Table 4. Overview of nucleotide 1896, 1762, and 1764 sequencing data with the deep sequencing analyses.

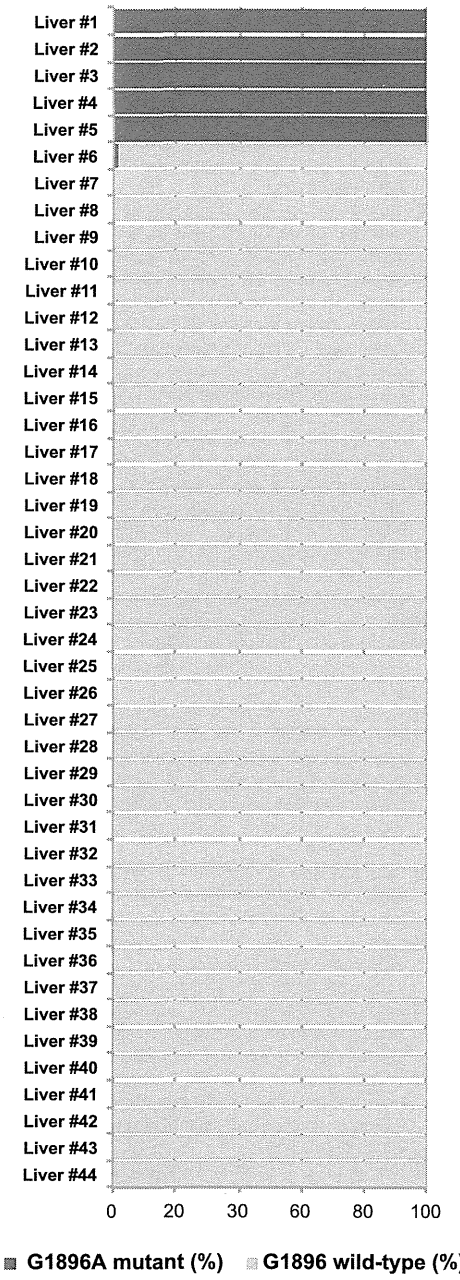
Case	G1896A		A1762T		G1764A	
	Base counts	(%)	Base counts	(%)	Base counts	(%)
Reactivation from occult HBV carrier status						
#1	1/10,833	(0.0)	0/6391	(0.0)	1/6491	(0.0)
#2	1/10,200	(0.0)	0/9213	(0.0)	3/9216	(0.0)
#3	8/27,694	(0.0)	1/16,506	(0.0)	4/16,851	(0.0)
#4	4/13,008	(0.0)	2/12,007	(0.0)	0/11,857	(0.0)
#5	0/6860	(0.0)	0/6175	(0.0)	0/6307	(0.0)
#6	273/31,622	(0.9)	8/29,996	(0.0)	4/30,400	(0.0)
#7	22/12,561	(0.2)	0/3405	(0.0)	1/3492	(0.0)
#8	1/11,500	(0.0)	0/4964	(0.0)	1/5089	(0.0)
#9	12,897/12,904	(100)	11,676/11,677	(100)	11,653/11,659	(100)
#10	11,432/11,444	(100)	1/6153	(0.0)	2/6217	(0.0)
#11	9533/9539	(99.9)	7669/7671	(100)	7681/7685	(99.9)
#12	10,944/10,945	(100)	2/10,874	(0.0)	1/11,325	(0.0)
#13*	9358/9411	(99.4)	2/10,900	(0.0)	0/11,298	(0.0)
#14*	11,174/11,179	(100)	0/6579	(0.0)	2/6773	(0.0)
Reactivation from HBsAg carrier status						
#15	734/12,544	(5.9)	7593/7596	(100)	7556/7570	(99.8)
#16	2/7469	(0.0)	0/6481	(0.0)	2/6618	(0.0)
#17	12,251/12,701	(96.5)	5110/5241	(97.5)	5180/5239	(98.9)
#18	9649/9660	(99.9)	0/10,026	(0.0)	0/10,069	(0.0)
#19	18,402/18,413	(99.9)	1/15,677	(0.0)	3/16,045	(0.0)
#20*	11,158/11,160	(100)	0/6671	(0.0)	3/6929	(0.0)

*Patients who developed fatal acute liver failure.

malignancy, we observed two patients without hematological malignancies who developed HBV reactivation. One case had colon cancer, with S-1 treatment triggering HBV exacerbation. Another case had psoriasis and received cyclosporine before the onset of HBV reactivation. Previously, we also reported a case of lethal *de novo* HBV hepatitis induced by adalimumab treatment for rheumatoid arthritis [26]. Thus, it is important to note that there is a risk of HBV reactivation in patients not only with hematological malignancies but also with solid tumors or non-cancerous diseases undergoing chemotherapy or immunosuppressive therapy. In addition, it is very important to regularly monitor HBV DNA levels to achieve the early administration of ETV before the onset of ALT elevation; however, the optimum frequency of HBV DNA testing in occult HBV carriers is not yet defined. A recent prospective study suggested that monthly monitoring of HBV DNA levels for lymphoma patients with resolved HBV infection might be a reasonable option during and after rituximab-CHOP chemotherapy [27].

To clarify the virological characteristics of HBV reactivation, we determined the genetic heterogeneity of viruses from patient sera. We found that the genetic complexity of the reactivated viruses in 14 patients with reactivation from occult HBV infection was significantly lower than that in six patients with reactivation from HBsAg carriers. There was no significant difference in circulating HBV DNA levels in serum after reactivation in both groups. The viral population in the sera of patients with reactivation from occult HBV infection was characterized by low heterogeneity, with nearly monoclonal viruses detected. We further examined the genetic complexity of latently infected HBV in the liver of 44 individuals with occult HBV infection. We found that the genetic heterogeneity of latently infected viruses in their livers was also very low. In one case we confirmed that the viral genome detected in serum after viral reactivation was almost identical to that in the latently infected liver before reactivation. These findings possibly suggest that the viral population in latently infected livers of occult HBV carriers is characterized by low heterogeneity, and the predominant viral clone increases in number under immunosuppressive conditions. The reason for the difference in the degree of genetic heterogeneity in the exacerbated viruses between patients with reactivation from occult infection and those with HBsAg carrier reactivation is unclear. One possibility is that the low levels of viral heterogeneity observed in occult HBV carriers are due to the relatively lower levels of viral replication compared with those of HBsAg carriers. Pollicino *et al.* demonstrated that the host immune system, not viral factors, likely plays a critical role in the strong suppression of viral replication and gene expression [28]. Since we could confirm the genetic homology of HBV DNA in the liver before reactivation and the serum after reactivation in only one case, further studies are required to determine the characteristics of the latent viruses in HBsAg-negative but anti-HBc-positive occult HBV carriers.

In this study, we found that 42.9% of cases that experienced HBV reactivation predominantly contained the G1896A pre-C variant in their sera. Infection with the G1896A variant was predominant in the liver of 11.4% of individuals with occult HBV infection. Patients acutely infected with the HBV G1896A pre-C variant have a high risk of developing ALF [16–18]. The G1896A variant is frequently detected in reactivated viruses in patients with reactivation from occult HBV infection that develop ALF [20]. We revealed that both patients who developed fatal ALF predominantly contained G1896A pre-C variants. The mechanism by which the G1896A mutation triggers the development of ALF



Ratio of G1896A mutant (%)	Number of G1896A mutant/total nucleotides
100	61,007/61,007
100	37,772/37,772
100	28,522/28,522
100	31,058/31,058
100	22,305/22,306
1.65	603/36,540
0.04	25/65,560
0.01	4/34,524
0	0/6250
0	0/3713
0	0/28,607
0	0/19,565
0	0/30,432
0	0/25,118
0	0/44,475
0	0/18,628
0	0/56,581
0	0/33,525
0	0/65,535
0	0/27,574
0	0/38,029
0	0/28,124
0	0/34,889
0	0/38,163
0	0/22,696
0	0/41,092
0	0/35,525
0	0/27,640
0	0/48,424
0	0/27,096
0	0/35,044
0	0/45,925
0	0/55,458
0	0/28,405
0	0/40,664
0	0/65,535
0	0/32,852
0	0/39,434
0	0/28,938
0	0/16,115
0	0/19,120
0	0/49,353
0	0/37,564
0	0/24,832

Fig. 3. Prevalence of G1896A pre-core mutants in the liver of 44 healthy occult HBV carriers. The ratio of G1896A mutants to wild-type G1896 for total reads is shown in the left panel. The number of G1896A mutants, total reads at nucleotide position 1896, and the proportion of G1896A mutants (%) are shown in the right panel. (This figure appears in colour on the web.)

remains unknown at present. Previous studies reported that the G1896A variant has increased replication activity compared with the wild-type strain in vitro [18,29], but we found no significant association between the levels of circulating HBV DNA and the ratios of wild-type/G1896A pre-C mutants in cases with reactivation from occult HBV infection. On the other hand, it is well recognized that HBeAg/anti-HBe serostatus is closely associated with the ratios of wild-type/G1896A pre-C mutants in patients with chronic HBV infection [30]. Interestingly, accumulating evidence suggests that G1896A mutations abrogating HBeAg synthesis remove the tolerogenic effect of HBeAg, leading to an enhanced immune response that contributes to ALF development [31]. We must also pay attention to the genotype of HBV in cases with viral reactivation. Among the 14 cases with reactivation from occult HBV infection, genotype B and C strains were detected in five and nine patients, respectively. Among them, three of five cases were negative for HBeAg but positive for anti-HBe (60%) in genotype B and three of nine (33.3%) were genotype C-infected patients, and both cases with developing ALF were negative for HBeAg and infected with genotype B.

Research Article

Previous studies demonstrated that HBV genotypes affect the liver disease outcome [32], and genotype B strain is frequently detected in patients developing ALF [18]. Thus, it is possible that the ratios of wild-type/G1896A pre-C mutants and viral genotype influence the pathophysiology of viral reactivation.

In conclusion, our findings suggest that HBV reactivation can occur during and after termination of chemotherapy or immunosuppressive therapy in occult HBV carriers with underlying hematological malignancies, solid tumors or non-cancerous diseases. Occult HBV infection and the resulting HBV reactivation is characterized by low genetic heterogeneity. It is unclear whether occult HBV carriers with the G1896A pre-C variant have an increased risk of developing HBV reactivation and fatal ALF. Further analysis with a larger cohort of patients is required to clarify the frequency and mechanisms of HBV reactivation and ALF in patients with occult HBV carrier status receiving chemotherapy or immunosuppressive therapy.

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Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Authors' contribution

Conceived and designed the experiments: TI, HM. Performed the experiments: TI, HM, HM. Analyzed the data: TI, YF, HM. Contributed reagents/materials/analysis tools: TI, YU, MU, TK, YO, SU, HM, TC. Wrote the paper: TI, YU, HM, TC.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhep.2014.04.033.

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Chronic Rejection Associated with Antiviral Therapy for Recurrent Hepatitis C after Living-Donor Liver Transplantation

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Background. Chronic rejection (CR) has been reported to be associated with antiviral therapy for recurrent hepatitis C in liver transplant (LT) recipients. The aims of this study were to clarify the details of antiviral therapy-associated CR after living-donor liver transplantation (LDLT) and to identify the factors associated with CR.

Methods. A retrospective chart review was performed on 125 recipients who had received antiviral therapy for recurrent hepatitis C after LDLT between January 2001 and September 2012. The characteristics of patients who developed CR during or within 6 months after antiviral therapy were compared with those of 76 patients who did not develop CR despite receiving antiviral therapy for more than 1 year.

Results. Seven of 125 (6%) patients developed CR during or within 6 months after the end of antiviral therapy. CR was diagnosed after a median (range) of 9 (1–16) months of antiviral therapy. In five patients, rejection progressed rapidly and resulted in death within 3 months after diagnosis. Analysis revealed two significant factors associated with CR: reduction of the immunosuppressant dose during antiviral therapy and a low fibrosis score as the indication for antiviral therapy.

Conclusions. CR developed in association with antiviral therapy for recurrent hepatitis C after LDLT. This complication may be prevented by ensuring that the immunosuppressant dose is not reduced during antiviral therapy.

Keywords: Chronic rejection, Hepatitis C, Liver transplantation, Living donor, Antiviral therapy.

(*Transplantation* 2013;00: 00–00)

Hepatitis C virus (HCV) infection, which leads to liver cirrhosis and hepatocellular carcinoma, is the most common indication for liver transplantation (LT) in Japan, the United States, and western Europe. Most patients who undergo LT for HCV-related liver disease develop recurrent viral infection, and 70% to 90% suffer from histologically proven recurrent hepatitis (*1–6*). The progression of recurrent hepatitis C is often rapid. Without appropriate antiviral therapy, 10% to 25% of patients develop cirrhosis within 5 years after transplantation, and this explains the relatively poor prognosis for HCV-positive recipients compared with HCV-negative recipients (*7*). Interferon (IFN)–based combination therapy is commonly administered to prevent the progression of hepatitis C after LT (*8, 9*), but its efficacy in LT recipients is limited. The mean (range) sustained virologic response (SVR) rate in patients with recurrent hepatitis C after LT is only 30% (8%–50%) (*10*). One reason for the low SVR rate is the high rate of treatment withdrawal, particularly because of the unique adverse effects of IFN therapy for transplant recipients, including chronic rejection (CR) (*11, 12*).

CR is characterized by progressive ductopenia, with atrophy and loss of the bile ducts in the portal tracts and by arteriopathy with foamy cell infiltration (*13–15*). A cholestatic liver enzyme pattern suggests the diagnosis of CR. If bile duct enlargement and/or hepatic artery changes are excluded by imaging studies as potential causes of abnormal liver function tests, then CR is confirmed or excluded by liver biopsy examination. The incidence of CR after LT is

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Y.U. participated in the research design, researched relevant data, performed the data analysis, and wrote the article. T.K., T.I., K.O., A.Y., Y.F., A.M., A.M.-H., and H.M. researched relevant data. H.H., T.C., and S.U. participated in the research design and article review.
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approximately 3% to 5%. This event does not simply represent end-stage acute cellular rejection (ACR), although the two may be temporally related. The pathogenesis of CR is not completely understood, although its association with donor-specific human leukocyte antigen antibodies was recently reported (*16*). Additional immunosuppressive therapy is unlikely to be beneficial for CR patients, particularly those with late disease in which bile duct loss affects more than 50% of the portal tracts, and retransplantation is required (*15*).

Several studies have suggested an association of CR with IFN-based antiviral therapy (*17–20*). Two recent reports found that CR was associated with antiviral therapy for recurrent hepatitis C after LT (*11, 12*). Stanca et al. (*12*) reported that 12 of 70 LT recipients with HCV infection treated with pegylated IFN (peg-IFN) and ribavirin developed CR. Their study indicated that ACR and CR are not strongly associated and that CR progresses rapidly, terminating in graft failure. Fernandez et al. (*11*) reported that 7 of 79 (9%) patients developed CR during antiviral therapy. They found that the use of cyclosporine in immunosuppression therapy, achievement of an SVR, and ribavirin discontinuation were factors associated with CR development.

Although the details of patients with antiviral therapy-associated CR after deceased-donor liver transplantation (DDLT) have been reported (*11, 12*), no study of antiviral therapy-associated CR in patients receiving living-donor liver transplantation (LDLT) has been published thus far. The features specific to LDLT, including blood-relative donors, posttransplantation liver regeneration, and ABO-incompatible LT, might result in characteristic differences between LDLT and DDLT patients.

We aimed to clarify the details of antiviral therapy-associated CR after LDLT and to identify the factors associated with CR.

RESULTS

Patient Characteristics and Treatment Outcomes

The study included 125 HCV-infected LT patients treated with standard IFN and/or peg-IFN in combination with ribavirin for recurrent hepatitis C after LDLT. Of these, 69 (55%) were men (median [range] age at the beginning of therapy, 57 [32–70] years). Most patients were infected with HCV genotype 1b (n=101 [81%]). The HCV genotype for the remaining patients was 2a (n=14), 2b (n=6), 3a+3b (n=1), and indeterminate (n=2). Genotype was not examined in one patient. The median (range) serum HCV RNA load at the beginning of antiviral therapy after LDLT was 3980 (31 to <69,000) kIU/mL. The median (range) donor age was 42 (19–65) years. Seventy-three (58%) donors were men, and 84 (67%) were blood relatives of the recipients. The graft type was the right lobe for 108 (86%) patients and the left lobe for 17 (14%) patients. The blood type combination was incompatible for 27 (22%) patients. Thirty-six (29%) patients had histologically diagnosed ACR before antiviral therapy, 16 of whom had moderate or severe ACR. No patient had shown ACR findings in the liver biopsy examination immediately before antiviral therapy. The median (range) time to treatment initiation after LDLT was 8.9 (1.1–72.4) months. Before treatment, necroinflammatory activity of levels A1, A2, and A3 based on the METAVIR score was found in 82 (66%), 40 (32%), and 3 (2%) patients, respectively. Fibrosis scores of F0, F1, F2, and F3 were found in 19 (15%), 82 (66%), 19 (15%), and 5 (4%) patients, respectively. Tacrolimus-based immunosuppression was administered to 117 (94%) patients and cyclosporine was administered to 7 (6%) patients. Mycophenolate mofetil (MMF) without calcineurin inhibitor (CNI) was administered to one patient because of renal failure at the beginning of antiviral therapy. In the patients who received tacrolimus, the mean (range) serum trough level at therapy initiation was 6.2 (2.0–12.7) ng/mL. In addition to CNIs, MMF and prednisolone were administered at the start of the antiviral treatment to 39 (31%) and 21 (17%) patients, respectively.

Of the 123 patients in whom the final treatment outcomes could be evaluated, 54 (44%) patients achieved SVR, 12 (10%) relapsed, 30 (24%) were nonresponders, and 27 (22%) withdrew from treatment. The remaining two patients were still undergoing treatment during the analysis.

Characteristics of Patients with Antiviral Therapy-Associated CR

Seven of 125 (6%) patients developed CR during or within 6 months after the end of antiviral therapy. The characteristics and clinical courses of these seven patients are shown in Table 1. Although four patients had a history of ACR before antiviral therapy was initiated (three of whom had moderate or severe ACR), three had no previous ACR episodes. The METAVIR score-based fibrosis level before antiviral therapy was F0 in three of the seven patients, F1 in three patients, and F2 in one patient, indicating that the antiviral therapy had been initiated at an early stage of fibrosis. The median (range) time from transplantation to initiation of antiviral therapy in these seven recipients was 9 (2–72) months. Tacrolimus was administered to five patients and cyclosporine was administered to one patient when the antiviral therapy was initiated. One patient did not receive a CNI because of renal failure (patient 7). Four patients received MMF, and one patient received prednisolone in combination with tacrolimus and MMF. The trough levels of tacrolimus and cyclosporine were within the therapeutic range. Standard amounts of immunosuppressant were therefore used for all patients, except for patient 7 who received MMF only. Immunosuppressant doses were reduced during therapy in five of seven patients. The tacrolimus dose was reduced for two patients (patients 2 and 3), as a result of which the blood trough level of tacrolimus decreased by approximately 2 ng/mL. In patient 3, MMF (500 mg/day) was also stopped during treatment. In patient 4, the MMF dose was reduced from 1000 to 250 mg per day, and prednisolone treatment (2.5 mg/day) was also terminated during treatment. In patient 5, MMF (1000 mg/day) was stopped immediately after initiation of antiviral therapy. Patient 6 received no CNI, and MMF dose was reduced from 500 to 250 mg per day during treatment. Three patients received standard IFN, and four received peg-IFN. Ribavirin was not administered to three patients immediately before the diagnosis of CR because of anemia.

CR was diagnosed after a median (range) of 9 (1–16) months of antiviral therapy. Two patients were diagnosed

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TABLE 1. Characteristics of patients with CR associated with antiviral therapy

Patient	1	2	3	4	5	6	7
Age (years)	62	41	45	67	50	59	49
Gender	Female	Male	Female	Female	Female	Male	Male
ABO mismatch with donor	Match	Match	Match	Mismatch	Match	Mismatch	Match
Relation to donor	Related	Related	Nonrelated	Related	Nonrelated	Nonrelated	Nonrelated
Graft type (lobe)	Right	Right	Right	Right	Left	Right	Right
Splenectomy	No	No	No	No	Yes	Yes	No
Previous ACR	Yes	Yes	Yes	No	Yes	No	No
Previous moderate/severe ACR	Yes	No	Yes	No	Yes	No	No
Previous steroid pulse	Yes	No	No	No	Yes	No	No
HCV genotype	1b	1b	1b	2a	1b	1b	1b
HCV RNA (kIU/mL) before IFN	>850	3620	1790	>5000	>5000	>5000	16,000
METAVIR score before IFN	A2 F2	A2 F0	A1 F0	A1 F1	A2 F1	A1 F0	A1 F1
Months from LT to IFN	13	2	5	13	7	9	72
Months from initiation of IFN to diagnosis of CR	9	1	16	10	15	8	7
Immunosuppressant at initiation of IFN	Tacrolimus	Tacrolimus,	Tacrolimus, MMF	Tacrolimus, MMF, PSL	Cyclosporine, MMF	Tacrolimus, MMF	MMF
Trough level of CNI	7.8	7.9	7.9	6.8	152	5.9	—
Reduction of immunosuppressant during IFN (reduced drugs)	No	Yes (tacrolimus)	Yes (tacrolimus, MMF)	Yes (MMF, PSL)	Yes (MMF)	No	Yes (MMF)
Type of IFN	Standard	Standard	Standard	Pegylated	Pegylated	Pegylated	Pegylated
Ribavirin discontinuation	No	No	Yes	Yes	No	No	Yes
IFN at diagnosis of CR	On treatment	On treatment	1 month after end of IFN	5 months after end of IFN	On treatment	On treatment	On treatment
At diagnosis of CR							
Liver biopsy	Foam cell arteriopathy, bile duct atrophy	Bile duct atrophy	Bile duct atrophy	Bile duct atrophy, bile duct loss	Bile duct atrophy, bile duct loss	Bile duct atrophy, bile duct loss	Foam cell arteriopathy, bile duct atrophy
AST (IU/L)	121	90	53	73	331	124	36
ALT (IU/L)	67	37	43	63	288	52	32
ALP (IU/L)	2034	906	494	1751	2143	528	1164
γ-GTP (IU/L)	561	768	155	209	515	27	1489
Bilirubin (mg/dL)	18.6	18.8	31.5	38.1	11.8	16.4	22.6
HCV RNA (kIU/mL)	Undetectable	460	Undetectable	Undetectable	16,000	Undetectable	0.40
Treatment for CR	Tacrolimus, MMF	Tacrolimus	Tacrolimus, steroid pulse, MMF	Tacrolimus, MMF, PSL	Tacrolimus, MMF, rapamycin, steroid pulse	Tacrolimus, steroid pulse, MMF	Tacrolimus, MMF, rapamycin, steroid pulse
Outcome	Died	Alive	Died	Died	Died	Died	Died
Months from diagnosis of CR to death	64	—	1	1	1	3	1

ALT, alanine aminotransferase; AST, aspartate aminotransferase; PSL, prednisolone.

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with CR after antiviral therapy was terminated. Antiviral therapy was discontinued in the remaining five patients. Of note, six patients were treated with IFN for more than 7 months, suggesting that long-term administration of IFN is associated with CR. Liver biopsy was performed for diagnosis of CR because of abnormal liver function tests in all cases. All patients with documented CR had high levels of alkaline phosphatase (ALP). Total bilirubin levels were extremely high (11.8–38.1 mg/dL) at diagnosis, suggesting a delayed diagnosis of CR. All liver biopsies showed atrophy affecting most bile ducts as well as hepatocanalicular cholestasis. Two patients (patients 1 and 7) showed foam cell obliterative arteriopathy. Bile duct loss was shown in 100%, 67%, and 20% of the portal tracts in patients 4, 5, and 6, respectively. In none of the seven patients was evidence of ACR found in the biopsy specimens. Hepatic artery or biliary tract obstruction or structuring was excluded by imaging in all patients.

Serum HCV RNA was undetectable in four patients at CR diagnosis and remained undetectable in all four patients during the follow-up period. Two of the four patients were considered to have SVR. Final outcomes could not be determined in the remaining two patients who died within 24 weeks after termination of treatment.

Various intensive treatment protocols were used for these seven patients after CR diagnosis, including increase of tacrolimus dose, addition or increase in MMF and/or prednisolone dose, administration of steroid pulse therapy, and inclusion of rapamycin in the therapy. CR progressed rapidly to liver failure in five patients (patients 3–7). These five patients died within 3 months after diagnosis of CR due to liver failure and infection. The liver damage in patient 1 gradually progressed to liver failure, and the patient died at 64 months after CR was diagnosed. Only one patient (patient 2) recovered from CR and survived, although a follow-up liver biopsy showed chronic hepatitis C.

Risk Factors of CR Associated with Antiviral Therapy

Factors associated with the development of CR during and after antiviral therapy were analyzed by comparing the features of 7 CR patients with those of 76 patients who did not develop CR despite receiving antiviral therapy for more than 1 year (Table 2). A reduction of the immunosuppressant dose during antiviral therapy (P=0.034) and a low fibrosis stage before antiviral therapy (P=0.045) were significantly associated with antiviral therapy-related CR. No significant associations were found with other variables, including donor factors, ribavirin discontinuation, and undetectable HCV RNA. The rate of previous ACR (P=0.065), rate of previous moderate or severe ACR (P=0.059), ALP level (P=0.121), and γ-glutamyl transpeptidase (γ-GTP) level (P=0.051) before antiviral therapy was higher in the patients who developed CR, but the differences from patients without CR were not significant.

DISCUSSION

Of the 125 patients, 7 (6%) who received antiviral therapy for hepatitis C after LDLT developed CR. CR progressed rapidly, resulting in death within 3 months after diagnosis, in 5 of these 7 patients.

The risk of rejection have been suggested to increase with IFN administration because of the drug's theoretical immunomodulatory actions, such as up-regulation of human leukocyte antigen class II antigens and induction of proinflammatory cytokines (*21*). Previous studies have reported that the frequency of CR in patients who received IFN was substantially higher compared with patients who did not receive antiviral therapy (*11, 12, 17*). In the present study, the rate of antiviral therapy-associated CR was 6%. This rate is high, because no CR occurred in the entire study period other than during or within 6 months after termination of antiviral therapy in the 230 HCV-positive recipients analyzed. Some cases showed sudden onset of CR after a long transplantation period in the absence of preexisting ACR, supporting the association of antiviral therapy with CR.

In our analysis, the two significant risk factors for CR were reduction of the immunosuppressant dose during antiviral therapy and low fibrosis score at antiviral therapy initiation. Additional characteristics associated with CR were elevated cholestatic enzyme levels at the time of diagnosis, onset of CR more than 7 months after treatment initiation (excluding one patient) and poor prognosis after the diagnosis. The MMF dose was reduced or stopped during antiviral therapy in four of five patients who had received MMF at the start of the treatment. We had initially tried to reduce the MMF dose during antiviral therapy, because MMF is known to suppress the bone marrow and could therefore augment the cytopenic effects of IFN and ribavirin. We had reduced immunosuppressant according to our reduction protocol even during antiviral therapy. Based on the data, we subsequently changed our strategy to maintaining the MMF dose and increasing the trough level of CNIs during antiviral therapy. The reason for the association between the low fibrosis score and CR is currently unclear. Although some institutions recommend early introduction of antiviral therapy (*8, 9*), our data suggest that antiviral therapy should not be administered to patients with no or mild fibrosis. On the contrary, it is reported that tolerance to therapy decreases significantly in patients with a fibrosis stage ≥3 on baseline liver biopsy (*22*). Therefore, the antiviral therapy should be initiated in patients with a fibrosis stage 2, as the recent review articles recommended (*23, 24*).

All our patients underwent LDLT, but no characteristics specific to LDLT, including blood-relative donors, graft size, and ABO incompatibility, were identified as risk factors for CR in our study. This appears to indicate that LDLT and DDLT patients do not differ with respect to antiviral therapy-associated CR.

Early diagnosis of CR, as well as prevention, is important for ensuring improved outcomes in LT recipients. CR was diagnosed in our patients after liver damage had already progressed. Histologic diagnosis of CR was difficult in all these cases, despite repeated liver biopsy examination. However, all the patients had elevated ALP and γ-GTP levels before jaundice was observed. CR should therefore be suspected when a cholestatic liver enzyme pattern develops during antiviral therapy for hepatitis C. When imaging has excluded large bile duct and/or hepatic artery changes as the

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TABLE 2. Risk factors for CR

	CR (n=7)	No CR (n=76)	P
Age at LT (years)	50 (41−67)	56 (36−69)	0.506[a]
Gender, male/female	3/4	44/32	0.352[b]
HCV genotype, 1/non-1	6/1	71/5	0.421[b]
Donor age at LT (years)	46 (28−60)	42 (21−65)	0.857[a]
Donor gender, male/female	4/3	40/36	0.568[b]
Sex mismatch, match/mismatch	0/7	26/50	0.064[b]
ABO mismatch, match/mismatch	5/2	59/17	0.507[b]
Relation to donor, related/nonrelated	3/4	48/28	0.254[b]
HLA-A matched number, 0/1/2/unknown	0/5/2/0	13/44/16/3	0.332[a]
HLA-B matched number, 0/1/2/unknown	2/4/1/0	21/47/5/3	0.778[a]
HLA-DR matched number, 0/1/2/unknown	3/3/1/0	18/47/8/3	0.487[a]
Graft type, left lobe/right lobe	1/6	9/67	0.608[b]
Splenectomy, yes/no	2/5	38/38	0.247[b]
Previous ACR, yes/no	4/3	17/59	0.065[b]
Previous moderate/severe ACR, yes/no	3/4	9/67	0.059[b]
Previous steroid pulse therapy, yes/no	2/5	8/68	0.198[b]
Months from LT to therapy	9.0 (1.8−72.4)	9.1 (2.2−68.8)	0.694[a]
Valuables at initiation of IFN			
Age (years)	55 (41−68)	57 (37−70)	0.599[a]
CNI tacrolimus/cyclosporine	5/1	71/5	0.376[b]
Trough level for tacrolimus (ng/mL)	7.3 (0−7.9)	6.2 (2.6−10.9)	0.641[a]
AST (IU/L)	68 (24−464)	76 (21−331)	0.908[a]
ALT (IU/L)	88 (25−354)	79 (20−392)	0.842[a]
ALP (IU/L)	878 (283−2977)	462 (168−2818)	0.121[a]
γ-GTP (IU/L)	317 (48−1623)	112 (15−1704)	0.051[a]
Bilirubin (mg/dL)	0.8 (0.3−10.4)	0.9 (0.3−4.6)	0.861[a]
Activity grade, A1/A2/A3	4/3/0	50/24/2	0.693[a]
Fibrosis stage, F0/F1/F2/F3	3/3/1/0	4/56/13/3	0.045[a]
Reduction of immunosuppressant during IFN, yes/no	5/2	22/54	0.034[b]
Ribavirin discontinuation during IFN, yes/no	3/4	26/50	0.468[b]
Undetectable HCV RNA during IFN, yes/no	4/3	51/25	0.439[b]

[a] Wilcoxon rank-sum test.
[b] Chi-square test.
Comparison was made between 7 patients with CR and 76 patients without CR despite receiving antiviral therapy for more than 1 year (No CR). Qualitative variables expressed as number. Quantitative variables expressed as median (range).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; HLA, human leukocyte antigen.

potential etiology of abnormal liver function, we believe that cessation of antiviral therapy and initiation of intensive immunosuppressive therapy should be considered, even without histologic confirmation of CR.

Some limitations of this study are its retrospective nature and relatively small sample size. Because the frequency of CR was low, the sample size was not adequate for multivariate analysis.

In conclusion, CR developed in association with antiviral therapy for recurrent hepatitis C after LDLT. Reduction of the immunosuppressant dose during antiviral therapy should be avoided and antiviral therapy should not be administered to patients with no or mild fibrosis to prevent antiviral therapy-associated CR. Early CR diagnosis should be suspected when a cholestatic liver enzyme pattern develops during antiviral therapy. In these cases, discontinuation of antiviral therapy and increase in the immunosuppressant dose are recommended when other causes of liver dysfunction are excluded.

MATERIALS AND METHODS

Patients

A total of 232 patients with HCV-related end-stage liver disease underwent LDLT at Kyoto University Hospital between March 1999 and September 2012. Two patients who received a liver graft from an identical twin were excluded from this study, because they did not require immunosuppression because of genetic identity. Of the remaining 230 patients, 157 patients were followed up for more than 6 months after LDLT in our hospital. Antiviral therapy was administered to 125 of the 157 patients with recurrent hepatitis C between January 2001 and September 2012. They were diagnosed with recurrent hepatitis C after LDLT via serum HCV RNA analysis and histologic evidence. The remaining 32 patients did not receive antiviral therapy for various reasons: serum HCV RNA negative after LDLT (n=4), no histologic hepatitis C recurrence in the follow-up period (n=13),

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no fibrosis seen by liver histology (n=8), and ongoing treatment for the other complications (n=7). CR was defined histologically according to the updated International Banff Schema for Liver Allograft Rejection with the following criteria: (a) the presence of bile duct atrophy/pyknosis affecting most of the bile ducts with or without bile duct loss, (b) convincing foam cell obliterative arteriopathy, or (c) bile duct loss affecting more than 50% of the portal tracts (*13*). Patients who were diagnosed with CR based on these diagnostic criteria during or within 6 months after terminating antiviral therapy were examined for antiviral therapy-associated CR. The clinical features of these 7 patients with CR were compared with those of 76 patients who did not have CR despite receiving antiviral therapy for more than 1 year to determine the risk factors for CR.

The study protocol was approved by the ethics committee at Kyoto University and performed in compliance with the Helsinki Declaration.

Treatment Protocol and Definition of Responses to Treatment

Between January 2001 and April 2004, 40 patients with recurrent hepatitis C after LDLT received treatment with IFN-α-2b plus ribavirin (*25*). From May 2004 to June 2011, patients received combination therapy with peg-IFN-α-2b plus ribavirin (*26*). Patients who acquired a negative serum HCV RNA status within 12 months after treatment initiation continued to receive the treatment for an additional 12 months. Patients who tested negative for serum HCV RNA for more than 6 months after completing IFN therapy were defined as having achieved SVR. For those who tested positive for serum HCV RNA after 12 months of treatment, therapy was discontinued or switched to maintenance therapy with low-dose peg-IFN (*27*), and patients were classified as having shown no response.

Histologic Assessment

Liver biopsy examination was performed when patients showed abnormal liver function tests, or at yearly intervals, with informed consent. Biopsy specimens were evaluated by two pathologists (H.H. and A.M.-H.) with extensive experience in the pathology of LT. Necroinflammatory activity (A0–A3) and fibrosis stage (F0–F4) were assessed using METAVIR scores (*28*).

Immunosuppression

Tacrolimus with low-dose steroid or MMF was administered to most patients for immunosuppression (*25*). The target whole blood lower level for tacrolimus was 10 to 15 ng/mL during the first 2 weeks, 10 ng/mL thereafter, and 5 to 8 ng/mL starting from the second month. Steroid therapy was initiated at a dose of 10 mg/kg methylprednisolone before graft reperfusion then tapered down from 1 mg/kg per day on days 1 to 3, to 0.5 mg/kg per day on days 4 to 6, and to 0.3 mg/kg per day on day 7. Subsequently, oral prednisolone was continued at 0.3 mg/kg per day until the end of the first month, and this was followed by 0.1 mg/kg per day until the end of the third month. After that, steroid administration was terminated. MMF was initiated at a starting dose of 10 to 15 mg/kg on day 1, which was gradually increased to a target dose of 30 mg/kg, and this was continued for 6 months. Thereafter, MMF administration was terminated. Four patients received cyclosporine microemulsions instead of tacrolimus. MMF and/or prednisolone was administered again to patients who experienced refractory rejection or required reduction of the tacrolimus or cyclosporine dose because of adverse events and then tapered down gradually. Twenty-seven patients who received ABO-incompatible transplants were treated with rituximab, plasma exchange, and hepatic artery or portal vein infusion with prostaglandin E1 and methylprednisolone (*29*).

Virologic Assays

HCV genotype was determined using a genotyping system based on polymerase chain reaction (PCR) to amplify the core region using genotype-specific primers (*30*). The serum HCV RNA load was evaluated before LDLT, before IFN treatment, once a month during treatment, and 24 weeks after treatment using PCR and an Amplicor HCV assay (Cobas Amplicor HCV Monitor; Roche Molecular Systems, Pleasanton, CA) until April 2008. A real-time PCR-based quantitation method for HCV (COBAS AmpliPrep/COBAS TaqMan HCV Test; Roche Molecular Systems) was used alternatively from May 2008.

Statistical Analysis

To evaluate the association between patient characteristics and CR, the characteristics were defined and compared between patients with and without CR. Medians and ranges were determined for continuous variables, and data were analyzed using the Wilcoxon rank-sum test. Categorical variables were expressed as counts, and data were analyzed using the chi-square test. A significance level of $P<0.05$ was considered significant. Statistical analyses were performed using PASW Statistics version 18.0.0 (SPSS, an IBM company).

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肝移植後の抗ウイルス療法の新展開

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索引用語：肝移植，C型肝炎，direct acting antiviral agents

1 はじめに

C型肝炎ウイルス(HCV)に起因する肝硬変・肝細胞癌が，本邦における成人の肝移植症例の中で最多の原疾患である[1]．HCV陽性レシピエントが肝移植を受けた場合には，ほぼ全例で移植後にC型肝炎が再発し，急速に進行するため，肝移植後の長期生存率は他の疾患と比較して低い[1]．そのため，肝移植後に抗HCV治療が必要となるが，これまで行われてきたペグインターフェロン・リバビリン治療の効果は低く有害事象が多いことが明らかになっている[2,3]．最近使用可能となっているdirect acting antiviral agents（DAA）を用いた治療は，移植後に使用される免疫抑制剤との薬物相互作用が問題となる．

本稿では，肝移植後C型肝炎再発の特徴と治療法，特にDAAを用いた治療の効果と安全性について述べる．

2 肝移植後C型肝炎の特徴

肝移植後C型肝炎は通常のC型肝炎とは異なる特殊な病態であり，以下の5つの特徴がある[2,3]．

1. C型肝炎再発

HCV陽性レシピエントに対して肝移植を行った際には，HCVが多く存在する肝臓が摘出され，未感染の正常肝が移植されるが，血中に残存するウイルスがグラフト肝に再感染する．現時点ではその再感染を予防する方法はなく，ほぼ全例で移植後血中HCV RNAが陽性となる[2]．さらに，通常の初感染の場合とは異なり，急性肝炎から治癒する症例はほとんどなく，ほぼ全例が慢性化し，大部分の症例で組織学的にも肝炎の再発が確認される(図1)．肝移植後C型肝炎は，HCV未感染の移植肝に血中のHCVが感染するため，肝臓にとっては初感染といえるが，レシピエントの免疫は持続感染状態であるという，非常に特殊な病態となる．

2. 高ウイルス量

肝移植直後はHCV RNA量は一時的に低下するが，すぐに移植肝に再感染し，多くの例において移植後1カ月程度で血中HCV RNA

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量が移植前の10～20倍にまで増加することが知られている[2].

3. 速い進行

肝移植後C型肝炎は進行が速いことが知られており，20～50％の症例が移植後5年以内という短い期間で肝硬変に進行することが知られている[3]．感染(肝移植)から肝硬変へと至るまでの平均期間は10年とされており，通常のC型肝炎の20～30年の経過と比較して明らかに短い期間で肝硬変に至る(図1)．そのため，HCV陽性肝移植症例の予後は他の疾患と比較して悪いことが明らかとなっている．

4. 胆汁うっ滞

肝移植後には，Fibrosing cholestatic hepatitis (FCH)と呼ばれる，特殊な病態を示すことがあり，注意が必要である．FCHは免疫抑制下に生じる原因不明の胆汁うっ滞性の肝障害を示す病態であり，HCV陽性レシピエントに対する肝移植例の7～15％程度に認めるとされている．本症は一度発症すると3～6カ月の経過で肝不全に進行し，極めて予後不良である．また，FCHと診断されなくても，肝移植後C型肝炎の特徴として，胆道系酵素(ALPとγ-GTP)の上昇を認めることが多く，速い肝線維化の進行に関与する[4]．

5. 肝移植後合併症の併存

肝移植後は拒絶反応や胆道系合併症，血管系合併症など多くの合併症が生じる可能性があり，これらが肝移植後C型肝炎の病態や治療に影響を与える．また，これらの疾患とC型肝炎再発との鑑別が臨床上困難な例が多いため，肝移植後C型肝炎再発の診断も非常に困難となる．

3 これまでの肝移植後C型肝炎治療

肝移植後C型肝炎の治療としては，これまでペグインターフェロンとリバビリンの併用療法が中心であった[2,3]．治療開始時期は各施設で異なり，C型肝炎再発の有無にかかわらず移植後数週間という早期から抗ウイルス治療を開始するpreemptive治療を行っている施設と，肝生検にてC型肝炎再発を確認してから治療を開始する慢性肝炎期治療を行っている施設とに分かれる(図1)．投与量は通常のC型慢性肝炎に準じて行われることが多いが，減量して開始している施設もある．投与期間は，本邦の多くの施設において，血中HCV RNAが陰性化してから1年間治療を継続する個別化延長治療が行われている[5]．肝移植後C型肝炎症例は，前述のように高ウイルス量であること，移植前に治療が行われ無効例が多く，遺伝子型1b型が多いこと，高齢者が多いこと，血球低下例が多いこと，免疫抑制剤などの影響で腎障害や糖尿病の合併症が多いこと，拒絶や胆管合併症などの移植後合併症が併存する場合があること，などの

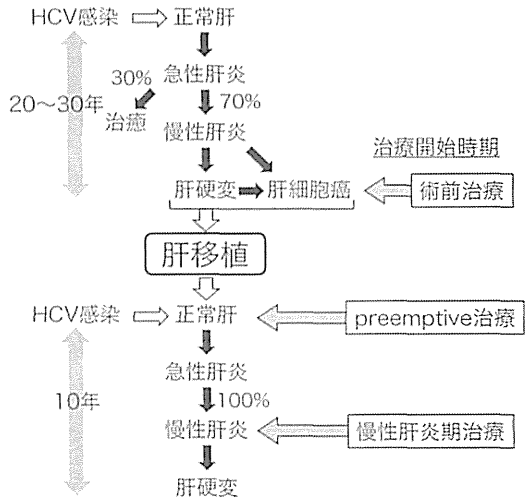

図1 肝移植後C型肝炎の経過と治療開始時期

通常のC型肝炎の経過と肝移植後のC型肝炎の経過を示す．肝移植後C型肝炎はほぼ全例で再発し，慢性肝炎へと移行する．その後，平均10年という短い期間で肝硬変へと進行することが特徴である．

理由から，治療抵抗例が多く有害事象の発生率も高い．これらの問題のため，肝移植後C型肝炎の治療成績は満足いくものではなく，HCVの排除に至るsustained virological response(SVR)例は全体の30％程度にとどまっている[6]．ウイルス排除にいたらない場合には，急速に肝硬変へと進行する場合があるため，本邦では多くの施設がウイルス非排除例に対してペグインターフェロンの少量長期維持投与を行っており，その線維化進行抑制効果が示されている[7]．

さらに，肝移植後症例に特有の有害事象として，インターフェロン治療によって拒絶反応が誘導される可能性が示唆されている．肝移植後に生じる拒絶反応の中で，慢性拒絶とPlasma cell hepatitis（*de novo*自己免疫性肝炎）の2つが肝移植後C型肝炎に対するインターフェロン治療中に問題になることが報告されている[8,9]．これらはいずれも放置すると肝不全へと進行する可能性が高いため，適切な免疫抑制剤の使用ならびに肝生検による早期診断，早期治療が必要である．

4 肝移植後C型肝炎に対する新規抗HCV薬の使用

HCVの機能タンパク質を標的としたNS3/4プロテアーゼ阻害剤，NS5A阻害剤，ならびにNS5Bポリメラーゼ阻害剤といったDAAと呼ばれる新規抗HCV薬が順次使用可能となっている．現時点で本邦にて使用可能であるDAAは，HCVのNS3/4Aプロテアーゼ阻害薬であるテラプレビル，シメプレビル，アスナプレビル，ならびにNS5A阻害剤であるダクラタスビルである．テラプレビルとシメプレビルはペグインターフェロンとリバビリンに加えた3剤併用療法として用いられる．また，アスナプレビルとダクラタスビルはこれら2剤の併用による，初のインターフェロンフリー治療である．これらの新規治療法によって，一般の（非肝移植症例の）C型慢性肝炎症例に対して優れた治療効果が報告されている．そのため，肝移植後C型肝炎治療効果の向上が期待されてきたが，大部分のDAAは薬物代謝酵素CYP3Aで代謝されることが明らかとなっている．すなわち，同様にCYP3Aによって代謝されるタクロリムスやシクロスポリンとの相互作用が予想される．実際に，最初に発売されたテラプレビルを健常人に使用した報告からは，タクロリムスやシクロスポリンの血中濃度の著明な上昇と半減期の延長が報告された[10]．これらのことから，肝移植後症例に対してDAAは容易に使用できず，薬物相互作用を克服する必要があることが明らかになっている．

1. テラプレビル＋ペグインターフェロン＋リバビリン治療

最近，肝移植後症例に対するテラプレビルを含む3剤併用療法の試みが国内外の各施設から報告されている[11-14]．多くの施設において，タクロリムスから薬物相互作用のより少ないシクロスポリンへとコンバートし，その後にテラプレビルを開始，シクロスポリンの血中濃度のモニタリングを行いながら内服量を減量して治療を行うという方法が用いられている．治療導入は可能であるものの，有害事象は多く，治療中の死亡例も報告されている[11,12]．最終的な治療効果はまだ少数施設からの報告のみであるが，SVR率は5％から82％まで施設間で大きく異なっている．

2. シメプレビル＋ペグインターフェロン＋リバビリン治療

その後使用可能となったシメプレビルは，タクロリムスやシクロスポリンの血中濃度への影響が少ないことが明らかになっている．

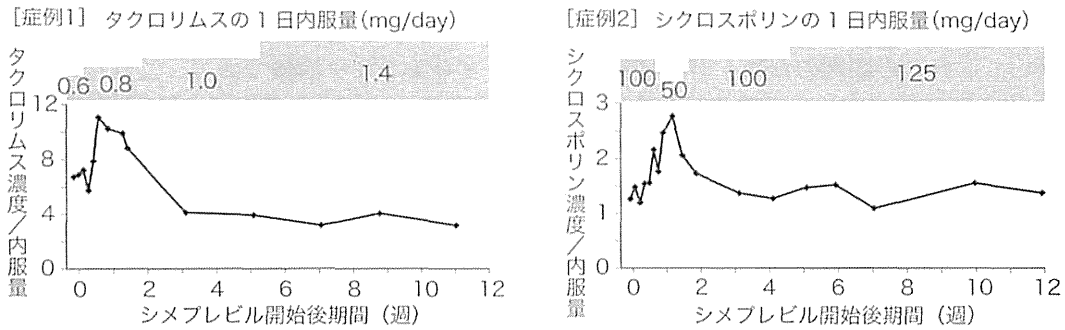

図2　シメプレビル開始後のカルシニューリン阻害剤の血中濃度/内服量比の変化

シメプレビル＋ペグインターフェロン＋リバビリン治療を行った2例のタクロリムスならびにシクロスポリンの血中濃度/内服量比の変化ならびに1日内服量の推移を示した．シメプレビル開始後2週間は，血中濃度/内服量比は上昇するが，3週目以降からは低下し，内服量の増量が必要であった．（文献15より改変）

そのため，テラプレビルの場合とは異なり，多くの施設でカルシニューリン阻害薬のコンバートは行わず，タクロリムスを内服している症例もそのままシメプレビルを開始している．実際に，タクロリムスならびにシクロスポリンの血中濃度の変化は少なく，血中濃度/投与量比は投与直後に1.0～1.3倍に増加するだけであった[15]．注意すべきはその後の血中濃度の低下であり，投与開始3週間後以降には血中濃度/投与量比が低下することが明らかになった(図2)[15]．これまでの各施設での経験から，テラプレビルと比較して有害事象が少ないことが明らかになっているが，効果はまだ不明である．

3. アスナプレビル＋ダクラタスビル治療

2014年9月より使用可能となったアスナプレビルとダクラタスビルによるインターフェロンフリー治療は，インターフェロンを用いないことから拒絶反応を含む有害事象軽減が期待される．しかしながら，免疫抑制剤との薬物相互作用の問題があることが明らかになっている．特に，シクロスポリンはアスナプレビルとの併用禁忌となっている．それは，シクロスポリンが有機アニオントランスポーター1B1（OATP1B1）を阻害するため，OATP1B1の基質であるアスナプレビルの肝臓への取り込みが減少して治療効果を減弱させるおそれがあるためである．タクロリムスとの併用は可能であり，ダクラタスビルとの併用で互いの血中濃度変化がほとんど認めないことが示されている．一方，アスナプレビルとの併用のデータは示されていない．アスナプレビルはCYP3A4の誘導作用があり，CYP3Aの基質であるため，相互作用による血中濃度変化の可能性があり，使用時には血中濃度のモニタリングを行う必要があると考えられる．実際の移植後症例への使用は開始されたばかりであり，その効果や安全性は現時点では不明である．

5　肝移植後C型肝炎治療の今後の展開

肝移植後症例に対するインターフェロンフリー治療の効果と安全性が明らかになれば，肝移植後症例に対する治療法は劇的に変化し，拒絶反応のリスクを考えずに安全に治療できる時代が到来することが期待できる．さらに，第二世代のインターフェロンフリー治療が使用可能となれば治療選択肢も増え，肝移植後C型肝炎との戦いは終わる可能性もある．しかしながら一方で，薬剤耐性ウイルス

出現の可能性も考慮に入れて治療を行う必要がある．使用可能となるDAAはNS3/4A阻害薬，NS5A阻害薬，NS5B阻害薬の3種類であり，これらの組み合わせによる治療となる．SVRに至らなかった場合には多剤耐性ウイルスが出現する可能性が高く，今後使用可能となる治療法の効果を減弱させることが危惧される．特に肝移植後症例は，薬物相互作用によって薬物血中濃度が変化する可能性があること，拒絶反応や胆管合併症などによって治療中断の可能性があることから，薬剤耐性ウイルス出現のリスクが高いと予想される．耐性ウイルス出現のないインターフェロンを用いた治療法も選択肢のひとつとして考慮すべきである．

さらに，移植前治療の可能性も再検討する必要があると考えられる．これまでも，肝移植前に抗HCV治療を行い，移植後の再感染を予防しようという治療法が試みられてきた[2]．しかしながら，移植例はほとんどが非代償性肝硬変であり，インターフェロンを含む治療法は副作用の問題から術前の治療は困難である場合が多かった．今後はインターフェロンフリー治療が使用可能となり，肝移植前の治療も可能となると考えられる．ただし，現在使用可能であるアスナプレビル＋ダクラタスビル治療は，非代償性肝硬変症例への投与が禁忌となっており，肝移植前には使用困難である．その理由は，Child-Pugh分類BまたはCの肝硬変患者にアスナプレビルを投与した場合に，その血中濃度が10～30倍に上昇するためであり，有害事象として肝機能障害が報告されていることも考慮に入れると移植前の投与はリスクが高いと考えられる．肝移植前治療は，第2世代インターフェロンフリー治療が非代償性肝硬変症例にも安全に投与できることが確認されてから行うべきであろう．生体肝移植ではほとんどの場合待機的手術であり，術前の計画的治療が可能であることから，移植前にSVRに至らなくても血中HCV-RNAが陰性化した後に肝移植を行うことによって，術後感染を予防できることが期待できる．

6 おわりに

肝移植後C型肝炎は，さまざまな特徴を持つ非常に特殊な病態である．さらに，免疫抑制剤がその病態や治療に影響を与える．そのため，肝移植後C型肝炎対策にはひと工夫必要であり，通常のC型肝炎治療と比べて考慮すべき点が多数ある．これらを克服して有効かつ安全な肝移植後治療法を確立し，加えてHCV再感染の予防が可能な肝移植前治療法を確立することによって，近い将来，C型肝硬変・肝細胞癌の肝移植成績が飛躍的に向上することが期待できる．

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報告

肝移植症例登録報告

日本肝移植研究会

Liver Transplantation in Japan
–Registry by the Japanese Liver Transplantation Society–

The Japanese Liver Transplantation Society

【Summary】

As of December 31, 2013, a total of 7,474 liver transplants have been performed at 66 institutions in Japan. This total included 7,255 living-donor transplants and 219 cadaveric-donor transplants (216 from heart-beating donors and 3 from non-heart-beating donors). The annual total of liver transplants in 2013 decreased to 408, from 422, in 2012. The number of liver transplants from living donors decreased to 369, from 381, whereas the number of liver transplants from heart-beating cadaveric donors did not change significantly. The most frequent indication was cholestatic disease, followed by neoplastic disease. As for the graft liver in living-donor cases, a right-lobe graft was the most popular (36%). Patient survival following transplantations from heart-beating donors (1 year, 85.9%; 3 years, 82.6%; 5 years, 81.3%; 10 years, 73.8%) was similar to those from living donors (1 year, 83.8%; 3 years, 79.6%; 5 years, 77.1%; 10 years, 71.9%; 15 years, 67.8%; 20 years, 66.1%). Graft survival was very much the same as patient survival. As for ABO-incompatible transplantation, new strategies, including portal vein infusion and rituximab prophylaxis, have been improving prognoses in adults as well as in children older than 3 years.

Keywords: Japanese Liver Transplantation Society, registry, cadaveric liver transplantation, living-donor liver transplantation, prognosis

I. はじめに

日本肝移植研究会は，1992年より肝移植症例の登録を開始し，1998年，2000年，そして2002年以降は毎年集計結果を誌上報告してきた[1-13]。今回2013年末までの肝移植症例の集計を終了したので，その結果を報告する。なお，2002年以降の報告[3-13]と同様，本邦で行われた肝移植のみについての報告である。

II. 対象と方法

初期にはレシピエント・ドナー合わせて25項目からなる登録用紙を年1回各施設に送付・回収する方法により登録業務を行ってきたが，よりリアルタイムでの移植症例の把握を目指し，2001年に登録法の改定を行った。すなわちレシピエント情報9項目のみよりなる一次登録用紙（「肝移植実施報告用紙」）をあらかじめ各移植施設に配布しておき，移植当日または翌日にこれに記入し事務局宛FAXしていただくこととした。このデータをもとに，年1回各施設に二次登録/予後調査用紙を送付・回収することにより，レシピエントおよびドナーについて残りの16項目のデータの追加を行った。

その後，2012年1月1日以降の症例を対象として，webでの登録に移行した。これに伴い登録項目が大幅に拡充されたので，2013年の報告は，まず第一報として，2012単年の移植例のみについて，詳細な報告を行った[14]。その後第二報として，2012年末までの全症例について，これまでと同様の形態での報告を予定していた。しかしながら，今回日本移植学会50周年の記念誌が発行されるのに伴い，2013年末までの全症例についての報告を，通常の年末ではなく早いタイミングで行わなければならないこととなった。そのため，上述の2013年の第二報と順序が逆になるが，ここに2014年の報告を行う次第である。

今回の集計対象は2013年末までに本邦で施行された肝移植である。旧制度で登録された2011年末まで

の肝移植と，新制度で2014年5月31日までに登録された肝移植のうち移植日が2013年末までのものを対象とした。

累積生存率はKaplan-Meier法で算出し，有意差の検定はlogrank testで行った。

＜協力施設：66施設＞

愛知医科大学2，旭川医科大学4，岩手医科大学47(1)，愛媛大学58，大阪医科大学34，大阪市立大学26，大阪大学227（18），岡山大学322（17），沖縄県立中部病院2，鹿児島大学1，神奈川県立こども医療センター61，金沢医科大学28，金沢大学71（2），関西医科大学29，北里大学8，九州大学512（12），京都大学1,681(37)，京都府立医科大学85(3)，熊本大学390(5)，久留米大学3，群馬大学52，慶應義塾大学210(2)，神戸市立医療センター中央市民病院45，神戸大学72（3），国立成育医療センター262（11），国立病院岡山医療センター6，国立病院水戸医療センター1，相模原協同病院2，自治医科大学245，島根大学1，順天堂大学70（4），昭和大学1，信州大学310（14），千葉大学50（2），筑波大学35，東京医科歯科大学6，東京医科大学58，東京慈恵会医科大学13，東京女子医科大学130，東京大学539(19)，東北大学164(4)，徳島大学24，獨協医科大学35，鳥取大学2，富山大学5，長崎大学192（1），名古屋市立大学54，名古屋大学198（19），奈良県立医科大学13，新潟大学114(3)，日本医科大学15，日本赤十字社医療センター46，日本大学23，兵庫医科大学18，弘前大学46，広島大学219（9），福岡大学10，福岡徳洲会病院1，福島県立医科大学47，藤田保健衛生大学47，北海道大学271(31)，松波総合病院25，三重大学142（2），山形大学1，山口大学4，横浜市立大学59

（数字は2013年末までの実施移植数。括弧内はそのうち死体移植の数）

Ⅲ. 結果と考察

総移植数は7,474であり，ドナー別では，死体移植が219(脳死移植216，心停止移植3)，生体移植が7,255であった(**表1**)。また，初回移植7,239，再移植223，再々移植12であった（死体移植がおのおの173，39，7，生体移植がおのおの7,066，184，5)。

生体・死体別の年次移植数の変遷を**表2**に示す。移植の総数は毎年着実に増加を続け2005年に570のピークに達した後，減少に転じた。2012年422，2013年408と減少傾向が続いている。1999年に開始された脳死移植の年次実施数は，改正法が年度半ばに施行された2010年に30と著明に増加したが，2011～2013

表1　本邦における肝移植数

Living-donor Transplantation	7,255
Cadaveric Transplantation	219
Heart Beating Donor	216
Non-heart Beating Donor	3
Primary Transplantation	7,239
Retransplantation	223
Third Transplantation	12

表2　本邦における肝移植数の推移（1964～2013年）

(Adults: ≧18 years)

Year	1964 – 1968	– 1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000
Living-donor	0	1	10	30 (2)	31	51 (2)	82 (6)	111 (10)	120 (22)	157 (48)	208 (90)	251 (142)	327 (188)
Cadaveric	1	0	0	0	0	1 (1)	0	0	0	0	0	2 (1)	6 (4)
Total	1	1	10	30 (2)	31	52 (3)	82 (6)	111 (10)	120 (22)	157 (48)	208 (90)	253 (143)	333 (192)

2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	Total
417 (264)	434 (292)	440 (300)	551 (426)	566 (446)	505 (383)	433 (303)	464 (326)	465 (324)	443 (299)	408 (264)	381 (256)	369 (253)	7,255 (4,646)
6 (3)	7 (4)	2 (1)	3 (3)	4 (4)	5 (5)	10 (9)	13 (13)	7 (7)	30 (27)	41 (36)	41 (34)	39 (37)	219 (189)
423 (267)	441 (296)	442 (301)	554 (429)	570 (450)	510 (388)	443 (312)	477 (339)	472 (331)	473 (326)	449 (300)	422 (290)	408 (290)	7,474 (4,835)

年は年間約40にとどまっている。なお，1964年，1968年，1993年の死体肝移植は，いずれも心停止ドナーからの移植である。**表2**の括弧内は18歳以上の大人の移植数を表わしている（本報告を通じて，18歳未満を小児，18歳以上を大人と定義して記載する）。

レシピエントの性別と年齢の分布は，**表3A**，**表3B**の通りであった。レシピエントの最低齢は生後9日（男，新生児ヘモクロマトーシス），最高齢は72歳11カ月（女，肝細胞癌/C型ウイルス性肝硬変）であった（いずれも生体移植）。

レシピエントの原疾患を死体，生体別に示す。死体肝移植は**表4A**の通りであり，2012年，2013年は急性肝不全の増加が目立った。脳死臓器提供数の増加により，急性肝不全症例が短い待機期間で移植を受けられるようになったためと思われる。生体肝移植は**表4B**の通りであり，胆汁うっ滞性疾患が最多を占め，その内訳では小児は胆道閉鎖症が，大人は原発性胆汁性肝硬変が，それぞれ最も多かった。胆汁うっ滞性疾患の「その他」には，肝内結石症7，短腸症候群による二次性胆汁性肝硬変5などがあった。また，何らかの治療/診断的手技の後に生じた二次性胆汁性肝硬変に対する移植が10，うち6は手術後であり，腹腔鏡下胆摘後の胆管損傷/狭窄4が含まれていた。肝細胞性疾患では，近年B型ウイルス性肝硬変が減少し，代わってアルコール性肝硬変が増加している。また，NASHも増加傾向にある（**表4C**）。腫瘍性疾患については肝細胞癌が大半を占めた。肝細胞癌に併存する慢性肝疾患の「その他」は，cryptogenic cirrhosis 41，自己免疫性肝炎9，胆道閉鎖症5などであった（正常肝4）。転移性肝腫瘍19のうち神経内分泌腫瘍の転移が16（原発巣は膵12，直腸3，胃1）と大半を占め，他は脳腫瘍，副腎癌，膵solid pseudopapillary tumorが各1であった。腫瘍性疾患の「その他」は，胆管細胞癌9，epithelioid hemangioendothelioma 7，肝血管肉腫と肝未分化肉腫と限局性結節性過形成が各1であった。なお，胆管細胞癌は1例を除き，摘出肝の病理的検索により移植後に初めて診断されたものである（併存疾患は原発性硬化性胆管炎4, Caroli病2, 胆道閉鎖症1，B型ウイルス性肝硬変1，cryptogenic cirrhosis 1）。急性肝不全の「その他」は，ヘモクロマトーシス6，熱中症1，毒キノコ摂取1，妊娠脂肪肝1，NASH 1，巨大甲状腺腫摘除術後1，家族性血球貪食性リンパ組織球症1であった。なお，いわゆるやせ薬によるものは薬剤性の項に含めた。代謝性疾患の「その他」は，プロピオン酸血症9，アミロイドーシス5，胆汁酸代謝異常症4，クリグラー・ナジャール病3，ミトコンドリアDNA枯渇症候群3，ポルフィリン症2，家族性高コレステロール血症2の他，アルギニン血症，アルギノコハク酸尿症，メープルシロップ尿症，Dubin-Johnson症候群各1であった。なお，**表4B**の一番下の「その他」の疾患群の中には，先天性肝線維症25，多発性肝嚢胞症19，特発性門脈圧亢進症8，GVHD（骨髄移植後）4，肝切除後の肝不全3（うち1例は生体肝提供術後）などがあった。

表5Aに死体移植の移植肝を示す。全肝移植が大半を占めたが，いわゆるmonosegment graft，外側区域graft，左葉graft，左葉＋尾状葉graft，右葉graft，右三区域graftも用いられた。**表5B**に生体移植の移植肝を示す。右葉graftが最も多く，外側区域graftがこれに次いだ。全肝グラフトはすべてドミノ移植によるものである。なお，ドミノ移植は合計41が施行されており（後述：**表8**），全肝以外のグラフトは，右葉11，左葉（＋尾状葉）7であった（うちsplitが3）。また，1人のレシピエントが2人のドナーから肝の提供を受けるいわゆる「dual graft」が2例あり，いずれも右葉と左葉を提供された。

ドナーの性別と年齢の分布は，死体移植は**表6A**の通りであった（延べ人数）。摘出肝のsplitが行われ2人のレシピエントに移植された事例があるので，実人数はこれより少なくなるが，詳細は割愛する。一方，生体ドナーは**表6B**の通りであった（延べ人数）。30歳代が最も多く，20歳代がこれに次いだ。最年少は17歳（息子4，母1，妹1），最高齢は70歳（祖母1，夫1）であった。前述のようにdual graftが2あったため，**表6B**の合計は，生体肝移植の総数7,255より2多い7,257になっている。なお，3人のドミノ移植のドナー（20歳代，50歳代，60歳代のいずれも男性）でsplitが行われているので，実人数で示せば，**表6B**は20歳代男性，50歳代男性，60歳代男性につきそれぞれ1を減じ，合計7,254名のドナーとなる。

生体ドナーの続柄を**表7**に示す（延べ人数）。小児では，両親が95%と大半を占めた。一方，大人では，子供（43%），配偶者（23%），兄弟姉妹（18%），両親（10%）の順に多かった。やはりdual graftのため，**表7**の合計は生体肝移植の総数7,255より2多い7,257になっている。また，3人のドミノ移植のドナーでsplitが行われているので，実人数で示せば，**表7**は合計7,254名のドナーとなる。なお，splitのドミノ移植