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CLINICAL STUDIES

Hepatocellular carcinoma and survival in patients with autoimmune hepatitis (Japanese National Hospital Organization-autoimmune hepatitis prospective study)

Kiyoshi Migita[1], Yukio Watanabe[1], Yuka Jiuchi[1], Yoko Nakamura[1], Akira Saito[1], Michiyasu Yagura[1], Hajime Ohta[1], Masaaki Shimada[1], Eiji Mita[1], Taizo Hijioka[1], Haruhiro Yamashita[1], Eiichi Takezaki[1], Toyokichi Muro[1], Hironori Sakai[1], Makoto Nakamuta[1], Seigo Abiru[1], Atsumasa Komori[1], Masahiro Ito[1], Hiroshi Yatsuhashi[1], Minoru Nakamura[1] and Hiromi Ishibashi[1] Japanese NHO-Liver-network study group

Clinical Research Center, NHO Nagasaki Medical Center, Omura, Japan

Keywords
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Correspondence
Kiyoshi Migita, MD, Clinical Research Center, NHO Nagasaki Medical Center, Kubara 2-1001-1, Omura 856-8652, Japan
Tel: +81 957 52 3121
Fax:+81 957 53 6675
e-mail: migita@nmc.hosp.go.jp

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Abstract

Background/Aims: Although the outcome of autoimmune hepatitis (AIH) is generally good, the natural course and likelihood of progression to cirrhosis or hepatocellular carcinoma (HCC) remain undefined, and may vary by region and population structure. Our aims were to evaluate risk factors that contribute to poor outcome and particularly development of HCC in a prospective multicentric cohort study of AIH. *Methods:* The study group comprised 193 Japanese patients with AIH who were prospectively followed up at annual intervals between 1995 and 2008. The mean follow-up period was 8.0 ± 4.5 years. *Results:* Twenty-one (10.9%) patients had cirrhosis at presentation and a further 15 (7.8%) developed cirrhosis during the follow-up period. Survival rates were 94.2% at 10 years and 89.3% at 15 years. HCC was diagnosed in seven of the 193 patients. The presence of cirrhosis at presentation was a risk factor for HCC according to a Cox proportional hazard model, and the HCC-free survival rate was significantly lower in those with cirrhosis compared to those without cirrhosis according to Kaplan–Meier analysis. *Conclusions:* Although the outcome of AIH is as good if not better among Japanese than for other populations, there was an increased risk of HCC in these patients. Cirrhosis at presentation was predictive of development of HCC in AIH in Japan.

Autoimmune hepatitis (AIH) is a chronic inflammatory disorder, dependent in part on autoimmune reactivity, that can cause cirrhosis and end-stage liver disease (1, 2). Current descriptions of features of AIH, derived mostly from Caucasian patients, cite a generally good outcome with 10- and 20-year survivals more than 80% (3). Similarly, the outcome among Japanese patients with AIH is generally good with a 10-year survival rate reported as 90% (4). In general, the natural history and course of AIH are largely defined by the degree of inflammatory activity at the onset of disease and the presence or development of cirrhosis (5). Hepatocellular carcinoma (HCC) complicating AIH is reported (6) but occurs rarely among Caucasian populations secondary to AIH (7): the true incidence remains uncertain and factors contributing to development of HCC in AIH are not fully elucidated. To better understand the natural history and outcome of AIH, and to establish comparisons of AIH among Japanese and Caucasian patients, a nation-wide multicentre cohort study was developed, and herein, we describe the clinical presentation, course and efficacy of treatment of 193 consecutive AIH patients enrolled in the Japanese National Hospital Organization (NHO)-AIH register. We particularly assessed risk factors for a fatal outcome and development of HCC.

Patients and methods

Study population

There were 212 patients initially enrolled in the register of the Japanese National Hospital Organization (NHO) liver-network study, contributed to medical facilities in Japan. Of these 212 patients, 193 were retained and prospectively followed between 1995 and 2008 as a multicentre cohort population. All patients satisfied the 1999 revised criteria of International Autoimmune Hepatitis Group (IAIHG) for a diagnosis of definite (114 cases) or probable (79 cases) AIH (8). Patients were excluded

from study if there was histological evidence of cholangitis or non-alcoholic steatohepatitis. Also, patients who were positive for hepatitis B virus (HBV)-surface antigen (HBsAg) or hepatitis C virus (HCV)-RNA were excluded and other causes of liver disease, such as excess alcohol, or drugs had been excluded by appropriate history and investigations. The study protocol was approved by the Ethics Committees of all institutes.

Clinical and histological assessments

Follow-up assessments were made at annual intervals. Standard laboratory tests of liver inflammation and function were measured at each assessment. Liver tissue from percutaneous biopsy performed at the referring facility was available for the majority of the patients at the time of entry (143/193, 74.1%) and at subsequent follow-up examination for some (39/193, 20.2%). The histological variables examined included degree of fibrosis (0; absent, 1; expansion of fibrosis to parenchyma, 2; portal-central or portal-portal bridging fibrosis, 3; presence of numerous fibrous septa, 4; multi-nodular cirrhosis). The histological diagnosis of cirrhosis required loss of normal lobular architecture, reconstruction of hepatic nodules and presence of regenerative nodules (9). Biopsy samples from AIH patients developing to HCC were examined in a blinded fashion by a dedicated pathologist (MI). Anti-nuclear antibodies (ANA) and smooth muscle antibodies (SMA) were measured by indirect immunofluorescence on HEp-2 cell respectively and cut-off titres for positivity were 1:40. Clinical relapse was defined as an increase of serum ALT levels to beyond three-fold of the upper limit of normal range (ULN) (10). Asymptomatic patients or patients with lower serum aminotransferase, total bilirubin or IgG were managed with ursodeoxycholic acid (UDCA) therapy alone, which was demonstrated to be effective in Japanese patients with type I autoimmune hepatitis (11).

Variables at study entry

Demographic and other characteristics of the 193 retained patients were recorded as a data-base at the initial assessment. Data included gender, age at diagnosis, time of onset of symptoms or other evidence of liver disease, markers of infection with hepatitis viruses HBV and HCV, alcohol intake, coexisting autoimmune diseases, serum levels of ALT, AST, alkaline phosphatase and bilirubin, platelet count and prothrombin time.

Occurrence of hepatocellular carcinoma

Abdominal ultrasound and serum alpha-fetoprotein determinations were performed annually. Viral hepatitis was excluded by testing for HBsAg and HCV-RNA by polymerase chain reaction (PCR). Subjects with antibodies to HCV were subsequently screened for HCV-RNA using nested PCR. A diagnosis of HCC was made based on the typical patterns on imaging studies, such as early-phase hyperattenuation and late-phase hypoattenuation by dynamic computerized tomography, magnetic resonance imaging and finally, by ultrasonography-guided tumour biopsy.

Statistical analysis

For quantitative data, analysis was performed using a Mann–Whitney test for comparison of two independent groups. Differences in proportions were analysed by the Fisher's exact test when the number of subjects was <5, and the chi-squared test for 2 × 2 tables when the number of subjects was >5. Prognostic factors for HCC were analysed using the univariate and multivariate Cox proportional hazard model with SPSS software (Chicago, Illinois, USA). The *P*-values of entering variables for multivariate Cox proportional hazard model were <0.1. Survival, related to follow-up time, was analysed using the Kaplan–Meier method and compared using the log-rank test. A value of $P \leq 0.05$ was considered statistically significant.

Results

Baseline data at entry

Of the original 212 recruited patients registered as AIH, 19 were excluded from analysis owing to loss of follow-up (Fig. 1). The retained 193 were considered eligible for the study. Table 1 presents the demographic data for the cohort at entry. The age at diagnosis ranged from 16 to 84 years (mean, 56.6 ± 13.9 years), greater than that in earlier studies on Caucasian patients, and female patients predominated (91.7%). In 51 patients (26.4%) there was concurrent symptomatic autoimmune disease, notably Hashimoto thyroiditis in 11, rheumatoid arthritis in 20, systemic lupus erythematosus in 6, Sjögren's syndrome in 14 and systemic sclerosis in 2. Regarding tests for autoantibodies, data for SMA were lacking in 105 and for ANA in 5. Of those tested, 158 (84.0%) gave positive tests (titre >1:40) for ANA and 36 (40.9%) for SMA. Regarding treatment, 144 patients (74.6%) had been treated with prednisolone, and 43 (22.3%) with ursodeoxycholic acid alone. Relapse occurred in 47 (24.4%) during the follow-up period.

Patient outcome and survival

Seven patients (3.6%) died as a result of complications of AIH. Liver-related death (HCC 2, ruptured oesophageal varices 1, hepatic failure 1) was confirmed in four AIH patients with cirrhosis at presentation (19.0%), one patient (hepatic failure) who subsequently developed cirrhosis during follow-up (6.7%) and one patient (fulminant hepatitis) without cirrhosis during the follow-up period (Fig. 1, Table 2). The overall survival in the AIH patients is shown in Figure 2. The calculated

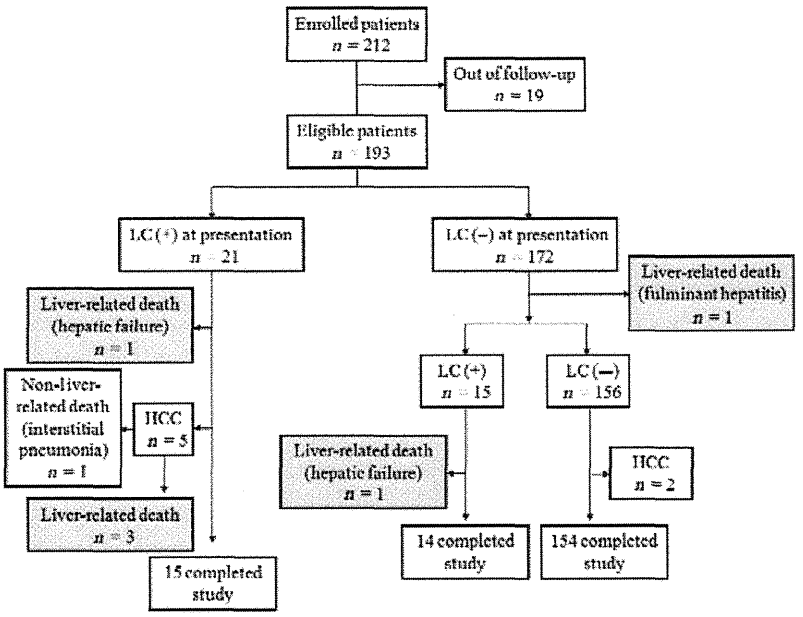

Fig. 1. Flow diagram of patient selection and clinical outcome of autoimmune hepatitis (AIH) patients in the present cohort study. C; cirrhosis, HCC; hepatocellular carcinoma.

Table 1. Baseline characteristics of AIH patients

	n = 193
Gender (male/female)	16/177
Mean age at presentation (years)	56.6 ± 13.9 (16–84)
Mean age	
Age ≥ 60year	98 (50.8%)
Age<60year	95 (49.2%)
Other autoimmune diseases	51 (26.4%)
Mean follow-up (years)	8.0 ± 4.5 (0.1–21)
Baseline Laboratory Values	
AST (<40IU/L)	392.00 ± 450.65 (29–2718)
ALT (<40IU/L)	408.55 ± 421.21 (18–2020)
ALP (<112U/L)	453.18 ± 270.04 (112–2135)
Bilirubin (mg/dl)	3.95 ± 5.66 (0.27–31.8)
Albumin (3.5–5.0g/L)	3.76 ± 0.61 (2.00–5.10)
IgG (500–1300mg/dl)	2517.49 ± 913.43 (210.2–5221)
Platelets (15–40×10^4μl)	19.25 ± 8.02 × 10^4 (2.00–57.00 × 10^4)
ANA+ (≥ 1:40)	158/188 (84.0%)
SMA+ (≥ 1:40)	36/88 (40.9%)
Cirrhosis at presentation	21 (10.9%)
Received treatment	
Mean PSL (mg/day)	28.71 ± 72.98 (0–1000)
PSL ≥ 20mg	126 (65.3%)
PSL alone	100 (51.8%)
PSL+UDCA	42 (21.8%)
PSL+Aza	2 (1.0%)
UDCA alone	43 (22.3%)
Relapse	47 (24.4%)

ALP, alkaline phosphate; ALT, alanine aminotransferase; ANA, antinuclear antibody; AST, aspartate aminotransferase; Aza, azathioprine; PSL, prednisolone; SMA, anti-smooth muscle antibody; UDCA, ursodeoxy cholic acid.

Table 2. Fatal outcome of patients with type 1 autoimmune hepatitis

	Cirrhosis at entry (*n* = 21)	Developed cirrhosis (*n* = 15)	No cirrhosis (*n* = 157)
Deaths (*n*)	5	2	5
Liver-related	4	1	1
HCC	2	0	0
Ruptured oesophageal varices	1	0	0
Fulminant hepatitis	0	0	1
Liver failure	1	1	0
Non-Liver-related	1	1	4
CVA	0	0	3
Lymphoma	0	1	0
Lung cancer	0	0	1
Interstitial pneumonia	1	0	0
Follow-up after cirrhosis (year)	10.0 ± 4.8	4.3 ± 3.9	NA
Total follow-up (year)	10.0 ± 4.8	9.5 ± 4.6	7.6 ± 4.4

Note: Numbers in parentheses represent percentages. Data are expressed as mean ± SD.
HCC, hepatocellular carcinoma; CVA, c erebrovascular accident.

survival for the entire cohort was 94.2% at 10 years and 89.3% at 15 years.

Development of HCC

Hepatocellular carcinoma was diagnosed in as many as seven of the 193 (3.6%) patients with AIH, in five

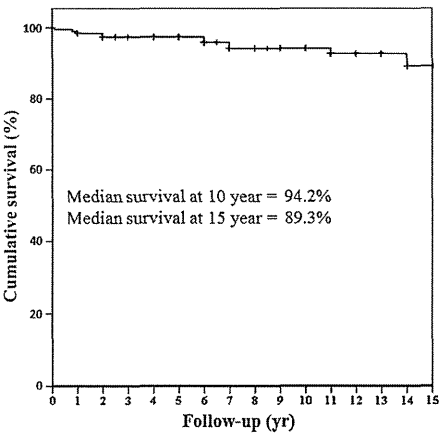

Fig. 2. Survival curve of Japanese patients with AIH. The 10-year survival was 94.2% and 15-year survival was 89.3%.

female patients (2.6%) and in two male patients (1.0%). The mean age at diagnosis was 62.0 ± 10.5 years (range, 43–73 years; median 65 years). Of the seven patients who developed HCC, five had antecedent cirrhosis (mean duration of cirrhosis, 9.0 ± 3.5 years; range, 4–12 years; median 11 years); two who developed HCC did not have antecedent cirrhosis (Fig. 1). Two patients with cirrhosis and HCC died within 2 years after the diagnosis of HCC and three were surviving at the last follow-up. HCC developed in seven patients. Table 3 summarizes the clinical features, and outcome of these HCC patients. At diagnosis of HCC, five patients had cirrhosis and the remaining two patients did not progress to cirrhosis. Overall, four patients died, among which two patients died of HCC, one of ruptured oesophageal varies and one of interstitial pneumonia. The mean survival time from HCC diagnosis was 3.2 ± 2.2 years in these four patients. The remaining three patients still alive and the mean duration from HCC diagnosis to the final surveillance was 5.0 ± 1.3 years.

Initial clinical features and laboratory data at diagnosis of AIH were compared between patients with and without HCC (Table 4). In patients who developed HCC, the frequencies of the association with cirrhosis were significantly higher. Also lower platelet counts, total bilirubin and ALT were observed in the group with HCC. Using time-dependent univariate analysis (Cox proportional hazard model), the following variables at

Table 3. Clinical features of 7 patients who developed HCC

Patient no	Case1	Case2	Case3	Case4	Case5	Case6	Case7
Age at diagnosis (years)	72	60	73	43	55	65	66
Gender	Female	Female	Female	Female	Male	Male	Female
Labo data at diagnosis							
Platelet (/μl)	126000	196000	172000	94000	129000	135000	44000
AST/ALT (IU/ml)	244/86	102/55	140/107	57/44	95/185	100/159	80/66
Cirrhosis at presentation	(+)	(–)	(+)	(+)	(+)	(–)	(+)
Liver histology at presentation							
Grading A score	NT	NT	A2	A2	A2	A2	A2
Staging F score	NT	NT	F3	F4	F4	Fl	F3
Other autoimmune diseases	SLE	RA	(+)	Chronic thyroiditis	(–)	(–)	(–)
Initial treatments	PSL30 mg/day	PSL2.5 mg/day	PSL30 mg/day	PSL40 mg/day	UDCA600 mg/day	PSL30 mg/day UDCA60 0 mg/day	PSL30 mg/day UDCA300 mg/day
Age at HCC diagnosis (years)	84	72	79	54	67	71	70
Duration from AIH diagnosis (years)	11.5	11.1	5.7	11.5	11.5	5.9	4.0
Cirrhosis at HCC diagnosis	(+)	(–)	(+)	(–)	(+)	(–)	(+)
Labo data at HCC diagnosis							
AST/ALT (IU/ml)	59/30	81/49	27/40	46/25	74/56	25/36	187/90
Treatments	TACE	Surgery TACE	TACE	TACE	TACE/RFA	TACE	TACE/PEIT
Survival (survival' death)	Death	Survival	Death	Death	Survival	Survival	Death
Cause of death	HCC		HCC	Interstitial pneumonia			Ruptured oesophageal varices
Survial time from HCC diagnosis (years)	2.0 year (death)	6.1 year (alive)	1.4 year (death)	6.4 year (death)	4.5 year (alive)	40 year (alive)	3.0 year (death)

HCC, hepatocellular carcinoma; PEIT, precutaneous ethanol injection therapy; RA, rheumatoid arthritis; RFA, radio frequency ablation; SLE, systemic lupus erythematosus; TACE, transcatheter arterial chemoembolization; UDCA, ursodeoxycholic acid.

Table 4. Baseline characteristics of AIH patients with or without HCC

	HCC (+) $n = 7$	HCC (−) $n = 186$	*P*
Mean age			
Age ≥ 60year	5	93	0.235
Age<60year	2	93	
Gender (male female)	215	14/172	0.106
Mean age at presentation (years)	62.0 ± 10.5 (43–73)	56.4 ± 14.0 (16–84)	0.321
Other autoimmune diseases	3 (42.9%)	48 (25.8%)	0.271
Mean follow-up (years)	12.9 ± 4.8 (7–18)	7.9 ± 4.4 (0.1–21)	0.011
Baseline laboratory values			
AST(<40IUL)	114.00 ± 54.59 (57–224)	402.44 ± 455.67 (29–2718)	0.050
ALT (<40IU/L)	100.29 = 53.65 (44–185)	420.15 ± 424.62 (18–2020)	0.014*
ALP (<112IU/L)	383.43 ± 197.06 (135–679)	455.89 ± 272.53 (112–2135)	0.611
Bilirubin (mg/dl)	0.74 ± 0.38 (0.3–1.5)	4.07 ± 5.73 (0.27–31.8)	0.015*
Albumin (3.5–5.0g/L)	3.66 ± 0.60 (2.7–4.4)	3.77 ± 0.61 (2.00–5.10)	0.633
IgG (500–1300mg/dl)	2842.43 ± 1008.55 (1480–4280)	2504.64 ± 910.22 (210.2–5211)	0.322
Platelets (15–40×10/μl)	12.80 ± 5.00 × 10^4 (4.40–19.60 × 10^4)	19.51 ± 8.02 × 10^4 (2.00–57.05 × 10^4)	0.015*
ANA+(≥ 1:40)	6/7 (85.7%)	152/181 (84.0%)	0.690
SMA+(≥ 1:40)	0/2	36/86 (41.9%)	0.346
HCV Ab (+)	1 (14.3%)	4 (2.2%)	0.175
Cirrhosis at presentation	5 (71.4%)	16 (8.6%)	<0.001**
Received treatment			
Mean PSL (mg/day)	23.21 ± 15.46 (0–40)	28.92 ± 74.30 (0–1000)	0.991
PSL ≥ 220mg	5 (71.4%)	117 (62.9%)	0.490
PSL aloilff	4 (57.1%)	89 (47.8%)	0.460
PSL+UDCA	2 (28.6%)	39 (21.0%)	0.459
PSL+Aza	0	2 (1.1%)	0.929
UDCA alone	1 (14.3%)	37 (19.9)	0.584
Relapse	4(57.1%)	43 (23.1)	0.061
Liver biopsy specimen available at presentation	(n = 5)	(n = 138)	
Stage of fibrosis			
F0	0	10 (7.2%)	
FI	1 (20.0%)	34 (24.6%)	
F2	0	35 (25.4%)	
F3	2 (40.0%)	31 (22.5%)	
F4	2 (40.0%)	7 (7.1%)	
ALT	(*n* = 6)	(*n* = 164)	
1year after first treatment	54.0 ± 27.31 (31–96)	44.7 ± 65.2 (6–521)	0.020*

*P < 0.05; **P < 0.01.

ALP, alkaline phosphate; ALT, alanine aminotransferase; ANA, anti-nuclear antibody; AST, aspartate aminotransferase; Aza, azithioprine; PSL, prednisolone; SMA, anti-smooth muscle antibody; UDCA, ursodeoxy cholic acid.

accession were associated with risk for HCC; male gender ($P = 0.033$) and the presence of cirrhosis ($P = 0.002$) at presentation (Table 5). By multivariate Cox analysis, the presence of cirrhosis (Hazard ratio 11.47, 95% CI 2.13–64.60, $P = 0.005$) was associated independently with risk for HCC (Table 6). These data suggest that male patients and cirrhosis at the onset are at particular risk for HCC.

HCC-free survival rates

Figure 3 presents Kaplan–Meier estimates for the cumulative HCC-free survival rate, based upon the presence or absence of cirrhosis at enrolment. The 15-year survival rate without HCC was 96.6% ±2.7 in AIH patients without cirrhosis, and 62.2% ±13.9 in those with cirrhosis. A log-rank test of the two curves showed a significant difference in that the HCC-free survival rate of AIH patients with cirrhosis was significantly lower than that of those without cirrhosis ($P < 0.0001$).

Discussion

Autoimmune hepatitis is a chronic progressive liver disease caused by immune-mediated destruction of hepatic parenchymal cells. Accurate diagnosis depends on a combination of features scored as recommended by the IAIHG (8), with critical criteria including interface hepatitis histologically, hypergammaglobulinemia and characteristic serum autoantibodies (1). The precise pathogenic processes that lead to AIH are uncertain, but likely depend on a genetic predisposition of the host to

Table 5. Variables associated with increased risk factor for HCC (Univariate Cox proportional hazard model)

Characteristics	Subgroup	HCC		HR (95% CI)	*P* value
		Yes (*n* = 7)	No (*n* = 186)		
Gender	Male	2 (28.6%)	14 (7.5%)	6.058 (1.151–31.869)	0.033
Age	<50	1 (14.3%)	51 (27.4%)	0.325 (0.039–2.707)	0.299
	50–59	1 (14.3%)	42 (22.6%)	0.496 (0.060–4.124)	0.517
	≥60	5 (71.4%)	93 (50.0%)	3.617 (0.699–18.723)	0.125
Other autoimmune disease	(+)	3 (42.9%)	48 (25.8%)	1.730 (0.386–7.750)	0.474
Cirrhosis at presentation	(+)	5 (71.4%)	16 (8.6%)	13.878 (2.670–72.142)	0.002
PSL	(−)	1 (14.3%)	55 (29.6%)	0.330 (0.040–2.748)	0.306
	1–19 mg/day	1 (14.3%)	13 (7.0%)	1.164 (0.138–9.827)	0.889
	20–39 mg/day	4 (57.1%)	60 (32.3%)	3.352 (0.750–14.994)	0.113
	≥40 mg/day	1 (14.3%)	57 (30.6%)	0.484 (0.058–4.053)	0.503
Relapse	(+)	4 (57.1%)	43 (23.1%)	3.789 (0.848–16.936)	0.081

HCC, hepatocellular carcinoma; HR, hazard ratio; PSL, prednisolone.

Table 6. Multivariate analysis of predictive factor for HCC in AIH patients (Cox proportional hazards model)

Variables	*P*	HR (95% CI)
Male gender	0.275	2.572 (0.472–14.008)
Cirrhosis at presentation	0.005	11.741 (2.134–64.602)

CI, confidence interval; HCC, hepatocellular carcinoma; HR, hazard ratio.

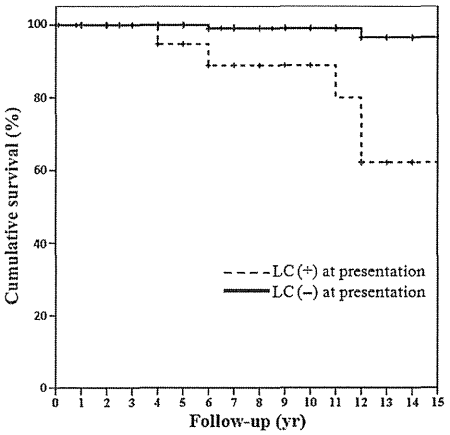

Fig. 3. Fifteen-year HCC-free survival for AIH patients with and without cirrhosis at entry. Kaplan–Meier survival curve comparing HCC-free survival among patients with or without cirrhosis at baseline. The 15-year HCC-free survival was 62.2% for patients with cirrhosis compared with 96.6% for patients without cirrhosis at baseline ($P < 0.0001$).

specific autoimmune reactivity to self-antigens with ensuing hepatic inflammation mediated by T-cell cytotoxicity mechanisms (12, 13).

Our present study has revealed some features of AIH particular to the Japanese population, as well as some differences in practice between Japanese and 'western'-trained internists. In our longitudinal multicentre study on Japanese AIH patients, 71% were treated with corticosteroid during the mean 8-year follow-up period giving an estimated 10-year survival rate as high as 94%. Thus, the survival for corticosteroid-treated type 1 AIH is generally good as in previous reports (3, 4). However, our study did not confirm previous experience that the incidence in AIH of HCC was low, since this did not pertain in patients with histological cirrhosis.

There are a number of reports that patients with chronic viral hepatitis, whether owing to HBV or HCV, are prone to develop HCC in contrast to its infrequency in AIH (7, 14). Park *et al.* (14) reported low incidence of HCC in Caucasian patients with AIH and cirrhosis, observing only one case of HCC among 88 patients with cirrhosis caused by AIH among a total of 212 patients overall, suggesting an incidence of HCC of about 0.1% per patient year (14). In our cohort of 193 patients with AIH, 21 (10.9%) patients suffered from cirrhosis at presentation and, during the follow-up period, seven developed HCC. The patients in our AIH cohort comprised a proportion with cirrhosis similar to that of Park but our follow-up time 8.0 ± 4.5 years was longer. Perhaps the true risk of HCC in AIH patients differs according to ethnicity of the population. Werner *et al.* (6) in the Swedish national-wide AIH cohort showed an overall increase in risk in AIH for all malignancies, mainly contributed to by hepatobiliary cancer. To reiterate, our data indicate that cirrhosis at presentation of AIH was indeed associated with a risk for occurrence of HCC, contrary to traditional belief (15), so that, although HCC occurrence in AIH is lower than that seen in viral-mediated liver disease (15, 16), long-standing cirrhosis may well be a significant risk factor for HCC even in AIH, as described previously (17).

Regarding gender as a risk factor for HCC, Montano-Loza *et al.* (17) reported that male gender and long-standing cirrhosis are combined risk factors for development of HCC in AIH patients. In the present

study, we found a correlation between gender and progression to HCC only in univariate Cox proportional hazard model and not in multivariate Cox proportional hazard model. Thus furthermore large-scale studies are needed in AIH to elucidate the link between gender and HCC.

Although previous concerns have been raised regarding occult viral hepatitis infection as being instrumental in the aetiology of HCC in AIH (18), all patients in our study were negative for serological markers of HBV infection and as well for persisting HCV infection. International diagnostic criteria of AIH (8) allocate negative points for positive HBV or HCV diagnostic tests. Although, seven had anti-HCV antibodies none of these had any evidence of active HCV infection (HCV-RNA negative), and all fulfilled the IAIHG criteria. Whilst seropositivity for HCV was not a statistically significant risk factor for HCC development, the unlikely possibility exists that pre-existing HCV infection was a contributing risk factor for HCC in a small subset of AIH patients. Recently, non-alcoholic steatohepatitis (NASH) has been recognized as an important cause of HCC even in Japan (19). In patients with risk factors for non-alcoholic fatty liver diseases (NAFLD), diagnosis of AIH was confirmed according to the liver histological findings (20). However, we could not rule out the possibility that NASH-related LC was included in our population completely.

In AIH, cirrhosis at presentation is reported to be an important prognostic factor (20, 21). Our study suggests that the long-term outcome for AIH patients with cirrhosis is relatively unfavourable, since the 15-year HCC-free survival rate was only 64% and further that HCC-related death partly contributed to the poorer outcome in AIH with cirrhosis. Consistent with this, Feld *et al.* (22) reported that AIH patients with cirrhosis at presentation had an inferior 10-year survival vs. those without cirrhosis [61.9% vs. 94.0%].

In conclusion, the outcome for Japanese patients with AIH is good, consistent with previous reports. However, 7 (3.6%) of 193 developed HCC and so, among Japanese AIH patients, HCC may not be an uncommon final event in patients with AIH. The presence of cirrhosis at presentation confers an increased risk for future HCC, albeit less commonly than with hepatitis virus-related liver diseases. These risk factors call for a regular screening strategy for HCC in AIH patients with cirrhosis.

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NHO-AIH study group: Kiyoshi Migita, Yuka Jiuchi, Seigo Abiru, Atsumasa Komori, Masahiro Ito, Hiroshi Yatsuhashi, Minoru Nakamura, Hiromi Ishibashi (NHO Nagasaki Medical Center), Yukio Watanabe, Yoko Nakamura (NHO Sagamihara National Hospital), Akira Saito (NHO Nishisaitama-chuo Hospital), Michiyasu Yagura (NHO Tokyo National Hospital), Hajime Ohta (NHO Kanazawa Medical Center), Masaaki Shimada (NHO Nagoya Medical Center), Eiji Mita (NHO Osaka National Hospital), Taizo Hijioka (NHO Osaka Minami Medical Center), Haruhiro Yamashita (NHO Okayama Medical Center), Eiichi Takezaki (NHO Higashi Hiroshima Medical Center), Toyokichi Muro (NHO Oita Medical Center), Hironori Sakai (NHO Beppu Medical Center), Makoto Nakamuta (NHO Kyushu Medical Center).

References

1. Krawitt EL. Autoimmune hepatitis. *N Engl J Med* 1996; **334**: 897–903.
2. Donaldson PT, Czaja AJ. Genetic effects on susceptibility, clinical expression, and treatment outcome of type 1 autoimmune hepatitis. *Clin Liver Dis* 2002; **6**: 707–25.
3. Czaja AJ. Treatment of autoimmune hepatitis. *Semin Liver Dis* 2002; **22**: 365–78.
4. Miyake Y, Iwasaki Y, Terada R, *et al.* Persistent normalization of serum alanine aminotransferase levels improves the prognosis of type 1 autoimmune hepatitis. *J Hepatol* 2005; **43**: 951–7.
5. Czaja AJ. Natural history, clinical features, and treatment of autoimmune hepatitis. *Semin Liver Dis* 1984; **4**: 1–12.
6. Werner M, Almer S, Prytz H, *et al.* Hepatic and extrahepatic malignancies in autoimmune hepatitis. A long-term follow-up in 473 Swedish patients *J Hepatol* 2009; **50**: 388–93.
7. Teufel A, Weinmann A, Centner C, *et al.* Hepatocellular carcinoma in patients with autoimmune hepatitis. *World J Gastroenterol* 2009; **15**: 578–82.
8. Alvarez F, Berg PA, Bianchi FB, *et al.* International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. *J Hepatol* 1999; **31**: 929–38.
9. Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. *Hepatology* 1994; **19**: 1513–20.
10. Manns MP, Czaja AJ, Gorham JD, *et al.*; American Association for the Study of Liver Diseases. Diagnosis and management of autoimmune hepatitis. *Hepatology* 2010; **51**: 2193–213.
11. Nakamura K, Yoneda M, Yokohama S, *et al.* Efficacy of ursodeoxycholic acid in Japanese patients with type 1 autoimmune hepatitis. *J Gastroenterol Hepatol* 1998; **13**: 490–5.
12. Ichiki Y, Aoki CA, Bowlus CL, *et al.* T cell immunity in autoimmune hepatitis. *Autoimmun Rev* 2005; **4**: 315–21.
13. Vergani D, Longhi MS, Bogdanos DP, Ma Y, Mieli-Vergani G. Autoimmune hepatitis. *Semin Immunopathol* 2009; **31**: 421–35.
14. Park SZ, Nagorney DM, Czaja AJ. Hepatocellular carcinoma in autoimmune hepatitis. *Dig Dis Sci* 2000; **45**: 1944–8.
15. Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology* 2004; **127**: S35–50.

16. Tsukuma H, Hiyama T, Tanaka S, *et al.* Risk factors for hepatocellular carcinoma among patients with chronic liver disease. *N Engl J Med* 1993; **328**: 1797–801.
17. Montano-Loza AJ, Carpenter HA, Czaja AJ. Predictive factors for hepatocellular carcinoma in type 1 autoimmune hepatitis. *Am J Gastroenterol* 2008; **103**: 1944–51.
18. Ryder SD, Koskinas J, Rizzi PM, *et al.* Hepatocellular carcinoma complicating autoimmune hepatitis: role of hepatitis C virus. *Hepatology* 1995; **22**: 718–22.
19. Michitaka K, Nishiguchi S, Aoyagi Y, *et al.*; Japan Etiology of Liver Cirrhosis Study Group. Etiology of liver cirrhosis in Japan: a nationwide survey. *J Gastroenterol* 2010; **45**: 86–94.
20. Yatsuji S, Hashimoto E, Kaneda H, *et al.* Diagnosing autoimmune hepatitis in nonalcoholic fatty liver disease: is the International Autoimmune Hepatitis Group scoring system useful? *J Gastroenterol* 2005; **40**: 1130–8.
21. Yeoman AD, Al-Chalabi T, Karani JB, *et al.* Evaluation of risk factors in the development of hepatocellular carcinoma in autoimmune hepatitis: implications for follow-up and screening. *Hepatology* 2008; **48**: 863–70.
22. Feld JJ, Dinh H, Arenovich T, *et al.* Autoimmune hepatitis: effect of symptoms and cirrhosis on natural history and outcome. *Hepatology* 2005; **42**: 53–62.

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Original article

Age and total ribavirin dose are independent predictors of relapse after interferon therapy in chronic hepatitis C revealed by data mining analysis

*Masayuki Kurosaki[1], Naoki Hiramatsu[2], Minoru Sakamoto[3], Yoshiyuki Suzuki[4], Manabu Iwasaki[5], Akihiro Tamori[6], Kentaro Matsuura[7], Sei Kakinuma[8], Fuminaka Sugauchi[9], Naoya Sakamoto[8], Mina Nakagawa[8], Hiroshi Yatsuhashi[10], Namiki Izumi[1]**

[1]Division of Gastroenterology and Hepatology, Musashino Red Cross Hospital, Tokyo, Japan
[2]Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine, Osaka, Japan
[3]First Department of Internal Medicine, University of Yamanashi, Yamanashi, Japan
[4]Department of Hepatology, Toranomon Hospital, Tokyo, Japan
[5]Department of Computer and Information Science, Seikei University, Tokyo, Japan
[6]Department of Hepatology, Osaka City University Medical School, Osaka, Japan
[7]Department of Gastroenterology and Metabolism, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan
[8]Department of Gastroenterology and Hepatology, Tokyo Medical and Dental University, Tokyo, Japan
[9]Department of Gastroenterology, Nagoya Koseiin Medical Welfare Center, Nagoya, Japan
[10]Clinical Research Center, National Nagasaki Medical Center, Nagasaki, Japan

*Corresponding author e-mail: nizumi@musashino.jrc.or.jp

Background: This study aimed to define factors associated with relapse among responders to pegylated interferon (PEG-IFN) plus ribavirin (RBV) therapy in chronic hepatitis C.

Methods: A cohort of genotype 1b chronic hepatitis C patients treated with PEG-IFN plus RBV and who had an undetectable HCV RNA by week 12 (n=951) were randomly assigned to model derivation (n=636) or internal validation (n=315) groups. An independent cohort (n=598) were used for an external validation. A decision tree model for relapse was explored using data mining analysis.

Results: The data mining analysis defined five subgroups of patients with variable rates of relapse ranging from 13% to 52%. The reproducibility of the model was confirmed by internal and external validations (r^2=0.79 and 0.83, respectively). Patients with undetectable HCV RNA at week 4 had the lowest risk of relapse (13%), followed by patients <60 years with undetectable HCV RNA at week 5–12 who received ≥3.0 g/kg of body weight of RBV (16%). Older patients with a total RBV dose <3.0 g/kg had the highest risk of relapse (52%). Higher RBV dose beyond 3.0 g/kg was associated with further decrease of relapse rate among patients <60 years (up to 11%) but not among older patients whose relapse rate remained stable around 30%.

Conclusions: Data mining analysis revealed that time to HCV RNA negativity, age and total RBV dose was associated with relapse. To prevent relapse, ≥3.0 g/kg of RBV should be administered. Higher dose of RBV may be beneficial in patients <60 years.

Introduction

The currently recommended therapy for chronic hepatitis C is a combination of pegylated interferon (PEG-IFN) plus ribavirin (RBV) [1]. This therapy is effective in 50% of patients with HCV genotype 1b [2,3]. The most reliable predictor of sustained virological response (SVR) is the response during early weeks of therapy. A satisfactory response to therapy in the early weeks is associated with a high rate of SVR [4–8]. A basic concept of response-guided therapy is to modify the duration of therapy according to the time to HCV RNA negativity. Extended therapy may be given to patients with delayed virological response [9–13]. Modification of duration of therapy or drug dose may also be necessary in patients with early virological

response (EVR), because approximately 20% of these patients experience relapse after the completion of 48 weeks of therapy. Recent reports have revealed that single nucleotide polymorphisms located near the *IL28B* gene are strongly associated with SVR or a null response to PEG-IFN plus RBV therapy [14–16]. However, single nucleotide polymorphisms located near the *IL28B* gene are not associated with relapse after EVR [17]. Identification of risk factors for relapse among patients with virological response may lead to more individualized therapy and improved SVR rate.

Decision tree analysis, a core component of data mining analysis, is a method that explores data to develop predictive models [18]. This method has been originally used in business and recently in medical fields [19–25]. Decision tree analysis was successfully used to build a predictive model of EVR [26] and SVR to PEG-IFN plus RBV combination therapy in chronic hepatitis C [17,27,28]. The results of the analysis are presented as a tree structure, which is easy to understand and use in clinical practice. Patients can be allocated into subgroups by simply following the flowchart form of the decision tree [29].

In the present study, we used decision tree analysis to identify predictors of relapse among patients who achieved EVR to PEG-IFN plus RBV therapy, and to define a more individualized therapeutic strategy beyond response-guided therapy.

Table 1. Background of study population

Characteristic	Value
Age, years	54.9 (10.8)
Gender	–
Male, *n* (%)	557 (59)
Female, *n* (%)	394 (41)
Body mass index, kg/m^2	23.2 (3.3)
Albumin, g/dl	4.1 (1.8)
Creatinine, mg/dl	0.7 (0.2)
AST, IU/l	60.6 (46.2)
ALT, IU/l	80.7 (77.2)
GGT, IU/l	52.0 (60.0)
White blood cell count, cells/μl	4,993 (1,363)
Haemoglobin, g/dl	15.9 (52.6)
Platelets, 10^9/l	174.4 (6.1)
HCV RNA, KIU/ml	1,655 (1,455)
Fibrosis stage	–
F1-2, *n* (%)	626 (66)
F3-4, *n* (%)	98 (10)
NA, *n* (%)	227 (24)
Time to HCV RNA negativity 4/8/12 weeks	–
4 Weeks, *n* (%)	233 (24)
8 Weeks, *n* (%)	386 (41)
12 Weeks, *n* (%)	332 (35)
Treatment duration, weeks	42 (13)
Total RBV dose, g/kg body weight	3.1 (1.3)
Total PEG-IFN dose, μg/kg body weight	62.5 (38.6)
Outcome	–
Relapse, *n* (%)	238 (25)
SVR, *n* (%)	713 (75)

Total *n*=951. Data are expressed as mean (SD) unless otherwise indicated. ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase; NA, not available; PEG-IFN, pegylated interferon; RBV, ribavirin; SVR: sustained virological response.

Methods

Patients

This is a multicentre retrospective cohort study involving Musashino Red Cross Hospital, Toranomon Hospital, Tokyo Medical and Dental University, Osaka University, Nagoya City University, Yamanashi University, Osaka City University, and their related hospitals. The inclusion criteria were chronic hepatitis C patients treated with PEG-IFN-α2b plus RBV, genotype 1b, pretreatment HCV RNA titre >100 KIU/ml as confirmed by quantitative PCR; Cobas Amplicor HCV Monitor version 2.0; Roche Diagnostic Systems, Pleasanton, CA, USA), an undetectable HCV RNA level within week 12 after the start of therapy, no coinfection with HBV or HIV, and no other causes of liver disease. Patients were treated with PEG-IFN-α2b (1.5 μg/kg) subcutaneously every week plus a daily weight-adjusted RBV dose (600 mg for patients weighing <60 kg, 800 mg for patients weighing 60–80 kg and 1,000 mg for patients weighing >80 kg). Dose reduction or discontinuation of PEG-IFN and RBV was considered based on the recommendations of the package inserts and the discretion of physicians at each university and hospital. The standard duration of therapy was set at 48 weeks, but extension of duration was allowed and implemented at the discretion of each physician. The duration of therapy was extended beyond 48 weeks in 118 patients (mean duration was 56.3 weeks, ranging from 49 to 72 weeks). Although the exact reason for the prolonged treatment in each case was not available, one reason may be that each physician tried to achieve high adherence of RBV by extending the duration of therapy. Another reason may be the late time point of HCV RNA negativity even within early virological response. Among 118 patients, time to HCV RNA negativity was between 9 to 12 weeks in 56% of patients.

A total of 951 patients fulfilled the study criteria. The baseline characteristics and representative laboratory test results are listed in Table 1. For analysis, patients were randomly assigned to either the model derivation (636 patients) or internal validation (315 patients) groups. There were no significant differences in the clinical backgrounds between these two groups. For external validation of the model, we collaborated with another multicentre study group consisting of 29 medical centres and hospitals belonging to the National

Hospital Organization (Japan). A dataset collected from 598 patients who were treated with PEG-IFN-α2b plus RBV and had undetectable HCV RNA within week 12 were used for external validation. Informed consent was obtained from each patient. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the institutional review committees of all concerned hospitals.

Laboratory tests

Haematological tests, blood chemistry and HCV RNA titre were analysed before therapy and at least once every month during therapy. Rapid virological response (RVR) was defined as an undetectable HCV RNA level at week 4, and complete early virological response (cEVR) was defined as an undetectable HCV RNA level at week 5 through week 12 after the start of therapy. SVR was defined as an undetectable HCV RNA level 24 weeks after the completion of therapy. Detection of HCV RNA level was based on qualitative PCR with a lower detection limit of 50 IU/ml (Amplicor; Roche Diagnostic Systems). A database of pretreatment variables included haematological tests (haemoglobin level, white blood cell count and platelet count), blood chemistry tests (serum levels of creatinine, albumin, aspartate aminotransferase, alanine aminotransferase, γ-glutamyltransferase, total cholesterol, triglycerides and HCV RNA titre), stage of histological fibrosis and patient characteristics (age, sex and body mass index). Post-treatment variables included time to HCV RNA negativity, calculated total RBV dose (g/kg of body weight), and calculated total PEG-IFN dose (μg/kg of body weight).

Statistical analysis

The Student's *t*-test was used for the univariable comparison of quantitative variables and Fisher's exact test was used for the comparison of qualitative variables. Logistic regression models with backward selection procedures were used for multivariable analysis of factors associated with relapse. IBM SPSS software version 18.0 (SPSS Inc., Chicago, IL, USA) was used for analysis. For the decision tree analysis [30], the data mining software IBM SPSS Modeler 14 (SPSS Inc.) was used, as reported previously [17,26–28]. The decision tree analysis, the core component of the data mining, belongs to a family of non-parametric regression methods based on binary recursive partitioning of data. In this analysis, the software automatically explored the database to determine optimal split variables to build a decision tree structure. A statistical search algorithm evaluate the model derivation group to determine the optimum variables and cutoff values and to yield the most significant division of patients into two subgroups that were as homogeneous as possible for the probability of relapse. Once patients were divided into 2 subgroups, the analysis was automatically repeated on each subgroup in the same way until either no additional significant variable was detected or the number of patients was <20. Finally all patients were classified into particular subgroups that are homogeneous with respect to the probabilities of relapse.

Results

The decision tree model for the prediction of relapse

The overall rate of relapse was 26% in the model derivation group. The decision tree analysis selected three variables that are associated with relapse: time to HCV RNA negativity, age and total RBV dose (Figure 1). Time to HCV RNA negativity was selected as the best predictor of relapse. The rate of relapse was 13% for patients with RVR compared to 30% for patients with cEVR. Among patients with cEVR, age was selected as the variable of second split. Patients <60 years had a lower probability of relapse (22%) compared with those ≥60 years (41%). The total RBV dose was selected as the third variable of split with an optimal cutoff of 3.0 g/kg of body weight. The rate of relapse was lower in patients who received ≥3.0 g/kg of body weight of RBV compared to patients who received <3.0 g/kg of body weight (among patients <60 years rates were 16% versus 32% and among patients ≥60 years rates were 26% versus 52%, respectively).

According to this decision tree, the patients were divided into five groups with different rates of relapse ranging from 13% to 52%. Patients with RVR had the lowest risk of relapse. Among patients with cEVR, patients <60 years who received ≥3.0 g/kg of body weight of RBV also had a low risk of relapse (16%). By contrast, patients who received <3.0 g/kg of body weight of RBV had higher than the average risk of relapse, especially in patients ≥60 years (52%).

Validation of the decision tree model

The decision tree model was validated using an internal validation group that was not included in the model derivation. The rates of relapse for each subgroup of patients were correlated closely between the model derivation and the internal validation group (r^2=0.79; Figure 2A). When validated using an external validation group, the rates of relapse for each subgroup of patients were again correlated closely between the model derivation and the external validation group. (r^2=0.83; Figure 2B).

Multivariable logistic regression analysis for factors associated with relapse

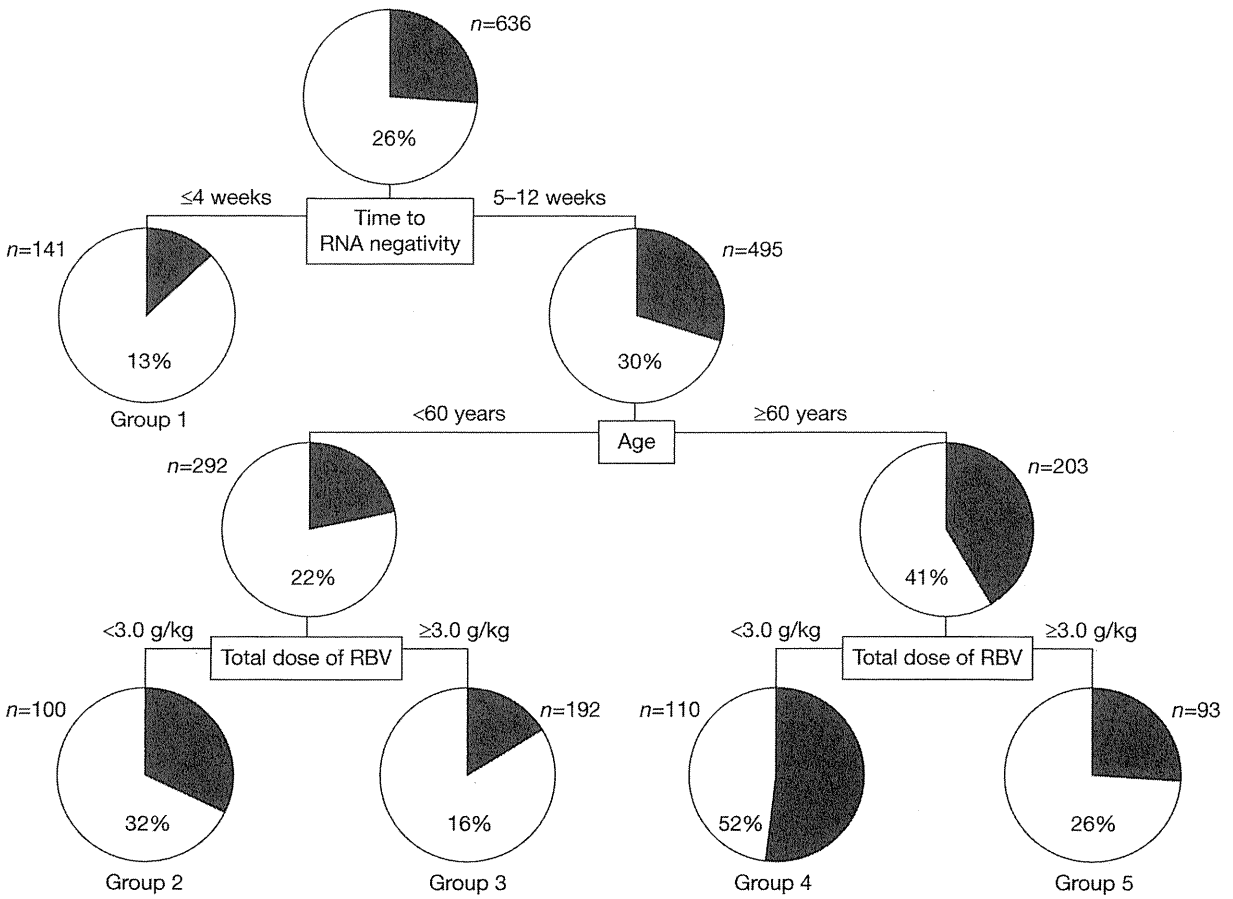
Univariable and multivariable analysis was performed using the combined population of model derivation and internal validation group. Univariable analysis found

that age, sex, serum levels of creatinine, haemoglobin, platelet count, HCV RNA titre, time to HCV RNA negativity, total PEG-IFN dose and total RBV dose were associated with relapse. Duration of therapy was not associated with reduction in relapse rate. Multivariable analysis including these factors showed that age, total RBV dose, serum level of creatinine, and time to HCV RNA negativity were independent predictors of relapse (Table 2). Creatinine was not selected as a splitting variable in data mining analysis probably due to the limitation to stop the analysis when the number of patients was <20. Using the combined population of model derivation and internal validation group, patients in each subgroup of decision tree model were further stratified by creatinine levels and the effect of creatinine level on relapse was analysed. Among patients with RVR, the rate of relapse did not differ between patients with creatinine levels of <0.7 g/dl and ≥0.7 g/dl and were 12% and 12%, respectively. Among patients with cEVR, the rate of relapse was higher in patients with creatinine levels of <0.7 g/dl compared to those with creatinine levels of ≥0.7 g/dl and were 39% versus 23%, respectively, for patients <60 years who received <3.0 g/kg of body weight of RBV, 19% versus 14% for patients <60 years who received ≥3.0 g/kg of body weight of RBV, 58% versus 41% for patients ≥60 years who received <3.0 g/kg of body weight of RBV, and 42% versus 26% for patients ≥60 years who received ≥3.0 g/kg of body weight of RBV.

Effect of age and total RBV dose on relapse among patients with cEVR

The effect of total RBV dose on relapse was analysed among patients with cEVR in a combined group of

Figure 1. The decision-tree model of relapse among patients with rapid virological response or complete early virological response

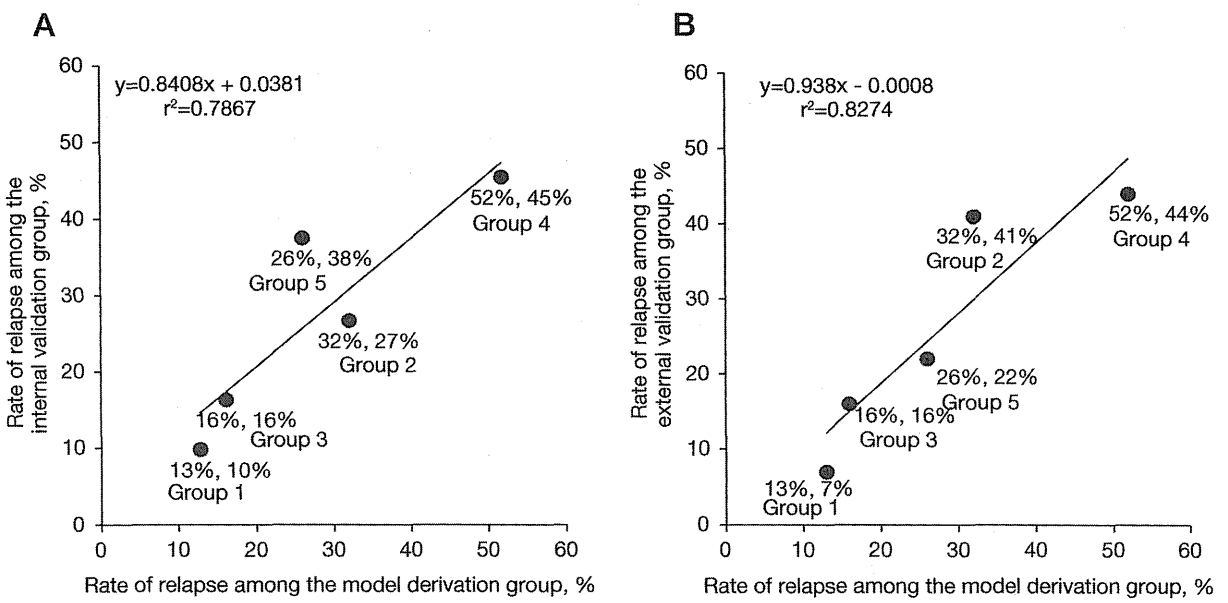


Boxes indicate the factors used for splitting and the cutoff values for the split. Pie charts indicate the rate of relapse for each group of patients after splitting. Terminal groups of patients discriminated by the analysis are numbered from 1 to 5. The rate of relapse was higher than average (>26%) in subgroups 2 and 4, where total ribavirin (RBV) dose was <3 g/kg of body weight.

model derivation and internal validation (n=718). The relapse rate decreased with an increase in RBV dose (Figure 3A). When patients were stratified into two groups according to age, the relapse rate decreased with an increase in RBV dose in patients <60 years. The relapse rate was lowest (11%) in patients <60 years who received ≥4.0 g/kg of body weight of RBV. By contrast, among patients ≥60 years, the relapse rate decreased with an increase in RBV dose up to 3.0 g/kg of body weight, but remained relatively stable despite a further increase in the RBV dose beyond 3.0 g/kg of body weight. The rate of relapse was 31% to 33% in patients who received ≥3.0 g/kg of body weight. Patients ≥60 years had higher relapse rate compared with patients <60 years after stratification by RBV dose (P=0.044 for RBV <2.5 g/kg, P=0.009 for RBV 2.5–2.9 g/kg, P=0.150 for RBV 3.0–3.4 g/kg, P=0.036 for RBV 3.5–3.9 g/kg and P=0.006 for RBV ≥4.0 g/kg).

To exclude the effect of the duration of therapy, patients who received 42–54 weeks of therapy were selected (n=544). Again, the relapse rate decreased with an increase in RBV dose in patients <60 years but remained stable despite a further increase in the RBV dose beyond 3.0 g/kg of body weight in patients ≥60 years (Figure 3B); in addition, patients ≥60 years had a higher relapse rate compared with younger patients after stratification by

Figure 2. Internal and external validation of the decision-tree model: subgroup-stratified comparison of the rate of relapse between the model derivation and validation groups



Each patient in the internal and external validation population was allocated to groups 1 to 5 following the flowchart of the decision tree. The rates of relapse were then calculated for each group and a graph was plotted. The rate of relapse in the (A) internal and (B) external validation groups are shown. The rates of relapse are shown as percentages below data points: the value on the left is from the model derivation group and on the right is from the validation group. The rates of relapse in each group of patients correlated closely between the model derivation group and the validation group (correlation coefficient: r^2=0.79 and 0.83, respectively).

Table 2. Multivariable analysis of factors associated with relapse among patients with RVR/cEVR

Factor	OR	95% CI	P-value
No-RVR	4.07	2.57–6.43	<0.0001
Total RBV dose <3.0 g/kg body weight	2.19	1.58 –3.03	<0.0001
Creatinine <0.7 g/dl	1.67	1.22–2.29	0.001
Age ≥60 years	2.37	1.73–3.24	<0.0001

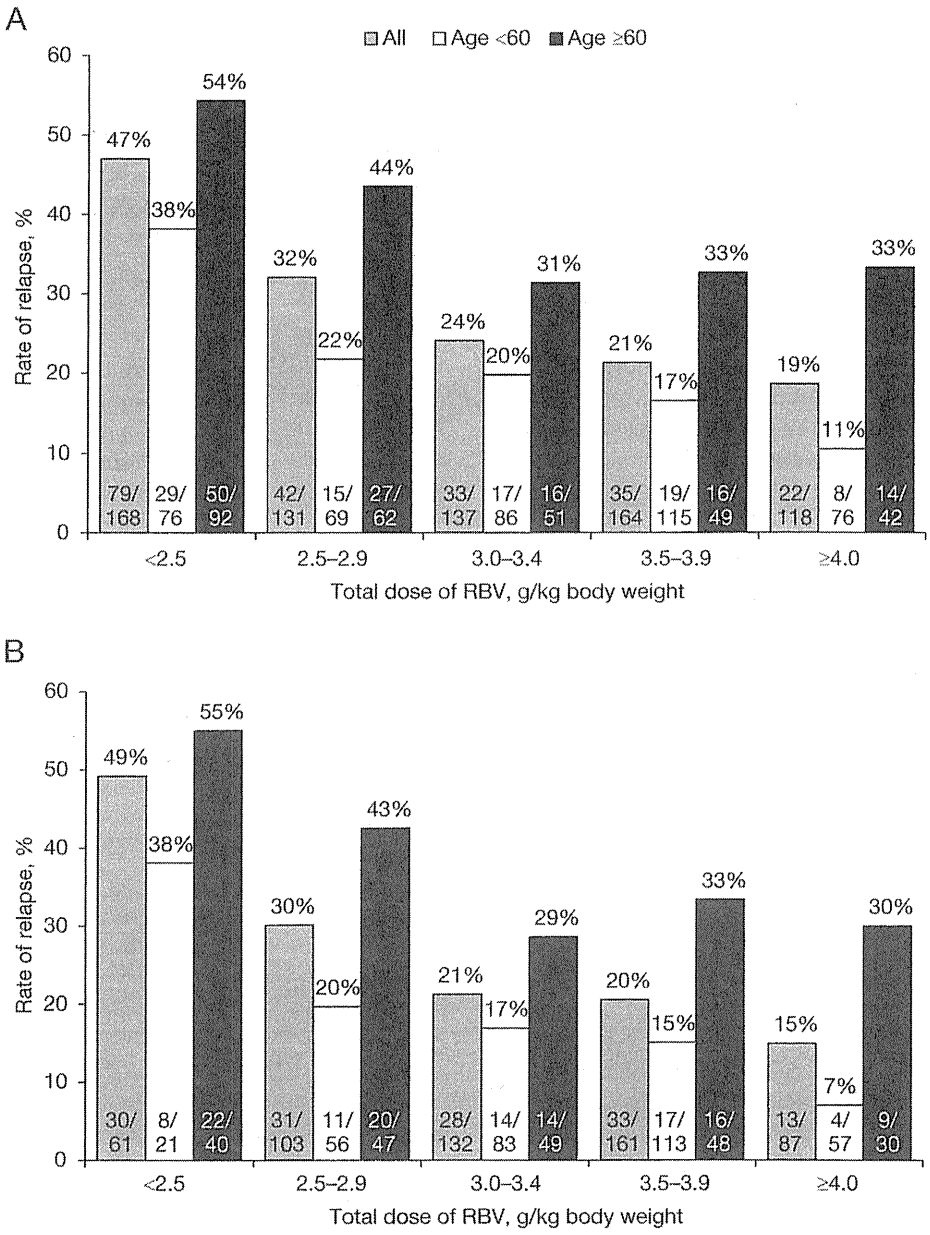
cEVR, complete early virological response (HCV-RNA-positive at week 4, but negative at week 12); RBV, ribavirin; RVR, rapid virological response (HCV-RNA-negative at week 4).

RBV dose (P=0.283 for RBV <2.5 g/kg, P=0.017 for RBV 2.5–2.9 g/kg, P=0.127 for RBV 3.0–3.4 g/kg, P=0.011 for RBV 3.5–3.9 g/kg and P=0.009 for RBV ≥4.0 g/kg).

Total dose of RBV was associated with relapse independently of PEG-IFN dose. The cutoff value of 58 zμg/kg of PEG-IFN was selected, which corresponds to the 80% of 1.5 μg/kg dose for 48 weeks. In patients who received <58 μg/kg of body weight of PEG-IFN, the rate of relapse for patients who received ≥3.0 g/kg or <3.0 g/kg of body weight of RBV was 24% and 42%, respectively. In patients who received ≥58 μg/kg of body weight of PEG-IFN, the rate of relapse for patients who received ≥3.0 g/kg or <3.0 g/kg of body weight of RBV was 21% and 38%, respectively.

The data mining analysis procedure did not select further split variables among RVR patients. However,

Figure 3. Correlation between the rate of relapse and total RBV dose among patients with cEVR after stratification by age



Association between the total ribavirin (RBV) dose and the rate of relapse among patients with complete early virological response (cEVR) is shown. (A) Higher dose of RBV was associated with reduced rate of relapse. (B) These associations were also confirmed in selected patients who received 42–54 weeks of therapy.

when analysed separately, the rate of relapse was also associated with age and total RBV dose among patients with RVR. The rate of relapse for patients who received ≥3.0 g/kg or <3.0 g/kg of body weight of RBV was 5% and 14%, respectively. The rate of relapse for patients <60 and ≥60 years was 9% and 18%, respectively. Collectively, the rate of relapse for patients <60 years who received ≥3.0 g/kg or <3.0 g/kg of body weight of RBV was 2% and 11%, respectively, whereas the rate of relapse for patients ≥60 years who received ≥3.0 g/kg or <3.0 g/kg of body weight of RBV was 12% and 20%, respectively.

Discussion

The result of the present study shows that older age and insufficient dose of RBV are significant and independent risk factors for relapse among patients with cEVR to PEG-IFN plus RBV. Older patients (≥60 years) who received a total RBV dose <3.0 g/kg of body weight had the highest risk of relapse (52%), whereas younger patients who received a total RBV dose ≥3.0 g/kg of body weight had the lowest risk of relapse (16%). The rate of relapse decreased depending on the total RBV dose in younger patients, but remained stable in older patients despite a further increase in the RBV dose beyond 3.0 g/kg of body weight. These findings imply that the target dose of total RBV can be set at 3.0 g/kg of body weight in patients who achieved cEVR, and further increase in RBV dose up to 4.0 g/kg of body weight or greater may be recommended in patients <60 years.

The associations between the drug adherence and virological response had been reported with inconsistent results. In an earlier study, patients who received >80% of the planned dose of PEG-IFN plus RBV for >80% of the planned duration of therapy had a higher rate of SVR compared to those who received a lesser dose (51% versus 34%) [31]. Consistent results were obtained in a study reporting that patients who received >80% of the planned dose of PEG-IFN and RBV within the first 12 weeks of therapy had a higher rate of EVR compared with those who received a lesser dose of both drugs (80% versus 33%) [4]. By contrast, a large-scale multicentre study showed that reducing the PEG-IFN dose during the first 20 weeks reduced SVR; however, reducing RBV did not affect SVR as long as RBV was not prematurely discontinued [32]. The reason for these inconsistencies is unclear. One reason may be the differences in the backgrounds of patients enrolled in the study, and hence the last study was limited to patients with advanced fibrosis and prior non-responders to PEG-IFN therapy. Because the probability of SVR is affected by virological response and relapse after response, the effect of drug dosing should be analysed separately with respect to these two factors.

In the present study, we focused on factors predictive of relapse after early virological response. According to the decision tree model, relapse was less likely in patients with RVR compared with cEVR. Among patients with cEVR, older patients (≥60 years) had a higher risk of relapse compared to younger patients (41% versus 22%). In addition, our results emphasized the effect of RBV dose for the prevention of relapse. In our study, a total RBV dose of ≥3.0 g/kg of body weight was repeatedly associated with a suppressed rate of relapse in the model derivation and validation groups. The rate of relapse in patients <60 years who received an RBV dose of <3.0 versus ≥3.0 g/kg of body weight in the model derivation, internal validation and external validation groups were 32% versus 16%, 27% versus 16%, and 41% versus 16%, respectively. The rate of relapse in patients ≥60 years who received an RBV dose of <3.0 versus ≥3.0 g/kg of body weight in the model derivation, internal validation and external validation groups were 52% versus 26%, 45% versus 38%, and 44% versus 22%, respectively. It has been reported that the rate of relapse is suppressed in 48 weeks of IFN plus RBV combination therapy compared to IFN monotherapy, indicating that RBV contributes to the increase in SVR by reducing relapse [2,3]. Another study, focused on the associations between the drug dose reduction and relapse in patients with virological response, found that maintaining RBV dose ≥12 mg/kg/day during 48 weeks of treatment, which can be translated into a total dose of 4.0 g/kg of body weight, suppressed relapse [33]. Results of the present study are in accordance with this report.

The importance of drug dosing on reduction in relapse is also supported by the findings that extending therapy from 48 to 72 weeks in patients with delayed virological response improved SVR rates by reducing relapse [9–13]. Apart from these clinical studies, in the real world of clinical practice, duration of therapy is extended – even in patients with cEVR – at the physician's discretion. The relationship between duration of therapy or RBV dose, and relapse among patients with cEVR and treated with various lengths of therapy has not been examined. In the combined group of our study, extending the duration of therapy was not associated with a reduction in relapse rate. Rather, the rate of relapse decreased depending on the total RBV dose. These findings suggest that acquiring a sufficient total RBV dose, either within 48 weeks or by extending the duration of therapy, is essential to prevent relapse among patients with cEVR. The limitation of the present study was that the mean duration of therapy was only 56.3 weeks in patients whose duration of therapy was extended beyond 48 weeks. It is probable that extended duration of therapy was not long enough for the prevention of relapse. Further studies with

longer durations of therapy are necessary to confirm the effect of extended duration of therapy on reduction of relapse among patients with cEVR.

Previous reports did not consider the effects of age in setting the optimal dose of RBV. In the present study, the relapse rate decreased with an increase in RBV dose from <2.5 to 3.0–3.5 g/kg of body weight, but remained relatively stable despite a further increase in the RBV dose in older patients. Thus, a total RBV dose ≥3.0 g/kg of body weight should be the target dose for patients ≥60 years with cEVR. By contrast, ≥3.0 g/kg of body weight of RBV was associated with lower risk of relapse in patients <60 with cEVR (16% versus 32%), and a further increase in RBV dose led to a more profound reduction in relapse rates, as low as 11% in patients who received ≥4.0 g/kg of body weight. Thus, a total dose of ≥4.0 g/kg of body weight or even greater should be the target dose in patients <60 years.

In the near future, more potent therapies, such as direct antiviral agents [34,35], may become available. These drugs require RBV and PEG-IFN in combination. However, not all patients may be able to tolerate this triple combination therapy due to adverse drug reactions, such as severe anaemia or skin eruption. In particular, it may be difficult to administer a full dose of triple drugs to older patients. Thus, personalizing the PEG-IFN and RBV combination therapy based on this model may be beneficial to patients who were intolerant to triple combination therapy.

In the present study creatinine was an independent predictor of relapse by multivariable logistic regression analysis. However creatinine was not selected as a splitting variable in decision tree, which may be due to the unique property of data mining analysis. In data mining analysis, limitation is imposed to stop the analysis when the number of patients is <20. This limitation is used to avoid dividing patients into too small subgroups which lead to the generation of rules that only apply to the model derivation population and not reproduced when applied to other populations. This phenomenon is called the over-fitting of the model. Due to this limitation, the variables selected in the data mining analysis are not necessarily identical to the variables that are significant by ordinary multivariable analysis. In a separate analysis, lower level of creatinine was associated with higher rate of relapse in each subgroup of patients with cEVR. The reason for this association is not clear, but lower creatinine level may be related to more efficient clearance of RBV leading to lower serum level of RBV. Further research is needed to confirm this speculation.

A potential limitation of the present study is that data mining analysis has an intrinsic risk of showing relationships that fit to the original dataset, but are not reproducible in different groups. Although internal and external validations showed that our model had high reproducibility, we recognized that further validation on a larger external validation cohort, especially in groups other than Japanese, may be necessary to further verify the reliability of our model.

In conclusion, we built a decision tree model for the prediction of relapse among patients with EVR to PEG-IFN plus RBV. The result of the present study shows that older age and insufficient dose of RBV are significant and independent risk factors for relapse. The target dose of total RBV can be set at 3.0 g/kg of body weight in patients who achieved cEVR. A further increase in RBV dose up to 4.0 g/kg of body weight may be warranted in patients <60 years.

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Disclosure statement

The authors declare no competing interests.

References

1. Strader DB, Wright T, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C. *Hepatology* 2004; **39**:1147–1171.
2. Fried MW, Shiffman ML, Reddy KR, *et al.* Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; **347**:975–982.
3. Manns MP, McHutchison JG, Gordon SC, *et al.* Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; **358**:958–965.
4. Davis GL, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. *Hepatology* 2003; **38**:645–652.
5. Lee SS, Ferenci P. Optimizing outcomes in patients with hepatitis C virus genotype 1 or 4. *Antivir Ther* 2008; **13 Suppl 1**:9–16.
6. Namiki I, Nishiguchi S, Hino K, *et al.* Management of hepatitis C: report of the consensus meeting at the 45th annual meeting of the Japan Society of Hepatology (2009). *Hepatol Res* 2010; **40**:347–368.
7. Jensen DM, Morgan TR, Marcellin P, *et al.* Early identification of HCV genotype 1 patients responding to 24 weeks peginterferon alpha-2a (40 kd)/ribavirin therapy. *Hepatology* 2006; **43**:954–960.
8. Yu ML, Dai CY, Huang JF, *et al.* Rapid virological response and treatment duration for chronic hepatitis C genotype 1 patients: a randomized trial. *Hepatology* 2008; **47**:1884–1893.
9. Berg T, von Wagner M, Nasser S, *et al.* Extended treatment duration for hepatitis C virus type 1: comparing 48 versus 72 weeks of peginterferon-alfa-2a plus ribavirin. *Gastroenterology* 2006; **130**:1086–1097.

10. Sánchez-Tapias JM, Diago M, Escartin P, *et al.* Peginterferon-alfa2a plus ribavirin for 48 versus 72 weeks in patients with detectable hepatitis C virus RNA at week 4 of treatment. *Gastroenterology* 2006; **131**:451–460.
11. Ferenci P, Laferl H, Scherzer TM, *et al.* Peginterferon alfa-2a/ribavirin for 48 or 72 weeks in hepatitis C genotypes 1 and 4 patients with slow virologic response. *Gastroenterology* 2010; **138**:503–512.e1.
12. Buti M, Lurie Y, Zakharova NG, *et al.* Randomized trial of peginterferon alfa-2b and ribavirin for 48 or 72 weeks in patients with hepatitis C virus genotype 1 and slow virologic response. *Hepatology* 2010; **52**:1201–1207.
13. Pearlman BL, Ehleben C, Saifee S. Treatment extension to 72 weeks of peginterferon and ribavirin in hepatitis c genotype 1-infected slow responders. *Hepatology* 2007; **46**:1688–1694.
14. Tanaka Y, Nishida N, Sugiyama M, *et al.* Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009; **41**:1105–1109.
15. Suppiah V, Moldovan M, Ahlenstiel G, *et al.* IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat Genet* 2009; **41**:1100–1104.
16. Ge D, Fellay J, Thompson AJ, *et al.* Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; **461**:399–401.
17. Kurosaki M, Tanaka Y, Nishida N, *et al.* Pre-treatment prediction of response to pegylated-interferon plus ribavirin for chronic hepatitis C using genetic polymorphism in IL28B and viral factors. *J Hepatol* 2011; **54**:439–448.
18. Breiman LJH, Friedman RA, Olshen CJ, Stone CM. *Classification and regression trees*. 1980. Belmont, CA: Wadsworth.
19. Garzotto M, Park Y, Mongoue-Tchokote S, *et al.* Recursive partitioning for risk stratification in men undergoing repeat prostate biopsies. *Cancer* 2005; **104**:1911–1917.
20. Miyaki K, Takei I, Watanabe K, Nakashima H, Omae K. Novel statistical classification model of type 2 diabetes mellitus patients for tailor-made prevention using data mining algorithm. *J Epidemiol* 2002; **12**:243–248.
21. Averbook BJ, Fu P, Rao JS, Mansour EG. A long-term analysis of 1018 patients with melanoma by classic Cox regression and tree-structured survival analysis at a major referral center: Implications on the future of cancer staging. *Surgery* 2002; **132**:589–604.
22. Leiter U, Buettner PG, Eigentler TK, Garbe C. Prognostic factors of thin cutaneous melanoma: an analysis of the central malignant melanoma registry of the German dermatological society. *J Clin Oncol* 2004; **22**:3660–3667.
23. Valera VA, Walter BA, Yokoyama N, *et al.* Prognostic groups in colorectal carcinoma patients based on tumor cell proliferation and classification and regression tree (CART) survival analysis. *Ann Surg Oncol* 2007; **14**:34–40.
24. Zlobec I, Steele R, Nigam N, Compton CC. A predictive model of rectal tumor response to preoperative radiotherapy using classification and regression tree methods. *Clin Cancer Res* 2005; **11**:5440–5443.
25. Baquerizo A, Anselmo D, Shackleton C, *et al.* Phosphorus ans an early predictive factor in patients with acute liver failure. *Transplantation* 2003; **75**:2007–2014.
26. Kurosaki M, Matsunaga K, Hirayama I, *et al.* A predictive model of response to peginterferon ribavirin in chronic hepatitis C using classification and regression tree analysis. *Hepatol Res* 2010; **40**:251–260.
27. Kurosaki M, Sakamoto N, Iwasaki M, *et al.* Pretreatment prediction of response to peginterferon plus ribavirin therapy in genotype 1 chronic hepatitis C using data mining analysis. *J Gastroenterol* 2011; **46**:401–409.
28. Kurosaki M, Sakamoto N, Iwasaki M, *et al.* Sequences in the interferon sensitivity determining region and core region of hepatitis C virus impact pretreatment prediction of response to peg-interferon plus ribavirin: data mining analysis. *J Med Virol* 2011; **83**:445–452.
29. LeBlanc M, Crowley J. A review of tree-based prognostic models. *Cancer Treat Res* 1995; **75**:113–124.
30. Segal MR, Bloch DA. A comparison of estimated proportional hazards models and regression trees. *Stat Med* 1989; **8**:539–550.
31. McHutchison JG, Manns M, Patel K, *et al.* Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. *Gastroenterology* 2002; **123**:1061–1069.
32. Shiffman ML, Ghany MG, Morgan TR, *et al.* Impact of reducing peginterferon alfa-2a and ribavirin dose during retreatment in patients with chronic hepatitis C. *Gastroenterology* 2007; **132**:103–112.
33. Hiramatsu N, Oze T, Yakushijin T, *et al.* Ribavirin dose reduction raises relapse rate dose-dependently in genotype 1 patients with hepatitis C responding to pegylated interferon alpha-2b plus ribavirin. *J Viral Hepat* 2009; **16**:586–594.
34. Hézode C, Forestier N, Dusheiko G, *et al.* Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. *N Engl J Med* 2009; **360**:1839–1850.
35. McHutchison JG, Everson GT, Gordon SC, *et al.* Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N Engl J Med* 2009; **360**:1827–1838.

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Original Article

Combination of hepatitis B viral antigens and DNA for prediction of relapse after discontinuation of nucleos(t)ide analogs in patients with chronic hepatitis B

Akihiro Matsumoto,[1] Eiji Tanaka,[1] Yoshiyuki Suzuki,[2] Mariko Kobayashi,[2] Yasuhito Tanaka,[4] Noboru Shinkai,[4] Shuhei Hige,[6] Hiroshi Yatsuhashi,[8] Shinya Nagaoka,[8] Kazuaki Chayama,[9] Masataka Tsuge,[9] Osamu Yokosuka,[10] Fumio Imazeki,[10] Shuhei Nishiguchi,[11] Masaki Saito,[11] Kei Fujiwara,[5] Nobuyuki Torii,[3] Naoki Hiramatsu,[12] Yoshiyasu Karino[7] and Hiromitsu Kumada[2]

[1]Department of Medicine, Shinshu University School of Medicine, Matsumoto, [2]Department of Hepatology, Toranomon Hospital, [3]Department of Internal Medicine and Gastroenterology, Tokyo Women's Medical University, Tokyo, [4]Department of Virology and Liver Unit, Nagoya City University Graduate School of Medical Sciences, [5]Gastroenterology Section, Nagoya Daini Red Cross Hospital, Nagoya, [6]Department of Gastroenterology and Hepatology, Graduate School of Medicine, Hokkaido University, [7]Department of Gastroenterology, Sapporo Kosei General Hospital, Sapporo, [8]The Clinical Research Center, NHO Nagasaki Medical Center, Omura, [9]Program for Biomedical Research, Division of Frontier Medical Science, Department of Medicine and Molecular Science, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, [10]Department of Medicine and Clinical Oncology, Graduate School of Medicine, Chiba University, Chiba, [11]Division of Hepatobiliary and Pancreatic Diseases, Department of Internal Medicine, Hyogo College of Medicine, Hyogo, and [12]Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine, Osaka, Japan

Aim: The factors associated with hepatitis recurrence after discontinuation of nucleos(t)ide analogs (NAs) in patients with chronic hepatitis B were analyzed to predict the risk of relapse more accurately.

Methods: A total of 126 patients who discontinued NA therapy were recruited retrospectively. The clinical conditions of a successful discontinuation were set as alanine aminotransferase (ALT) below 30 IU/L and serum hepatitis B virus (HBV) DNA below 4.0 log copies/mL.

Results: Relapse of hepatitis B were judged to occur when maximal serum ALT became higher than 79 IU/L or when maximal serum HBV DNA surpassed 5.7 log copies/mL following NA discontinuation since these values corresponded with mean values of ALT (30 IU/L) and HBV DNA (4.0 log copies/mL), respectively. At least 90% of patients with either detectable hepatitis B e antigen or serum HBV DNA higher than 3.0 log copies/mL at the time of NA discontinuation relapsed within one year. In the remaining patients, higher levels of both hepatitis B surface and core-related antigens at the time of discontinuation, as well as a shorter course of NA treatment, were significantly associated with relapse by multivariate analysis.

Conclusions: It appears that negative results for hepatitis B e antigen and serum HBV DNA lower than 3.0 log copies/mL are essential for successful NA discontinuation, which may be attained by a longer treatment period. Levels of hepatitis B surface and core-related antigens are also significant factors independently associated with relapse of hepatitis.

Key words: discontinuation, hepatitis B core-related antigen, hepatitis B surface antigen, nucleos(t)ide analogs, relapse of hepatitis

INTRODUCTION

HEPATITIS B VIRUS (HBV) infection is a major health concern that has an estimated 350 to 400 million carriers worldwide. Chronic infection with HBV can cause chronic hepatitis, and may eventually develop into liver cirrhosis and hepatocellular carcinoma.[1–3] Over the last decade, major advances in the treatment of chronic hepatitis B have been made with nucleos(t)ide

Correspondence: Professor Eiji Tanaka, Department of Medicine, Gastroenterology Division, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan. Email: etanaka@shinshu-u.ac.jp

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analogs (NAs) such as lamivudine (LVD), adefovir dipivoxil (ADV), and entecavir (ETV).[4] NAs are orally administered and are associated with low rates of adverse effects. Treatment with NAs shows strong suppression of HBV replication and consequently rapid improvement of elevated ALT levels. Furthermore, these drugs have been reported to lower the risk of complicating cirrhosis and hepatocellular carcinoma,[5–7] and so NAs are becoming widely used to treat patients with chronic hepatitis B. On the other hand, NAs carry the risk of developing drug-resistance;[8] drug-resistant viruses emerging during treatment may be associated with hepatitis flare-ups. Hepatitis B patients are also required to undergo prolonged treatment with NAs because early discontinuance often leads to relapse of hepatitis and ensuing hepatic failure following rises in alanine aminotransferase (ALT) level.[9,10]

Serum HBV DNA is normally used to monitor the antiviral effect of NAs. HBV DNA decreases rapidly and becomes undetectable in the majority of patients who are treated with NAs,[11–13] but relapse after discontinuation is not rare.[14–17] Since it is also true that favorable virological and biochemical responses to NAs may continue indefinitely in some patients,[9,15] reliable markers that can predict relapse of hepatitis after NA discontinuation are needed. Such markers would benefit not only patients who are considering discontinuation of NA treatment, but also clinicians, hospitals, and the medical economy.

In the present study, we assessed several factors associated with relapse of hepatitis after discontinuation of NAs in patients with chronic hepatitis B, including hepatitis B viral antigens, which have been reported as new and promising markers for monitoring the effect of antiviral agents, such as interferon and NAs.

METHODS

Patients

A TOTAL OF 126 patients with chronic hepatitis B who underwent and completed NA treatment between 2000 and 2010 were enrolled in this study. Patients were recruited retrospectively from 11 hospitals across Japan (Toranomon Hospital, Hokkaido University Hospital, Nagoya City University Hospital, Shinshu University Hospital, Hiroshima University Hospital, National Hospital Organization Nagasaki Medical Center, Chiba University Hospital, The Hospital of Hyogo College of Medicine, Japanese Red Cross Nagoya Daini Hospital, and Tokyo Women's Medical University Hospital, Sapporo Kosei General Hospital) and met the following conditions: (i) serum ALT higher than 30 IU/L and serum HBV DNA higher than 4.0 log copies/mL were observed at least twice within the 6 months prior to administration of NAs; (ii) stored serum samples at initiation and discontinuation of NAs were available for measurements of viral markers; (iii) clinical outcomes were followed for at least 6 months after the discontinuation of NAs; and (iv) tests for hepatitis C and human immunodeficiency virus antibodies were negative. Hepatitis B surface antigen (HBsAg) was confirmed to be positive on at least two occasions at least 6 months apart in all patients before treatment. Patients complicated with hepatocellular carcinoma or signs of hepatic failure at treatment discontinuation were excluded from the study. Our cohort consisted of 83 men and 43 women with a median age of 46 (range, 19 to 79) years when NA administration was discontinued. Hepatitis B e antigen (HBeAg) was positive in 64 patients (51%) at the initiation of treatment and in 24 patients (19%) at its discontinuation. HBV genotype was A in two (2%) patients, B in five (4%), C in 102 (81%), and undetermined in 17 (13%). Thirty-five of the 126 patients in this study were younger than 35 years old. Although not recommended as the first line treatment for this group by Japanese guidelines,[18] NA treatment was commenced since chronic active hepatitis had been persisting in all cases irrespective of their HBeAg status (26 positive and nine negative) at the initiation of treatment.

The decision to discontinue NAs was made by individual physicians using similar, but not uniform, conditions. Four patients who halted NAs for financial reasons were included. No patient underwent interferon treatment during or after NA treatment. The decision to recommence NA administration was also made by individual physicians, essentially when relapse of hepatitis became obvious. With few exceptions, patients were seen at least once a month during the first year after discontinuation of NAs, and at least once every several months afterwards. Stored serum samples were kept frozen at −20°C or below until assayed. This study was approved by the Ethics Committees of all participating institutions.

Hepatitis B viral markers

Serological markers for HBV, including HBsAg, HBeAg, and antibody to HBe (anti-HBe) were tested using commercially available enzyme immunoassay kits (Abbott Japan Co., Ltd, Tokyo, Japan; Fujirebio Inc., Tokyo, Japan; and/or Sysmex Co., Kobe, Japan) at each hospital. Quantitative measurement of HBsAg[19] was done using a chemiluminescence enzyme immunoassay

(CLEIA)-based HISCL HBsAg assay manufactured by Sysmex Corporation (Kobe, Japan). The assay had a quantitative range of −1.5 to 3.3 log IU/mL. End titer was determined by diluting samples with normal human serum when initial results exceeded the upper limit of the assay range.

Serum concentration of HBV DNA was determined using an Amplicor HBV monitor kit (Roche, Tokyo, Japan),[20] which had a quantitative range of 2.6 to 7.6 log copies/mL. Serum HBV DNA was also determined using a COBAS TaqMan HBV kit (Roche, Tokyo, Japan)[21] with a quantitative range of 2.1 to 9.0 log copies/mL in 43 patients whose serum samples were available at the time of NA discontinuation. According to the manufacturer's instructions, detection of a positive signal below the quantitative range was described as a positive signal, and no signal detection was described as a negative signal. Six HBV genotypes (A–F) were evaluated according to the restriction patterns of DNA fragments from the method reported by Mizokami *et al.*[22]

Serum hepatitis B core-related antigen (HBcrAg) levels were measured using a CLEIA HBcrAg assay kit with a fully automated Lumipulse System analyzer (Fujirebio Inc., Tokyo, Japan) as described previously.[23,24] Briefly, 150 μL of serum was incubated with pretreatment solution and then added to a ferrite microparticle suspension in an assay cartridge. Ferrite particles were coated with a monoclonal antibody mixture against denatured HBcAg, HBeAg, and the 22 kDa precore protein. After incubation and washing, further incubation was carried out with alkaline phosphatase conjugated with two kinds of monoclonal antibodies against denatured HBcAg, HBeAg, and the 22 kDa precore protein. Following washing, a substrate solution was added to the test cartridge and then incubated. The relative chemiluminescence intensity was measured, and HBcrAg concentration was calculated by a standard curve generated using recombinant pro-HBeAg. The immunoreactivity of pro-HBeAg at 10 fg/mL was defined as 1 U/mL. We expressed HBcrAg in terms of log U/mL, with a quantitative range set at 3.0 to 6.8 log U/mL.

Statistical analyses

A linear regression model was used to examine for associations between mean and maximal values of both ALT and HBV DNA. Correlations between variables were calculated using the Spearman's rank correction correlation coefficient test. Each cut-off value was decided using receiver operating characteristic curve (ROC) analysis and results were evaluated by measuring the area under the curve (AUC). The Fisher's exact and Pearson's χ^2 tests were adopted to test for differences between subgroups of patients. To compare continuous data, the Mann–Whitney *U*-test was used. The Kaplan–Meier method was used to estimate rates of non-relapse observations, and the log-rank test was used to test hypotheses concerning differences in non-relapse observations between selected groups. Multivariate analyses were performed using the Cox regression model. Variables associated with a *P*-value < 0.2 in univariate analyses were included in a stepwise Cox regression analysis to identify independent factors associated with relapse of hepatitis after discontinuation of NAs. All tests were performed using the IBM SPSS Statistics Desktop for Japan ver. 19.0 (IBM Japan Inc., Tokyo, Japan). *P*-values of less than 0.05 were considered to be statistically significant.

RESULTS

Definition of hepatitis relapse after discontinuation of NAs

THE CLINICAL CONDITIONS of a successful discontinuation of NAs were set at serum HBV DNA below 4.0 log copies/mL and ALT below 30 IU/L according to the Japanese guidelines for the treatment of hepatitis B.[18] However, these criteria could not be directly applied to our cohort as post-therapy fluctuations in ALT and HBV DNA were difficult to evaluate consistently. In total, 26 (76%) of 34 patients with successful discontinuation of NAs showed transient abnormal levels of ALT and/or HBV DNA, especially during the early phase after cessation. We therefore used mean and maximal values of these markers to evaluate relapse of hepatitis B in this study; mean values were used to evaluate relapse of hepatitis as a whole, and maximal values were used to dynamically assess relapse during the follow-up period after NA discontinuation. Both ALT and HBV DNA were measured 11.0 times per year on average during the first year and 4.1 times per year on average thereafter.

The mean values of HBV DNA were significantly ($P < 0.001$) correlated with maximal values with a correlation coefficient of 0.853. Similarly, the mean values of ALT were significantly ($P < 0.001$) correlated with maximal values with a correlation coefficient of 0.940 (Fig. 1). The mean HBV DNA value of 4.0 log copies/mL corresponded to a maximal HBV DNA value of 5.7 by ROC analysis (AUC = 0.930, $P < 0.001$), and the mean ALT value of 30 IU/L corresponded to a maximal ALT value of 79 IU/L (AUC = 0.988, $P < 0.001$). These results suggested that patients having serum HBV DNA higher