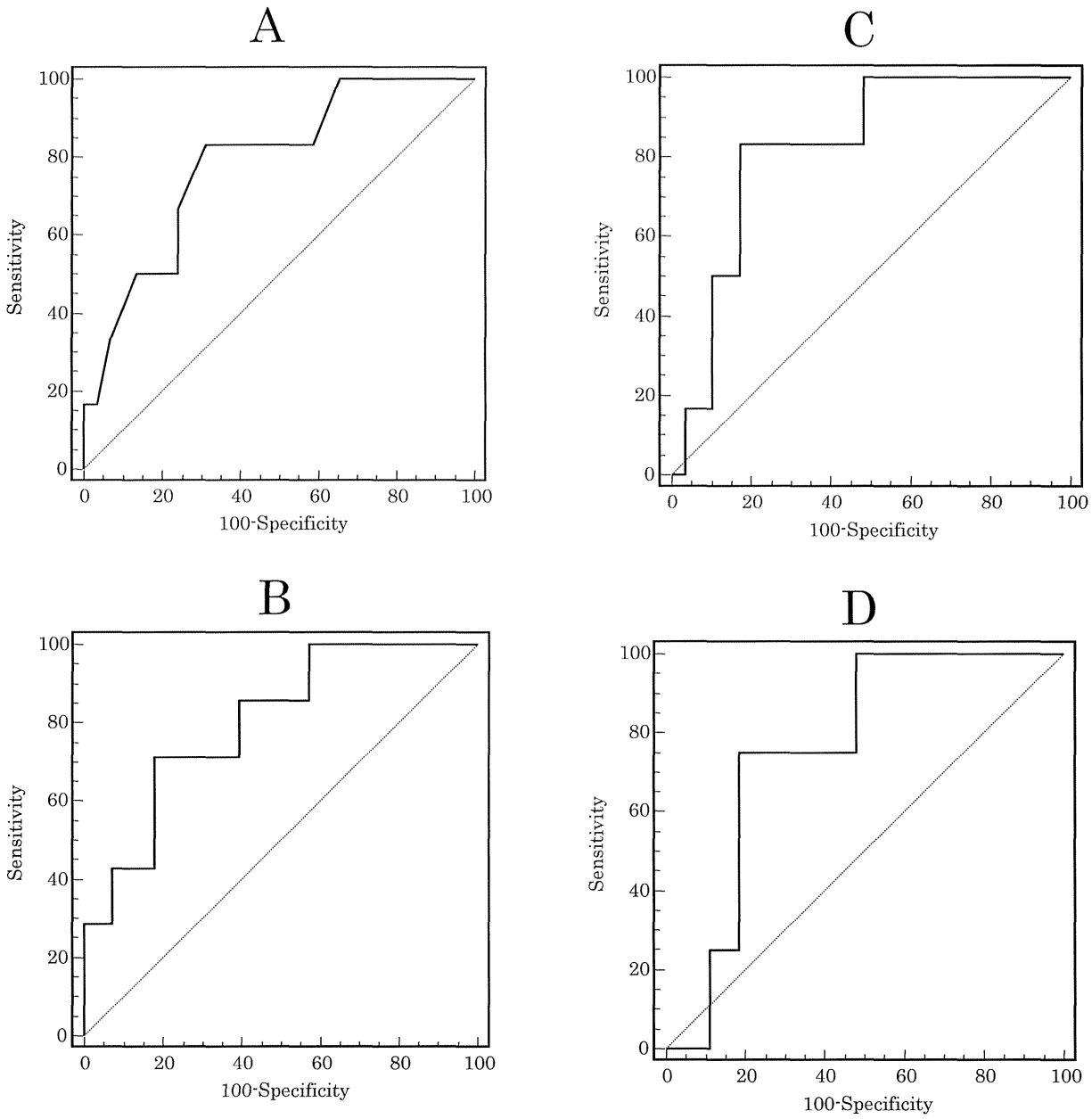


Figure 3. ROC curves for pregnant recipients: (A) age at the diagnosis of pregnancy and pregnancy-induced hypertension, (B) interval from LT to pregnancy and fetal growth restriction, (C) interval from LT to pregnancy and pregnancy-induced hypertension, and (D) interval from LT to pregnancy and extremely low birth weight.

higher than the incidence in recipients who were less than 33 years old at the diagnosis of pregnancy (*P* value = 0.0278 according to Fisher's exact test).

Relationships between the interval from LDLT to pregnancy and delivery outcomes were studied with ROC curves. The AUC was 0.801 (95% CI = 0.632-0.916) for fetal growth restriction (Fig. 3B). The optimal cutoff value was 1096 days (sensitivity = 71.4%, specificity = 82.1%). The AUC was 0.822 (95% CI = 0.656-0.930) for pregnancy-induced hypertension (Fig. 3C). The optimal cutoff value was 1096 days (sensitivity = 83.3%, specificity = 82.8%). The AUC was 0.759 (95% CI = 0.573-0.893) for extremely low birth weight (Fig. 3D). The optimal cutoff value was 1096 days (sensitivity = 75.0, specificity = 81.5%). No significant relationship was found between the interval and other factors, including spontaneous abortion, fetal death, liver dysfunction, and preterm delivery.

The obstetric complications and delivery outcomes were compared for 10 pregnancies for which the interval from LT to pregnancy was <3 years (the early group) and 25 pregnancies for which this interval was ≥3 years (the late group) because the optimal cutoff value was 1096 days according to the analysis using ROC curves (Table 2). The 3 pregnancies for which

artificial abortions were performed in the first trimester were excluded from this comparison. The mean age at pregnancy was significantly higher for the early group versus the late group. The proportions of recipients with fetal growth restriction and pregnancy-induced hypertension were significantly higher in the early group versus the late group. The proportion of neonates with extremely low birth weight was significantly higher in the early group versus the late group.

The incidence of pregnancy-induced hypertension in recipients in the early group who were 33 years old or older at the diagnosis of pregnancy (5/8 pregnancies or 62.5%) was significantly higher than the incidence in recipients in the late group who were less than 33 years old at the diagnosis of pregnancy (1/19 pregnancies or 5.3%, $P = 0.0037$) and the incidence in recipients in the late group who were 33 years old or older at the diagnosis of pregnancy (0/6 pregnancies, $P = 0.031$); the incidence of pregnancy-induced hypertension was highest in recipients in the early group who were 33 years old or older at the diagnosis of pregnancy (interval from LDLT to pregnancy < 3 years).

## DISCUSSION

An increased risk of complications, including prematurity, low birth weight, pregnancy-induced hypertension, renal dysfunction, and cesarean delivery, has been reported in previous studies of pregnancy in LT recipients (most patients have undergone cadaveric LT).[1-24]

In this study, pregnancy-induced hypertension developed during 6 pregnancies (17.1%) in 5 recipients. Shiozaki et al.[29] reported that pregnancy-induced hypertension was present in 1.2% of pregnancies (2802/241,292) in the Japan Society of Obstetrics and Genecology database. The incidence of pregnancy-induced hypertension seems to be higher in LDLT recipients versus the general population. Several studies have reported that pregnancy-induced hypertension is common among LT recipients (11%-43%).[1,3-6,10,11,13,17,20,23,24] The incidence of pregnancy-induced hypertension in LDLT recipients (17.1%) was similar to the incidence in cadaveric LT recipients. On the other hand, pregnancy-induced hypertension did not occur in 1 recipient (2 pregnancies) who did not receive immunosuppressive drugs during pregnancy. This complication has been shown to occur more frequently in LT recipients with renal dysfunction.[11,12] Although no relationship between the mean trough levels of tacrolimus and pregnancy-induced hypertension was observed in this study, underlying renal dysfunction[11] and the vasoconstrictive effects of calcineurin inhibitors may affect hypertension. In addition, it is necessary to pay attention when the recipient's age at the diagnosis of pregnancy is ≥33 years.

In this study, a spontaneous abortion ended 1 pregnancy (1 recipient), and fetal death ended 3 pregnancies (3 recipients). Coffin et al.[23] reported that infants of LT recipients had a 3-fold risk of complications, most notably fetal death (6% versus 2% in controls). Among 241 pregnancies in LT recipients described in the National Transplantation Pregnancy Registry in 2008,[3] 19.2% and 2.1% ended in spontaneous abortions and stillbirths, respectively. The maternal and fetal conditions might affect the rates of spontaneous abortion and fetal death. Another adverse fetal outcome noted in this study was fetal growth restriction in 7 pregnancies (20.0%). The incidence of complications appears to be higher in these individuals versus the general population.[23] However, the mechanisms underlying the high incidences of spontaneous abortion, fetal death, and fetal growth restriction are unclear.

Several previous studies have reported a high incidence of preterm delivery (14%-53%).[1,3-6,8-10,13,14,17,18,20,23,24] In this study, preterm delivery (<37 weeks) occurred in 10 pregnancies (32.3%). The proportion of preterm deliveries seemed to be high because the database of the Japan Society of Obstetrics and Genecology indicated that the rate of threatened premature delivery was 2.34%.[30] Preterm delivery might be related to maternal conditions such as hypertension and fetal conditions such as fetal growth restriction.

Several previous studies have shown that cesarean delivery is more common among transplant recipients.[4-6,10,13,15-17,20,23,24] In this study, cesarean delivery was performed for 12 of 31 pregnancies (38.7%). The indications for cesarean delivery included pregnancy-induced hypertension, hypotonic contraction during labor, transient bradycardia, ileus, multiple previous abdominal operations, previous cesarean delivery, and the recipient's will. Thus, it is likely that the high rate of cesarean delivery was attributable to pregnancy complications rather than LT itself.

Acute rejection is an important problem during and after pregnancy because rejection may induce graft loss. In fact, the National Transplantation Pregnancy Registry (2006) reported that 7% of pregnancies were complicated by acute rejection, and 8% of individuals lost their grafts within 2 years of delivery.[1] Other studies have reported that rejection rates during pregnancy are 0% to 17%.[2-6,9,10,13,15-17,20,23] It has been reported that rejection episodes up to 3 months after delivery are a risk factor for graft loss after delivery.[5,7] Kainz et al.[31] reported that rejection was followed by preeclampsia, renal impairment, and infection. In this study, acute rejection occurred in 2 recipients during pregnancy and in 3 recipients after delivery (within 4 months of delivery), although these patients had no renal dysfunction. All recipients were successfully treated with an increased dose of tacrolimus and/or the addition of corticosteroids or MMF, and graft loss did not occur. Thus, adequate treatment for acute rejection can prevent graft loss, although close follow-up of pregnant recipients is necessary even after delivery, especially when the recipients have renal dysfunction.

Congenital malformations in live-born neonates have been reported to occur in 3% of the

nontransplant population.[32] In transplant recipients, the incidence of congenital malformations has been reported to be 4% with corticosteroids,[32] 7% with azathioprine,[32] 3% with cyclosporine,[33] and 4% with tacrolimus.[14] Kainz et al.[31] reported that 4 neonates presented with malformations among 100 pregnancies in which the mother was treated with tacrolimus. In the present series, most recipients received tacrolimus-based therapy, and 2 of the 31 neonates (6.4%) had congenital malformations (tetralogy of Fallot and hypospadias). A higher incidence of structural malformations was observed with MMF exposure during pregnancy.[34] This agent is classified as pregnancy category D (there is positive evidence of fatal risk to humans, but potential benefits may warrant the use of the drug in pregnant women despite the potential risk; there is evidence of fetal risk).[35] No structural defects have been reported with early-pregnancy sirolimus exposure to date. In this study, artificial abortions were performed in 2 recipients to whom MMF or sirolimus was administered. Calcineurin inhibitors are classified as pregnancy category C (animal reproductive studies have shown an adverse effect on the fetus or are lacking, and there are no adequate and well-controlled studies in humans, but the potential benefits may warrant the use of the drug in pregnant women despite the potential risks; fetal risk cannot be ruled out).[35] Thus, calcineurin inhibitor–based therapy, including cyclosporine and tacrolimus, is favorable for pregnant recipients.

Although there is no established optimal interval between LT and pregnancy, a report from the National Transplantation Pregnancy Registry and the American Society of Transplantation recommended that LT recipients wait a minimum of 1 year before conception to stabilize graft function and immunosuppressant dosage. Christopher et al.[16] reported that pregnancies occurring within 1 year of LT had an increased incidence of prematurity, low birth weight, and acute rejection in comparison with those occurring more than 1 year after LT. Nagy et al.[15] reported that the risk of complications during pregnancy is low when liver LT recipients become pregnant more than 2 years after LT because the recipients have stable and normal hepatic function and normal renal function, and immunosuppressive therapy is at a maintenance dosage. The results of the National Transplantation Pregnancy Registry (2008) showed that the incidence of very-low-birth-weight neonates in pregnancies within 2 years of LT was higher than the incidence in pregnancies more than 5 years after LT.[3] A higher incidence of rejection was also reported for recipients who were pregnant 1 to 2 years after LT. These results indicate better outcomes for recipients and infants with pregnancies occurring at least 2 years after LT. In this study, the incidences of fetal growth restriction, pregnancy-induced hypertension, and neonates with extremely low birth weights were significantly higher in the early group (<3 years after LDLT) versus the late group (≥3 years after LDLT). In addition, the incidence of pregnancy-induced hypertension was higher for recipients who were 33 years old or older at the diagnosis of pregnancy versus recipients who were less than 33 years old. Thus, it is necessary to pay careful attention to complications during pregnancy when a recipient becomes pregnant within 3 years of LDLT, particularly if the age at the diagnosis of pregnancy is ≥33 years.

The pregnancy outcomes of LDLT recipients were similar to those of cadaveric LT recipients. Although most pregnancy outcomes are favorable, special attention should be given to obstetric complications such as pregnancy-induced hypertension, spontaneous abortion, fetal death, fetal growth restriction, preterm delivery, cesarean delivery, and acute rejection. It is difficult to draw definitive conclusions from this study because the number of recipients in this study was too small, and this survey might not reflect all pregnant recipients. Thus, it is necessary to analyze the outcomes after pregnancy in larger studies with prospective registration to establish and improve the clinical management of pregnancy in LT recipients.

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# Successful Telaprevir Treatment in Combination of Cyclosporine against Recurrence of Hepatitis C in the Japanese Liver Transplant Patients

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**Telaprevir (TVR) is a protease inhibitor used in combination with pegylated interferon alfa-2b and ribavirin for hepatitis C, and TVR strongly inhibits CYP3A4 and CYP3A5. We reported successful TVR treatment of liver transplant patients with recurrence of hepatitis C during receiving immunosuppressive therapy. Before initiation of triple therapy, all patients switched from tacrolimus to cyclosporine, which has a lower inhibitory effect on CYP3A4 and CYP3A5 than tacrolimus. To avoid graft failure, we measured the cyclosporine blood concentrations at 0, 2, and 6 h after administration to maintain the target level (150–200 ng/mL) within 1 week after initiation of TVR and adjusted the dose of cyclosporine. The dose of cyclosporine was decreased 0.24–0.40 fold in all patients after initiation of TVR treatment. In 3 patients, the dose of TVR was decreased two-thirds of starting dose because of adverse effects, including anorexia and skin rash. However, the HCV RNA level rapidly decreased to undetectable levels within 1 month. Furthermore, all patients completed the TVR therapy in 12 weeks and did not experience liver graft rejection. In addition, we found the rapid elimination of inhibitory effect of TVR on the disposition of cyclospirne in the all four cases and therefore, rapid increase in the dosage of cyclosporine would be required immediately after the end of TVR administration. These results suggest that frequent measurement of cyclosporine levels was important for successful TVR triple therapy and prevention of rejection.**

**Key words** telaprevir; cyclosporine; drug interaction; hepatitis C; liver transplantation

Telaprevir (TVR), a protease inhibitor, is a new drug to treat hepatitis C.[1–3] Triple therapy with pegylated interferon alpha 2b (PEG-IFN $\alpha$-2b), ribavirin, and TVR for 12 weeks and double therapy with PEG-IFN $\alpha$-2b and ribavirin for 12 weeks strongly affects the hepatitis C virus (HCV), and 73.0% of patients achieve sustained viral responses (SVRs).[4] TVR is metabolized by CYP 3A4 and CYP3A5 and strongly inhibits CYP3A4 and CYP3A5.[5,6] Therefore, TVR has strong drug interactions with immunosuppressants like tacrolimus and cyclosporine.[7]

Some hepatitis C patients develop liver cirrhosis or hepatocellular carcinoma, which require a liver transplant. After liver transplantation, they have to take immunosuppressive agents to prevent graft loss, and the blood concentrations of these drugs have to be carefully monitored. However, patients can show recurrence of hepatitis C even after liver transplantation.[8,9] Therefore, it is difficult to control the blood concentration of immunosuppressive agents with TVR triple therapy in patients with recurrence of HCV infection. To prevent graft rejection and treat hepatitis C, we carefully adjusted the dose of immunosuppressive agents to maintain the target blood concentrations.

This case report describes successful treatment of transplant patients with recurrence of hepatitis C with TVR, PEG-IFN $\alpha$-2b, and ribavirin triple therapy to prevent liver graft rejection. We carefully controlled the blood concentration of immunosuppressive agents to that of the target level by frequent measurement.

## MATERIALS AND METHODS

**Treatment Protocol** The day of initial administration of TVR was set as Day 1. The primary immunosuppressive agent was changed from tacrolimus to cyclosporine around one week before the initiation of TVR administration. The target trough and $C_2$ level of cyclosporine was set between 150 ng/mL and 200 ng/mL and between 600 ng/mL and 800 ng/mL, respectively, beginning around 2 weeks of TVR administration. TVR 750 mg was orally administered twice daily. Around day 7, PEG-IFN $\alpha$-2b and ribavirin were added to start the triple therapy with TVR. The administration of TVR was terminated after 12 weeks, and then combination treatment with PEG-IFN $\alpha$-2b and ribavirin was continued for 24 weeks. All periods of HCV treatment were 24 weeks.

**Blood Samples** Blood samples for measuring the levels of immunosuppressive agents were collected immediately before the morning dosage and at 2 h and 6 h after the administration between days 1 and 7. After day 8, the blood samples were collected for measuring the morning trough level of immunosuppressants.

The authors declare no conflict of interest.

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**Measurements of Blood Concentration of Tacrolimus and Cyclosporine** Whole blood concentrations of tacrolimus and cyclosporine were determined using chemiluminescence immunoassay (CLIA) using an ARCHITECT® i2000SR analyzer (Abbott Laboratories, Chicago, IL, U.S.A.) and affinity column-mediated immunoassay (ACMIA) using Dimension® (Siemens Healthcare Diagnostics Inc., Newark, DE, U.S.A.), respectively, according to the manufacturer's instructions.

**Ethics** This study was conducted in accordance with the Declaration of Helsinki and its amendments, and the study protocol was approved by the Kyoto University Graduate School and Faculty of Medicine Ethics Committee. Written informed consent was obtained from each patient.

## CASE REPORTS

Although TVR strongly inhibits the dispositions of tacrolimus and cyclosporine, the interaction between TVR and cyclosporine is relatively mild compared to that between TVR and tacrolimus.[7] In addition, the clinical efficiency of cyclosporine was found to be similar with tacrolimus in the 39 living donor liver transplant patients.[10] Therefore, we carefully switched the primary immunosuppressant from tacrolimus to cyclosporine about a week before the initiation of TVR administration to avoid toxicity from calcineurin inhibitors. In addition, the dosage of TVR was decreased to 1500 mg/bid, because previous studies in Japan indicated that the standard dose of TVR 2250 mg/tid was toxic to Japanese patients.[11,12]

The clinical characteristics of the patients in this study are summarized in Table 1. All patients showed a recurrence of HCV genotype 1b after liver transplantation. The median (range) of duration between liver transplantation and initiation of TVR treatment was 21 (1–75) months.

**Case I**: A 62-year-old man who underwent cadaveric donor liver transplantation because of hepatocellular carcinoma after HCV-related liver cirrhosis. HCV RNA was detected in his serum after transplantation; therefore, he was administered anti-HCV therapy with PEG-IFN $\alpha$-2b and ribavirin at the 2nd post-transplant month. Because of fatigue and nausea, the double therapy (consisting of PEG-IFN $\alpha$-2b and ribavirin) was interrupted twice; once between months 9 and 10 and once between months 45 and 67. Then, the double therapy for recurrence HCV was withdrawn 75 months after liver transplantation. Before initiation of triple therapy (consisting of TVR, PEG-IFN $\alpha$-2b, and ribavirin), the calcineurin inhibitor was switched from tacrolimus to cyclosporine about a week before the administration of TVR. The dosage and trough concentration of cyclosporine at the day before the administration of TVR were 150 mg/bid and 212 ng/mL, respectively (Figs. 1A, B). To avoid an excessive increase in the blood concentration of cyclosporine, the dosage of cyclosporine was reduced to one-third of the original on the day of TVR administration (1500 mg/bid). Although the blood concentration of cyclosporine varied, dosage adjustment was carefully performed on the basis of the blood concentration of cyclosporine in the morning during the two weeks after initiation of TVR therapy. On day 7 of TVR therapy, PEG-IFN $\alpha$-2b (100 $\mu$g/week) and ribavirin (400 mg/bid) were also added to constitute the triple therapy. The levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) decreased to normal levels during the first 7 d with TVR, without PEG-IFN $\alpha$-2b and ribavirin. The levels of uric acid, serum creatinine, and blood urea nitrogen (BUN) moderately increased; an increase in the dosage of allopurinol from 100 mg/d to 200 mg/d was effective against TVR-related kidney injury. The patient successfully completed the 12-week regimen of the triple therapy; subsequently, the administration of TVR was discontinued. Immediately after the termination of TVR administration, the dosage of cyclosporine was increased to 100 mg/bid to maintain a sufficient trough level to prevent rejection. Over the course of 100 d, the patient did not experience any severe adverse reactions related to TVR and cyclosporine and such as graft rejection.

**Case II**: A 50-year-old man who underwent living-donor liver transplantation from his offspring because of HCV-related liver cirrhosis. Because the serum HCV RNA level rapidly increased after the transplantation, he received liver-supporting therapy with monoammonium glycyrrhizinate for 1 month. The calcineurin inhibitor was carefully switched from tacrolimus to cyclosporine about 10 d before the admin-

Table 1. Pretreatment Profile and Clinical Characteristics

| Case number | I | II | III | IV |
|---|---|---|---|---|
| Male/Female | Male | Male | Female | Female |
| Age (years) | 62 | 50 | 67 | 68 |
| Body weight (kg) | 68.6 | 120.2 | 53.4 | 43.0 |
| Primary disease for transplantation | HCC | LC | LC, HCC | LC, HCC |
| Milan criteria for HCC treatment | Within | | Within | Within |
| ABO blood type (donor/recipient) | O/O | A/A | B/B | O/O |
| Donor | Cadaveric | Offspring | Spouse | Offspring |
| HCV genotype | 1b | 1b | 1b | 1b |
| Months after liver transplantation | 77 | 22 | 74 | 37 |
| Months after transplantation recurrence of HCV | 75 | 1 | 69 | 33 |
| Post-transplant anti-HCV treatment | PEG-IFN, RBV | Not treated | PEG-IFN, RBV | PEG-IFN, RBV |
| Duration of post-transplant anti-HCV treatment (months) | 2–8, 11–44, 68–71 | | 8–22 | 5–15 |
| Outcome | Withdraw | | SVR* | Withdraw |
| Positive conversion of HCV after SVR (month after liver transplantation) | | | 66 | |

HCC, hepatocellular carcinoma; LC, liver cirrhosis; HCV, hepatitis C virus; LT, liver transplantation; PEG-IFN, pegylated interferon alfa-2b; RBV, ribavirin; SVR, sustained viral response.

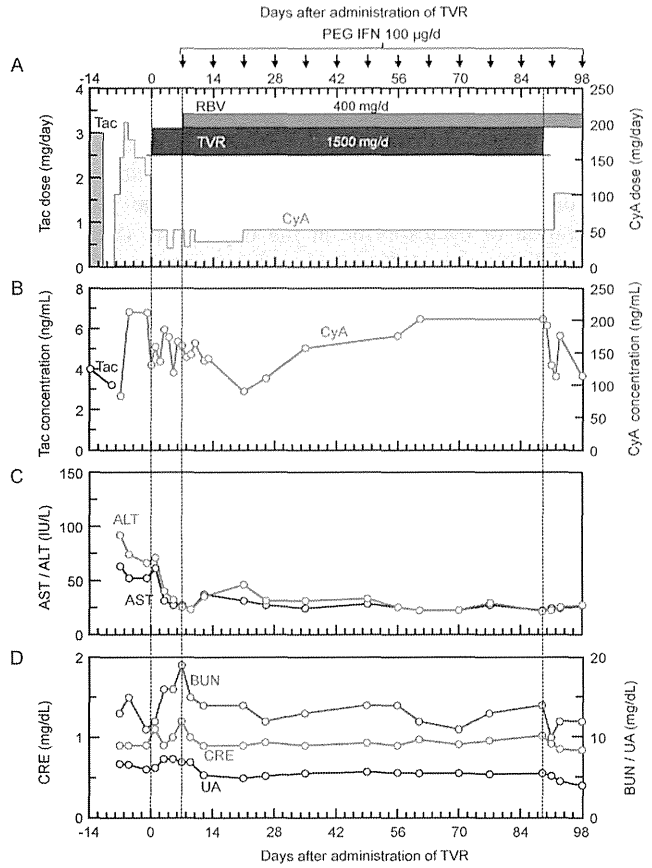


Fig. 1. Dosage of Immunosuppressants and TVR (A); Blood Concentrations of Immunosuppressants (B); Monitoring of Levels of Transaminases (C); Creatinine, Blood Urea Nitrogen, and Uric Acid Levels (D) in Case I

(A) The daily doses of tacrolimus (black) and cyclosporine (red) were documented. (B) The blood concentration of tacrolimus (black) and cyclosporine (red) were quantified by chemiluminescence immunoassay (CLIA) and affinity column-mediated immunoassay (ACMIA), respectively. (C) Aspartate aminotransferase (black) and alanine aminotransferase (red) values were determined. (D) Creatinine (red), blood urea nitrogen (blue), and uric acid (black) values were determined. TVR, telaprevir; Tac, tacrolimus; CyA, cyclosporine; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CRE, creatinine; BUN, blood urea nitrogen; UA, uric acid.

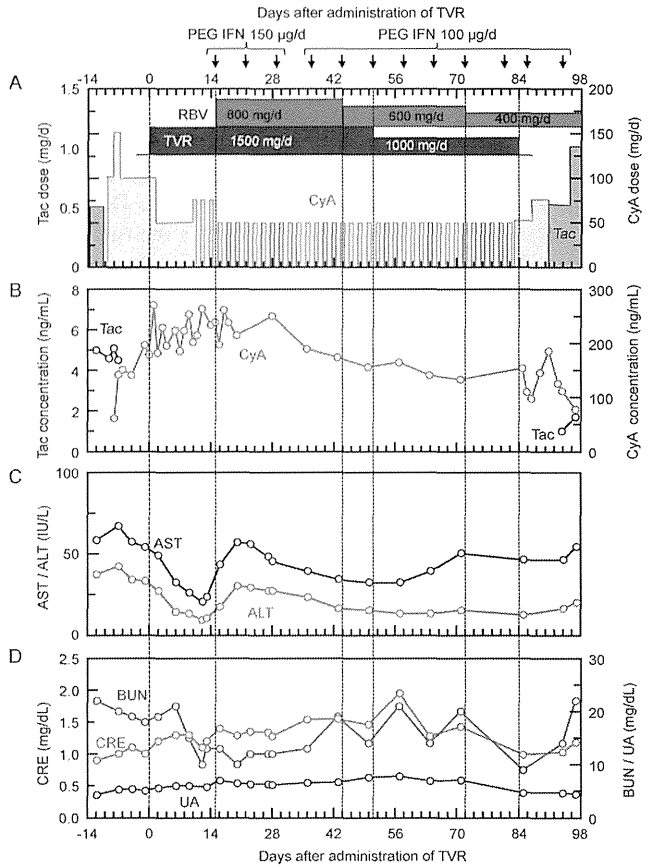


Fig. 2. Dosage of Immunosuppressants and TVR (A); Blood Concentrations of Immunosuppressants (B); Monitoring of Levels of Transaminases (C); Creatinine, Blood Urea Nitrogen, and Uric Acid Levels (D) in Case II

(A) The daily doses of tacrolimus (black) and cyclosporine (red) were documented. (B) The blood concentration of tacrolimus (black) and cyclosporine (red) were quantified by CLIA and ACMIA, respectively. (C) Aspartate aminotransferase (black) and alanine aminotransferase (red) values were determined. (D) Creatinine (red), blood urea nitrogen (blue), and uric acid (black) values were determined. TVR, telaprevir; Tac, tacrolimus; CyA, cyclosporine; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CRE, creatinine; BUN, blood urea nitrogen; UA, uric acid.

istration of TVR. Before TVR administration (1500 mg/bid), the dosage and trough concentration of cyclosporine were 100 mg/bid and 197 ng/mL, respectively (Figs. 2A, B). Because of interactions between cyclosporine and TVR, the dosage of cyclosporine was reduced to half on day 1 of TVR administration. We carefully adjusted the dosage of cyclosporine on the basis of the measurement of morning blood concentration during 3 weeks after administration of TVR, because of the unstable pharmacokinetics of cyclosporine. Finally, the dosage of cyclosporine was 50 mg/qod and the trough level of cyclosporine was 215 ng/mL at day 21. Hepatorenal syndrome and diabetic nephropathy deteriorated his renal function at surgery; therefore, the initiation of PEG-IFN $\alpha$-2b (150 $\mu$g/week) and ribavirin (800 mg/bid) was carefully set back to day 14 to avoid further kidney dysfunction. Fatigue and anorexia worsened from 1 month after initiation of TVR treatment, and the patient developed intense pruritus on his back from day 51 after TVR treatment. Because of these complications, the dose of TVR was decreased to 1000 mg/bid; subsequently, these adverse reactions subsided. Although the blood concentration of cyclosporine decreased to 141 ng/mL at the 20th day after reducing the TVR dosage, the dosage of cyclosporine of 50 mg on alternate days (qod) was unchanged without any rejection episode. On the 19th day, the uric acid level was 7.0 mg/dL; therefore, the patient was administered febuxostat (10 mg/d). Moreover, the serum creatinine level moderately increased, but it decreased after reduction of TVR dose. The patient completed the 12-week TVR therapy. After the end of TVR administration, the dosage of cyclosporine was gradually increased from 50 mg/qod to 50 mg/qd, and finally to 75 mg/qd to maintain the target trough level. At that point, the blood concentration of cyclosporine was stable. The calcineurin inhibitor was switched again from cyclosporine to tacrolimus. Over the course of 100 d, the patient showed no rejection episode.

**Case III**: A 67-year-old woman who underwent living-donor liver transplantation from her spouse because of hepatocellular carcinoma after HCV-related liver cirrhosis, which was within the Milan criteria. The patient experienced a recurrence of hepatitis C at 6 months after transplantation; therefore, she was treated with PEG-IFN $\alpha$-2b and ribavirin for 14 months and achieved SVR. At the 66th post-transplant month after achieving SVR, she experienced relapse of HCV infection and was followed-up without PEG-IFN $\alpha$-2b treat-

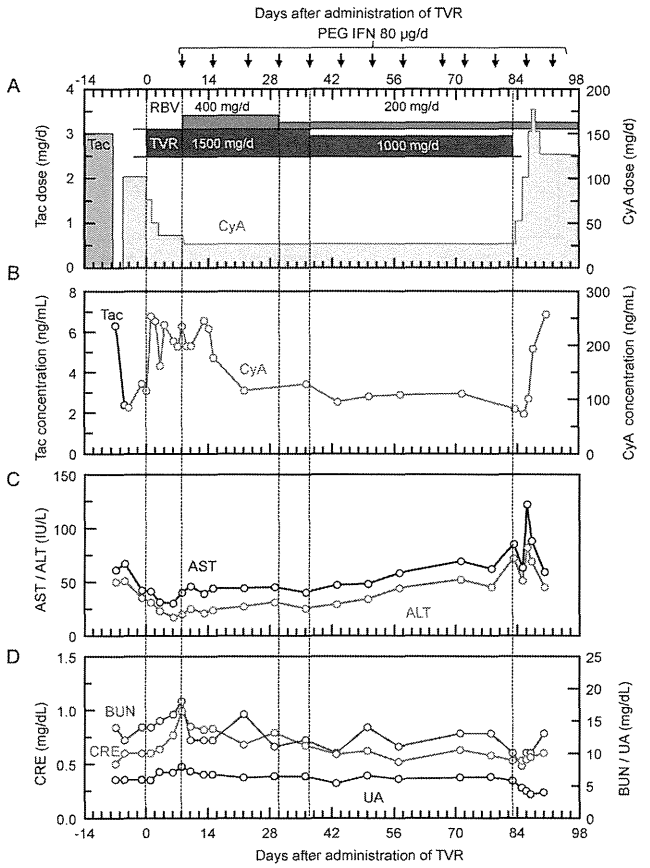


Fig. 3. Dosage of Immunosuppressants and TVR (A); Blood Concentrations of Immunosuppressants (B); Monitoring of Levels of Transaminases (C); Creatinine, Blood Urea Nitrogen, and Uric Acid Levels (D) in Case III

(A) The daily doses of tacrolimus (black) and cyclosporine (red) were documented. (B) The blood concentration of tacrolimus (black) and cyclosporine (red) were quantified by CLIA and ACMIA, respectively. (C) Aspartate aminotransferase (black) and alanine aminotransferase (red) values were determined. (D) Creatinine (red), blood urea nitrogen (blue) and uric acid (black) values were determined. TVR, telaprevir; Tac, tacrolimus; CyA, cyclosporine; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CRE, creatinine; BUN, blood urea nitrogen; UA, uric acid.

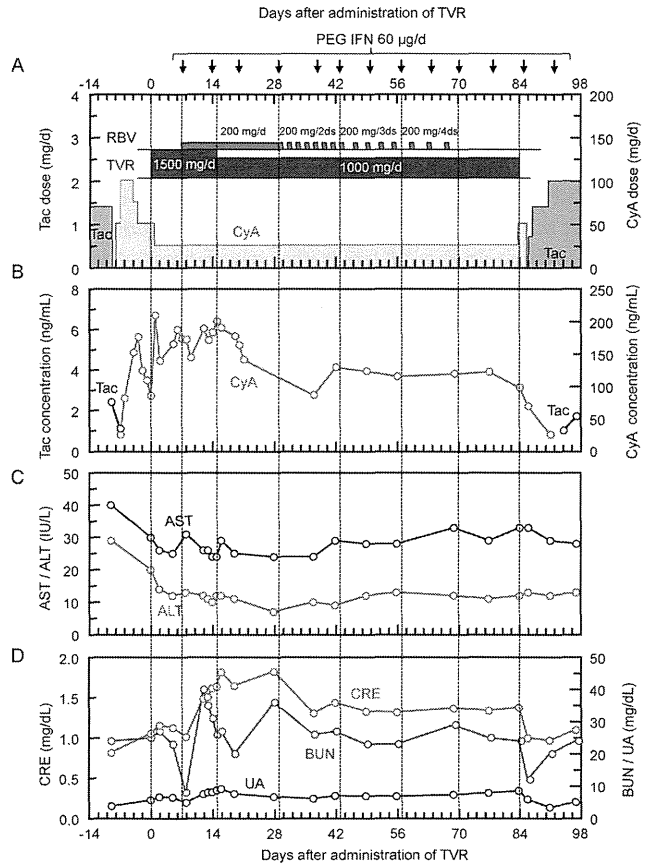


Fig. 4. Dosage of Immunosuppressants and TVR (A); Blood Concentrations of Immunosuppressants (B); Monitoring of Levels of Transaminases (C); Creatinine, Blood Urea Nitrogen, and Uric Acid Levels (D) in Case IV

(A) The daily doses of tacrolimus (black) and cyclosporine (red) were documented. (B) The blood concentration of tacrolimus (black) and cyclosporine (red) were quantified by CLIA and ACMIA, respectively. (C) Aspartate aminotransferase (black) and alanine aminotransferase (red) values were determined. (D) Creatinine (red), blood urea nitrogen (blue) and uric acid (black) values were determined. TVR, telaprevir; Tac, tacrolimus; CyA, cyclosporine; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CRE, creatinine; BUN, blood urea nitrogen; UA, uric acid.

ment. Before treatment with TVR, PEG-IFN $\alpha$-2b, and ribavirin, the calcineurin inhibitor was switched from tacrolimus to cyclosporine about a week before TVR administration. The dosage and trough concentration of cyclosporine at the day before the administration of TVR were 100 mg/bid and 129 ng/mL, respectively (Figs. 3A, B). To achieve the target trough concentration of cyclosporine (150–200 ng/mL), the dosage of cyclosporine was gradually decreased from 100 mg/bid to 35 mg/qd within 4 d after initiation of TVR administration (1500 mg/bid). The dosage of cyclosporine decreased to 25 mg/qd when the trough concentration of cyclosporine was greater than 200 ng/mL. On day 8 of TVR therapy, PEG-IFN $\alpha$-2b (80 $\mu$g/week) and ribavirin (400 mg/bid) were also added to constitute the triple therapy. The levels of AST and ALT decreased during the first 7 d with TVR without PEG-IFN $\alpha$-2b or ribavirin. Administration of allopurinol (50 mg/d) was effective against TVR-related kidney injury such as temporary increases in the levels of uric acid, serum creatinine, and BUN (Fig. 3D). Because of anemia and anorexia, the doses of TVR and ribavirin were decreased to 1000 mg/bid on day 37 and 200 mg/day on day 30, respectively. Subsequently, the severity of these adverse decreased. The patient completed the 12-week TVR therapy. After the final day of TVR administration, the dose of cyclosporine was gradually increased from 25 mg/qd to 50 mg/bid, and finally to 100 mg/bid to maintain the target trough level. At that point, the blood concentration of cyclosporine was stable at the dosage of 125 mg/bid. Over the course of 100 d, the patient showed no rejection episode.

**Case IV**: A 68-year-old woman who underwent living-donor liver transplantation from her offspring because of hepatocellular carcinoma and liver cirrhosis. The patient experienced hepatitis C recurrence at 5 months after transplantation, and was immediately treated with PEG-IFN $\alpha$-2b and ribavirin. However, she was withdrawn from this therapy because of nausea at 15 months after liver transplantation. The calcineurin inhibitor was switched from tacrolimus to cyclosporine about a week before the administration of TVR. The dosage and trough concentration of cyclosporine at the day before TVR administration were 50 mg/bid and 109 ng/mL, respectively (Figs. 4A, B). To achieve the target trough concentration of cyclosporine (150–200 ng/mL), the dosage of cyclosporine was decreased from 50 mg/bid to 25 mg/qd at the first day of TVR administration (1500 mg/bid). At Day 7 of TVR therapy, PEG-IFN $\alpha$-2b (60 $\mu$g/wk) and ribavirin (200 mg/d) were also

added to constitute the triple therapy. The levels of AST and ALT returned to normal during the first 7 d of TVR treatment without PEG-IFN and ribavirin. The serum creatinine level increased from 1.02 mg/dL to 1.49 mg/dL within 4 d, and BUN and uric acid levels also increased; therefore, the administration of febuxostat (10 mg/d) was initiated. Because renal dysfunction worsened despite the temporal effect of febuxostat, the dosage of TVR was decreased to 1000 mg/bid on day 15, and the dosage of febuxostat was increased to 20 mg/d on day 16. Because of the reduced TVR dosage, the blood concentration of cyclosporine decreased to 141 ng/mL in 7 d. Because the blood concentration of cyclosporine was around the lower limit of the target window, and the renal dysfunction was not completely ameliorated, the dosage of cyclosporine was maintained at 25 mg/qd. After successful completion of the 12-week TVR treatment, the calcineurin inhibitor was switched back from cyclosporine to tacrolimus. The dosage of tacrolimus was set at 1.4 mg/bid. The dosage of ribavirin was adjusted considering adverse reactions such as a skin rash on her back and a decrease in hemoglobin levels. The patient noticed the skin rash on her back on day 23 of TVR treatment, and her hemoglobin level decreased from 10.3 g/dL to 9.1 g/dL within 9 d. These adverse reactions were presumably caused by ribavirin; therefore, the dosage of ribavirin was decreased to 200 mg/qod on day 29. The dosage of ribavirin was further decreased to 200 mg every 3 d on day 43, 200 mg every 4 d on day 57, and finally, she was withdrawn from ribavirin on day 70. No signs of rejection were observed over the course of 100 d of TVR administration.

In Case I, the average cyclosporine doses during 7 d before and after beginning administration of TVR were 157.1 mg/bid and 46.4 mg/qd, respectively. In the case of all patients, the dosage of cyclosporine decreased by about 60% after initiation of administration of TVR. The data of concentration/dose ratios (C/D ratio, (ng/mL)/mg) of cyclosporine from all study patients are shown in Fig. 5. When TVR therapy was initiated, the C/D ratio increased 3.04-fold from the −1st week to the 1st week. In addition, the C/D ratio of cyclosporine during the 2nd week significantly increased ($p<0.0001$). On the other hand, after completion of TVR therapy, the C/D ratio decreased from 4.90 to 2.91.

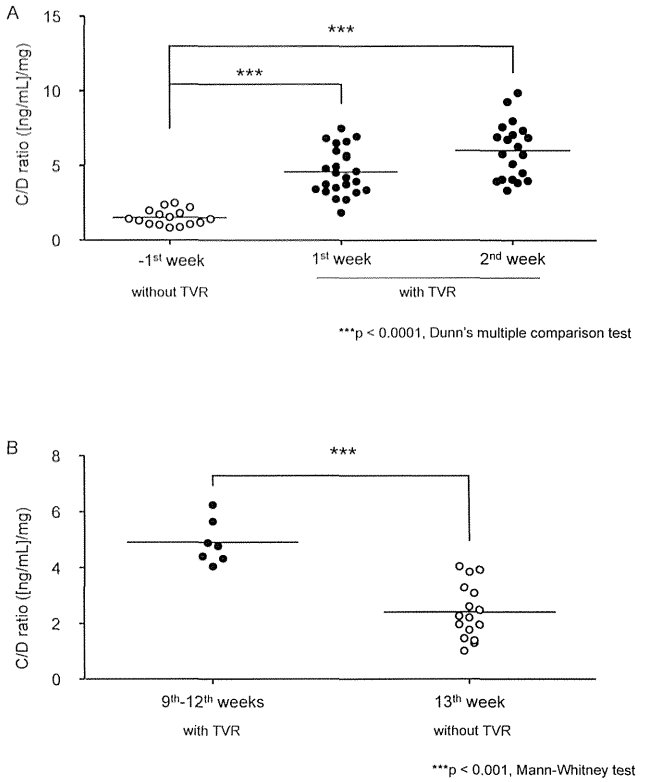


Fig. 5. Influence of Telaprevir (TVR) on C/D Ratio of Cyclosporine

Concentration/dose (C/D) ratio of cyclosporine was documented within TVR combination therapy beginning (A) and TVR combination therapy ending (B) in this study. ***$p<0.001$, significant difference between groups.

## DISCUSSION

Previously, the standard therapy for liver transplantation patients with chronic hepatitis C has been double combination therapy with PEG-IFN $\alpha$ and ribavirin.[13–16] Recently, TVR is a new drug with strong efficacy against hepatitis C when administrated in combination with PEG-IFN and ribavirin.[17,18] TVR has strong interactions with drugs metabolized by CYP3A4 and CYP3A5.[5,6] Liver transplant patients have to take immunosuppressive agents such as tacrolimus and cyclosporine to avoid graft failure, and these agents are metabolized by CYP3A4 and CYP3A5. The blood concentrations of tacrolimus and cyclosporine increase with the administration of TVR. Therefore, to control the blood concentration of tacrolimus and cyclosporine, treatment with the new TVR therapy is important to prevent recurrence of hepatitis C in patients after liver transplantation. Garg *et al.* showed TVR increased the area under the curve of tacrolimus and cyclosporine to 70.3-fold and 4.11-fold, respectively.[7] Therefore, we switched the immunosuppressive agent from tacrolimus to cyclosporine in this study. On the basis of our predictions, the dosage of cyclosporine was decreased after initiation of TVR administration, and the blood concentration of cyclosporine was unstable. Moreover, the average dosage of cyclosporine decreased to 0.24–0.40-fold before initiation of TVR (data not shown). To maintain the target trough level of cyclosporine, we measured the blood concentration of cyclosporine 3 times a day during the 1st week of TVR administration. This was important to prevent graft rejection. In Case II, the peak blood concentration of cyclosporine was low and the trough level was difficult to decrease. To avoid liver graft rejection, the $C_2$ blood concentration was maintained at the target level.[19] On the other hand, the trough level of cyclosporine was kept low to prevent adverse effects on the kidney.[20,21] Therefore, we determined that the dosage of cyclosporine would not be 25 mg/d, but rather 50 mg/qod. Thus, we controlled not only the total dosage of cyclosporine but also the timing of administration.[20] The dosage adjustment of cyclosporine immediately after the end of TVR treatment is an important issue, because the rapid reduction of blood level of cyclosporine should lead the risk of acute rejection. In the present study, we found the rapid elimination of inhibitory effect of TVR on the disposition of cyclospirne in the all four cases (Fig. 5B). Based on these findings, rapid increase in the dosage of cyclosporine would be required to maintain the immunosuppressive effect throughout the anti HCV therapy with or without TVR.

Recently, we reported the drug interactions with tacrolimus in patients after living-donor liver transplantation.[22–24] Uesugi *et al.* showed that the CYP3A5 genotype in the graft liver and native intestine had an effect on the blood concentration

Table 2. Transition of HCV RNA before and after the TVR Treatment

| Case | Before (days before TVR treatment)[a] | Time after TVR treatment (week) | | |
|---|---|---|---|---|
| | | 1 | 2 | 4 |
| | | (log IU/mL) | | |
| Case I | 7.2 (7) | 4.7 | 3.0 | 1.6 |
| Case II | 6.5 (12) | 2.3 | N.D.[b] | N.D. |
| Case III | 6.1 (6) | 2.3 | 1.2> | N.D. |
| Case IV | 4.5 (8) | 1.2> | N.D. | N.D. |

TVR, telaprevir; HCV, hepatitis C virus. *a*) The latest day before administration of TVR. *b*) N.D., HCV RNA was not detected by real-time PCR.

of tacrolimus.[25] TVR inhibits not only CYP3A4 but also CYP3A5. We did not examine the CYP3A5 genotype in this study. However, the differences in the CYP3A5 genotype may affect the blood concentration of tacrolimus. Recently, Zheng *et al.* showed a correlation between CYP3A5 genotype and the risk of cyclosporine-induced nephrotoxicity.[26] Therefore, the genotype of CYP3A5 may be important in implementing TVR therapy.

Almost all patients experienced HCV recurrence, and some underwent re-transplantation because of HCV-related liver cirrhosis or hepatocellular carcinoma.[27,28] The disease progression of hepatitis C is faster in graft liver than in healthy liver,[8,9] and the rate of SVR is lower in transplant patients than in patients who did not receive liver transplants.[29] In this study, all patients completed the 12-week TVR triple therapy. In all patients, the HCV RNA levels immediately decreased to the baseline levels (Table 2). This indicated that PEG-IFN $\alpha$-2b, ribavirin, and TVR therapy were effective against hepatitis C. Moreover, HCV RNA levels decreased and liver function markedly improved with TVR alone. However, SVR was detected 24 weeks after combination therapy with PEG-IFN $\alpha$-2b, ribavirin, and TVR.[4,17,30,31] Therefore, these results did not suggest SVR, but these might be steps to SVR.

The dose of TVR had to be reduced in cases II, III, and IV because of severe adverse effects. TVR is known to elicit a severe rash. In Case IV, the level of hemoglobin decreased, and the dosage of ribavirin was gradually decreased. Cases II and IV showed skin rash, and Cases II and III experienced malaise or anorexia. The latter adverse effects were caused not only by TVR but also by PEG-IFN. Therefore, the adverse reactions might be a synergistic effect of triple therapy. To achieve successful outcome with the PEG-IFN, ribavirin, and TVR triple therapy, maintaining these adverse reactions become less bad may be key points to continuing this therapy. The difference between Case I and the others was the level of HCV RNA after administration of TVR. In Case I, the HCV RNA level rapidly decreased; however, these levels decreased almost immediately in Cases II–IV. In addition, the HCV RNA level in Case IV decreased to limits of detection with only TVR (Table 2). The connection between these adverse effects and therapeutic benefit of this therapy was difficult to determine. Small doses of TVR, for example 1000 mg/d, might be beneficial to treat HCV and to avoid adverse effects in Cases II, III, and IV. Therefore, dose reduction from the standard dose (2250 mg/d) is suitable for Japanese HCV-recurrence patients after liver transplantation.[12] In addition, the association between TVR blood level profile and its adverse reactions should be examined in future to individualized dosage adjustment of TVR.

In all patients, the serum creatinine levels were temporarily increased at the 1st week of TVR administration. However, in Case IV, during TVR treatment, the serum creatinine remained at a high level ($1.41 \pm 0.25$ mg/dL). Cyclosporine or TVR might cause renal dysfunction; therefore, cyclosporine was replaced with tacrolimus immediately after TVR therapy. To treat avoid adverse effects of cyclosporine and the risk of re-control of cyclosporine, triple therapy should be administered with tacrolimus as the immunosuppressive agent.

In conclusion, we could treat patients with recurrence of hepatitis C after liver transplantation by TVR therapy to avoid liver graft rejection. Controlling the drug interaction between TVR and cyclosporine was the most important aspect to achieving both treatment of hepatitis C and prevention of liver graft rejection. We selected cyclosporine instead of tacrolimus as the immunosuppressive agent and carefully adjusted the dosage of cyclosporine by frequent measurement of blood concentration. It was risky to change the immunosuppressive agent from tacrolimus to cyclosporine for graft rejection. Therefore, in the future, we will need to control the blood concentration of tacrolimus, which has a strong interaction with TVR, to treat HCV with TVR triple therapy.

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