and mechanisms of HBV reactivation and ALF in patients with occult HBV carrier status receiving chemotherapy or immunosuppressive therapy.

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References

[1] Wands JR, Chura CM, Roll FJ, Maddrey WC. Serial studies of hepatitis-associated antigen and antibody in patients receiving antitumor chemotherapy for myeloproliferative and lymphoproliferative disorders. Gastroenterology 1975;68:105-112.

[2] Galbraith RM, Eddleston AL, Williams R, Zuckerman AJ. Fulminant hepatic failure in leukaemia and choriocarcinoma related to withdrawal of cytotoxic drug therapy. Lancet 1975;2:528-530.

[3] Hoofnagle JH, Dusheiko GM, Schafer DF, Jones EA, Micetich KC, Young RC, et al. Reactivation of chronic hepatitis B virus infection by cancer chemotherapy. Annals of internal medicine 1982;96:447-449.

[4] Lok AS, Liang RH, Chiu EK, Wong KL, Chan TK, Todd D. Reactivation of hepatitis B virus replication in patients receiving cytotoxic therapy. Report of a prospective study. Gastroenterology 1991;100:182-188.

[5] Dervite I, Hober D, Morel P. Acute hepatitis B in a patient with antibodies to hepatitis B surface antigen who was receiving rituximab. The New England journal of medicine 2001;344:68-69.

[6] Hui CK, Cheung WW, Zhang HY, Au WY, Yueng YH, Leung AY, et al. Kinetics and risk of de novo hepatitis B infection in HBsAg-negative patients undergoing cytotoxic chemotherapy. Gastroenterology 2006;131:59-68.

[7] Mason AL, Xu L, Guo L, Kuhns M, Perrillo RP. Molecular basis for persistent hepatitis B virus infection in the liver after clearance of serum hepatitis B surface antigen. Hepatology 1998;27:1736-1742.

[8] Raimondo G, Allain JP, Brunetto MR, Buendia MA, Chen DS, Colombo M, et al. Statements from the Taormina expert meeting on occult hepatitis B virus infection. Journal of hepatology 2008;49:652-657.

[9] Uemoto S, Sugiyama K, Marusawa H, Inomata Y, Asonuma K, Egawa H, et al. Transmission of hepatitis B virus from hepatitis B core antibody-positive donors in living related liver transplants. Transplantation 1998;65:494-499.

[10] Marusawa H, Uemoto S, Hijikata M, Ueda Y, Tanaka K, Shimotohno K, et al. Latent hepatitis B virus infection in healthy individuals with antibodies to hepatitis B core antigen. Hepatology 2000;31:488-495.

[11] Marusawa H, Imoto S, Ueda Y, Chiba T. Reactivation of latently infected hepatitis B virus in a leukemia patient with antibodies to hepatitis B core antigen. Journal of gastroenterology 2001;36:633-636.

[12] Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology 2007;45:507-539.

[13] Kusumoto S, Tanaka Y, Mizokami M, Ueda R. Reactivation of hepatitis B virus following systemic chemotherapy for malignant lymphoma. International journal of hematology 2009;90:13-23.

[14] Rodriguez C, Chevaliez S, Bensadoun P, Pawlotsky JM. Characterization of the dynamics of hepatitis B virus resistance to adefovir by ultra-deep pyrosequencing. Hepatology 2013;58:890-901.

[15] Nishijima N, Marusawa H, Ueda Y, Takahashi K, Nasu A, Osaki Y, et al. Dynamics of Hepatitis B Virus Quasispecies in Association with Nucleos(t)ide Analogue Treatment Determined by Ultra-Deep Sequencing. PloS one 2012;7:e35052.

[16] Omata M, Ehata T, Yokosuka O, Hosoda K, Ohto M. Mutations in the precore region of hepatitis B virus DNA in patients with fulminant and severe hepatitis. The New England journal of medicine 1991;324:1699-1704.

[17] Carman WF, Fagan EA, Hadziyannis S, Karayiannis P, Tassopoulos NC, Williams R, et al. Association of a precore genomic variant of hepatitis B virus with fulminant hepatitis. Hepatology 1991;14:219-222.

[18] Ozasa A, Tanaka Y, Orito E, Sugiyama M, Kang JH, Hige S, et al. Influence of genotypes and precore mutations on fulminant or chronic outcome of acute hepatitis B virus infection. Hepatology 2006;44:326-334.

[19] Steinberg JL, Yeo W, Zhong S, Chan JY, Tam JS, Chan PK, et al. Hepatitis B virus reactivation in patients undergoing cytotoxic chemotherapy for solid tumours: precore/core mutations may play an important role. Journal of medical virology 2000;60:249-255.

[20] Umemura T, Tanaka E, Kiyosawa K, Kumada H. Mortality secondary to fulminant hepatic

failure in patients with prior resolution of hepatitis B virus infection in Japan. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2008;47:e52-56.

[21] Marusawa H, Osaki Y, Kimura T, Ito K, Yamashita Y, Eguchi T, et al. High prevalence of anti-hepatitis B virus serological markers in patients with hepatitis C virus related chronic liver disease in Japan. Gut 1999;45:284-288.

[22] Cholongitas E, Papatheodoridis GV, Burroughs AK. Liver grafts from anti-hepatitis B core positive donors: a systematic review. Journal of hepatology 2010;52:272-279.

[23] Chevaliez S, Rodriguez C, Pawlotsky JM. New virologic tools for management of chronic hepatitis B and C. Gastroenterology 2012;142:1303-1313 e1301.

[24] Polson J, Lee WM. AASLD position paper: the management of acute liver failure. Hepatology 2005;41:1179-1197.

[25] Sato S, Suzuki K, Akahane Y, Akamatsu K, Akiyama K, Yunomura K, et al. Hepatitis B virus strains with mutations in the core promoter in patients with fulminant hepatitis. Annals of internal medicine 1995;122:241-248.

[26] Matsumoto T, Marusawa H, Dogaki M, Suginoshita Y, Inokuma T. Adalimumab-induced lethal hepatitis B virus reactivation in an HBsAg-negative patient with clinically resolved hepatitis B virus infection. Liver international : official journal of the International Association for the Study of the Liver 2010;30:1241-1242.

[27] Hsu C, Tsou HH, Lin SJ, Wang MC, Yao M, Hwang WL, et al. Chemotherapy-induced hepatitis B reactivation in lymphoma patients with resolved HBV infection: A prospective study. Hepatology 2014, *in press*.

[28] Pollicino T, Raffa G, Costantino L, Lisa A, Campello C, Squadrito G, et al. Molecular and functional analysis of occult hepatitis B virus isolates from patients with hepatocellular carcinoma. Hepatology 2007;45:277-285.

[29] Scaglioni PP, Melegari M, Wands JR. Biologic properties of hepatitis B viral genomes with mutations in the precore promoter and precore open reading frame. Virology 1997;233:374-381.

[30] Hadziyannis SJ, Vassilopoulos D. Hepatitis B e antigen-negative chronic hepatitis B. Hepatology 2001;34:617-624.

[31] Milich DR, Chen MK, Hughes JL, Jones JE. The secreted hepatitis B precore antigen can modulate the immune response to the nucleocapsid: a mechanism for persistence. J Immunol 1998;160:2013-2021.

[32] Chu CJ, Lok AS. Clinical significance of hepatitis B virus genotypes. Hepatology 2002;35:1274-1276.

Figure legends

Fig. 1. Representative clinical courses of patients with reactivation from occult HBV infection.

Serial serum ALT (solid lines), HBV DNA (dashed lines) and HBV serology of four cases that developed HBV reactivation after (cases #1) or during (cases #3, #11 and #14) chemotherapy or immunosuppressive therapy. All cases were treated with entecavir (ETV) immediately after diagnosis of HBV reactivation. BMT, bone marrow transplantation; FK506, tacrolimus; MEL, melphalan; Op, operation; PSL, prednisolone; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone.

Fig. 2. Comparison of viral genetic heterogeneity in patients with reactivation from occult HBV and HBsAg carrier status.

Comparison of viral genetic heterogeneity expressed as the Shannon entropy value among representative patients with reactivation from occult HBV infection (A) and reactivation from HBsAg carriers (B). The total number of different nucleotides from the representative HBV reference sequences (mismatch bases) (C), and the mean Shannon entropy values (D) in both groups. preC-C, pre-core–core; preS, pre-surface; P, polymerase; S, surface.

Fig. 3. Prevalence of G1896A pre-core mutants in the liver of 44 healthy occult HBV carriers.

The ratio of G1896A mutants (red) to wild-type G1896 (yellow) 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.

Fig. 1. Inuzuka et al.

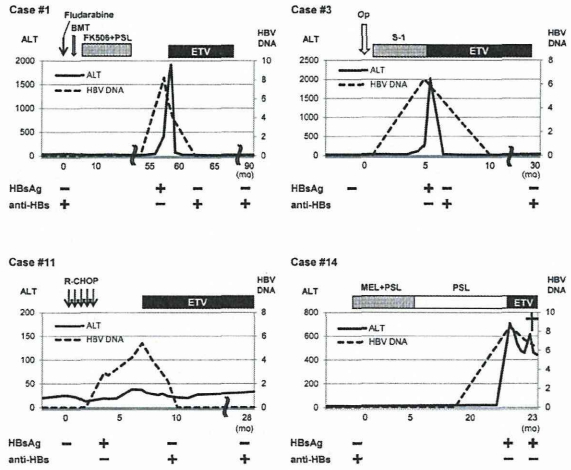

Fig. 2. Inuzuka et al.

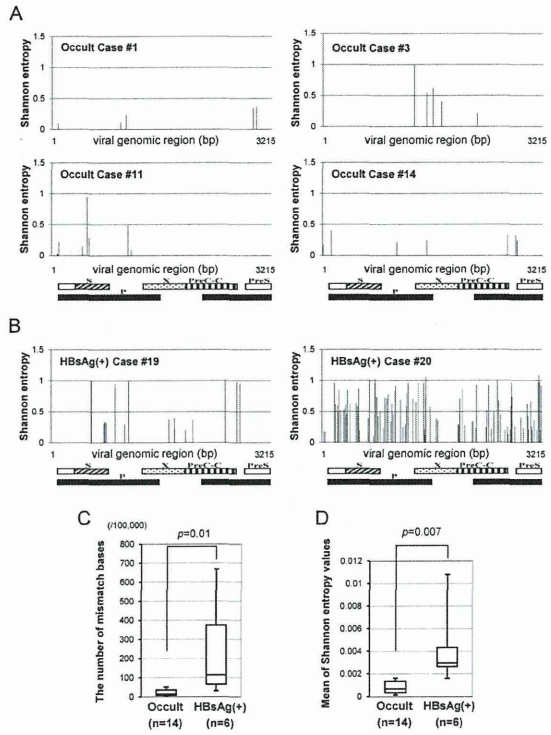

Fig. 3. Inuzuka et al.

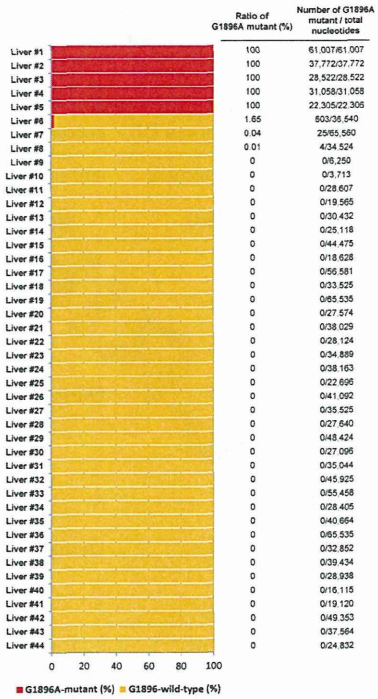

Table 1. Clinical characteristics of patients with reactivation from occult HBV and HBsAg carrier status BEFORE viral exacerbation

Case	Age/ Sex	Anti-HBs	Primary disease	Treatment	Use of steroids	HSCT	Period between HBV reactivation and start of treatment (months)	Period between HBV reactivation and end of treatment (months)
Reactivation from occult HBV carrier status								
#1	48M	+	ML	Fludarabine	+	+	57.7	39.8
#2	25M	−	AML	IDA+AraC	+	+	27.0	19.2
#3	59M	Unknown	Colon cancer	S-1	−	−	3.6	During treatment
#4	61M	Unknown	ML	R-CHASE	+	+	13.8	9.5
#5	64M	−	MM	MP→CAD	+	+	13.6	6.4
#6	72M	−	ML	MTX+AraC →Rituximab	+	−	10.9	During treatment
#7	78M	Unknown	ML	R-CVP	+	−	34.7	34.2
#8	66M	Unknown	MM	MP	+	−	49.1	6.6
#9	61F	−	ML	R-FND	+	−	1.0	During treatment
#10	66M	Unknown	Psoriasis	Cyclosporine	−	−	37.8	During treatment
#11	79F	Unknown	ML	R-CHOP	+	−	3.7	During treatment
#12	81F	−	ML	R-CVP	+	−	11.2	7.6
#13	84F	Unknown	ML	R-CHOP	+	−	17.4	During treatment
#14	87F	+	MM	MP	+	−	23.1	During treatment
							median: 15.6	median: 9.5
Reactivation from HBsAg carrier status								
#15	32F	−	Sjögren synd.	PSL	+	−	15.1	During treatment
#16	63F	−	Raynaud's dis.	PSL	+	−	20.4	During treatment
#17	42F	−	Aortitis synd.	PSL	+	−	122.2	During treatment
#18	59M	−	Lung cancer	Chemotherapy[a]	+	−	17.9	During treatment
#19	54M	−	RA	MTX+PSL	+	−	11.5	During treatment
#20	72M	−	RA	Bucillamine	−	−	6.7	During treatment
							median: 16.5	

[a]carboplatin, paclitaxel → docetaxel → gemcitabine, vinorelbine → cisplatin, irinotecan

AML, acute myeloid leukemia; AraC, cytarabine; dis, disease; CAD, cyclophosphamide, doxorubicin, dexamethasone; F, female; HBsAg, hepatitis B surface antigen; HSCT, hematopoietic stem cell transplantation; IDA, idarubicin; M, male; ML, malignant lymphoma; MM, multiple myeloma; MP, melphalan, prednisolone; MTX, methotrexate; PSL, prednisolone; RA, rheumatoid arthritis; R-CHASE, rituximab, cyclophosphamide, cytosine arabinoside, etoposide, dexamethasone; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine,

prednisolone; R-CVP, rituximab, cyclophosphamide, doxorubicin, prednisolone; synd, syndrome; R-FND, rituximab, fludarabine, mitoxantrone, dexamethasone.

Table 2. Clinical courses of patients with reactivation from occult HBV and HBsAg carrier status AFTER viral exacerbation

Case	At diagnosis of HBV reactivation				ETV treatment*	Period to HBsAg disappearance**
	HBV Genotype	HBeAg/ anti-HBe	HBV DNA level (log copies/mL)	ALT[a] level (IU/mL)		(months)
Reactivation from occult HBV carrier status						
#1	C	+/−	8.2	1,915	+	13.3
#2	C	+/−	6.2	24	+	2.8
#3	C	+/−	6.4	2,019	+	0.6
#4	C	+/−	8.3	720	+	3.1
#5	C	+/−	5.4	681	n.t.	−
#6	C	+/−	8.4	15	+	−
#7	B	+/−	7.7	1,983	+	2.9
#8	B	+/−	6.2	97	+	−
#9	C	−/+	5.0	18	+	1.7
#10	C	−/+	6.6	2,028	+	0.9
#11	C	−/+	5.4	38	+	13.5
#12	B	−/+	9.0	503	+	10.5
#13	B	−/+	6.5	623	+	−
#14	B	−/+	8.5	705	+	−
			median: 6.6	median: 652		median: 2.9
Reactivation from HBsAg carrier status						
#15	C	+/−	8.8	499	+	−
#16	C	+/−	7.1	1,740	+	−
#17	C	−/+	7.8	628	+	−
#18	C	−/+	5.5	1,674	+	−
#19	B	−/+	5.8	619	+	−
#20	C	−/+	8.8	813	+	0.4
			median: 7.5	median: 716		

ALT, alanine aminotransferase; anti-HBe, antibodies to hepatitis B e antigen; ETV, entecavir; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; n.t., not treated

* All patients except case #5 were treated with ETV immediately after diagnosis of HBV reactivation to suppress viral activity.

** Period (months) between ETV administration and HBsAg disappearance

[a] normal range 10-42 IU/L.

Table 3. Mean mutation rate of the reactivated HBV clones in patients with reactivation from occult HBV and HBsAg carrier status

	Occult HBV carrier status (n=14)	HBsAg carrier status (n=6)
Average aligned reads	605,890	630,253
Average aligned nucleotides	52,814,651	52,812,297
Average coverage	16,712	16,632
Mutation rate* (%)	0.015	0.114

Mutation rate * (%): the ratio of total different nucleotides from the representative HBV reference sequences.

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)	0/6,391	(0.0)	1/6,491	(0.0)
#2	1/10,200	(0.0)	0/9,213	(0.0)	3/9,216	(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	0/6,860	(0.0)	0/6,175	(0.0)	0/6,307	(0.0)
#6	273/31,622	(0.9)	8/29,996	(0.0)	4/30,400	(0.0)
#7	22/12,561	(0.2)	0/3,405	(0.0)	1/3,492	(0.0)
#8	1/11,500	(0.0)	0/4,964	(0.0)	1/5,089	(0.0)
#9	12,897/12,904	(100)	11,676/11,677	(100)	11,653/11,659	(100)
#10	11,432/11,444	(100)	1/6,153	(0.0)	2/6,217	(0.0)
#11	9,533/9,539	(99.9)	7,669/7,671	(100)	7,681/7,685	(99.9)
#12	10,944/10,945	(100)	2/10,874	(0.0)	1/11,325	(0.0)
#13*	9,358/9,411	(99.4)	2/10,900	(0.0)	0/11,298	(0.0)
#14*	11,174/11,179	(100)	0/6,579	(0.0)	2/6,773	(0.0)
Reactivation from HBsAg carrier status						
#15	734/12,544	(5.9)	7,593/7,596	(100)	7,556/7,570	(99.8)
#16	2/7,469	(0.0)	0/6,481	(0.0)	2/6,618	(0.0)
#17	12,251/12,701	(96.5)	5,110/5,241	(97.5)	5,180/5,239	(98.9)
#18	9,649/9,660	(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)	0/6,671	(0.0)	3/6,929	(0.0)

* Patients who developed fatal acute liver failure.

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Pretransplant Serum Hepatitis C Virus RNA Levels Predict Response to Antiviral Treatment after Living Donor Liver Transplantation

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Abstract

Background: Given the limited efficacy and high adverse event rate associated with treatment of recurrent hepatitis C after liver transplantation, an individualized treatment strategy should be considered. The aim of this study was to identify predictors of response to antiviral therapy for hepatitis C after living donor liver transplantation (LDLT) and to study the associated adverse events.

Methods: A retrospective chart review was performed on 125 hepatitis C virus (HCV)-positive LDLT recipients who received interferon plus ribavirin and/or peginterferon plus ribavirin therapy at Kyoto University between January 2001 and June 2011.

Results: Serum HCV RNA reached undetectable levels within 48 weeks in 77 (62%) of 125 patients, and these patients were defined as showing virological response (VR). Of 117 patients, 50 (43%) achieved sustained VR (SVR). Predictive factors associated with both VR and SVR by univariate analysis included low pretransplant serum HCV RNA levels, a non-1 HCV genotype, and low pretreatment serum HCV RNA levels. In addition, LDLT from ABO-mismatched donors was significantly associated with VR, and white cell and neutrophil counts before interferon therapy were associated with SVR. Multivariate analysis showed that 2 variables–pretransplant serum HCV RNA level less than 500 kIU/mL and a non-1 HCV genotype–remained in models of both VR and SVR and that an ABO mismatch was associated with VR. No variables with a significant effect on treatment withdrawal were found.

Conclusions: Virological response to antiviral therapy in patients with hepatitis C recurring after LDLT can be predicted prior to transplant, based on pretransplant serum HCV-RNA levels and HCV genotype. LDLT from ABO-mismatched donors may contribute to more efficacious interferon therapy.

Trial Registration: UMIN-CTR UMIN000003286.

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Introduction

Hepatitis C virus (HCV) infection, leading to liver cirrhosis and hepatocellular carcinoma, is the leading indications for liver transplantation in Japan, the United States, and Western Europe. However, almost all patients who undergo liver transplantation for HCV-related liver disease develop recurrent viral infection, and 70–90% of patients suffer from histologically proven recurrent hepatitis [1,2,3,4,5,6]. The progression of recurrent hepatitis C is often accelerated and, without appropriate antiviral therapy, 10–25% of patients develop cirrhosis within 5 years after transplantation, resulting in poorer prognoses for HCV-positive recipients than HCV-negative recipients [7]. To prevent the progression of hepatitis C after liver transplantation, interferon-based combination therapy is commonly administered [8,9]. However, its efficacy in liver transplant recipients is limited, with the mean sustained virological response (SVR) rate among patients with recurrent hepatitis C after liver transplantation being only 30% (range, 8–50%) [10]. One of the reasons for the low SVR rate is the high rate of treatment withdrawal. Several severe adverse events have been reported in transplant recipients after interferon therapy,

including chronic rejection and *de novo* autoimmune hepatitis [11,12,13].

To improve the efficacy of anti-HCV treatment in patients with hepatitis C after liver transplantation, an individualized treatment strategy based on efficacy prediction and adverse events should be attempted. In several studies, an analysis of predictors associated with SVR was conducted in patients with recurrent hepatitis C after deceased donor liver transplantation (DDLT) [10,14,15,16,17,18,19,20]. In these studies, variables most frequently associated with SVR were early virological response (EVR) at 3 months of therapy, HCV genotype 2, adherence to therapy, and baseline viremia [14,15,16,17,18,19,20]. Of these factors, EVR and adherence to therapy can only be recognized after the initiation of treatment. However, to enable decisions on treatment indications and strategy, predictors of response that are available before initiation of therapy are more valuable. Thus, an individualized treatment strategy could be based on the identification of baseline predictive factors before interferon therapy. Moreover, no study of factors predictive of response to the interferon therapy in patients with recurrent hepatitis C after living donor liver transplantation (LDLT) has been reported so far. Characteristics specific to LDLT, including blood-relative donors, post-transplant liver regeneration, and ABO-incompatible liver transplantation, might cause the antiviral effects of interferon therapy in these patients to differ from those who received DDLT.

The direct-acting antiviral agents telaprevir and boceprevir recently became available for clinical use. The results of clinical trials of these agents in combination with peginterferon plus ribavirin in nontransplant patients with HCV were promising [21,22,23,24]. SVR rates to telaprevir-based combination therapy were significantly higher than those to the peginterferon-ribavirin combination. The efficacy in the patients who had suffered a relapse after a previous treatment by peginterferon plus ribavirin was especially striking [21,24]. The SVR rate to telaprevir based-therapy in patients who had a previous relapse was more than 80%, while that in patients who had no response to previous treatment was around 30% [24]. These results suggest that patients who show a virological response (VR) to peginterferon plus ribavirin are expected to achieve SVR after telaprevir-based therapy. Therefore, identification of factors predictive of virological response to peginterferon plus ribavirin should also prove useful when making the clinical decision about telaprevir usage. In liver transplant recipients, the use of telaprevir and boceprevir poses risks because of their inhibitory action on the enzyme cytochrome P450 3A, responsible for the metabolism of both tacrolimus and cyclosporine. In fact, the phase I study of telaprevir in healthy individuals revealed that it significantly increased the blood concentrations of both tacrolimus and cyclosporine [25]. Therefore, the selection of the patients for whom telaprevir is prescribed is especially important in liver transplant recipients.

Recently, a polymorphism in the interleukin-28B (IL28B) gene region, encoding interferon-lambda 3, was identified as a strong predictive factor for response to antiviral treatment in nontransplant patients with hepatitis C [26,27,28]. In post-transplant patients, the IL28B polymorphism in both recipients and donors was shown to be associated with response to antiviral treatment [29,30]. In addition, HCV-RNA mutations, including those affecting amino acid (aa) residues 70 and 91 in the core region of HCV and those in the interferon sensitivity determining region (ISDR) in nonstructural protein 5A (NS5A), were also demonstrated to be predictors of response to interferon therapy in transplant recipients, as well as in nontransplant settings [31,32,33]. These factors could be used to predict response to antiviral therapy, but these are presently not part of a routine clinical examination and require special techniques not covered by health insurance. Moreover, probing individual genetic information poses potential ethical issues.

The aims of this study were, therefore, to identify noninvasively obtained regular baseline factors associated with VR, SVR, and treatment withdrawal, in order to elucidate the factors associated purely with response to interferon therapy, to identify the valuables related to final outcomes, and to clarify the factors associated with adverse events.

Methods

A retrospective chart review was performed for all HCV-positive liver transplant patients who received antiviral therapy with standard interferon and/or pegylated interferon in combination with ribavirin after liver transplantation at Kyoto University between January 2001 and June 2011.

Patients

Between March 1999 and June 2011, 214 HCV-positive recipients underwent LDLT at Kyoto University. Of these, 157 patients were followed up for more than 6 months after LDLT in our hospital. Anti-viral therapy was administered to 125 of the 157 patients with recurrent hepatitis C between January 2001 and June 2011. The remaining 32 patients did not receive anti-viral therapy for various reasons: serum HCV-RNA negative after LDLT ($n = 4$), no histological hepatitis C recurrence in the follow-up period ($n = 13$), no fibrosis seen by liver histology ($n = 8$), and ongoing treatment for the other complications ($n = 7$). HCV RNA concentrations and histological evidence were used to diagnose patients with recurrent hepatitis C after LDLT. These patients were given combination therapies with interferon plus ribavirin and/or peginterferon plus ribavirin at Kyoto University between January 2001 and June 2011. The study protocol was approved by the Ethics Committee at Kyoto University and performed in compliance with the Helsinki Declaration. Written informed consent for participation in this study was not obtained, because this study is an observational study without use of human specimen. Our institutional review board waived the need for written informed consent from the participants of the initial study.

Treatment Protocol and Definition of Responses to Treatment

Between January 2001 and April 2004, patients with recurrent hepatitis C after LDLT received treatment with interferon-α-2b (3 or 6 mega units, 3 times/week) plus ribavirin (400–800 mg/day orally), for the first 6 months. This was followed by interferon monotherapy for 6 months [34]. Forty patients received this treatment. Of the 40 patients, 14 patients achieved SVR and 9 withdrew from the treatment protocol. The remaining 17 patients, including 2 who relapsed and 15 nonresponders were retreated by the following protocol with peginterferon and rebavirin. Between May 2004 and June 2011, patients received combination therapy with peginterferon-α-2b (1.5 μg/kg) plus ribavirin (400–800 mg/day orally) [35]. 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 before treatment termination. Total 102 patients, including 17 patients who had previously treated with standard interferon plus ribavirin and did not achieve SVR, were treated with this treatment protocol. Patients who were negative for serum HCV RNA for more than 6 months after completion of interferon therapy were defined as having achieved SVR. If serum HCV RNA was positive after 12 months of treatment, therapy was discontinued or

switched to maintenance therapy with low-dose peginterferon [36], and the patient was classified as having shown no response. Treatment was discontinued in patients with severe adverse events. Additionally, peginterferon treatment was discontinued when neutrophil and platelet counts fell below 500/μL and 30000/μL, respectively, and ribavirin was discontinued when hemoglobin levels fell below 8 g/dL.

We studied the final outcomes of the treatment with peginterferon plus ribavirin (n = 102) and with standard interferon plus ribavirin (n = 23).

Histological Assessment

Liver biopsies were performed when patients' alanine aminotransferase (ALT) levels were more than twice the normal upper limit, or at yearly intervals, with informed consent. Biopsy specimens were evaluated by 2 pathologists (H.H. and A.M-H.) with extensive experience in the pathology of liver transplantation. Necroinflammatory activity (A0–A3) and fibrosis stage (F0–F4) were assessed using METAVIR scores [37,38]. Activity was graded as A0 (no activity), A1 (mild activity), A2 (moderate activity), or A3 (severe activity); Fibrosis was staged as F0 (no fibrosis), F1 (mild fibrosis), F2 (moderate fibrosis), F3 (severe fibrosis), or F4 (cirrhosis).

Immunosuppression

Tacrolimus and low-dose steroid therapy were administered to induce immunosuppression in most patients [34]. Four patients received cyclosporine microemulsions instead of tacrolimus. Mycophenolate mofetil was administered to patients who experienced refractory rejection or required reduction of tacrolimus or cyclosporine doses due to adverse events. Patients who received ABO blood-type incompatible transplants were treated with rituximab, plasma exchange, and hepatic artery or portal vein infusion with prostaglandin E1 and methylprednisolone [39].

Virological Assays

HCV genotype was determined using a genotyping system based on polymerase chain reaction (PCR) to amplify the core region using genotype-specific PCR primers [40]. Serum HCV RNA load was evaluated before LDLT, before interferon 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, USA) until April 2008, or a real-time PCR-based quantitation method for HCV (COBAS AmpliPrep/COBAS TaqMan HCV Test, Roche Molecular Systems, Pleasanton, CA, USA) from May 2008. Detection of amino acid substitutions in the HCV core region was performed using the method reported previously [31].

Statistical Analysis

To evaluate the association between the patient characteristics and the outcomes (VR, SVR, or withdrawal), the Wald test was performed based on a logistic regression model. Multivariate logistic regression analysis with backward variable selection was used to identify independent and significant predictors for the outcomes, and to estimate the odds ratio (OR) ant its 95% confidence interval (CI). A p-value of 0.05 was used for variable selection and was regarded as significant. Statistical analyses were performed using SAS version 9.2 (SAS Institute Inc., Cary NC).

Results

Patient Characteristics

This study included 125 HCV-infected liver transplant patients treated with standard interferon and/or pegylated interferon in combination with ribavirin for recurrent hepatitis C after LDLT. Of the 125 patients, 69 (55%) were male, and the median age was 57 years (range: 15–70) at the beginning of the therapy. Most patients were infected with HCV genotype 1b (n = 103, 82%). HCV genotypes of the remaining patients were 2a (n = 13), 2b (n = 5), 3a plus 3b (n = 1), not determined (n = 2), and not examined (n = 1). Median serum HCV RNA load was 410 kIU/mL (range: <0.5–5000<kIU/mL) before LDLT, and 3260 kIU/mL (range: 31–69000<kIU/mL) at the beginning of the interferon therapy after LDLT. The median donor age was 41 (range: 19–65) years. Seventy-two donors (58%) were male, and 86 (69%) were related to the recipients. The graft type was the right lobe in 109 patients (87%), and the left lobe in 16 patients (13%). The blood type combination was incompatible in 26 patients (21%). The median time to treatment initiation after LDLT was 9.0 months (1.1–85.3 months). Before treatment, the necroinflammatory activity was A1 or greater in all patients, and 104 patients (83%) had a fibrosis score of F1 or greater (METAVIR score). Tacrolimus-based immunosuppression was used in 116 patients (93%). Among patients receiving tacrolimus for immunosuppression, the mean serum trough level was 6.0 ng/mL (range: 2.0–12.7) at the initiation of interferon therapy. In addition to calcineurin inhibitors, mycophenolate mofetil and prednisolone were used at the initiation of the interferon treatment in 36 (29%) and 19 (15%) patients, respectively.

Efficacy of Interferon Therapy

Of the 125 patients who received interferon therapy, serum HCV RNA reached undetectable levels (less than 0.05 kIU/mL) within 48 weeks in 77 patients (62%) (Figure 1). These patients were defined as showing virological response (VR). Of the remaining 48 patients, 2 patients received treatment for less than 48 weeks, and 15 patients withdrew from the treatment protocol within 48 weeks because of worsening of liver function (n = 5), recurrent hepatocellular carcinoma (n = 2), ascites (n = 2), anemia (n = 1), leucopenia (n = 1), brain hemorrhage (n = 1), biliary complication (n = 1), sepsis (n = 1), or myocardial infarction (n = 1). The remaining 31 patients with detectable HCV RNA in the serum 48 weeks after the initiation of the treatment were placed in the non-VR group. All patients in the non-VR group received peginterferon plus ribavirin therapy, including 9 patients who had previously treated with standard interferon plus ribavirin and did not achieve SVR. Of the patients with VR, 11 discontinued the treatment protocol within 24 weeks after serum HCV-RNA became negative, and 6 patients are still under treatment. The reasons for discontinuation were biliary complications (n = 2), worsening of liver function (n = 2), general fatigue (n = 2), recurrent hepatocellular carcinoma (n = 1), leucopenia (n = 1), hemoptysis (n = 1), brain tumor (n = 1), and depression (n = 1). Of 60 patients who achieved VR and completed the treatment protocol, 50 achieved SVR and 10 relapsed. None of the non-VR patients achieved VR even after more than 48 weeks of treatment, and were classified as nonresponder (NR).

In summary, among the 117 patients in whom the final outcomes of the treatment could be evaluated, 50 patients (43%) achieved SVR, and the remaining 67 patients, including 10 who relapsed (9%), 31 NR (26%), and 26 withdrawals (22%), were classified as non-SVR.

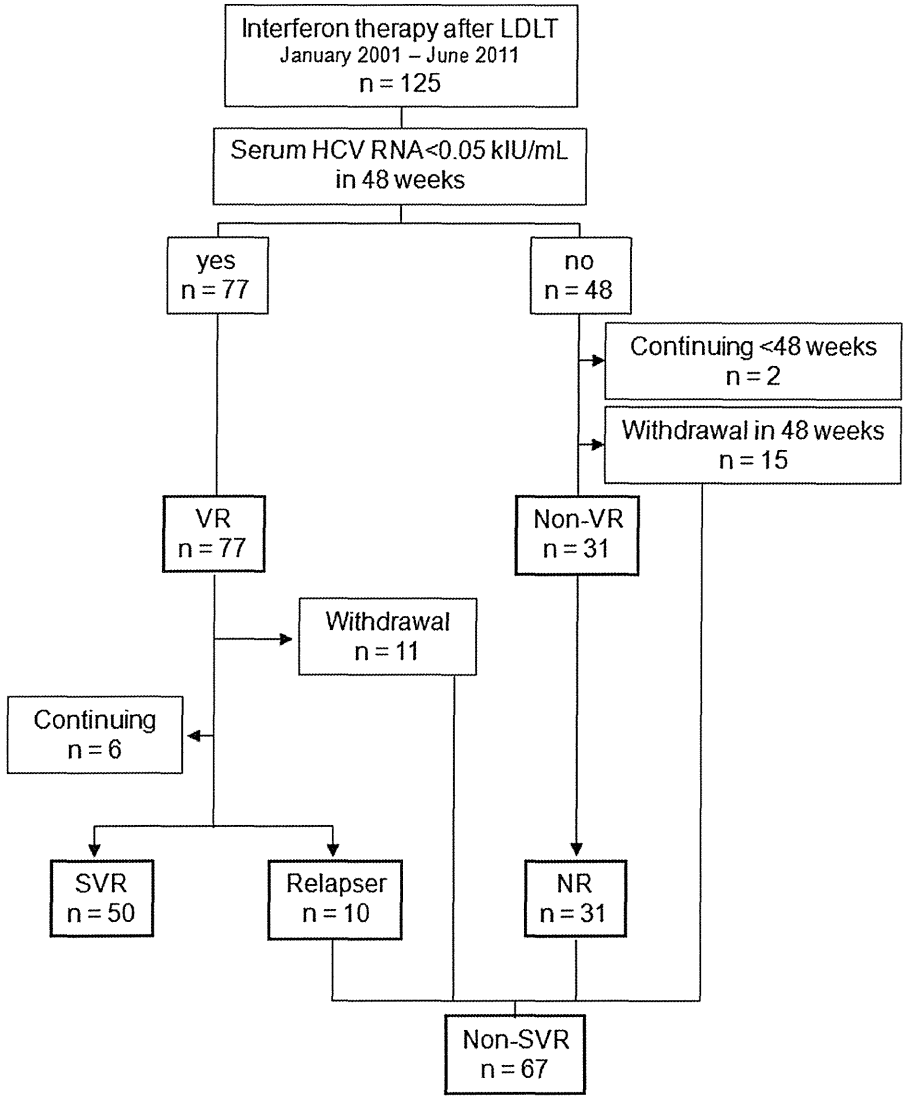

Figure 1. Flow diagram showing the outcome of interferon therapy in patients with recurrent hepatitis C after living donor liver transplantation (LDLT) and indicating the classification of patients in this study. N, number of patients; VR, virological response; SVR, sustained virological response; NR, nonresponder.
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Factors Predictive of Virological Response

Factors that could predict virological response were analyzed by comparing patients in the VR (n = 77) and non-VR (n = 31) groups (Table 1). Univariate analysis demonstrated that a low pretransplant serum HCV RNA level (less than 500 kIU/mL, $P<0.001$; and less than 1000 kIU/mL, $P<0.001$), an ABO-mismatched donor ($P=0.036$), HCV genotype (non-1, $P=0.001$), and a low pretreatment serum HCV RNA level (less than 5000 kIU/mL, $P=0.020$) were significantly associated with VR. There were no significant associations with any other variables, including donor factors. Multivariate analysis revealed that the 3 variables that retained a significant association in the model were a pretransplant serum HCV RNA level less than 500 kIU/mL [odds ratio (OR): 0.178, 95% confidence interval (CI): 0.054–0.535, $P=0.001$], a non-1 HCV genotype (OR: 0.087, 95% CI: 0.000–0.589, $P=0.008$), and an ABO-mismatched donor (OR: 5.492, 95% CI: 1.004–58.06, $P=0.049$) (Table 2). All 20 patients with a non-1 HCV genotype achieved VR, while VR rate in patients with the HCV genotype 1 was 65% (57 out of 88 patients). In the patients with HCV genotype 1, VR rate was 80% (36 of 45 patients) when pretransplant serum HCV-RNA level was less than 500 kIU/mL and 42% (15 of 36 patients) when it was 500 kIU/mL or more. Among 22 recipients from ABO-mismatched donors, 20 patients (91%) showed VR, while 57 (66%) out of 86 patients who underwent LDLT from an ABO-matched (identical and compatible) donor achieved VR.

Factors Predictive of SVR

The same variables were analyzed to clarify factors that predicted SVR by comparing patients in the SVR (n = 50) and non-SVR (n = 67) groups (Table 1). By univariate analysis, the same variables that had a significant association with VR were identified as significant predictive factors for SVR–low pretransplant serum HCV RNA levels (less than 100 kIU/mL, $P=0.028$;