(http://links.lww.com/TP/A890). Univariate analyses showed that MELD score ≥20 (72.1% vs. 89.7% for 2-year graft survival; $P<0.01$) and donor age ≥45 years (77.8% vs. 89.0%; $P<0.01$) were significantly associated with poor graft outcome. The cutoff values for MELD, donor age, and ischemic time were obtained using receiver operating characteristic curve analysis. GV/standard liver volume (SLV) did not have significant impact on graft survival. Multivariate analyses also showed that MELD score ≥20 (hazard ratio, 2.9; 95% confidence interval, 1.6–5.2; $P<0.01$) and donor age ≥45 years (hazard ratio, 4.8; 95% confidence interval, 2.2–5.3; $P<0.01$) were independently associated with reduced graft survival.

Significance of D-MELD for Predicting In-Hospital Mortality

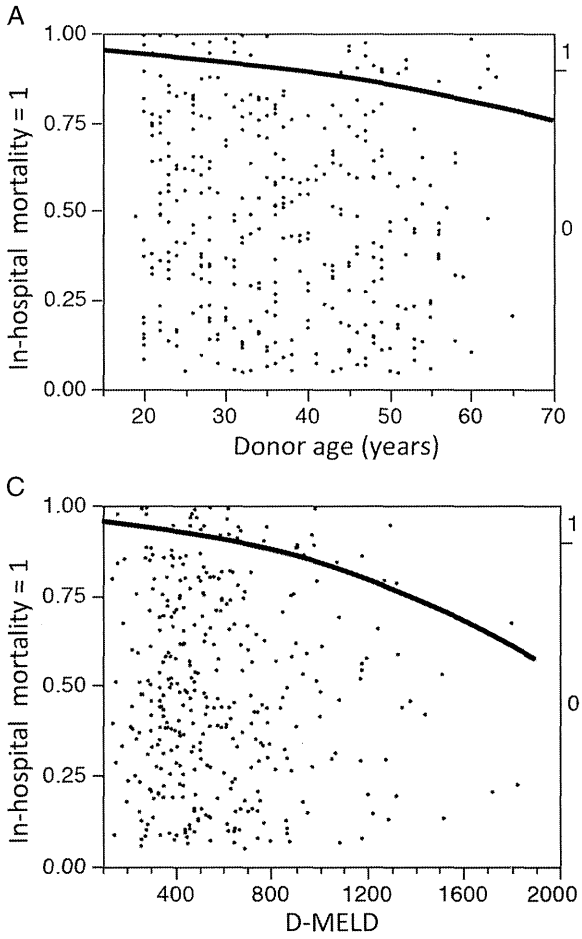
Cumulative logistic probability plots showed that there were significant relationships between graft survival and donor age, MELD score, and D-MELD score, with downward sloping fit lines (Fig. 1A–C). D-MELD (χ^2=8.31; Prob>χ^2= 0.004) demonstrated the strongest association: there was a progressively diminishing probability of graft survival as D-MELD increased. Receiver operating characteristic curve analysis showed that a D-MELD score of 462 was the optimal cutoff for discriminating in-hospital mortality after LDLT. The area under the receiver operating characteristic curve for this value was 0.65 (see **SDC 2**, http://links.lww.com/TP/A890).

Classifying D-MELD

The mean D-MELD score was 583±299 (median, 506; range, 138–1824; Fig. 2A). Guided by the receiver operating characteristic curve analysis and Kaplan–Meier method (*3*), we chose cutoff values of 450 and 900 to best differentiate survival and therefore define D-MELD classes: Class A (≤449; n=142; 40.0%), Class B (450–899; n=163; 45.9%), and Class C (≥900; n=50; 14.1%). The mean D-MELD score was 334±79 in Class A, 623±123 in Class B, and 1160±231 in Class C, respectively. Kaplan–Meier survival curves were used to evaluate graft survival for each D-MELD class. The cumulative 2-year graft survival rate was 94.1% in Class A, 85.3% in Class B, and 63.1% in Class C. The differences between the classes were all statistically significant ($P<0.01$; Fig. 2B).

Comparison between D-MELD Classes

Clinical parameters, including recipient and donor operative and postoperative, were compared for each D-MELD class. D-MELD Class C was characterized by more advanced



Whole model test

X-model	Log Likelihood	DF	Chi Square	Prob>ChiSq
Donor age	114.2	1	4.77	0.029
MELD	114.3	1	4.49	0.034
D-MELD	112.4	1	8.31	0.004

FIGURE 1. Logistic regression analysis of (A) MELD score, (B) donor age, and (C) D-MELD score plotted against graft survival. Although all curves demonstrated a significant relationship, a steeper curve was seen with D-MELD, indicating a stronger relationship.

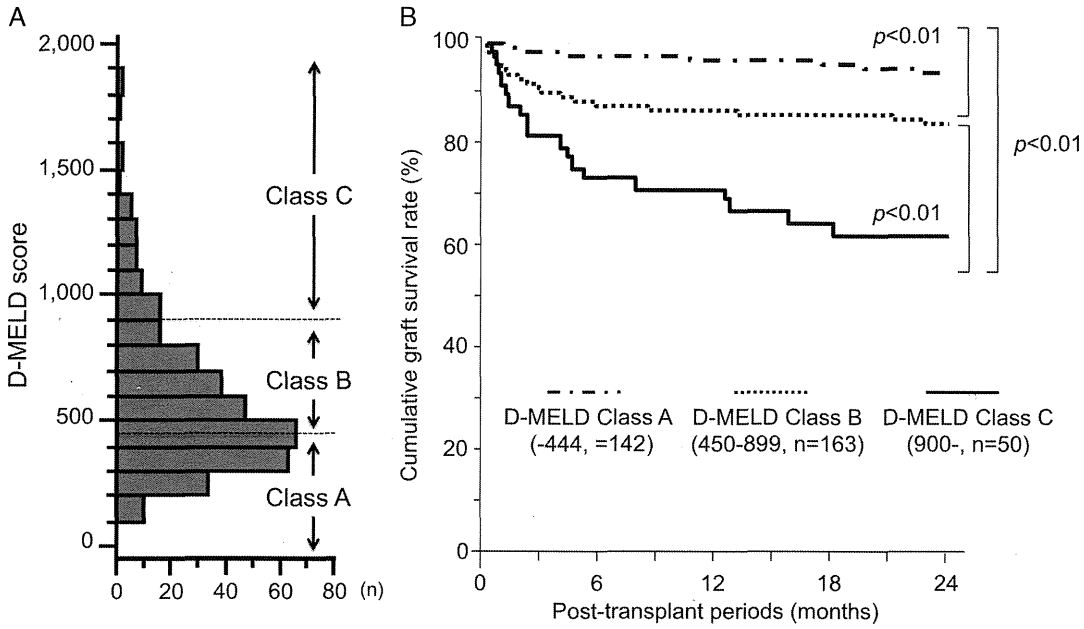


FIGURE 2. A, distribution of LDLT by D-MELD scoring. B, cumulative graft survival rate by D-MELD Classes A, B, and C.

liver disease, comprising a larger proportion of Child–Pugh Class C patients ($P<0.01$), significantly higher MELD scores ($P<0.01$), younger age of recipients ($P<0.01$), and increased use of right lobe grafts ($P<0.01$) procured from significantly older donors ($P<0.01$) with larger GV ($P=0.03$) and larger GV/SLV ($P<0.01$) compared with the other classes (Table 1). As a consequence of the increased use of right lobe grafts in D-MELD Class C patients ($P<0.01$), cold and warm ischemic times were significantly prolonged ($P<0.01$ for both). Intraoperative blood loss was also significantly greater in D-MELD Class C patients. Postoperatively, there were no significant differences in the rates of acute rejection or vascular and biliary complications; however, advanced D-MELD class was characterized by an increased incidence of primary graft dysfunction with bacterial sepsis and in-hospital mortality.

Hepatitis C Subpopulation

Patients were also subdivided based on their hepatitis C status: 168 (47.3%) were hepatitis C virus (HCV) positive. In HCV-positive subgroups, the cumulative 2-year graft survival rate was 93.5% in D-MELD Class A (n=80), 84.9% in Class B (n=6), and 44.2% in Class C (n=19), respectively. Six of the 19 HCV-positive D-MELD Class C patients (31.5%) had primary graft dysfunction and 3 (15.7%) had aggressive hepatitis C recurrence. The graft survival rate in D-MELD Class C was significantly lower compared with the other classes ($P<0.01$ for both; Fig. 3A).

In HCV-negative subgroups, the cumulative 2-year graft survival rate was 94.3% in D-MELD Class A (n=57), 86.7% in Class B (n=93), and 73.5% in Class C (n=31). The primary disease without HCV included primary biliary cirrhosis (n=57), hepatitis B (n=37), nonalcoholic steatohepatitis (n=22), primary sclerosing cholangitis (n=15), alcoholic cirrhosis (n=13), biliary atresia (n=12), autoimmune hepatitis (n=7), giant hemangioma (n=2), hemangioendothelioma (n=2), Wilson disease (n=2), secondary biliary cirrhosis (n=2), and others (n=16). The graft survival rate in D-MELD Class C was significantly lower compared with the other classes ($P<0.01$ for both). The cumulative graft survival rate was significantly inferior in hepatitis C–positive D-MELD Class C patients than those who were HCV negative ($P=0.04$; Fig. 3B).

DISCUSSION

The principal utility of the D-MELD score is to improve the clinical decision-making process when considering high-risk donor–recipient combinations for DDLT (*3*). Although the graft properties and selection processes were quite different, our study shows that D-MELD can also be used to evaluate the risk of in-hospital mortality after LDLT in certain donor–recipient combinations. The rationale for evaluating the risk of in-hospital mortality after LDLT is to inform the decision about whether the risks of major hepatectomy in a healthy donor outweigh the potential benefits to the recipient (*9, 10*); it is essential to ensure the optimal outcome for donors and recipients.

Our findings show that D-MELD can be used as a tool to inform risk-benefit decisions when planning LDLT in various donor–recipient combinations. For example, if the MELD score of a recipient with decompensated cirrhosis is 15 and the donor is 52 years old, the D-MELD score is 780, indicating an acceptable expected 24-month graft survival rate of 85%. If the MELD score of a recipient is 28 and the donor is 52 years old, the D-MELD score is 1456 and expected 24-month graft survival is 63%. If the same recipient had cirrhosis due to hepatitis C, the expected 24-month graft survival would be less than 50%. In the last scenario, we think LDLT is not justified or would need to be performed after very extensive discussion with the donor and recipient.

GV of the grafts, selected under institutional guideline and bias, did not appear to have a significant influence on

TABLE 1. D-MELD classes and operative and postoperative characteristics of donors and recipients

D-MELD	Class A (≤449; n=142)	Class B (450–899; n=163)	Class C (≥900; n=50)	*P*
D-MELD score	334±79	623±123	1,160±231	<0.01
Recipient age (years)	53.5±10.7	53.1±11.7	45.3±11.4	<0.01
Child–Pugh Class C (%)	64 (45.1)	118 (72.4)	45 (90.0)	<0.01
MELD score	11.7±3.5	16.7±4.0	24.3±6.5	<0.01
Total bilirubin (mg/dL)	3.0±3.1	6.8±7.2	16.4±13.2	<0.01
Prothrombin time INR	1.4±0.2	1.5±0.3	1.9±0.5	<0.01
Creatinine (mg/dL)	0.7±0.3	0.7±0.3	1.3±1.6	<0.01
Hepatitis C	80 (56.4)	69 (42.3)	19 (38.0)	0.02
Hepatocellular carcinoma	93 (53.5)	68 (39.1)	13 (7.5)	<0.01
Donor age (years)	30.3±9.7	38.9±9.9	48.7±6.9	<0.01
Left lobe graft (%)	101 (71.1)	94 (57.6)	17 (34.0)	<0.01
GV (g)	466±103	485±106	511±108	0.03
GV/SLV (%)	40.3±8.4	42.3±8.1	44.4±8.5	<0.01
Blood type incompatibility (%)	8 (5.6)	8 (4.9)	2 (4.0)	0.89
Splenectomy (%)	70 (49.3)	95 (58.3)	29 (58.0)	0.25
Cold ischemic time (min)	84±56	94±52	123±77	<0.01
Warm ischemic time (min)	39±10	43±15	44±12	<0.01
PVP at laparotomy (mmHg)	23.2±5.9	25.3±5.4	25.1±26.2	<0.01
PVP at closure (mmHg)	17.0±4.0	17.1±4.8	17.1±3.5	0.94
HA flow (mL/min)	112±78	122±122	117±55	0.10
PV flow (L/min)	1.6±0.6	1.7±0.7	1.7±0.6	0.09
Operative time (min)	789±178	810±117	843±216	0.17
Blood loss (L)	4.2±3.6	9.3±20.1	11.8±16.7	<0.01
Acute rejection (%)	22 (15.5)	19 (11.7)	7 (14.0)	0.61
Biliary stenosis (%)	31 (21.8)	30 (18.4)	11 (22.0)	0.72
HA thrombosis (%)	0 (0.0)	2 (1.2)	1 (2.0)	0.32
PV thrombosis (%)	3 (2.1)	4 (2.5)	1 (2.0)	0.97
CMV antigenemia (%)	22 (15.5)	34 (20.9)	15 (30.0)	0.08
Bacterial sepsis (%)	10 (7.1)	18 (11.0)	12 (24.0)	<0.01
Total bilirubin on day 14 (mg/dL)	4.9±5.9	7.1±8.4	14.0±10.6	<0.01
Primary graft dysfunction (%)	5 (3.5)	20 (12.3)	18 (36.0)	<0.01
Early graft mortality (%)	5 (3.5)	18 (11.0)	13 (26.0)	<0.01
Mean hospital stay (days)	35.9±20.1	42.9±32.1	56.1±41.2	<0.01

CMV, cytomegalovirus; HA, hepatic artery; INR, international normalized ratio; PV, portal vein.

short-term graft survival in our cohort, although this association has been reported before (*4*). It just does not mean a graft with GV/SLV<20% works. Therefore, we only used the product of donor age and MELD score to calculate D-MELD, in accordance with the method described for DDLT (*3*). To the best of our knowledge, all institutions at which LDLT is performed have lower limits for donated GV and remnant donor liver volume that include a safety margin: GV/SLV >30% to 40% or graft recipient weight ratio >0.6 to 0.8 for transplanted GV and remnant donor liver volume >30% to 35% of total liver volume (*4–7, 11–13*). Our finding that GV was not among the risk factors for in-hospital mortality indicates that these lower limits are effective (*4–7*). We have previously shown that use of a larger GV as a partial LDLT graft did not confer a survival advantage if GV exceeded 35% (*14*). Therefore, with careful donor and recipient selection for LDLT, in which an appropriately sized graft under selection bias with institutional cutoff for lower limit without steatosis is transplanted without a prolonged ischemic time, it is logical that donor age should be the most important contributing factor to graft outcome (*4*). Nevertheless, regarding the impact of graft size on graft survivals especially for sick patients, larger multicenter studies, with different institutional cutoffs for LDLT donors, should confirm or disprove the role of graft size in prognostic tools.

In the previous studies of D-MELD in DDLT, the cutoff for donor–recipient combinations with the highest risk for graft loss was 1600 to 1700 compared with 900 in our analysis (*3, 15, 16*). The balance of ethical issues that need to be considered in LDLT, which apply differently to DDLT, could explain this difference. For example, DDLT may be indicated for recipients in very poor condition and there are no concerns about risk to the donor. In LDLT, donation should be performed only if the risk to the donor is justified by the expectation of an acceptable outcome for the recipient, as stated by the Vancouver Forum in 2005 (*17*). A limit to the age of donors may also be a contributing factor: although donations for LDLT are not usually accepted from

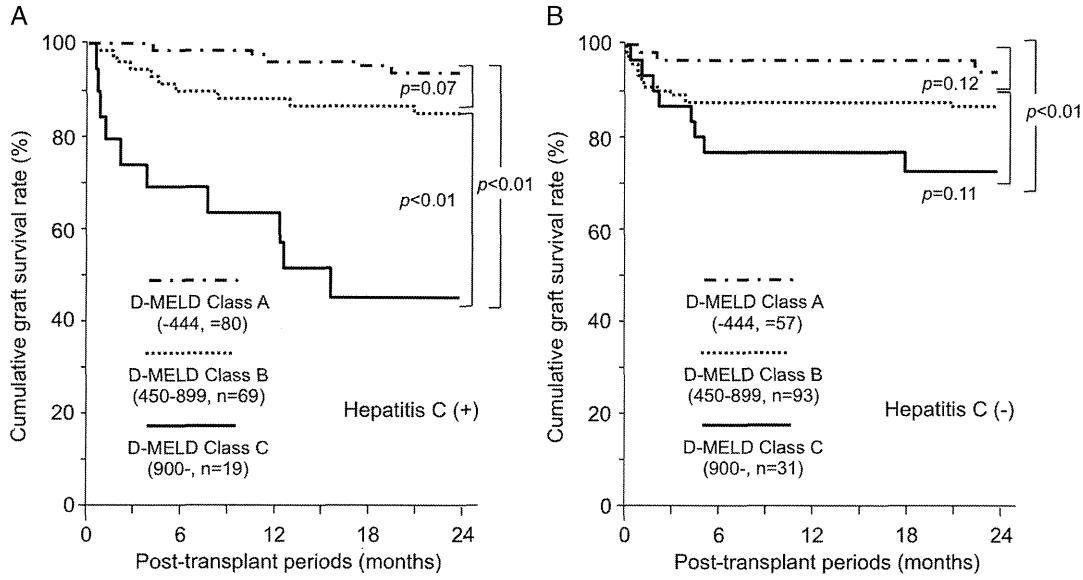


FIGURE 3. Cumulative graft survival rate by D-MELD classes in (A) hepatitis C patients and (B) non–hepatitis C patients.

those aged over 65 years due to increased risks to donor and recipient, this does not necessarily apply in DDLT (*18*).

Regarding recipient factors, it is well recognized that disease severity is the most significant factor that determines graft outcomes (*6, 7*). Although MELD score, Child–Pugh score or grade, or clinical symptoms and signs such as intractable ascites or encephalopathy have been used to reflect the manifestation of severe hepatic disease in these reports, only MELD score has the precision to accurately predict outcome. Another advantage is that D-MELD can also be calculated before surgery, whereas some risk stratification systems rely on perioperative or postoperative data (*3*). Thus, we think D-MELD is made from donor age and MELD score, both of which are continuous variables known before LDLT and significant factors for graft outcomes.

Comparison of patients allocated to the three D-MELD Classes was also illuminating. We found that recipients in D-MELD Class C were more likely to have portal venous hypertension at laparotomy than the other classes. As a consequence of this observation, and the advanced age of donors and poorer condition of the recipients, more right lobe grafts were used. Although postoperative portal venous pressures (PVP) were comparable with the other groups, the incidences of primary graft dysfunction, bacterial sepsis, and graft loss were still greater. Patients with primary graft dysfunction, characterized by hyperbilirubinemia, tend to have bacterial sepsis, and the finding is consistent with a previous report (*5, 19*). This finding should guide clinical practice, as the influences of advanced donor age and poor recipient condition could not be overcome by selecting larger GVs or by intentional decompression of portal hypertension (*5*). Thus, to achieve acceptable outcomes in adult-to-adult LDLT for chronic liver disease, a reasonable approach would be to undertake surgery in patients with D-MELD scores<900. Given our findings, it can be argued that LDLT is only indicated for patients with a D-MELD score in excess of 900 if they are HCV negative.

The negative impact of HCV infection on graft outcomes in D-MELD Class C patients is a particularly important finding and concurs with previous reports (*21, 24*). Onaca et al. found that HCV-positive patients with a MELD score in excess of 25 had significantly worse graft survival (*20*). The negative influence of advanced donor age in HCV-positive recipients has also been reported (*21, 22*). In LDLT, Yoshida et al. found that both positive HCV status and advanced donor age were significant risk factors for graft loss in patients with high MELD scores (*22*). As D-MELD score includes donor age, it follows that it effectively predicts outcome after LDLT in HCV-positive patients. Unless results can be improved, high-risk combinations with D-MELD >900 especially in HCV patients should probably not undergo LDLT.

The main limitation of this study is that data were collected and analyses were performed retrospectively. Reports from other centers are also necessary to help generalize our findings. Further studies with larger numbers of patients are required to characterize the very high-risk subgroup of patients in D-MELD Class C.

In conclusion, D-MELD score is a useful predictor of in-hospital mortality, enabling simple and reliable evaluation of donor–recipient matching for LDLT in adults.

MATERIALS AND METHODS

Patients

We reviewed the records of 358 adults with chronic liver disease who underwent LDLT at Kyushu University Hospital, Japan, between July 1998 and May 2013. We did not include patients with acute liver failure in this study, as their clinical features may have introduced confounding variables into our analysis (*23*). Two patients who underwent auxiliary LDLT and one patient who underwent dual graft LDLT were excluded; therefore, 355 patients were included in the analysis.

MELD score was calculated without exception points, and D-MELD score was calculated as the product of donor age and laboratory-based MELD score (capped at 40 years) as described previously by Halldorson et al. (*3*).

The primary outcome was graft survival measured in terms of in-hospital mortality and 2-year cumulative graft survival.

Graft Selection and Surgical Procedures

Grafts were selected as described previously (*24*). Left lobe grafts were used as the primary graft type if the desired GV/SLV was ≥35%. Right lobe grafts were used if the simulated GV/SLV of the left lobe graft was <35% and the donor's remnant liver volume was ≥35%. Other factors, such as anatomical variations and recipient condition, were also taken into account to achieve the optimal outcome for each patient.

The surgical procedures used in donors and recipients have been described previously (*25–27*). Briefly, procured living-donor grafts were preserved in University of Wisconsin solution (Viaspan; DuPont, Wilmington, DE). After recipient hepatectomy, the grafts were transplanted in a piggyback fashion. Arterial reconstruction was performed under a microscope. Splenectomy was usually performed to address high PVP as described previously (*27*). Major spontaneous portosystemic shunt ligation was also performed (*28*). The reasons for aggressive inflow control with favorable outcomes were described before (*14*). Biliary reconstruction was performed by duct-to-duct biliary anastomosis whenever possible.

Posttransplantation Medical Care

The basic immunosuppression protocol consisted of tacrolimus or cyclosporine with mycophenolate mofetil and steroids, gradually tapered to calcineurin monotherapy within 1 year of LDLT (*14*).

Primary graft dysfunction was defined as graft insufficiency with possible in-hospital mortality, without technical, anatomical, immunologic, or hepatitis-related issues (*5*). Graft insufficiency was defined as hyperbilirubinemia (total serum bilirubin ≥20 mg/dL), occurring at least 7 days after surgery and persisting for ≥7 consecutive days (*5*). Bacterial sepsis was defined as the isolation of bacteria other than common skin contaminants from a single blood culture within 3 months of transplantation, along with clinical symptoms, including pyrexia, shivering, dyspnea, altered mental status, tachycardia, or hypotension. In-hospital mortality was defined as graft loss during the hospitalization for LDLT surgery, and the mean period from LDLT to in-hospital mortality (n=36) was 0.17±0.11 months.

Statistical Analysis

Variables were analyzed using the chi-square test for categorical values or the Mann–Whitney test for continuous variables. Cumulative survival analyses were determined using the Kaplan–Meier method with the log-rank test and Cox proportional hazards multivariate model. Values are expressed as mean±SD. Only statistically significant variables were used in multivariate analyses. $P<0.05$ was considered statistically significant.

Cumulative probability plots for logistic regression were performed by plotting in-hospital mortality of LDLT (y-axis) as the categorical response against the continuous variables (x-axis) including MELD score, donor age, and D-MELD score. Receiver operating characteristic curve analysis was also performed to identify the optimal cutoff of D-MELD score for discriminating in-hospital mortality. All statistical analyses were performed using JMP version 7.0.1 (SAS Institute, Cary, NC).

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Reduced-Dose Telaprevir-Based Triple Antiviral Therapy for Recurrent Hepatitis C After Living Donor Liver Transplantation

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Introduction. The feasibility of telaprevir-based triple therapy for recurrent hepatitis C after liver transplantation (LT) has not been evaluated in Asian patients.

Methods. Eleven Japanese patients received reduced-dose telaprevir (1500 mg) and adjusted-dose cyclosporine after LT. Six patients were nonresponders and three were transient responders to dual therapy.

Results. Rapid viral response, early viral response, end of treatment response, and sustained viral response were achieved in 27.3%, 90.9%, 90.9%, and 81.8% of patients, respectively. One patient had viral breakthrough at week 8 with a T54A mutation in NS3. Deep sequence analysis showed that the T54A mutation reverted to wild-type after stopping telaprevir administration. Seven patients developed severe anemia, and six received blood transfusions (4–20 U). Their hemoglobin and estimated glomerular filtration rate remained significantly lower than pretreatment values at 36 weeks after treatment. Four patients developed plasma cell hepatitis after completing telaprevir treatment, and it was treated by increasing the immunosuppressants. Although the cyclosporine level/dose ratio was 2.7 times higher at week 4 than before treatment, it was 0.7 times lower at week 36.

Conclusions. Reduced-dosed telaprevir-based triple antiviral therapy achieved a high viral clearance rate in Japanese patients after LT. Major adverse events included severe anemia, renal dysfunction, and plasma cell hepatitis.

Keywords: Telaprevir, Interferon, Ribavirin, Liver transplantation, Hepatitis C.

(*Transplantation* 2014;98: 994–999)

Although end-stage liver disease secondary to hepatitis C virus (HCV) is the leading indication for liver transplantation (LT), HCV reinfection is an universal event (*1, 2*). Pegylated-interferon (Peg-IFN)/ribavirin has long been the only treatment option for this disease (*3*). However, it is less effective in post-LT settings because only one third of patients treated with Peg-IFN/ribavirin achieved sustained viral response (SVR) (*3*).

Telaprevir, a potent NS3/4A serine protease inhibitor, was recently approved for use in combination with Peg-IFN/ribavirin (triple therapy) for the treatment of patients with genotype 1 HCV infection, and it significantly increased the SVR rate (*4*). However, telaprevir and other protease inhibitors exhibit some safety problems because they exhibit drug-drug interactions with calcineurin inhibitors (CNIs) (*5*). Another limitation of telaprevir-based triple therapy after LT is the adherence to or the durability of treatment, mainly because many patients develop severe anemia (*6–10*). In a recent French study of telaprevir-based triple therapy for LT patients, the discontinuation rate was 58% and the SVR_{12} rate was 20% (*7*). These issues highlight the need for more efficient modes of telaprevir-based triple therapy in post-LT patients. In Japan, we have used reduced-dose telaprevir, reduced-dose ribavirin, and standard-dose Peg-IFNα2b for the treatment of recurrent HCV after living donor liver transplantation (LDLT).

In the current report, we describe the outcomes of reduced-dose telaprevir-based triple therapy in Japanese patients with recurrent HCV after LDLT.

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The authors declare no conflicts of interest.

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T.I. participated in study conception and design and drafting of the article. T.Y. participated in the analysis of data. M.K. participated in study design. S.Y., T.F., Y.M., and S.N. participated in viral RNA analyses. S.I. participated in data collection. K.S. participated in approval of the article. Y.M. participated in the final approval of the article.

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RESULTS

Patient Characteristics

Eleven patients received telaprevir-based triple antiviral treatment (Table 1). The mean age and body surface area of the patients were 58.9±4.5 years and 1.66±0.15 m^2, respectively. Right lobe, left lobe, and posterior segment grafts were implanted in five patients, five patients, and one patient, respectively. All of the patients underwent simultaneous splenectomy during LDLT. Of nine patients (81.8%) who had previously received IFN, six were nonresponders and three were transient responders.

The HCV genotype was type 1b and the HCV-RNA titer was more than 5 $\log_{10}$IU/mL in all of the patients. The mean HCV-RNA titer before starting telaprevir was 6.2±0.8 $\log_{10}$IU/mL. The interleukin (IL)-28B (rs8099917) haplotype was TT in both donor and recipient in eight patients (72.7%) and was TG in both or either donor or recipient in the other three (27.3%) patients. The inosine triphosphate pyrophosphatase (ITPA; rs1127354) haplotype was CC in all of the recipients, except in patient 6. The HCV core protein was wild-type in six (54.5%) patients (R at amino acid [AA] 70 and L for AA91). All of the patients received cyclosporine A (CsA) instead of tacrolimus. The mean Peg-IFN and ribavirin dosages were 1.44±0.14 μg/kg/wk and 5.7±1.8 mg/kg/d, respectively. All of the patients received 1500 mg of telaprevir per day.

Treatment Outcomes and Adverse Events

HCV-RNA was undetectable in 10 patients (90.9%). The rapid viral response at week 4, early viral response (EVR) at week 12, and end-of-treatment response rates at week 24 were 27.3% (n=3), 90.9% (n=10), and 90.9% (n=10), respectively. Because patient 11 experienced viral relapse (transient response) at 4 weeks after treatment, the SVR_{24} rate was 81.8% (Fig. 1A).

Patient 4 experienced viral breakthrough at 8 weeks after treatment (Fig. 1B). In this patient, the serum HCV-RNA titer decreased below the lower limit of quantification (1.08 $\log_{10}$IU/mL). However, the HCV-RNA titer was 1.10 $\log_{10}$IU/mL at week 8 and subsequently increased to 2.36 $\log_{10}$IU/mL. Therefore, all telaprevir-based treatment regimens were discontinued. Because direct-sequence analysis of serum samples showed a T54A mutation in the NS3

TABLE 1. Eleven patients treated with telaprevir-based antiviral treatment after living donor liver transplantation

Patient no.	1	2	3	4	5	6	7	8	9	10	11
Age/sex	52 M	63 M	70 M	59 M	64 F	56 M	58 M	62 F	55 F	60 F	49 F
Height (cm)	173	165	171	171	155	171	164	155	156	154	155
Weight (kg)	85	62	77	68	57	63	49	48	55	59	54
Body surface area (m^2)	1.99	1.68	1.89	1.79	1.55	1.74	1.52	1.44	1.54	1.57	1.51
Graft type	Right	Left	Left	Right	Left	Right	Right	Left	Left	Right	Posterior
Splenectomy	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Previous IFN treatment	TR	NR	NR	NR	TR	NR	—	TR	NR	—	NR
HCV genotype	1b	1b	1b	1b	1b	1b	1b	1b	1b	1b	1b
HCV-RNA ($\log_{10}$IU/mL)	6.4	5.3	5.4	5.8	5.7	7.0	7.4	6.0	6.7	7.9	5.1
Recipient IL-28B	TT	TG	TT	TT	TG	TT	TG	TT	TT	TT	TT
Donor IL-28B	TT	TG	TT	TT	TG	TT	TT	TT	TT	TT	TT
Recipient ITPA	CC	CC	CC	CC	CC	AC	CC	CC	CC	CC	CC
Core AA70 (wild: R)	R	Q	R	R	Q	Q	R	R	R	R	N/A
Core AA91 (wild: L)	L	L	L	L	M	M	L	L	L	M	N/A
Fibrosis stage	2	2	2	1	2	1	1	2	1	1	2
CNI	CsA	CsA	CsA	CsA	CsA	CsA	CsA	CsA	CsA	CsA	CsA
MMF (mg)	1,000	0	0	500	500	0	0	1,000	1,000	0	0
Peg-IFN (μg)	130	90	100	90	100	100	75	50	80	90	70
TVR (mg)	1,500	1,500	1,500	1,500	1,500	1,500	1,500	1,500	1,500	1,500	1,500
RBV (mg)	500	200	800	400	200	600	200	200	300	400	200
RBV dose reduction	Yes	Yes	Yes	No	No	Yes	Yes	No	Yes	No	Yes
Transfusion	Yes (4 U)	No	No	No	No	Yes (4 U)	Yes (6 U)	Yes (20 U)	Yes (12 U)	No	Yes (4 U)
Growth factors	No	No	No	No	No	No	No	No	No	No	No
Skin rash	Grade 1	No	No	No	No	Grade 1	No	No	No	No	No
Plasma cell hepatitis	Yes	No	No	No	Yes	No	No	No	Yes	No	Yes
Unquantifiable RNA	4	2	2	3	3	5	7	2	2	2	2
Undetectable RNA	5	5	4	No	4	11	8	6	4	8	8
Early viral clearance	EVR	EVR	RVR	NR	RVR	EVR	EVR	EVR	RVR	EVR	EVR
Viral outcomes	SVR_{24}	SVR_{24}	SVR_{24}	NR	SVR_{24}	SVR_{24}	SVR_{24}	SVR_{24}	SVR_{24}	SVR_{24}	TR

AA, amino acid; CNI, calcineurin inhibitor; CsA, cyclosporine A; EVR, early viral response; HCV, hepatitis C virus; LDLT; MMF, mycophenolate mofetil; NR, nonresponder; Peg-IFN, pegylated interferon; RBV, ribavirin; RVR, rapid viral response; TR, transient responder; TVR, telaprevir.

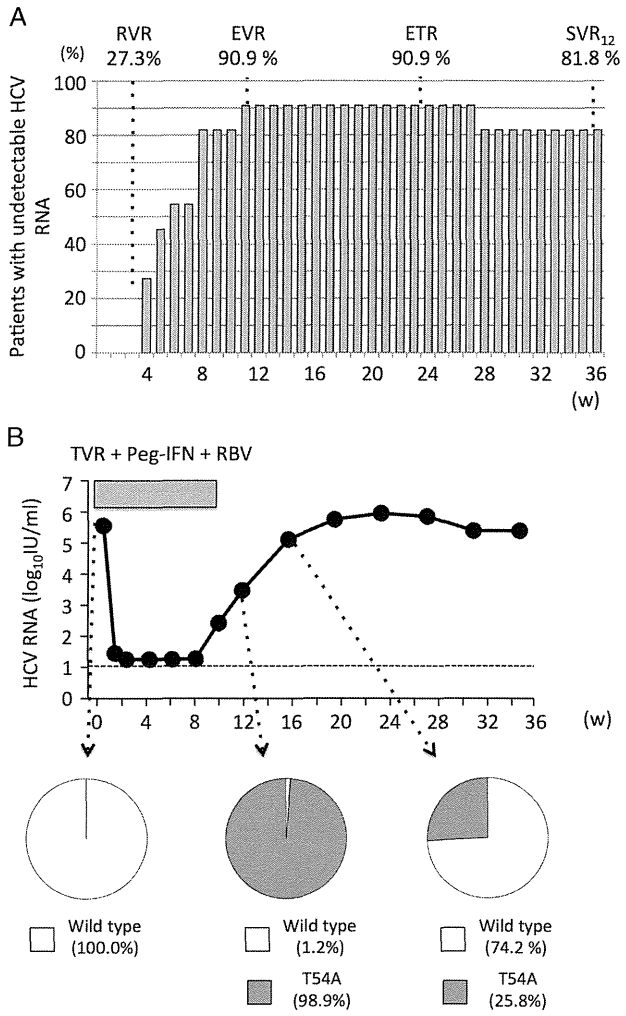

FIGURE 1. Viral response rates after treatment with telaprevir-based triple antiviral therapy. A, patients with undetectable HCV-RNA over the telaprevir-based antiviral treatment. B, one patient (patient 4) had viral breakthrough with a T54A mutation in the NS3 region. ETR, end of treatment response; EVR, early viral response; HCV, hepatitis C virus; Peg-IFN, pegylated interferon; RBV, ribavirin; RVR, rapid viral response.

region at week 10, we performed deep-sequence analysis and detected a high frequency (98.9%) of the T54A mutation. Four weeks after treatment discontinuation, deep sequence analysis showed that the major strain had reverted to wild-type (74.2%). The T54A mutation could not be detected at week 36.

Adverse Events

Anemia (Hb <10 g/dL) was observed in all 11 recipients. Severe anemia (Hb <8.5 g/dL) occurred in seven (63.6%) patients, and six received blood transfusion (4–20 U; Table 1). The Hb levels before treatment and at weeks 12 (end of telaprevir), 24 (end of treatment), and 36 were 13.1±1.9, 8.6±1.3, 9.2±1.1, and 11.5±1.3 g/dL, respectively (Fig. 2A). Hemoglobin (Hb) levels at weeks 12 (P<0.001), 24 (P<0.001), and 36 (P=0.015) were significantly lower than the pretreatment levels, although a significant recovery was observed at week 36 (P<0.001) after completing telaprevir therapy.

The estimated glomerular filtration rate (eGFR) before treatment and at weeks 12, 24, and 36 was 71.8±6.2, 52.8±9.7, 65.8±7.7, and 63.2±8.0 mL/min/1.73 m^2, respectively (Fig. 2B). None of the patients developed acute renal failure. The eGFR at weeks 12 (P<0.001), 24 (P=0.018), and 36 (P=0.011) were significantly lower than the pretreatment level, although a significant recovery was observed at week 36 (P=0.026) after completing telaprevir administration.

Grade 1 skin rash was observed in two patients and was treated with topical steroids. Plasma cell hepatitis (PCH) occurred in four patients (patients 1, 5, 9, and 11). Patient 1 developed moderate PCH with acute rejection at week 21 and was successfully treated by discontinuing IFN/ribavirin and administering a steroid pulse of 1 g of methylprednisolone, which was then down-titrated from 200 mg/d to 20 mg/d in 1 week, followed by 20 mg/d of oral prednisolone. The Cyclosporin A (CsA) trough level was increased to 200 to 250 ng/mL, and daily 2000 mg of mycophenolate mofetil (MMF) was added. Patient 5 developed moderate PCH at week 31, 7 weeks after completing telaprevir-based treatment. Plasma cell hepatitis was successfully treated with a steroid pulse, an increase in the CsA level, and the addition of MMF. Patient 9 developed mild PCH at Week 14. It was successfully treated by increasing the CsA level and daily oral prednisolone (5 mg/d). Patient 11 developed mild PCH at week 21, and it was treated by increasing the CsA level and the addition of oral prednisolone

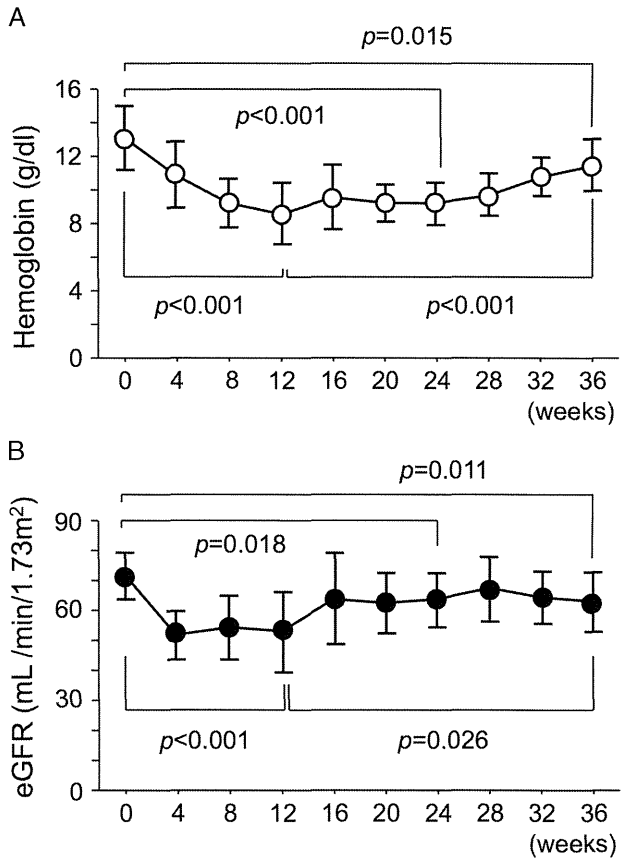

FIGURE 2. Changes in hemoglobin (A) and eGFR (B) in patients treated with telaprevir-based antiviral triple therapy. eGFR, estimated glomerular filtration rate; TPV, telaprevir.

(5 mg/d). Therefore, PCH was successfully treated in all four patients. Three of these patients (patients 1, 5, and 9) remained negative for HCV-RNA and achieved SVR_{24}. Patient 11 experienced viral relapse at week 28.

CsA Metabolism

The mean daily CsA dose and trough level were 80±22 mg/d and 132±36 ng/dL, respectively, before treatment; 28±4 mg/d and 81±22 ng/dL, respectively, at week 12; 89±23 mg/d and 95±27 ng/dL, respectively, at week 24; and 93±19 mg/d and 120±337 ng/dL, respectively, at week 36 (Fig. 3A and B). The blood CsA levels at weeks 24 (P=0.013) and 36 (P=0.048) were significantly lower than the pretreatment levels. The CsA level/dose ratio was 1.9±0.9 ng/mL·mg before treatment, 5.1±1.2 ng/mL·mg at week 4, 3.2±1.2 ng/mL·mg at week 12, 1.2±0.6 ng/mL·mg at week 24, and 1.3±0.5 ng/mL·mg at week 36 (Fig. 3C). The CsA level/dose ratio at week 4 was 2.7 times higher than the pretreatment value (P<0.001), but it decreased significantly at week 36 relative to the pretreatment level (P=0.025).

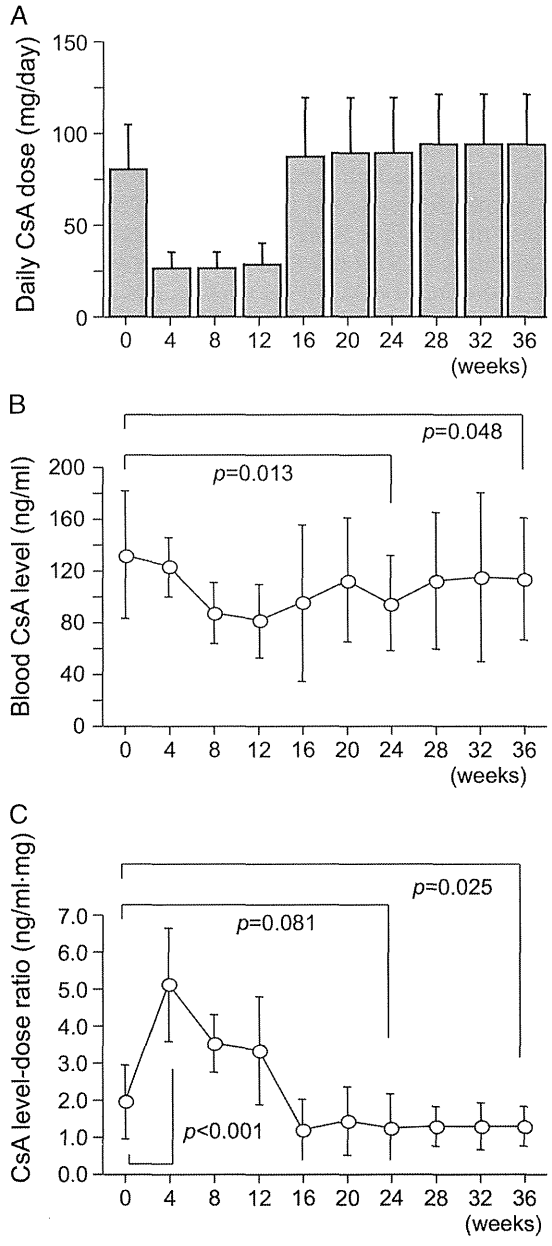

FIGURE 3. Daily CsA dose (A), blood CsA level (B), and CsA level/dose ratio (C) in patients treated with telaprevir-based triple antiviral therapy. CsA, cyclosporine A.

DISCUSSION

The addition of a protease inhibitor to Peg-IFN/ribavirin rapidly suppressed HCV replication in post-LT patients. In the current cohort, 81.8% (9/11) of the patients had HCV-RNA below the lower limit of quantification (<1.08 $\log_{10}$IU/mL) at week 4 and 90.9% achieved complete EVR at week 12. The early viral clearance achieved by telaprevir was greater than that reported (45%–55% for conventional Peg-IFN/ribavirin therapy after LT) (*2, 3*). Despite administration of telaprevir at a reduced dose, the EVR and SVR_{24} rates in our study (90.9% and 81.8%, respectively) were superior to those in previous studies, in which the EVR was 67% in a study by Pungpapong et al. (*8*) and the SVR_{12} was 20% in a study by Coilly et al. (*7*). However, it may be necessary to measure the blood trough level of telaprevir and determine the relationship between the telaprevir dose and clinical outcomes.

Only one patient (patient 4) experienced viral breakthrough during telaprevir therapy, although the HCV-RNA titer was less than 1.08 $\log_{10}$IU/mL between weeks 2 and 6. This patient had a mutation (T54A) in the NS3 region, conferring resistance to telaprevir. Nevertheless, the titer of the mutated variant decreased after discontinuing telaprevir. HCV exhibits high rates of replication and mutation, and a high error in replication results in the emergence of multiple viral populations called quasispecies (*11, 12*). Although various mutations are generated during telaprevir therapy, the T54A mutant became dominant quasispecies. After discontinuing telaprevir and reducing the pressure on HCV to T-cell–mediated immunity, the T54A quasispecies was replaced with a wild-type species because the wild-type species is most suitable for viral survival (*11–13*). Theoretically, a telaprevir-resistant mutant could only be prevented by increasing the IFN dose, which increases unselective immune pressure, or by the addition of other direct-acting antiviral agents, similar to the treatment of human immunodeficiency virus (*14*). Unlike human immunodeficiency virus infection, in which resistant mutants generated during unsuccessful antiviral treatment are archived in immune system, no such long-lived reservoir has been shown for HCV (*12*). Nevertheless, immediate treatment discontinuation, aimed for preventing possible long-lived resistant mutants, is recommended for those with breakthrough or transient response and is recommended in the current era with multiple developments in direct-acting agents for HCV (*15*).

We have previously reported that a combination of donor and recipient IL-28B haplotypes has a significant effect on HCV clearance after LDLT (*16*). The rs8099917 genotypes of TG or GG in the donor or recipient are associated with very low SVR rates. In the current series, three recipients had the TG genotype, and all three achieved SVR_{24}. Although four patients had mutations in the HCV core protein (AA70 and/or AA91), all of them achieved SVR_{24}. Thus, our results suggest that telaprevir-based triple

antiviral therapy may be suitable for patients with high-risk mutations (TG or GG in IL-28B or HCV core protein mutations), although the present results are still preliminary and warrant confirmation.

Although some pharmacokinetic studies have highlighted possible safety issues when using telaprevir in combination with a CNI in posttransplant settings (*5–8*), these combinations, especially those based on CsA, can be managed with careful monitoring and dose adjustments (*6–10*). In those studies, the CsA dose was reduced to 50% of the initial dose within 12 hr (*6*), by 75% to 100% within 24 hr (*8*), and by 25% to 50% in the current study, although the target trough levels were similar in all of these studies (about 80–150 ng/mL). As shown in Figure 3, the CsA trough level/dose ratio had increased at week 4, but decreased thereafter, reaching a stable value by week 8. Therefore, we suggest that CsA should be administered at 25% of the standard dose at week 0 and increased to 50% of the standard dose at week 4.

Another interesting finding in this study was that the CsA trough level/dose ratio was significantly lower at week 36 than at pretreatment, although this is several weeks after completing telaprevir therapy. Therefore, pharmacokinetic studies after completion of continuous telaprevir administration are necessary to elucidate this issue and to determine whether the induction of cytochrome P4503A is involved in these effects. Kugelmas et al. (*17*) investigated the relationship between viral clearance and the pharmacokinetics of a CNI and showed that HCV clearance improved hepatic microsomal function, resulting in lower immunosuppression levels, and that the mean decrease in the CNI level after viral clearance was 32% in responders and less than 1% in nonresponders. Considering these findings, we think that the high incidence of PCH during or after telaprevir-based triple therapy could be due to the changes in CsA clearance. Levitsky et al. (*18*) recently reported that immune-mediated graft dysfunction including plasma cell hepatitis caused by IFN-based antiviral tratment has significant poor graft outcomes, hence meticulous attention to maintain appropriate CNI levels during potent antiviral treatments such as a telaprevir-based one is warranted.

The major adverse events associated with telaprevir in our study were severe anemia and moderate renal impairment, similar to previous reports (*7, 8*), although we used a reduced dose of telaprevir. It is thought that the mechanism involved in severe anemia in telaprevir-based triple therapy involves an increase in the level of ribavirin in the blood because its renal excretion is reduced as a consequence of telaprevir-induced renal impairment (*19*). Ribavirin excretion could be further impaired in patients treated with nephrotoxic CNIs, resulting in more severe hemolysis and anemia. Although we anticipated that anemia and the renal impairment observed at week 12 of telaprevir therapy would recover at or shortly after the end of treatment, significant reductions in Hb and eGFR were still apparent at this time, although they had improved slightly since the end of telaprevir administration (*20*). Longer studies are necessary to assess the recovery of Hb and eGFR over time. Hara et al. (*21*) compared the effects of 1500 or 2250 mg of telaprevir per day in Japanese nontransplant patients and found that 1500 mg of telaprevir was associated with a much smaller decrease in Hb and an increase in serum creatinine, with comparable viral clearance to 2250 mg of telaprevir. Ogawa et al. (*22*) reported that 4 weeks of telaprevir at a weight-adjusted dose was associated with severe anemia and that the initial dose had a small effect on the treatment outcomes. Thus, in Japanese or Asian patients, who have a smaller body surface area than Caucasian patients, 1500 mg of telaprevir could be safe and effective in clinical practice, especially in posttransplant settings in patients with renal impairment.

The high SVR rates obtained in the current study could be attributed to the combination of three factors including less advanced fibrosis (no patients having fibrosis stage 3 or higher), more patients with IL-28B major haplotype (TT for rs8099917, 72.7%), and tailored dose settings of antiviral agents to prevent withdrawal for intolerance. The patient backgrounds of the series reported by Coilly et al. (*7*) and Pungpapong et al. (*8*) included more patients with advanced fibrosis stage (42% and 57% for fibrosis stage 3 or higher), fewer patients with IL-28B major haplotype (5% and 9% for CC, rs12979860), and treatment dropouts in 16% and 26% because of adverse events, respectively. Although telaprevir-based triple therapy has potent antiviral potential, acceptable outcomes could be obtained under its application to patients with feasible conditions.

This study has some limitations including the smaller number of patients in a special setting (i.e., after LDLT) and potential bias in the selection of candidate patients. Although this study was conducted at a single center, this approach allowed the researchers to monitor the patients carefully and to maintain consistent patient management, which may not be possible in multicenter studies.

In conclusion, reduced-dose telaprevir-based antiviral triple therapy achieved a high SVR rate in Japanese LDLT patients. The major adverse effects of this treatment regimen included severe anemia, renal dysfunction, and PCH, which must be addressed in future studies.

MATERIALS AND METHODS

Patients

Patients were considered for telaprevir-based triple antiviral treatment if posttransplant biopsy of the allograft showed recurrent hepatitis C with stage 2 fibrosis or higher or grade 2 lobular hepatitis or higher according to Scheuer's classification system (*23*) or cholestatic hepatitis (*24*). Patients with (i) recent acute cellular rejection or hepatic decompensation, (ii) Hb less than 10.0 g/L, (iii) eGFR (mL/min/1.73 m^2) of 0.808 (coefficient for Japanese population) × 175 × serum creatinine$^{-1.154}$ × age $^{-0.203}$ × 0.742 (if female) (*25*), or (iv) non–genotype 1 HCV were ineligible for triple antiviral therapy. The surgical procedures used for the donors and recipients are described elsewhere (*26*). In our institute, splenectomy is performed in recipients with hepatitis C to prevent pancytopenia during antiviral therapy (*27*). All procedures, including LDLT, were approved by the Ethics and Indications Committee of Kyushu University.

Antiviral Therapy Regimen

Antiviral therapy was indicated for patients at 3 months after LDLT or longer if the immunosuppression level was stable with a low CNI dose and MMF without steroids. All patients received a combination treatment of telaprevir (Telavic; Mitsubishi Tanabe Pharma, Co., Osaka, Japan), Peg-IFNα2b (Peg-Intron; MSD K.K., Tokyo, Japan), and ribavirin (Rebetol; MSD, Tokyo, Japan) for 12 weeks, followed by an additional 12 weeks of Peg-IFNα2b and ribavirin. Telaprevir (500 mg) was administrated three times a day after each meal (total daily dose, 1500 mg/d), representing a reduction in the original dose (2250 mg) (*4*). Peg-IFNα2b was injected subcutaneously once weekly at a dose of 1.5 μg/kg. Ribavirin was administered orally at a reduced dosage of 200 to 600 mg/d based on the patient's body weight (200 mg/d for patients weighing <60 kg,

400 mg/d for patients weighing 60–80 kg, and 600 mg/d for patients weighing >80 kg). Severe anemia during treatment was defined as Hb less than 8.5 g/dL. The ribavirin dose was to be reduced by 200 mg if Hb decreased to less than 10.0 g/dL and was reduced by a further 200 mg if Hb was less than 8.5 g/dL. Blood transfusion was performed for patients with severe anemia (Hb <8.5 g/dL). Treatments were discontinued for patients with viral breakthrough during treatment.

Administration of CNIs During Antiviral Therapy

CsA was exclusively used as the CNI of choice during telaprevir-based triple therapy. Patients were hospitalized for 2 weeks from 1 day before starting telaprevir for clinical monitoring and for achieving stable control of the CsA level. On the day of starting telaprevir, the CsA dose was reduced to 25% of the original dose. The trough level was measured every day, and the dose was adjusted to maintain a trough level of 100 to 150 ng/ml. After a few weeks of inpatient monitoring, the patients were transferred to an outpatient clinic, and the CsA level was biweekly until week 12, followed by weekly measurement after stopping telaprevir.

HCV-RNA Level

The serum HCV-RNA titer was determined using a real-time HCV assay (AccuGene HCV; Abbott Molecular, Inc., Des Plaines, IL). The lower and higher quantification limits of this assay are 1.08 and 8.00 $\log_{10}$IU/mL, respectively. The virological response was categorized as a rapid viral response, EVR, end-of-treatment response, or SVR_{24}, as an undetectable HCV-RNA at 4, 12, 24, and 24 weeks after the end of treatment, respectively.

IL-28B and ITPA Haplotyping

The IL-28B haplotype was determined in donors and recipients using rs8099917 as a representative single nuclear polymorphisms for IL-28B (*15*), and the recipient's ITPA haplotype was determined using rs1127354 (*28*). DNA was extracted from a biopsy or explanted liver tissue, and genotyping was performed using TaqMan GTX press Master Mix (Life Technologies, Inc., Tokyo, Japan) in accordance with the manufacturer's instructions.

Deep Sequencing of HCV-RNA Mutations at NS3

RNA was extracted from sera and reverse transcribed to complementary DNA using an RNeasy minikit (Qiagen, Valencia, CA) and PrimeScript RT reagent kit (Takara Bio, Shiga, Japan). Then, the NS3 region of the HCV genome was amplified by nested polymerase chain reaction using specific primers (*13*). The three amplified DNA fragments were separated on 1% agarose gels, and the products were purified using a QIAquick Gel Extraction kit (Qiagen). The amplified products were sequenced with the MiSeq system (Illumina, San Diego, CA). Quality filtering, read mapping, and variant detection were performed using CLC Genomics Workbench software (CLC Bio; Qiagen).

Statistical Analysis

Values are expressed as means±standard deviation. Paired variables were compared using Student paired *t* test. Values of $P<0.05$ were considered statistically significant.

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ORIGINAL ARTICLE

Strategies to treat interferon-induced graft dysfunction after living donor liver transplantation for hepatitis C

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Abstract
Purpose Interferon-induced graft dysfunction (IGD) is a poorly defined, unrecognized, but potentially serious condition for patients receiving antiviral drugs after liver transplantation for hepatitis C.
Methods We evaluated the characteristics of 80 patients who received pegylated interferon-based antiviral treatment for hepatitis C after living donor liver transplantation (LDLT).
Results Eight patients experienced IGD either during ($n = 6$) or after completing ($n = 2$) antiviral treatment. Pathological diagnosis included acute cellular rejection (ACR, $n = 1$), plasma cell hepatitis (PCH, $n = 2$), PCH plus ACR ($n = 3$), and chronic rejection (CR, $n = 2$). One patient with CR initially presented with PCH plus ACR and the other presented with ACR; both had apparent cholestasis. The six patients with ACR or PCH without cholestasis were successfully treated by discontinuing antiviral treatment and increasing immunosuppression, including steroids. By contrast, both of the patients with CR and cholestasis experienced graft loss, despite aggressive treatment. Univariate analysis showed that pegylated interferon-α2a-based treatment (75 vs. 26.4 %, $p < 0.01$) was the only significant factor for IGD, and was associated with decreased 5-year graft survival (93.4 vs. 71.4 %, $p = 0.04$).
Conclusions IGD is a serious condition during or even after antiviral treatment for hepatitis C after LDLT. Early recognition, diagnosis, discontinuation of interferon, and introduction of steroid-based treatment may help to save the graft.

Keywords Hepatitis C · Interferon · Liver transplantation · Autoimmune hepatitis · Rejection

Abbreviations

ACR	Acute cellular rejection
CNI	Calcineurin inhibitor
CR	Chronic rejection
HCV	Hepatitis C virus
IFN	Interferon
IGD	Interferon-induced graft dysfunction
LDLT	Living donor liver transplantation
MMF	Mycophenolate mofetil
Peg	Pegylated
PCH	Plasma cell hepatitis

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RBV Ribavirin
SVR Sustained viral response
VR Viral response

Introduction

Reinfection of hepatitis C virus (HCV) is a universal event after liver transplantation (LT) for HCV, and the success of post-transplant antiviral treatment has been reported to be a determinant of graft survival [1]. However, the outcomes of antiviral treatment with pegylated (Peg) interferon (IFN) and ribavirin (RBV) for these patients are often unsatisfactory, with a sustained viral response (SVR) of ~30 % [1–3]. Moreover, adherence to treatment for these patients is frequently poor, and early withdrawal or discontinuation of Peg-IFN and RBV often occurs because of adverse events [1–3].

Among IFN-induced adverse events, interferon-induced graft dysfunction (IGD) is increasingly being recognized as a disease entity among patients given Peg-IFN-based antiviral treatment for hepatitis C after LT [4–6]. IGD may be caused by a potent immunomodulatory effect of exogenous IFN, and may result in uncontrollable graft dysfunction and graft loss, even after HCV itself is cleared [4]. Although IGD might present as plasma cell hepatitis (PCH), acute cellular rejection (ACR), and chronic rejection (CR), alone or in combination, relationships between each outcome and their pathophysiologic features are unclear [6].

In this study, we reviewed the pathological and clinical course of patients with IGD, after antiviral treatment, after living donor liver transplantation (LDLT). Our objective was to identify possible risk factors and ways of overcoming this disorder.

Materials and methods

Patients

LDLT was performed for 99 patients with hepatitis C between January 2004 and July 2012 at Kyushu University Hospital, with approval from the Ethics and Indications Committee of Kyushu University. Of these, 80 received a Peg-IFN-based antiviral treatment to treat recurrent hepatitis C after transplantation. The surgical procedures performed on the donors and recipients are described in more detail elsewhere [7]. Splenectomy was performed for 63 recipients (78.7 %), to prevent pancytopenia associated with Peg-IFN-based antiviral treatments [8]. IGD was defined as graft dysfunction during or after IFN treatment for hepatitis C with pathological diagnosis of PCH, ACR, or CR, or a combination of these findings.

Immunosuppression

The post-transplant immunosuppression protocol is described elsewhere [7]. Briefly, immunosuppression induction consisted of triple therapy with calcineurin inhibitors (CNIs), including tacrolimus or cyclosporine, together with mycophenolate mofetil (MMF) and steroids. The steroids were gradually tapered off within three months after LDLT. Patients were usually using a CNI with or without MMF at the time of starting antiviral treatment. During antiviral treatment, target tacrolimus and cyclosporine levels were 5–8 and 100–200 ng/ml, respectively. The target immunosuppression level was not reduced or changed during antiviral treatment.

Antiviral therapy

Antiviral treatment was indicated for recurrent hepatitis C characterized by HCV-RNA seropositivity with abnormal liver function tests and histological evidence of recurrent hepatitis C. Antiviral treatment is usually started >6 months after LDLT, when immunosuppression treatment with CNI with or without MMF has stabilized [9], except for patients with cholestatic hepatitis, a severe form of recurrent hepatitis C that usually occurs <6 months after transplantation and for which mortality is high.

Peg-IFNα2b with RBV (Pegintron with Rebetol; Merck, Whitehouse Station, NJ, USA) was used as the primary treatment for recurrent hepatitis C after LDLT. Peg-IFNα2b was started at a dose of 1.0–1.5 μg/kg/week with 200–800 mg/day RBV. The planned duration of treatment was 48 weeks after achieving VR. Peg-IFNα2a with RBV (Pegasys with Copegus; Chugai Pharmaceutical, Chuo-ku, Tokyo, Japan) was used for patients with a poor decrease in their HCV-RNA levels during Peg-IFNα2b with RBV treatment. Peg-IFN-α2a was started at a dose of 90–180 μg/week with 200–800 mg/day RBV. No growth factor, including granulocyte colony stimulating factor or erythropoietin, was used.

The serum HCV-RNA level was determined by real-time HCV assay (AccuGene HCV; Abbott Molecular, Des Plaines, IL, USA). The lower and higher limits of quantification were 1.08 and 8.0 log IU/ml, respectively. DNA was obtained from biopsy or explanted liver tissue from the donors and the recipients, and subjected to rs8099917 genotyping by use of TaqMan GTX press Master Mix (Life Technologies, Tokyo, Japan) [9, 10].

Pathological diagnosis and treatment of ACR, PCH, and CR

Patients with abnormal liver function test results, including elevated serum transaminase with or without total bilirubin,

are indicated for radiological studies to rule out vascular or biliary complications. Patients without anatomical complications underwent percutaneous liver biopsy. Pathological diagnosis of ACR was based on the presence of mixed lymphocyte aggregations with bile duct damage and vascular endotheliitis [11]. Mild ACR was treated by increasing the CNI dose. Moderate or severe ACR was treated by intravenous administration of 1,000–2,000 mg methylprednisolone, followed by 20 mg oral prednisolone with increases in the CNI and MMF doses. Pathological diagnosis of PCH was based on the presence of plasma cell aggregations without apparent bile duct damage or endotheliitis [4, 5, 11]. It was treated by intravenous administration of 200–500 mg methylprednisolone followed by 40–60 mg oral prednisolone, which was gradually tapered to 5 mg over several months. If a biopsy showed prominent plasma cell aggregations together with bile duct damage or endotheliitis, it was referred to as PCH plus ACR. Pathological diagnosis of CR was based on bile duct loss, Glissonean or central venous fibrosis progression without cellularity, portal venous peliosis, and foamy hepatic arterial cells [4, 5, 11]. CR was treated as described for ACR.

Statistical analysis

Values are expressed as the mean ± standard deviation. Variables were analyzed by using χ^2 tests for categorical values or the Mann–Whitney test for continuous variables. Cumulative survival analysis was conducted by use of the Kaplan–Meier method with the log-rank test. Values of $p < 0.05$ were considered statistically significant.

Results

Clinical course of IGD

The characteristics of the eight patients with IGD either during ($n = 6$) or after ($n = 2$) Peg-IFN-based antiviral treatment for hepatitis C are summarized in Table 1. The final pathological diagnosis included ACR ($n = 1$), PCH ($n = 2$), PCH plus ACR ($n = 3$), and CR ($n = 2$). The initial diagnosis of the two patients with CR was ACR (#7) and PCH plus ACR (#8). The mean age of the patients with IGD was 54.8 ± 6.7 years, and half of the patients were male. Six patients (75.0 %) received Peg-IFNα2a-based treatment, and four of these developed IGD after switching from Peg-IFNα2b to Peg-IFNα2a. One patient (#1) developed IGD during Peg-IFNα2a and RBV therapy, and the other (#5) developed IGD during triple therapy, which included telaprevir. HCV-RNA was

Table 1 Characteristics of patients with interferon-induced graft dysfunction

Case	Age, sex	IFN protocol	Timing	HCV-RNA	T.Bil (mg/dl)	AST (IU/L)	GGT (IU/L)	Diagnosis	Treatments	Outcome
#1	45, M	Peg-IFNα2b + RBV	4 m after induction of treatment	–	1.3	138	270	ACR	Discontinue Peg-IFN Increase CNI	Alive, 95 m
#2	54, F	Peg-IFNα2b + RBV → Peg-IFNα2a + RBV	1 m after conversion of treatment	+	1.0	232	188	ACR	Discontinue Peg-IFN Increase CNI	Alive, 29 m
#3	64, F	Peg-IFNα2a + RBV	8 m after completing 16 m of treatment	–	0.6	70	19	PCH	Steroid pulse	Alive, 5 m
#4	62, F	Peg-IFNα2a + RBV	3 m after completing 21 m of treatment	–	0.5	105	87	PCH	Steroid pulse	Alive, 5 m
#5	48, M	Peg-IFNα2b + RBV +Telaprevir	5 m after induction of treatment	–	1.3	78	158	PCH + ACR	Discontinue Peg-IFN Steroid pulse Increase CNI/MMF	Alive, 6 m
#6	56, F	Peg-IFNα2b + RBV → Peg-IFNα2a + RBV	1 m after conversion of treatment	–	0.8	166	84	PCH + ACR	Discontinue Peg-IFN Steroid pulse Increase CNI/MMF	Alive, 40 m
#7	51, M	Peg-IFNα2b + RBV → Peg-IFNα2a + RBV	4 m after conversion of treatment	–	8.0	133	329	PCH + ACR → CR	Discontinue Peg-IFN Steroid pulse Increase CNI/MMF	Graft loss, 5 m
#8	59, M	Peg-IFNα2b + RBV → Peg-IFNα2a + RBV	1 m after conversion of treatment	– (+)	8.5	238	111	ACR → CR	Discontinue Peg-IFN Steroid pulse Increase CNI/MMF	Graft loss, 13 m

ACR acute cellular rejection, *AST* aspartate transaminase, *CNI* calcineurin inhibitor, *CR* chronic rejection, *GGT* gamma-glutamyl transpeptidase, *HCV* hepatitis C virus, *MMF* mycophenolate mofetil, *PCH* autoimmune hepatitis, *Peg-IFN* pegylated interferon, *RBV* ribavirin, *T.Bil* total bilirubin

Table 2 Risk factors for interferon-induced graft dysfunction

Variables	IGD		*p* value
	No ($n = 72$)	Yes ($n = 8$)	
Recipient age (years)	57.1 ± 8.8	54.8 ± 6.7	0.51
Recipient sex, male	40 (55.6)	4 (50.0)	0.70
Donor age (years)	33.9 ± 8.4	33.9 ± 9.7	0.92
Donor sex, male	50 (69.4)	4 (50.0)	0.26
Left lobe graft	38 (52.7)	2 (25.0)	0.13
Graft volume standard liver volume ratio (%)	41.4 ± 7.7	42.1 ± 2.9	0.79
Splenectomy	55 (76.4)	8 (100.0)	0.12
Tacrolimus	29 (40.3)	2 (25.0)	0.40
History of acute rejection	8 (11.1)	1 (12.5)	0.90
History of bile duct stenosis	16 (22.2)	0 (0.0)	0.14
HCV-RNA titer at LDLT (log IU/ml)	5.6 ± 0.7	5.6 ± 0.6	0.96
History of IFN before LDLT	34 (47.2)	3 (37.5)	0.60
HCV genotype 1b	60 (83.3)	7 (87.5)	0.76
Donor rs8099917 genotype, T/T	53 (73.6)	6 (75.0)	0.78
Recipient rs8099917 genotype, T/T	50 (70.4)	6 (75.0)	0.93
Time from LDLT to Peg-IFN induction (m)	6.8 ± 5.7	5.4 ± 4.5	0.42
Duration of Peg-IFN treatment (m)	20.8 ± 18.0	11.5 ± 6.9	0.15
Use of Peg-IFN-α2a (%)	19 (26.4)	6 (75.0)	<0.01
Daily ribavirin dosage (mg/day)	447 ± 235	550 ± 233	0.26
Viral response	47 (65.3)	7 (87.5)	0.20
Sustained viral response	39 (54.9)	6 (75.0)	0.27
Cumulative 5-year graft survival rate (%)	93.4	71.4	0.04

HCV hepatitis C virus, *IGD* interferon-induced graft dysfunction, *LDLT* living donor liver transplantation, *Peg-IFN* pegylated interferon

negative at the time of IGD diagnosis for all but one patient (#2). Patient #8 was found to have positive HCV-RNA a second time during use of increased immunosuppression for treatment of IGD. The mean total bilirubin level was 0.9 ± 0.3 mg/dl for patients without CR (#1–6) and 8.3 ± 0.3 mg/dl for patients with CR (#7, 8).

Treatments for IGD included discontinuation of Peg-IFN-based antiviral treatment, if used at the time, and an increase in the immunosuppression protocol, including steroids. For patients with ACR, a combination of a steroid bolus with an increase in CNI dose and re-administration of MMF was the main treatment of choice. For patient #2, a steroid bolus was not given because she had mild ACR, and she was successfully treated by increasing the CNI dose. For the patients with PCH without ACR, a steroid bolus followed by a maintenance dose had successful outcomes (#3, 4). The patients without CR (#1–6) did not experience graft loss, and five (83.3 %) of these patients, the exception being patient #2, had SVR.

Both patients with CR (#7, 8) presented with hyperbilirubinemia and an initial pathological diagnosis of ACR (#7) and PCH plus ACR (#8). At diagnosis of CR, Peg-IFN-based antiviral treatment was immediately discontinued, followed by a bolus steroid dose, increased CNI dose, and re-administration of MMF. Both patients became positive for cytomegalovirus during the treatment, and both suffered from progressive cholestasis that did not respond to treatment, resulting in the diagnosis of CR on follow-up biopsies and graft loss.

Pathological findings

The pathological findings of IGD are summarized by PCH, ACR, and CR or their combinations. The initial biopsy of patient #6 (Fig. 1) with PCH plus ACR with elevated transaminases and negative HCV-RNA during Peg-IFNα2a therapy revealed prominent aggregation of plasma cells with interface hepatitis (Fig. 1a) and portal venulitis (Fig 1b), indicating PCH plus ACR. A steroid bolus was given together with an increase in the CNI dose and re-administration of MMF. Follow-up biopsy two weeks after the initial biopsy revealed remarkable regression of plasma cell aggregation with minor remnant bile duct damage (Fig. 1c) and normal hepatocyte architecture (Fig. 1d).

The initial biopsy of patient #7 (Fig. 2), whose total bilirubin level was 8.0 mg/dl, revealed prominent plasma cell aggregation with bile duct damage and endotheliitis (Fig. 2a), indicative of PCH plus ACR. The hepatocytes also showed cellular cholestasis (Fig. 2b). One month after starting treatment, the patient's total bilirubin level was 12.9 mg/dl and a follow-up biopsy revealed a dense fibrous formation in periportal zone 1, together with the loss of bile ducts and remnant plasma cells, which was indicative of

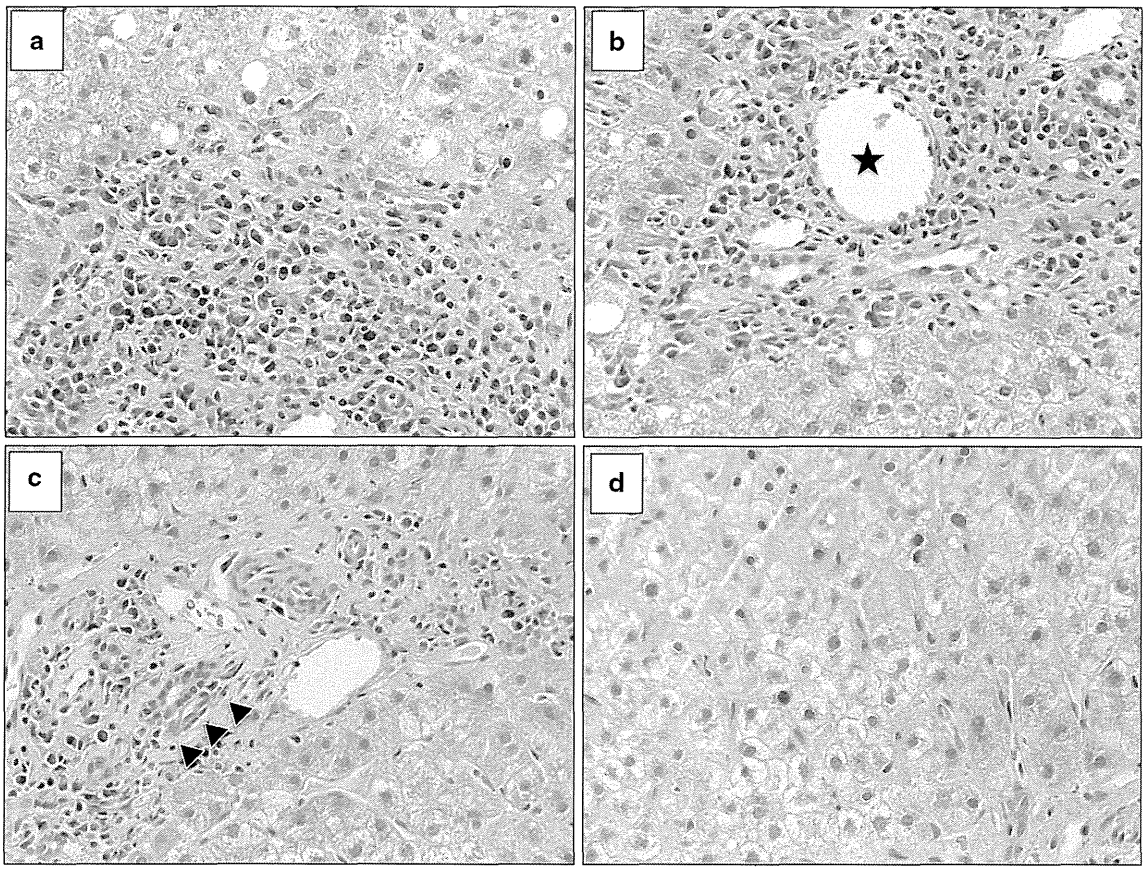

Fig. 1 Biopsies of patient #6 with plasma cell hepatitis plus acute cellular rejection taken before (**a**, **b**) and after (**c**, **d**) treatment. *Black star* portal endotheliitis; *black arrowheads* minimally damaged bile ducts

CR (Fig. 2c). Hepatocyte ballooning was observed together with cellular and canalicular cholestasis (Fig. 2d).

The initial biopsy of patient #8 (Fig. 3), whose total bilirubin level was 8.5 mg/dl, revealed prominent mixed lymphocyte aggregation and bile duct damage, indicative of ACR (Fig. 3a). Hepatic lobules with acidophilic bodies, cellular cholestasis, and lymphocyte aggregation in the Glissonean area bordered by a fibrous area, suggested progression to CR (Fig. 3b). Three weeks after starting treatment, the patient developed progressive icterus and total bilirubin increased to 17.5 mg/dl. The follow-up biopsy revealed loss of bile ducts and arteries with prominent peliosis, indicating sinusoidal obstruction in the fibrous Glissonean area, which was indicative of CR (Fig. 3c). Minor lymphocyte aggregation was also observed (Fig. 3d).

Factors associated with IGD

Univariate analysis showed that only Peg-IFN-α2a-based treatment (75 vs. 26.4 %, $p < 0.01$) was a significant risk factor for IGD. Other factors, including splenectomy, the type of CNI, rs8099917 genotype, and the duration of antiviral treatment, were not significantly associated with the occurrence of IGD. Cumulative five-year graft survival was 93.4 % for patients without IGD ($n = 72$) versus 71.4 % for patients with IGD ($p = 0.04$) (Table 2).

Discussion

IGD is a form of immune-mediated hepatitis that occurs as a complication after IFN-based antiviral treatment for hepatitis C after LT [4–6, 12–16]. Although IGD presents as ACR, PCH, or CR, or a combination of these, its etiology, pathophysiology, clinical presentation, clinical course, and treatment strategies are still unclear [5]. Recently, Levitsky et al. [4] performed the largest case–control study to date, which included 52 patients with PGD. They found that no previous history of IFN treatment, use of Peg-IFNα-2a, and PCH features in pretreatment liver biopsy are risk factors for IGD and for graft outcomes among patients with IGD, even if SVR is achieved. Notably, these findings were worse than for patients without IGD. Their most important finding was that graft survival for hepatitis C-positive grafts was significantly better than for grafts with IGD and SVR.

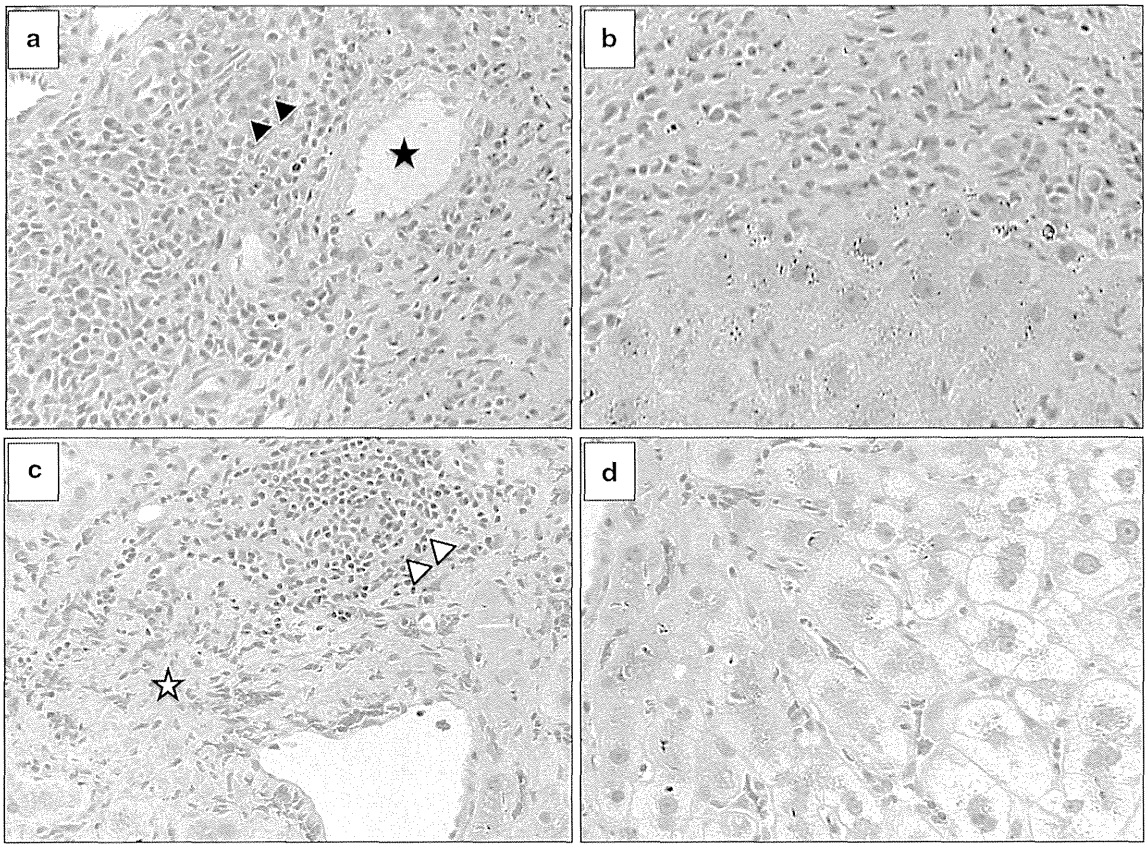

Fig. 2 Biopsies of patient #7 with plasma cell hepatitis plus acute cellular rejection taken before treatment (**a**, **b**) and at diagnosis of chronic rejection one month after starting treatment (**c**, **d**). *Black star* portal endotheliitis; *black arrowhead* minimally damaged bile duct; *white star* fibrous portal area; *white arrowhead* vanished bile ducts

Because IGD is a very serious condition that usually results in graft loss, even after achieving SVR, very aggressive treatment is essential.

In this context, an important finding of our study is that early diagnosis followed by aggressive treatment may result in favorable outcomes. In fact, we first started to recognize the presence and severity of IGD in 2007, at the time of the first reports of IGD during antiviral treatment for hepatitis C after LT [12–14]. In the mid 2,000s, transplant surgeons and hepatologists focused on adjustment or reduction of immunosuppression protocols for hepatitis C after LT, including use of a steroid-free regimen or rapid steroid tapering, to cope with aggressive hepatitis C recurrence and inevitable graft fibrosis [17–19]. In 2007, patients #7 and #8 received Peg-IFN-α2b for 48 weeks with VR, after non-early VR, and were converted to Peg-IFN-α2a-based treatment to bolster the antiviral treatment regimen and, hopefully, achieve SVR. However, because of difficulty in pathologically differentiating between IGD and recurrent hepatitis C, and considering the requirement for opposite treatments for these diseases, the common actions involved stopping IFN therapy and administering a bolus steroid dose instead [20–22]. Unfortunately, it took more than one month to start the required treatments for patients #7 and #8. We have since become more cautious of IGD and have started to treat IGD more aggressively; this has led to early treatment success in subsequent patients. In other words, we have experienced a type of learning curve in the treatment of hepatitis C after LT.

Although conversion from Peg-IFN-α2b to α2b is not a universal treatment of choice, we have tried such conversion for refractory hepatitis C after LDLT, on the basis of conjugated bulky poly(ethylene glycol) and the longer elimination half-life of Peg-IFN-α2b, with acceptable outcomes for transient responders or relapsed patients [23]. Herrine et al. [24] treated 124 non-transplant hepatitis C patients with poor response to Peg-IFN-α2b-based treatment, resulting in SVR for 37 % of patients after conversion to Peg-IFN-α2a with RBV. Nevertheless, the results from our series suggest that conversion for patients with VR on Peg-IFN-α2b increases the risk of IGD.

In our series, splenectomy was performed on 78.7 % of patients, and a clear association between splenectomy and IGD was not observed. Since 2005 when Peg-IFN became available in Japan, we started to perform splenectomy aggressively to prevent pancytopenia during antiviral

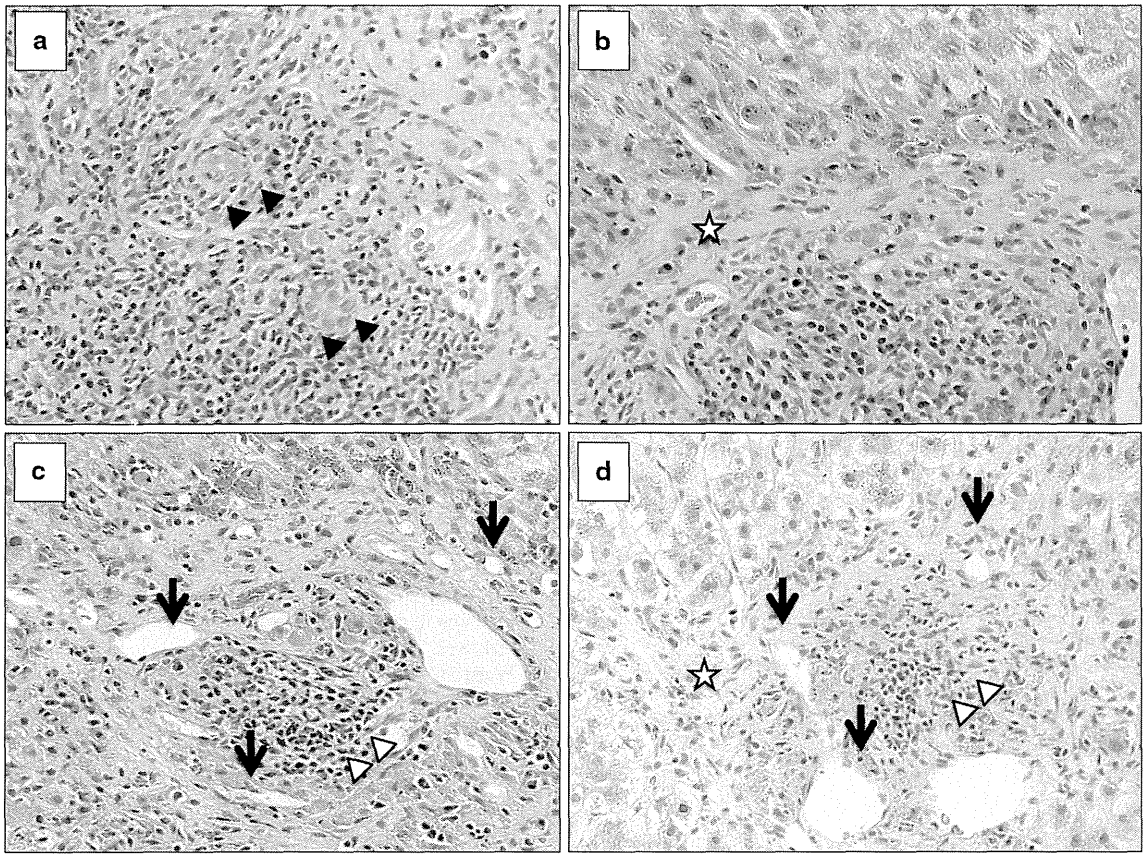

Fig. 3 Biopsies of patient #8 with acute cellular rejection before starting treatment (**a**, **b**) and at diagnosis of chronic rejection three weeks after starting treatment (**c**, **d**). *Black arrow* portal peliosis; *black arrowhead* minimally damaged bile duct; *white star* fibrous portal area; *white arrowheads* vanished bile ducts

treatments for hepatitis C after LDLT. We reported that splenectomy improved SVR among patients carrying rs8099917 minor haplotypes and protected against anemia and thrombocytopenia during the course of Peg-IFN therapy [25].

Among the three histological types of IGD, PCH is the most common. In the report by Letvitsky et al. [4], 86.5 % of patients with IGD had plasma cell infiltration, although they defined PCH as plasma cells accounting for >30 % of infiltrated cells. However, IGD seems to differ from pure de-novo autoimmune hepatitis and could be a form of rejection [15]. Fiel et al. [15] reported that in 82 % of their patients PCH developed after recent lowering of maintenance immunosuppression (47 %) or subtherapeutic CNI levels (35 %). Therefore, the rejection process might target hepatocytes in PCH, which is characterized by interface hepatitis and centrilobular hepatitis. In many studies [12–16], outcomes were significantly worse for patients with PCH than for patients without PCH. In contrast, ACR or CR, which are less frequent forms of IGD, could be diagnosed on the basis of conventional pathological findings, including mixed lymphocyte aggregation and damage to biliary epithelial and endothelial cells for ACR, and ductopenia with or without damage to the small arteries for CR [11]. Thus, because IGD is a combination of these pathologic categories and findings, the pathological features of IGD could be summarized as plasma cell-rich immune cell infiltrates with or without biliary or endothelial cell damage, with the possibility of progressing to bile duct loss.

Discontinuation of IFN and an increase in CNI dose without the use of steroids could be recommended for treating IGD in HCV-positive patients, because steroid use could be followed by an outbreak of HCV, as reported by Fiel et al. [15]. However, a greater proportion of patients with IGD may be negative rather than positive for HCV [13], as in the current series. Merli et al. [6] hypothesized that loss of the therapeutic target (i.e., HCV antigen) for the immune system may lead to increased sensitivity of aberrant antigens to the transplanted graft. If IGD is diagnosed when HCV is negative, the treatment is relatively straightforward and involves discontinuation of IFN, administration of a bolus steroid dose, and an increase in the CNI dose, as in the treatment of ACR. It is imperative that hepatologists realize that IGD becomes irreversible once cholestasis emerges with features of CR. Therefore, early recognition, diagnosis, and treatment are crucial. Moreover, use of a stable and more effective

immunosuppression protocol during induction and maintenance of IFN treatment of hepatitis C after LT is an important strategy to prevent the onset of IGD during IFN, and ensure there is a simple and safe method of treating recurrent hepatitis C.

In conclusion, IGD is a serious condition that may occur during or even after antiviral treatment for hepatitis C after LDLT. Early recognition, diagnosis, discontinuation of IFN, and introduction of a steroid-based treatment could help to save the graft.

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Compliance with ethical requirements and Conflict of interest All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients before inclusion in the study. No identifying information is included in this article. Toru Ikegami, Huanlin Wang, Takeo Toshima, Ken Shirabe, Tomoharu Yoshizumi, Shinichi Aishima, Takasuke Fukuhara, Norihiro Furusyo, Kazuhiro Kotoh, Shinji Shimoda, and Yoshihiko Maehara declare that they have no conflict of interest.

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ORIGINAL ARTICLE

Clinical Outcomes of Living Donor Liver Transplantation for Patients 65 Years Old or Older With Preserved Performance Status

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The purpose of this study was to determine the outcomes of living donor liver transplantation (LDLT) for elderly recipients. We reviewed 411 adult-to-adult LDLT cases, including 46 recipients who were 65 years old or older and 365 recipients who were less than 65 years old. The elderly group had a higher proportion of females ($P = 0.04$) and a smaller body surface area ($P < 0.001$) and more frequently underwent transplantation because of hepatitis C ($P < 0.001$) or hepatocellular carcinoma ($P < 0.001$). Elderly patients had less advanced liver disease with lower Model for End-Stage Liver Disease (MELD) scores ($P = 0.02$) and preserved health without the need for prolonged hospitalization ($P < 0.01$). The transplanted graft volume/standard liver volume ratios were similar for the 2 groups ($P = 0.22$). The elderly group had fewer episodes of acute rejection ($P = 0.03$) but had more neuropsychiatric complications ($P = 0.01$). The 5- and 10-year graft survival rates were comparable for the elderly group (89.8% and 77.8%, respectively) and the younger group (79.4% and 72.9%, respectively; $P = 0.21$). Seven recipients were 70 years old or older, and they had a mean MELD score of 15.6 ± 5.2; 6 of these patients were treated as outpatients before LDLT. All were alive after LDLT and showed good compliance with medical management with a mean follow-up of 5.7 ± 3.0 years. In conclusion, LDLT can be safely performed and has acceptable long-term outcomes for low-risk elderly recipients with preserved performance status. ***Liver Transpl 20:408-415, 2014.*** **© 2014 AASLD.**

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The aging of the general population and advances in the medical management of chronic liver diseases have resulted in an increase in life expectancy,[1] and this in turn has resulted in an increase in the number of elderly patients possibly eligible for liver transplantation (LT).[2] Although we have previously reported that recipients who are 60 years old or older can have acceptable outcomes, the recent increase in transplant candidates who are 65 to 70 years old is remarkable as reported by the National Center for Health Statistics.[3] Previous reports concerning deceased donor liver transplantation (DDLT) have indicated that advanced age itself is not a contraindication, but there might be issues with DDLT for high-risk recipients, who may have very poor outcomes.[5]

In Japan, the predominant mode of LT for patients with liver diseases is living donor liver transplantation (LDLT), even though deceased donor organ transplantation was legalized in 1997 (with revisions in the eligibility criteria made in 2009).[6] There are 2 major differences between LDLT and DDLT: the first is that LDLT does not cut down the public donor source for DDLT, and the other is ethical issues in LDLT associated with the surgical risk for healthy donors.[7,8] Therefore, LDLT for elderly

Abbreviations: ACR, acute cellular rejection; DDLT, deceased donor liver transplantation; GV, graft volume; HCC, hepatocellular carcinoma; LDLT, living donor liver transplantation; LT, liver transplantation; MELD, Model for End-Stage Liver Disease; SLV, standard liver volume.

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