groups. According to the European Society of Parenteral and Enteral Nutrition guidelines, recommended daily energy and protein intake for cirrhosis patients are 35–40 kcal/kg and 1.2–1.5 g protein/kg, respectively.[47] These recommendations should be evaluated with further studies on dietary education at our hospital. Third, the subpopulation with a high BMI (≥25 kg/m^2) may have mild ascites and edema, although we excluded patients with overt ascites and edema on clinical and ultrasonographic findings. Thus, BMI could have been overestimated in the present study. The relationship between high BMI and the degree of ascites and edema should also be further studied.

In conclusion, the results of present study showed that oral supplementation with BCAA granules may be associated with a reduced incidence of HCC in patients with HCV-related cirrhosis regardless of obesity. Reduction of oxidative stress, anti-angiogenesis and improvement of immune function are possible mechanisms by which BCAA may be involved in suppression of hepatocarcinogenesis. Although the results of the present study require validation in prospective trials, they support the necessity of BCAA supplementation in the management of patients with HCV-related cirrhosis.

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Research Article

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Non-hypervascular hypointense nodules detected by Gd-EOB-DTPA-enhanced MRI are a risk factor for recurrence of HCC after hepatectomy

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Background & Aims: The gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA)-enhanced magnetic resonance imaging (MRI) often depicts non-hypervascular hypointense hepatic nodules during the hepatobiliary phase in patients with hepatocellular carcinoma (HCC). It is unclear whether the presence of these nodules is associated with HCC recurrence after hepatectomy. We conducted a prospective observational study to investigate the impact of the presence of non-hypervascular hypointense hepatic nodules on the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI on the recurrence of HCC after hepatectomy.

Methods: A total of 77 patients who underwent hepatectomy for primary, non-recurrent, hypervascular HCC were prospectively followed up after hepatectomy. Post-operative recurrence rates were compared according to the presence of non-hypervascular hypointense nodules on preoperative Gd-EOB-DTPA-enhanced MRI.

Results: Recurrence rates after hepatectomy were higher in patients with non-hypervascular hypointense nodules (risk ratio 1.9396 [1.3615–2.7222]) and the presence of non-hypervascular hypointense nodules was an independent factor associated with postoperative recurrence (risk ratio 2.1767 [1.5089–3.1105]) along with HCC differentiation and portal vein invasion. While no differences were found in the rate of intrahepatic metastasis recurrence based on the preoperative presence of non-hypervascular hypointense hepatic nodules, the rate of multicentric recurrence was significantly higher in patients with preoperative non-hypervascular hypointense hepatic nodules.

Conclusions: Patients with preoperative non-hypervascular hypointense hepatic nodules detected during the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI are at higher risk of HCC recurrence after hepatectomy, mainly due to multicentric recurrence.

Keywords: Hepatocellular carcinoma; Gd-EOB-DTPA-enhanced MRI; Non-hypervascular hypointense nodule; Hepatobiliary phase; Hepatectomy; Recurrence.
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Abbreviations: Gd-EOB-DTPA, gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid; MRI, magnetic resonance imaging; HCC, hepatocellular carcinoma; US, ultrasonography; MDCT, multidetector-row computed tomography; TFE, turbo field echo; CTHA, computed tomography during hepatic arteriography.

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide and the third most common cause of cancer-related death [1,2]. In Japan, HCC is the third and fifth most common cause of death from cancer in men and women, respectively [3]. Tremendous efforts have been made to improve various imaging techniques, including ultrasonography (US), multidetector-row computed tomography (MDCT) [4,5], and magnetic resonance imaging (MRI) [6], for the detection of hepatic nodules, including small early-stage HCC tumors in high-risk patients under surveillance.

The liver-specific contrast agent gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA), which is taken up by hepatocytes, has been in clinical use for dynamic MRI studies since February 2008 in Japan. Gd-EOB-DTPA provides both dynamic and liver-specific hepatobiliary MR images [7–10]. In the hepatobiliary phase, hepatic lesions that lack normally functioning hepatocytes are imaged as an absence of hepatocyte-selective enhancement as compared with normal parenchyma [10,11]. The use of Gd-EOB-DTPA-enhanced MRI increases detection of concurrent non-hypervascular hepatic nodules as hypointense nodules during the hepatobiliary phase in patients with HCC. It is controversial whether the presence of these non-hypervascular hepatic nodules detected in patients with typical hypervascular HCC lesions has an impact on the recurrence of HCC after treatment.

In the present study, we attempted to evaluate the impact of concurrent non-hypervascular hepatic nodules detected as hypointense nodules during the hepatobiliary phase of Gd-EOB-

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Table 1. Comparison of clinical characteristics of study patients based on the presence of non-hypervascular hypointense nodules detected during the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI (n = 77).

	Non-hypervascular hypointense nodule (+) (n = 18)	Non-hypervascular hypointense nodule (-) (n = 59)	*p* value
Age (mean ± SD, yr) (range)	65.8 ± 9.0 (46-76)	69.1 ± 7.0 (53-82)	0.2727
Sex (female/male)	3 (16.7)/15 (83.3)	18 (30.5)/41 (69.5)	0.3921
Etiology (HBV/HCV/non-HBV, non-HCV)	2 (11.1)/11 (61.1)/5 (27.8)	9 (15.3)/39 (66.1)/11 (18.6)	0.6796
Child-Pugh class (A/B)*	17 (94.4)/1 (5.6)	58 (98.3)/1 (1.7)	0.9474
Albumin (mean ± SD, g/dl)	3.91 ± 0.51	4.08 ± 0.32	0.1664
Total bilirubin (mean ± SD, mg/dl)	0.88 ± 0.36	0.84 ± 0.33	0.7296
15-minute ICG retention rate (%)	18.1 ± 5.4	16.0 ± 6.7	0.2405
Prothrombin (%)	95.3 ± 15.6	95.1 ± 11.2	0.9105
Platelet count (x10^3/ml)	132 ± 47	152 ± 66	0.5433
Tumor size (mean ± SD, cm) (range)	2.52 ± 0.99 (1.3-4.7)	2.84 ± 1.54 (1.0-8.6)	0.6600
Number of tumors (single/multiple)	15 (83.3)/3 (16.7)	53 (89.8)/6 (10.2)	0.7358
Portal vein invasion (absent/present)**	17 (94.4)/1 (5.6)	50 (84.7)/9 (15.3)	0.4989
Differentiation (well-/moderately or poorly)**	7 (38.9)/11 (61.1)	21 (35.6)/38 (64.4)	0.9999
Growth pattern (expansive/infiltrative)**	14 (77.8)/4 (22.2)	52 (88.1)/7 (11.9)	0.4718
Follow-up period (months) (median, range)	31.3 (9.4-53.9)	34.9 (8.5-55.4)	0.4200

Percentages are in parentheses.
HBV, hepatitis B virus; HCV, hepatitis C virus; ICG, indocyanine green test.
* Child-Pugh class A includes patients without cirrhosis.
** Evaluated by pathologic examination based on resected specimens.

DTPA-enhanced MRI on postoperative recurrence in patients who underwent hepatectomy with curative intent for HCC.

Cancer

Materials and methods

Patients, treatment and follow-up

This prospective study was conducted after the approval by the hospital institutional review board and carried out in compliance with the Helsinki Declaration. Patient enrollment was carried out between February 2008 and December 2011. A total of 102 patients underwent hepatectomy as a curative treatment for primary, non-recurrent HCC during the study period at Ogaki Municipal Hospital. Gd-EOB-DTPA-enhanced MRI could not be performed prior to hepatectomy in 25 patients, including 11 patients who had been referred from another institution only for hepatectomy and 14 patients who could not receive examination due to metal implants, history of allergy to contrast medium, tattoos, or claustrophobia. The remaining 77 patients who underwent Gd-EOB-DTPA-enhanced MRI within 2 weeks prior to hepatectomy were studied. The initial diagnosis of HCC before treatment was based on appropriate imaging characteristics according to criteria of the guidelines by the American Association for the Study of Liver Diseases [12,13]. The final diagnosis of HCC was confirmed by pathologic diagnosis of resected specimens.

Decisions regarding individual treatments were based on Japanese treatment guidelines for HCC [14]. In all patients, HCC tumors were resected with ample margins; enucleation of tumors without margins was not performed.

After hepatectomy, all patients were prospectively followed from 8.5 months to 55.4 months (median follow-up, 34.1 months) until the end of September 2012 at our institution, with US and either MDCT or MRI every 3–6 months. Regular monitoring of serum tumor markers (alpha-fetoprotein, *Lens culinaris* agglutinin-reactive alpha-fetoprotein, and des-gamma-carboxy prothrombin) was performed every 3 months. When an elevation in tumor markers was detected, additional imaging (usually MDCT or MRI) was performed to check for HCC recurrence. Recurrence was diagnosed by pathologic examination of resected specimens when patients underwent re-hepatectomy. In the remaining patients, HCC was diagnosed by appropriate imaging characteristics according to criteria of the guidelines by the American Association for the Study of Liver Diseases [12,13]. Recurrent HCC was categorized into two groups prior to the study, as intrahepatic metastasis recurrence or multicentric recurrence according to a previous study [15,16]. Intrahepatic metastasis recurrence was defined as recurrent tumors consisting of moderately or poorly differentiated HCC with the same or lower degree of differentiation than the primary tumors on pathologic examination or hypervascular tumor without non-hypervascular peripheral regions in a same hepatic segment on imaging examination. Multicentric recurrence was defined according to previously reported criteria with some modifications [17,18] as follows: (i) the recurrent tumor consists of well-differentiated HCC occurring in a different hepatic segment, than moderately or poorly differentiated pre-existing HCCs; (ii) both the primary and recurrent tumors are well-differentiated HCCs; and (iii) the recurrent tumor contained regions of dysplastic nodules in peripheral areas based on pathologic examination or contained non-hypervascular regions in peripheral areas of hypervascular tumor on imaging examination.

Preoperative imaging examinations of liver nodules by gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid-enhanced MRI and confirmation of non-hypervascular hypointense hepatic nodules

All patients underwent Gd-EOB-DTPA-enhanced MRI within 2 weeks of hepatectomy. MRI was performed using a 1.5-T whole-body MRI system (Intera Achieva 1.5T NOVA; Philips Medical Systems) with a phased-array body coil as the receiver coil. T1-weighted sequences were acquired with the following parameters: T1-weighted turbo field echo (TFE) in-phase and opposed-phase transverse (TE, opposed-phase 2.3, in-phase 4.6; flip angle, 12°; matrix size, 256 × 512; scan percentage, 70) with 3.5-mm section thickness, a 0-mm intersection gap, and a 38-cm field of view. After intravenous injection of Gd-EOB-DTPA (Primovist; Bayer Schering Pharma, Osaka, Japan), T1-weighted transverse gradient-echo sequences (high-resolution isotropic volume examination [THRIVE] with spectral presaturation with inversion recovery [SPIR], 4/1.8; flip angle, 12°; matrix size, 256 × 512; scan percentage, 78.54) with 3.5-mm section thickness, a 0-mm intersection gap, and a 38-cm field of view were obtained. Gd-EOB-DTPA was administered intravenously as a bolus at a rate of 2 ml/s (0.1 ml/kg, maximum dose of 10 ml) through an intravenous cubital line (20–22 gauge), which was flushed with 20 ml of saline using a power injector (Sonic Shot; Nemoto Kyorindo, Tokyo, Japan). The timing for dynamic arterial phase imaging was determined using

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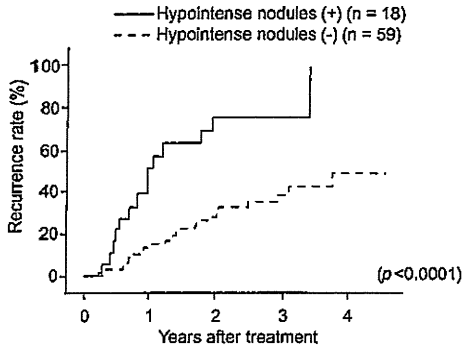

Patients at risk					
Hypointense nodules (+)	18	17	14	7	2
Hypointense nodules (-)	59	58	47	29	16

Fig. 1. Overall recurrence rate after hepatectomy in patients with or without concurrent non-hypervascular hypointense hepatic nodules detected during the hepatobiliary phase of preoperative Gd-EOB-DTPA-enhanced MRI.

MR fluoroscopic bolus detection of the descending aorta (Bolus Trak; Philips Medical Systems). The mean delay times (time interval between the start of bolus administration and the start of image acquisition) for the arterial, portal, and delayed phases were 20, 60, and 180 s, respectively. Immediately after the dynamic study, a respiration-triggered single-shot T2-weighted sequence, with a reduction factor of 4 (1200/100; flip angle, 90°; matrix size, 400 × 512) with 7-mm section thickness, a 1-mm intersection gap, and a 38-cm field of view, was obtained with SPIR. The 20-min-delayed hepatobiliary phase [19] was obtained with a T1-weighted TFE sequence (TR/TE, 4/1.8; flip angle, 12°; matrix size, 256 × 512) with 3.5-mm section thickness, a 0-mm intersection gap, and a 38-cm field of view. All the sequences were obtained with parallel imaging (SENSE). Hypointense hepatic nodules during the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI were nodules greater than 3.5 mm with low-intensity.

Prior to hepatectomy, all patients underwent CT during hepatic arteriography (CTHA) [20–22] to evaluate the intranodular blood supply, and to confirm the hypervascularity of HCC lesions and the lack of hypervascularity of non-hypervascular hepatic nodules.

All imaging findings were evaluated by a radiologist (Y.S.) and a hepatologist (H.T.) independently, blind to the clinical data. When the imaging assessment was discordant between two reviewers, consensus was made through the discussion.

Statistical analyses

Differences in percentages between groups were analyzed using the Chi-square test. Differences in mean quantitative values were analyzed by the Mann–Whitney *U* test. The date of hepatectomy was defined as time zero for calculations of recurrence rates. In the analysis of the overall recurrence rate, patients in whom HCC did not recur were censored, and those in whom HCC recurred were not censored. In the analysis of the intrahepatic metastasis recurrence rate, patients in whom HCC did not recur or patients with multicentric HCC recurrence were censored, and those in whom HCC recurred as intrahepatic metastases were not censored. In the analysis of the multicentric recurrence rate, patients in whom HCC did not recur were censored and patients with multicentric HCC recurrence were not censored, while those in whom HCC recurred as intrahepatic metastases were excluded from the analysis. The Kaplan–Meier method [23] was used to calculate recurrence rates, and the log-rank test [24] was used to analyze differences.

The Cox proportional hazards model [25] was used for univariate and multivariate analyses of factors related to recurrence. Variables analyzed included patient age and sex, Child-Pugh class (A/B), tumor size, number of tumors (single/multiple), differentiation of resected HCC (well-differentiated/moderately or

Table 2. Univariate and multivariate analyses of factors associated with post-operative recurrence in HCC patients (n = 77).

Factor	Univariate analysis		Multivariate analysis	
	Risk ratio (95% CI)	*p* value	Risk ratio (95% CI)	*p* value
Age	0.9943 (0.9535-1.0396)	0.7974	-	
Sex				
Male	1			
Female	1.0068 (0.6818-1.4290)	0.9711	-	
Child-Pugh class*				
A	1			
B	0.0428 (0.0198-1.5669)	0.2068	-	
Tumor size	0.9376 (0.7179-1.1700)	0.5935	-	
Number of tumors				
Single	1			
Multiple	1.0419 (0.5669-1.6643)	0.8792	-	
Differentiation**				
Well-	1		1	
Moderately/poorly	1.5871 (1.0958-2.4354)	0.0134	1.6536 (1.1381-2.5445)	0.0073
Growth pattern**				
Expansive	1			
Infiltrative	1.1101 (0.6798-1.6625)	0.6487	-	
Portal vein invasion**				
Absent	1		1	
Present	1.5659 (1.0161-2.2813)	0.0428	1.7818 (1.1388-2.6597)	0.0134
Non-hypervascular hypointense nodules				
Absent	1		1	
Present	1.9396 (1.3615-2.7222)	0.0004	2.1767 (1.5089-3.1105)	0.0001

CI, confidence interval.
* Child-Pugh class A includes patients without cirrhosis.
** Evaluated by pathologic examination of resected specimens.

Cancer

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poorly differentiated), growth pattern of resected HCC (expansive growth/infiltrative growth), portal vein invasion of resected HCC (absent/present), and presence of non-hypervascular hypointense nodules on the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI (absent/present). Data analyses were performed using JMP statistical software, version 6.0 (Macintosh version; SAS Institute, Cary, NC). All p values were derived from 2-tailed tests, with $p < 0.05$ accepted as statistically significant.

Results

Patients characteristics and imaging findings

Patients consisted of 56 males and 21 females with a mean age of 68.3 ± 7.6 years (range, 46–82 years). A total of 40 non-hypervascular hypointense hepatic nodules were identified during the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI in 28 of 77 patients (36.4%). The size of non-hypervascular hypointense nodules was 1.17 ± 0.38 cm (range, 0.4–2.1 cm). Two of 40 non-hypervascular hypointense hepatic nodules (5.0%) were identified by T2-weighted sequence as high-intensity nodules. The other 38 non-hypervascular hypointense hepatic nodules were not identified either by T1- and T2-weighted sequences. Two nodules were located in segment II of the liver, 7 in III, 1 in IV, 10 in V, 6 in VI, 4 in VII, and 10 in VIII, respectively. Among 28 patients with non-hypervascular hypointense nodules, 19 patients had one non-hypervascular hypointense nodule, 6 patients had 2 nodules, and the remaining 3 patients had 3 nodules. Non-hypervascular hypointense nodules were resected along with HCC lesions during hepatectomy in 10 patients, because they were included within the intended area of resection. Therefore, we categorized these 10 patients and the 49 patients in whom non-hypervascular hypointense nodules were not detected by preoperative Gd-EOB-DTPA-enhanced MRI as the hypointense nodule (−) group and the remaining 18 patients who had residual hypointense nodules after hepatectomy as the hypointense nodule (+) group. Of 13 hypointense nodules in 10 patients resected along with HCC at hepatectomy, 3 nodules were diagnosed as well-differentiated HCC and the remaining 10 nodules were diagnosed as dysplastic nodules on pathologic examination.

Table 1 compares the preoperative characteristics of the study patients. No differences were found in patient age and sex, etiology, liver function, and tumor progression as evaluated by preoperative imaging examinations and by post-operative pathologic examinations. Multiple HCC nodules were resected in 6 patients (10.2%) of the hypointense nodule (−) group and 3 patients (16.7%) of the hypointense nodule (+) group, without difference in proportions. No difference was observed in the length of follow-up period.

Recurrence rate after hepatectomy according to the presence of non-hypervascular hypointense nodules detected during preoperative gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid-enhanced MRI

We determined the recurrence rate in patients after hepatectomy with curative intent based on the presence of non-hypervascular hypointense hepatic nodules identified during the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI (Fig. 1). The recurrence rate was significantly higher in patients in the hypointense nodule (+) group than in the hypointense nodule (−) group ($p < 0.0001$). In univariate analysis, HCC differentiation and portal vein invasion were identified as factors associated with the rate of recurrence after hepatectomy along with preoperative non-hypervascular hypointense nodules by Gd-EOB-DTPA-enhanced MRI. In multivariate analysis, these factors were confirmed to be independently associated with the rate of recurrence (Table 2). Among 18 patients in the hypointense nodule (+) group, recurrence was observed in 7 of 11 patients with one non-hypervascular hypointense nodule, whereas recurrence was observed in all 7 patients with multiple non-hypovascular hypointense nodules.

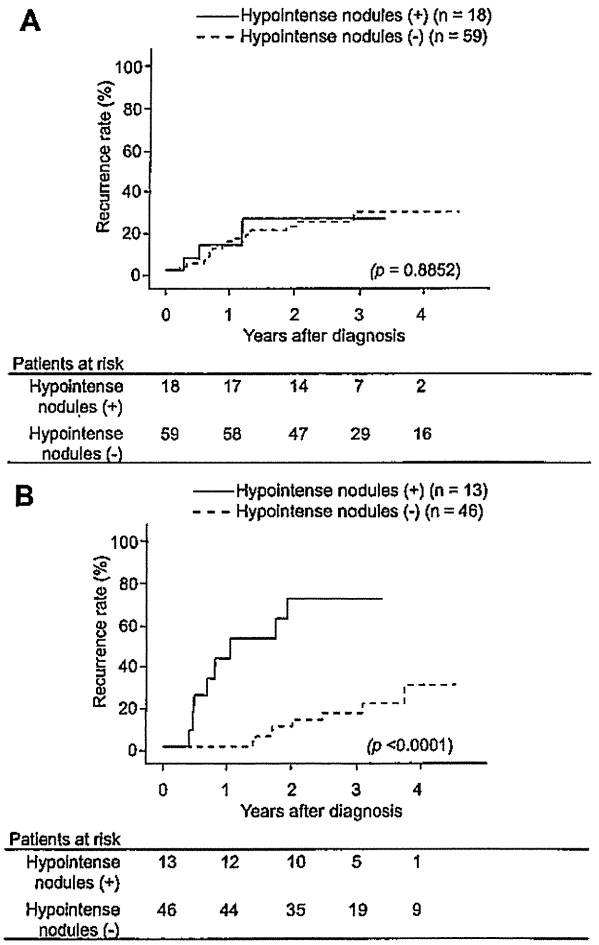

Fig. 2. Recurrence rate after hepatectomy according to the patterns of recurrence. (A) Rates of intrahepatic metastasis recurrence after hepatectomy in patients with or without concurrent non-hypervascular hypointense hepatic nodules detected during the hepatobiliary phase of preoperative Gd-EOB-DTPA-enhanced MRI. (B) Rates of multicentric recurrence after hepatectomy in patients with or without concurrent non-hypervascular hypointense hepatic nodules detected during the hepatobiliary phase of preoperative Gd-EOB-DTPA-enhanced MRI, among 59 patients, excluding 16 patients with intrahepatic metastasis recurrence.

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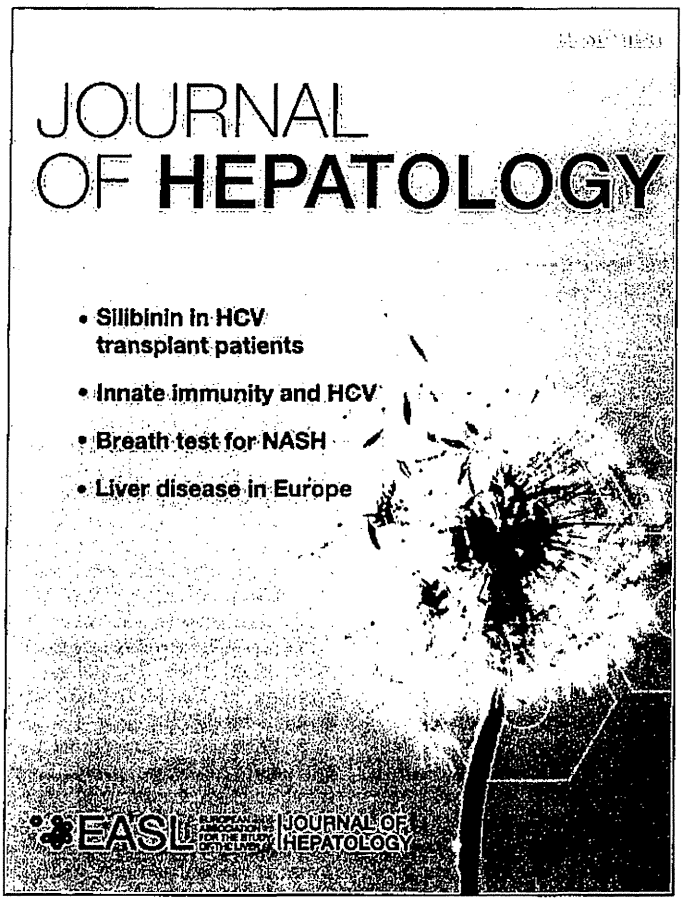

Research Article

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Effect of nucleos(t)ide analogue therapy on hepatocarcinogenesis in chronic hepatitis B patients: A propensity score analysis

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Background & Aims: Some patients with chronic hepatitis B virus (HBV) infection progress to hepatocellular carcinoma (HCC). However, the long-term effect of nucleos(t)ide analogue (NA) therapy on progression to HCC is unclear.

Methods: Therefore, we compared chronic hepatitis B patients who received NA therapy to those who did not, using a propensity analysis.

Results: Of 785 consecutive HBV carriers between 1998 and 2008, 117 patients who received NA therapy and 117 patients who did not, were selected by eligibility criteria and propensity score matching. Factors associated with the development of HCC were analyzed. In the follow-up period, HCC developed in 57 of 234 patients (24.4%). Factors significantly associated with the incidence of HCC, as determined by Cox proportional hazards models, include higher age (hazard ratio, 4.36 [95% confidence interval, 1.33–14.29], $p = 0.015$), NA treatment (0.28 [0.13–0.62], $p = 0.002$), basal core promoter (BCP) mutations (12.74 [1.74–93.11], $p = 0.012$), high HBV core-related antigen (HBcrAg) (2.77 [1.07–7.17], $p = 0.036$), and high gamma glutamyl transpeptidase levels (2.76 [1.49–5.12], $p = 0.001$).

Conclusions: NA therapy reduced the risk of HCC compared with untreated controls. Higher serum levels of HBcrAg and BCP mutations are associated with progression to HCC, independent of NA therapy.

Introduction

An estimated 350 million individuals worldwide are chronically infected with hepatitis B virus (HBV), of whom 1 million die annually from HBV-related liver disease [1]. Chronic HBV infection is recognized as a major risk factor for the development of hepatocellular carcinoma (HCC) [1,2]. Hepatitis B surface antigen (HBsAg)-positive patients have a 70-fold increased risk of developing HCC compared to HBsAg seronegative counterparts [3,4]. HBV infection is endemic in Southeast Asia, China, Taiwan, Korea, and sub-Saharan Africa, where up to 85–95% of patients with HCC are HBsAg positive [5]. HCC is the third and fifth leading cause of cancer death in men and women, respectively, and the number of deaths and the mortality rate from HCC have greatly increased in Japan since 1975 [6]. Hepatitis C virus (HCV)-related HCC accounts for 75% of all HCCs in Japan and HBV-related HCC accounts for 15% [6].

In 2004, Liaw *et al.* reported a significant reduction in HCC in 651 adults receiving lamivudine after adjustment for baseline variables (hazard ratio, 0.49 [95% confidence interval (95% CI), 0.25–0.99], $p = 0.047$) [7]. However, the results were not significant after exclusion of 5 patients who developed HCC within 1 year of randomization (0.47 [0.22–1.00], $p = 0.052$). Therefore, in 2009, the National Institutes of Health Consensus Development Conference concluded that there was insufficient evidence to assess whether nucleos(t)ide analogue (NA) therapy can prevent the development of HCC [8].

The long-term use of lamivudine has not been recommended because of tyrosine–methionine–aspartate–aspartate (YMDD) mutations, which have occasionally been associated with severe and even fatal flares of hepatitis [9,10]. Therefore, adefovir dipivoxil should be added immediately in patients with virological or biochemical breakthroughs or no response. Currently, there are 2 nucleoside agents (lamivudine, entecavir) and 1 nucleotide agent (adefovir dipivoxil) available for treatment of HBV infection in Japan. The agent with the higher genetic barrier to resistance, entecavir, is considered the initial drug of choice [11]. Recently, 3 studies on lamivudine suggested that long-term sustained viral suppression was associated with a reduced likelihood of developing HCC [12–14].

In this study, we sought to determine if NA therapy was associated with a reduction in the development of HCC. Since the validity of treatment effects in observational studies may be limited by selection bias and confounding factors, we performed a propensity analysis [15].

Keywords: HBcrAg; BCP; Gamma-GTP; Average integration value; HBV DNA.
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Abbreviations: HCC, hepatocellular carcinoma; HBV, hepatitis B virus; NA, nucleos(t)ide analogue; HBcrAg, HBV core-related antigen; BCP, basal core promoter; gamma-GTP, gamma glutamyl transpeptidase.

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Materials and methods

Patient selection

The study protocol was approved by the Institutional Ethics Committee of Ogaki Municipal Hospital in January 2011, and was in compliance with the Declaration of Helsinki. Written informed consent for the use of stored serum samples for the study was obtained from all patients.

Between 1998 and 2008, 1220 consecutive HBsAg-positive patients, who visited the Department of Gastroenterology and Hepatology at Ogaki Municipal Hospital, were prospectively enrolled in our HCC surveillance program. Of these, 785 patients met the following inclusion criteria: HBsAg positive for more than 6 months, no evidence of HCV co-infection, exclusion of other causes of chronic liver disease (alcohol consumption >80 g/day, hepatotoxic drugs, autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, or Wilson's disease), follow-up duration of greater than 3 years, no evidence of HCC for at least 1 year from the start of the follow-up period, receiving no interferon treatment, and receiving NA therapy for more than 1 year before the detection of HCC (Fig. 1). In patients on NA therapy, the date of NA therapy initiation was considered the starting point of the follow-up period.

Of these 785 patients, 148 received NA therapy (NA group) and 637 patients did not receive NA therapy (non-NA group) during the follow-up period. To reduce the confounding effects of covariates, we used propensity scores to match NA patients to unique non-NA patients. Six covariates including age, sex, HBV DNA concentration, hepatitis B e antigen (HBeAg), platelet count, and alanine aminotransferase (ALT) activity were taken into account at the start of follow-up. We computed the propensity score by using logistic regression with the independent variable including age (≤40 years or >40 years), sex (female or male), HBV DNA concentration (≤5.0 log copies/ml or >5.0 log copies/ml), HBeAg (negative or positive), platelet count (>150 × 10^3/m^3 or ≤150 × 10^3/m^3), and ALT activity (≤40 IU/ml or >40 IU/ml), as shown in previous reported cut-off values according to the indication for NA therapy [16–19]. This model yielded a *c* statistic of 0.85 (95% confidence interval [CI], 0.82–0.88), indicating very good ability of the propensity score model to predict treatment status. We sought to match each patient who received NA therapy to a patient who did not receive NA therapy, having a propensity by using greedy 5–1 digit matching [20]. Once this threshold was exceeded, a patient with NA therapy was excluded. This score ranged from 0.09198 to 0.98967 and, in effect, represented the probability that a patient would be receiving NA. We were able to match 117 patients with NA therapy to 117 unique patients without NA therapy. The follow-up period ended on 31 December, 2011 or the date when HCC occurrence was identified.

Surveillance and diagnosis

All patients were followed up at our hospital at least every 6 months. During each follow-up examination, platelet count, ALT, gamma glutamyl transpeptidase (gamma-GTP), total bilirubin, alkaline phosphatase (ALP), albumin, and alpha-fetoprotein (AFP) levels were measured. We used commercially available kits to test blood samples for HBsAg, HBeAg, and anti-HBe (Abbott Japan Co., Ltd., Tokyo, Japan). Before November 2007, the serum HBV DNA concentration was monitored by a polymerase chain reaction assay (COBAS Amplicor HBV monitor test, Roche Diagnostics K. K., Tokyo, Japan) with a lower detection limit of approximately 2.6 log copies/ml, and after December 2007, it was monitored with another polymerase chain reaction assay (COBAS AmpliPrep-COBAS TaqMan HBV Test, Roche Diagnostics K. K.), with a lower detection limit of approximately 2.1 log copies/ml. HBV genotyping was performed as described previously [21]. Serum levels of HBV core-related antigen (HBcrAg) were measured using a chemiluminescence enzyme immunoassay (CLEIA) as described previously [22,23]. Precore nucleotide 1896 and basal core promoter (BCP) dinucleotide 1762/1764 were determined using the line probe assay (INNO-LiPA HBV PreCore assay; Innogenetics NV) [24,25]. The probes were designed to determine the nucleotides at position 1896 (G *vs.* A) in the precore region and positions 1762 (A *vs.* T) and 1764 (G *vs.* A and G *vs.* T) in the BCP region. A line probe assay was used to identify any emergence of YMDD mutations (INNO-LiPA HBV DR assay; Innogenetics NV).

Platelet count, ALT, gamma-GTP, total bilirubin, ALP, albumin, AFP, and HBV DNA values were expressed as average integration values [26,27] after the start of follow-up.

According to the Clinical Practice Guidelines for Hepatocellular Carcinoma in Japan [28], we performed ultrasound (US) and monitoring of 3 biomarkers (AFP, *Lens culinaris* agglutinin-reactive fraction of alpha-fetoprotein [AFP-L3], and des-gamma-carboxy prothrombin [DCP]) every 3–4 months, and dynamic magnetic resonance imaging (MRI) every 12 months, for patients with cirrhosis under surveillance. For patients with chronic hepatitis, we performed US and monitoring of the 3 biomarkers every 6 months. Histological examinations were performed in 91 out of 234 patients. Among them, cirrhosis was diagnosed in 32 patients. In the remaining 143 patients, the diagnosis of cirrhosis was made according to typical US findings, e.g., superficial nodularity, a coarse parenchymal echo pattern, and signs of portal hypertension (splenomegaly >120 mm, dilated portal vein diameter >12 mm, patent collateral veins, or ascites) [29–31]. Patients who did not satisfy these criteria were classified as having chronic hepatitis. One hundred and forty-two patients were diagnosed with chronic hepatitis and 92 patients with cirrhosis. For diagnostic confirmation of HCC, patients underwent dynamic MRI. A histological diagnosis of HCC was made in 28 patients (surgical specimen, 23 patients; US-guided needle biopsy specimen, 5 patients). The remaining 29 patients were diagnosed with HCC based on typical dynamic MRI findings, including hypervascularity in the arterial phase with washout in the portal venous or delayed phase [32].

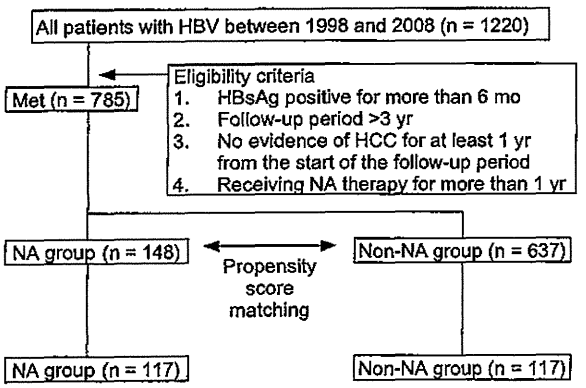

Fig. 1. Flowchart of the patient selection process.

Treatments

In the NA group, 117 patients received NA therapy including 18 patients with lamivudine, 28 patients with lamivudine and adefovir dipivoxil, and 71 patients with entecavir. The indications for NA therapy followed the guidelines of the American Association for the Study of Liver Diseases (AASLD), the European Association for the Study of the Liver (EASL), or the Asian Pacific Association for the Study of the Liver (APASL) [33–35]. In contrast, of the 117 patients not on NA therapy, 104 did not receive treatment before NA was not yet approved in Japan and the remaining 13 patients declined NA therapy.

Statistical analysis

Continuous variables are expressed as medians (range). The Mann–Whitney *U* test was used for continuous variables, and the Chi-square test with Yates' correction or Fisher's exact test was used for categorical variables. Actuarial analysis of the cumulative incidence of hepatocarcinogenesis was performed using the Kaplan–Meier method, and differences were tested with the log-rank test. The Cox proportional hazards model and the forward selection method were used to estimate the relative risk of HCC associated with age (≤40 years or >40 years), sex (female or male), treatment (NA or no NA), HBsAg (≤3.0 log IU/ml or >3.0 log IU/ml), HBV DNA level (≤5.0 log copies/ml or >5.0 log copies/ml), HBeAg (negative or positive), precore region (wild type or mutant), BCP (wild type or mutant type), HBcrAg (≤3.0 logU/ml or >3.0 logU/ml), platelet count (>150 × 10^3/m^3 or ≤150 × 10^3/m^3), ALT (≤40 IU/ml or >40 IU/ml), total bilirubin, gamma-GTP, ALP, albumin, and AFP (≤10 ng/ml or >10 ng/ml) for univariate and multivariate analyses. We used the minimum or maximum of the reference values at our institution as cut-off values for total bilirubin, gamma-GTP, ALP, and albumin. We conducted a sensitivity analysis to determine the magnitude of an unmeasured confounder [36].

We considered *p* values of 0.05 or less to be significant. Statistical analysis was performed with SPSS, version 18.0 for Windows (International Business Machines Corporation, Tokyo, Japan).

Table 1. Baseline characteristics of all patients.

	NA group (n = 148)	Non-NA group (n = 637)	*p* value	Standardized difference in %
Age (yr)	53 (26-81)	48 (4-85)	<0.0001	40.6
Sex (female/male)	60/88	285/352	0.5378	6.1
Genotype (A/B/C/D/F/n.d.)	2/5/137/0/1/2	24/60/389/2/0/162	<0.0001	37.6
HBsAg (log_{10} IU/ml)	3.5 (-0.1-5.5)	3.3 (-1.3-7.9)	<0.0001	53.8
HBV DNA (log_{10} copies/ml)	7.0 (2.6-9.6)	3.8 (2.3-9.9)	<0.0001	99.9
HBeAg (±)	76/72	151/486	<0.0001	62.8
Precore region (W/M/n.d.)	30/109/9	88/381/168	0.4652	0.0
BCP (W/M/n.d.)	33/123/10	135/279/205	0.0074	27.3
HBcrAg (log_{10} U/ml)	5.9 (2.9-7.0)	3.0 (2.9-7.0)	<0.0001	96.7
Platelet count ($x10^3/m^3$)	150 (32-388)	188 (37-503)	<0.0001	-59.7
ALT (IU/ml)	65 (7-1088)	26 (5-3410)	<0.0001	44.1
AFP (ng/ml)	3.9 (0.8-3363)	2.9 (0.8-3686)	0.0062	-6.2
Cirrhosis (presence/absence)	62/86	91/546	<0.0001	59.1
Child-Pugh classification (A/B)	132/16	618/19	0.0002	32.7
Follow-up duration (yr)	12.8 (3.1-19.6)	13.7 (3.1-20.0)	0.1565	-16.9
Administration period (yr)	6.5 (1.5-11.0)	-	-	-
Propensity score	0.58093 (0.09198-0.98686)	0.95253 (0.12913-0.98967)	<0.0001	-132.3

NA, nucleos(t)ide analogue; n.d., not done; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; W, wild type; M, mutant type; BCP, basal core promoter; HBcrAg, hepatitis B core-related antigen; ALT, alanine aminotransferase; AFP, alpha-fetoprotein; Child-Pugh classification, reference no [50], Standardized difference in%; $100(X_{NA} - X_{non\text{-}NA})/([S^2_{NA} + S^2_{non\text{-}NA}]/2)^{1/2}$, where for each covariate X_{NA} and $X_{non\text{-}NA}$ are the sample means in NA and non-NA groups, respectively, and S^2_{NA} and $S^2_{non\text{-}NA}$ are the corresponding sample variances.

Results

Patient characteristics

Table 1 shows baseline characteristics of all 785 patients before propensity matching. There were significant differences in age, HBV genotype, HBsAg, HBV DNA concentration, presence of HBeAg, BCP mutations, HBcrAg, platelet counts, ALT level, AFP level, presence of cirrhosis, and Child-Pugh classification. The baseline characteristics of the 234 study patients after propensity matching are summarized in Table 2. There are no significant differences in age, sex, HBV genotype, HBsAg, HBV DNA concentration, presence of HBeAg, precore region mutations, BCP mutations, platelet counts, ALT concentration, Child-Pugh classification, and follow-up duration. HBcrAg concentration was significantly higher in the NA group than in the non-NA group. NA was administered a median of 6.1 years (range: 1.5–10.7 years).

Factors associated with the incidence of hepatocarcinogenesis

Factors associated with the incidence of HCC as determined by the Cox proportional hazard models and the forward selection method were analyzed in all 785 patients. High age (hazard ratio, 6.43 [95% CI, 2.71–15.26], p <0.001), male sex (3.43 [1.67–7.02], p = 0.002), NA treatment (0.28 [0.21–0.85], p = 0.017), BCP mutation (19.96 [2.27–141.90], p = 0.03), high HBcrAg levels (8.21 [3.40–19.85], p <0.001), and high AFP levels (2.49 [1.43–4.34], p = 0.001) were significantly associated with the incidence of HCC.

HCC developed in 57 of 234 patients (24.4%) during follow-up after propensity matching. The 5-year, 7-year, and 10-year cumulative incidences of HCC were 9.6%, 20.4%, and 33.4%, respectively. The 5-year, 7-year, and 10-year cumulative incidences of HCC were 2.7%, 3.3%, and 3.3%, respectively, in patients on NA therapy (n = 117) and 11.3%, 26.0%, and 40.0% in patients not on NA therapy (n = 117). Hepatocarcinogenesis occurred at significantly higher rates in the non-NA group (p = 0.0094, Fig. 2). The 5-year, 7-year, and 10-year cumulative incidences of HCC were 0.0%, 0.0%, and 0.0%, respectively, in patients with wild type BCP (n = 38) and 11.0%, 25.2%, and 41.9% in patients with mutant BCP (n = 112; p = 0.0006, Fig. 3). Factors associated with the incidence of HCC as determined by the Cox proportional hazard models and the forward selection method are listed in Table 3. Higher age (hazard ratio, 4.36 [95% CI, 1.33–14.29], p = 0.015), NA treatment (0.28 [0.13–0.62], p = 0.002), BCP mutation (12.74 [1.74–93.11], p = 0.012), high HBcrAg levels (2.77 [1.07–7.17], p = 0.036), and high gamma-GTP levels (2.76 [1.49–5.12], p = 0.001) were significantly associated with the incidence of HCC. In addition, 2 patients died due to hepatic failure during the follow-up period in the non-NA group.

The sensitivity analysis found that the observed relationship between NA treatment and HCC incidence could be diminished by the unmeasured confounder that the high prevalence of the unmeasured confounder is greater in the non-NA group than in the NA group. For example, suppose a binary unmeasured confounder that increased the hazard of HCC incidence (hazard ratio, 1.50) was present in 40% of those who were treated with NA and 80% of those who were not treated with NA. Then, the study's result would become less extreme and would no longer be statistically significant (hazard ratio under sensitivity analysis, 0.48 [95% CI, 0.22–1.05]).

Follow-up data of various parameters in patients on or not on NA therapy

For this analysis, we used the average integration value during the follow-up period (Table 4). ALT, gamma-GTP, ALP, AFP, and

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Table 2. Baseline characteristics of patients on NA therapy and propensity-matched controls.

	NA group (n = 117)	Non-NA group (n = 117)	*p* value	Standardized difference in %
Age (yr)	52 (27-77)	52 (21-77)	0.9223	1.7
Sex (female/male)	44/73	45/72	0.8929	6.1
Genotype (A/B/C/n.d.)	1/4/109/3	4/7/85/21	0.1232	26.8
HBsAg (log_{10} IU/ml)	3.6 (0.9-5.5)	3.6 (0.9-7.9)	0.1440	29.9
HBV DNA (log_{10} copies/ml)	6.7 (2.6-9.6)	6.5 (2.3-9.6)	0.1273	20.5
HBeAg (±)	57/60	58/59	0.8960	2.0
Precore region (W/M/n.d.)	22/87/8	16/75/26	0.6399	5.1
BCP (W/M/n.d.)	22/88/7	17/70/30	0.9359	0.0
HBcrAg (log_{10} U/ml)	5.9 (2.9-7.0)	4.9 (2.9-7.0)	0.0022	41.2
Platelet count (x10^3/m^3)	143 (32-262)	146 (37-396)	0.6340	-12.1
ALT (IU/ml)	68 (7-1088)	55 (9-3410)	0.0977	1.9
AFP (ng/ml)	2.8 (0.8-402)	3.9 (0.8-1010)	0.3118	-13.5
Cirrhosis (presence/absence)	48/69	44/73	0.6882	6.1
Child-Pugh classification (A/B)	108/9	104/13	0.5024	3.1
Follow-up duration (yr)	12.3 (3.1-19.4)	11.6 (3.1-18.3)	0.7346	-4.5
Administration period (yr)	6.1 (1.5-10.7)	-	-	-
Propensity score	0.65895 (0.11449-0.96977)	0.65895 (0.12913-0.96989)	0.9931	0.0

NA, nucleos(t)ide analogue; n.d., not done; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; W, wild type; M, mutant type; BCP, basal core promoter; HBcrAg, hepatitis B core-related antigen; ALT, alanine aminotransferase; AFP, alpha-fetoprotein; Child-Pugh classification, reference no [50], Standardized difference in%; $100(X_{NA} - X_{non\text{-}NA})/([s^2_{NA} + s^2_{non\text{-}NA}]/2)^{1/2}$, where for each covariate X_{NA} and $X_{non\text{-}NA}$ are the sample means in NA and non-NA groups, respectively, and s^2_{NA} and $s^2_{non\text{-}NA}$ are the corresponding sample variances.

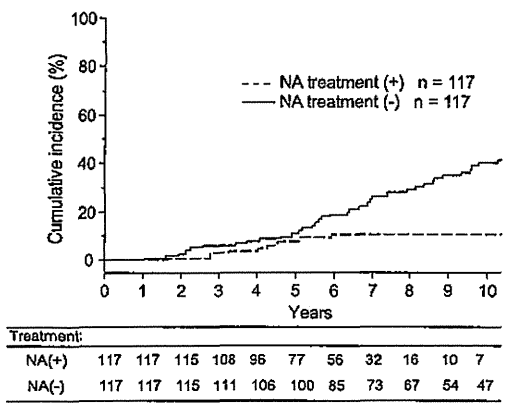

Fig. 2. Incidence of hepatocellular carcinoma (HCC) according to nucleos(t)ide analogue (NA) treatment status. The NA group had a significantly higher rate of progression to HCC than the non-NA group ($p = 0.0094$).

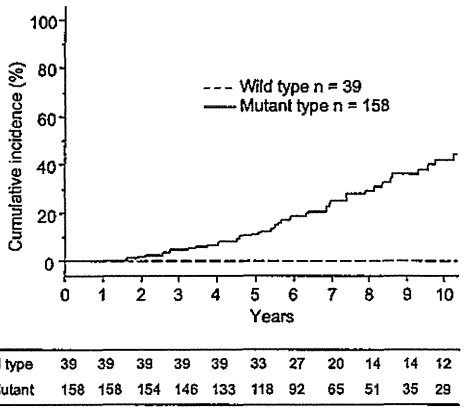

Fig. 3. Incidence of hepatocellular carcinoma (HCC) according to basal core promoter (BCP) mutations. Patients with mutant-type BCP had a significantly higher rate of progression to HCC than those with wild type BCP ($p = 0.0006$).

HBV DNA levels were significantly lower in patients on NA therapy than in patients not on NA therapy. In contrast, platelet counts and albumin levels were significantly higher in patients on NA therapy than in patients not on NA therapy.

Discussion

Our study shows that long-term NA maintenance therapy is associated with the suppression of progression to HCC. Liaw *et al.* reported that lamivudine decreased the risk of HCC in cirrhotic patients [7]. However, it is unclear whether the observed decreased risk of HCC with NA therapy was due to the short observation period in their study. It is very difficult to prove the preventive effect of NA on the development of HCC, because randomized control studies are not ethically possible. In this study, patients on NA therapy were compared to propensity score-matched untreated controls. In these control patients, NA therapy had not yet been approved or was not routinely used for chronic hepatitis B at the time, or was declined by the patient. As opposed to the entire population, these propensity-matched patients were well matched to patients on NA; significant differences included higher HBcrAg levels in the NA group.

Large community-based studies have confirmed that advanced age, male sex, HBeAg positivity, low platelet count,

Table 3. Factors associated with progression to hepatocellular carcinoma among propensity-matched patients (Cox proportional hazard model).

		Adjusted hazard ratio (95% CI)	*p* value
Age (yr)	≤40	1	0.015
	>40	4.36 (1.33-14.29)	
Treatment	no NA	1	0.002
	NA	0.28 (0.13-0.62)	
BCP	wild-type	1	0.012
	mutant-type	12.74 (1.74-93.11)	
HBcrAg (log_{10} U/ml)	≤3.0	1	0.036
	>3.0	2.77 (1.07-7.17)	
γ-GTP (IU/L)	≤56	1	0.001
	>56	2.76 (1.49-5.12)	

NA, nucleos(t)ide analogue; BCP, basal core promoter; HBcrAg, hepatitis B core-related antigen; γ-GTP, gamma glutamyl transpeptidase.

higher ALT levels, elevated AFP levels, and presence of cirrhosis are factors associated with the development of cirrhosis and HCC [17,18]. Platelet count is a useful surrogate marker for the diagnosis of cirrhosis [37]. All subjects were not histologically diagnosed in this study. Therefore, we selected platelet count as a marker of hepatic fibrosis instead of cirrhosis. An elevated ALT level indicates the presence of active disease, and persistently elevated AFP levels are a reflection of an enhanced regenerative state in the liver [16]. In the REVEAL study, a high HBV DNA load was associated with an increased rate of HCC development [17]. A direct correlation was observed between baseline HBV DNA levels and the incidence of HCC, independent of serum ALT concentration. In a model that integrated baseline and follow-up HBV DNA levels, the cumulative incidence of HCC ranged from 1.3% in patients with undetectable levels of HBV DNA to 14.9% in patients with HBV DNA levels greater than or equal to 10^6 copies/ml. Therefore, we have selected factors, such as age, sex, HBeAg serostatus, HBV DNA concentration, platelet count, and ALT for propensity matching.

Although the exact mechanisms of hepatocarcinogenesis by HBV remain unclear, two mechanisms have been proposed [38,39]. One mechanism involves chronic necroinflammation of hepatocytes, cellular injury, and hepatocyte regeneration [40]. The other mechanism involves the direct carcinogenicity of HBV through chromosomal integration [41]. Complete and sustained viral suppression by NA might block both pathways and prevent the development of HCC. It is well known that the rate of HCC is significantly higher in patients with virological breakthrough or no response. In our study, when virological or biochemical breakthrough was observed and the YMDD mutation was detected in patients on lamivudine, adefovir dipivoxil was immediately added. In patients with cirrhosis, especially in the decompensated stage, sustained viral response on NA therapy was not necessarily associated with a preventative effect against the development of HCC, even though the incidence was lower than in a group not on NA [14]. It is not surprising that viral suppression decreased but did not eliminate the risk of HCC, because HBV DNA may have already integrated into the host genome before the initiation of therapy and may have resulted in genomic alternations, chromosomal instability, or both [42,43].

It is reported that patients with HBV genotype C infection have higher HBV DNA levels, higher frequency of pre-S deletions, higher prevalence of BCP T1762/A1764 mutations, and significantly higher chances of developing HCC [16,44–46]. In our study, T1762/A1764 mutations were observed in 158 (80.2%) out of 197 patients and were associated with a higher risk of developing HCC (adjusted hazard ratio, 12.740 [95% CI 1.743–93.108]), independent of NA therapy. However, the BCP T1762/A1764 mutations were detected in HCC patients from Asia and Africa, where HBV genotype C infection is predominant [16].

HBcrAg is a new HBV marker that reflects HBV load and corresponds to HBV DNA levels [21]. HBcrAg is comprised of HBV core antigen (HBcAg) and HBeAg; both are products of the pre-core/core gene and share the first 149 amino acids of HBcAg. The HBcrAg assay measures HBcAg and HBeAg simultaneously by using monoclonal antibodies that recognize both denatured HBcAg and HBeAg [47]. Serum HBcrAg concentration is well correlated with intrahepatic levels of covalently closed circular DNA (cccDNA) [48]. It is reported that HBcrAg is a useful marker for guiding cessation of NA therapy and evaluation of disease activity [21,49]. In our study, elevated serum HBcrAg concentration was associated with a higher risk of developing HCC (adjusted hazard ratio, 2.767 [95% CI 1.067–7.172]). This is the first report demonstrating a relationship between HBcrAg and HCC.

The present study has several limitations. The retrospective design might have introduced an unintended bias. The propensity matching method was adopted to reduce the confounding effects of covariates. Characteristics of patients who did or did not receive NA therapy were similar except for HBcrAg concentration.

Table 4. Average integration values of various parameters in patients who did or did not receive NA therapy.

	NA group (n = 117)	Non-NA group (n = 117)	*p* value
Platelet count (x10^3/m^3)	17.0 (3.3-37.2)	14.8 (3.3-296)	0.0060
ALT (IU/ml)	28.2 (8.5-88.9)	39.1 (12.2-737.5)	<0.0001
γ-GTP (IU/L)	27.0 (10.9-267.6)	36.2 (9.5-269.7)	0.0427
Total bilirubin (mg/dl)	0.7 (0.3-2.0)	0.7 (0.3-2.6)	0.1554
ALP (IU/L)	242.7 (113.5-1028.8)	265.2 (140.5-1247.6)	0.0127
Albumin (g/dl)	4.4 (3.0-5.0)	4.0 (2.4-4.8)	<0.0001
Alpha-fetoprotein (ng/ml)	2.2 (0.8-106.0)	4.5 (0.9-723.8)	<0.0001
HBV DNA (log_{10} copies/ml)	2.5 (2.1-8.9)	4.6 (2.1-9.3)	<0.0001

NA, nucleos(t)ide analogue; ALT, alanine aminotransferase; γ-GTP, gamma glutamyl transpeptidase; ALP, alkaline phosphatase; HBV, hepatitis B virus.

However, the non-NA group included many historical cases when NA therapy was not yet available. In addition, the HBV DNA assay used between 1998 and 2007 was not the most sensitive one.

In conclusion, NA therapy reduces the risk of HCC compared with untreated controls. Higher serum HBcrAg levels and BCP mutations are associated with development of HCC, independent of NA therapy.

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Conflict of interest

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

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Baseline factors and very early viral response (week 1) for predicting sustained virological response in telaprevir-based triple combination therapy for Japanese genotype 1b chronic hepatitis C patients: a multicenter study

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Abstract
Background Genetic polymorphisms near *Interleukin 28B* (*IL28B*) (rs8099917) and a rapid virological response (RVR) have been reported as predictors for a sustained virological response (SVR) to telaprevir (TVR)-based triple combination therapy. However, the association between SVR and viral kinetics earlier than week 4 after initiation of therapy remains unclear. Thus, we evaluated the SVR prediction ability of baseline factors and reduced hepatitis C virus (HCV) RNA levels at week 1 after the initiation of TVR-based therapy in Japanese genotype-1b chronic hepatitis C (CHC) patients.

Methods A total of 156 Japanese CHC patients received a 24-week regimen of TVR-based therapy. Baseline factors and reduction in HCV RNA levels at weeks 1 and 4 after the initiation of therapy were analyzed for SVR prediction.
Results Multiple logistic regression analysis for SVR in TVR-based therapy identified the *IL28B* TT genotype, a reduction of $\geq$4.7 $\log_{10}$IU/mL in HCV RNA levels at week 1, RVR, and treatment-naïve/relapse. Whereas the SVR rate was higher than 90 % regardless of the reduction in HCV RNA levels at week 1 in patients with the TT genotype, a reduction of $\geq$4.7 $\log_{10}$IU/mL in HCV RNA levels at week 1 was the strongest predictor of SVR in patients with the non-TT genotype, as determined by multiple logistic regression analysis ($P = 0.0043$).
Conclusions The *IL28B* TT genotype is the most important baseline factor for predicting SVR, and a $\geq$4.7 $\log_{10}$IU/mL reduction in HCV RNA at week 1 is a useful very early on-treatment predictor of SVR, especially in the non-TT genotype.

Keywords Chronic hepatitis C · Reduction in HCV RNA at week 1 · Telaprevir · *IL28B*

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Introduction

In 2011, the first-generation direct-acting antiviral agents telaprevir (TVR) and boceprevir (BOC) were approved for treatment of chronic hepatitis C (CHC) patients with hepatitis C virus (HCV) genotype 1 in the United States (US), Canada, and the European Union (EU). Triple combination therapy with TVR or BOC, PEG-interferon (PEG-IFN), and ribavirin (RBV) is the current standard of care for genotype 1 CHC patients [1]. In Japan, TVR, which is a nonstructural (NS) 3/4A serine protease inhibitor, was

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approved in September 2011 and has been marketed since November 2011. In treatment-naïve genotype 1 CHC patients, TVR-based triple combination therapy for a shortened period was reported to remarkably improve the rate of sustained virological response (SVR) compared with PEG-IFN and RBV alone [2–4]. In treatment-experienced patients, the effect of TVR-based triple combination therapy reportedly depends on the response to PEG-IFN and RBV combination therapy [5–16].

Pivotal genome-wide association studies have found that genetic variations near the interleukin 28B (*IL28B*) gene (rs8099917 and rs12979860) are strongly associated with the treatment outcome of PEG-IFN and RBV combination therapy [17–19]. We previously confirmed that the *IL28B* single-nucleotide polymorphism (SNP) genotype was the strongest factor contributing to SVR in PEG-IFN and RBV combination therapy [20–23]. These genetic variations appear to be strong predictors of SVR to a 24-week regimen of TVR-based triple therapy, as well as PEG-IFN and ribavirin combination therapy [7, 11, 14–16, 24].

Two guidelines for treatment of genotype 1 CHC patients, which were based on the results of the clinical trials of a 24-week regimen of TVR-based triple therapy for Japanese patients [4, 10], provided recommendations for patient selection for TVR-based therapy [25]. Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis published by the Ministry of Health, Labour and Welfare of Japan, and Japan Society of Hepatology guidelines provided recommendations of a 24-week regimen of TVR-based triple therapy for Japanese genotype 1 CHC patients. These recommendations are based on baseline factors, including patient's age, sex, *IL28B* genotype, core amino acid substitution at position 70, previous treatment history and response, stage of fibrosis, viral load, and baseline hemoglobin level [25, 26].

In addition to the baseline predictive factors, changes in HCV RNA levels after the start of therapy are predictive for treatment outcomes. A rapid virological response (RVR), defined as undetectable serum HCV RNA at week 4 after the start of therapy, and an extended rapid virological response, defined as undetectable serum HCV RNA at both weeks 4 and 12, were also reported as significant predictors of TVR-based treatment outcome [7, 11, 15, 16, 27]. However, the association between SVR and viral kinetics earlier than 4 weeks after initiating TVR-based triple combination therapy remains unclear. RVR was achieved in only approximately 3–11 % of cases receiving PEG-IFN and RBV combination therapy [2, 3, 6, 27, 28]. In contrast, RVR was achieved in approximately 61–84 % of cases receiving TVR-based triple combination therapy [2–6, 8, 10, 11, 15, 16, 27, 29]. It is therefore important to determine whether viral kinetics earlier than week 4 after the start of therapy is predictive for SVR in TVR-based triple combination therapy?

TVR-based triple combination therapy remarkably improves the SVR rate in CHC patients with the difficult-to-treat HCV genotype 1. However, some patients still fail to achieve SVR. Adverse events occurred more frequently and were more severe in patients treated with TVR-based therapy than in those treated with PEG-IFN and RBV alone [2–6]. Additionally, TVR-based therapy is expensive. In clinical practice, the determination of predictive factors of successful treatment outcome as early as possible is necessary for preventing unnecessary treatment in addition to physical and economic burden. Thus, in this prospective, multicenter study, we evaluated the clinical relevance of baseline predictors and the reduction in HCV RNA levels at week 1 after starting therapy for predicting SVR in a 24-week regimen of TVR-based triple combination therapy for genotype 1b CHC patients.

Methods

Patients, treatment, and definition of outcomes

Between December 2011 and September 2012, 156 Japanese genotype 1b monoinfected CHC patients were enrolled in this multicenter study at Shinmatsudo Central General Hospital, Kurume University School of Medicine, Kagawa Prefectural Central Hospital, Jikei University School of Medicine Kashiwa Hospital, and Ogaki Municipal Hospital. The inclusion criteria for the study included persistently positive sera for HCV RNA for > 6 months as determined using the quantitative real-time PCR method (COBAS AmpliPrep/COBAS TaqMan HCV test, Roche Diagnostics, Tokyo, Japan), HCV RNA ≥ 5.0 $\log_{10}$IU/mL in treatment-naïve patients, age of 18–75 years, and body weight >35 kg at the time of entry into the study. Exclusion criteria were: (1) decompensated cirrhosis; (2) positive for hepatitis B surface antigen or antibodies against human immunodeficiency virus; (3) previous or current development of hepatocellular carcinoma; (4) co-existence of other liver diseases, such as autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, Wilson disease, and alcoholic liver disease; (5) renal disease or creatinine clearance $\leq$ 50 mL/min at baseline; (6) hemoglobin level < 12 g/dL, white blood cell count $< 2000/\mu$L, neutrophil count $< 1500/\mu$L, and platelet count $< 8.0 \times 10^4/\mu$L at baseline; (7) depression, schizophrenia or its history, or history of suicide attempts, (8) pregnancy in progress or planned for either partner during the study period. For 114 of 156 (73.1 %) patients, liver biopsy was conducted within 12 months of enrollment. The presence or absence of cirrhosis was established according to the Metavir score

Table 1 Patient profiles

Number of patients	156
Sex (male/female)	78/78
Age (years)	58.4 ± 10.3
Body weight (kg)	61.8 ± 12.8
Body mass index (kg/m^2)	23.7 ± 3.5
Absence or presence of cirrhosis (non-cirrhosis/cirrhosis)	120/36
Response to previous treatment (treatment-naïve/relapsers/partial responders/null responders)	78/50/14/14
rs8099917 (TT/TG/GG)	106/48/2
Core amino acid substitution 70 (wild-type/mutant-type)	97/59
ISDR of NS5A (wild-type/non-wild-type)	138/18
White blood cells (/μL)	4972 ± 1542
Hemoglobin (g/dL)	14.2 ± 1.4
Platelets (×10^4/μL)	17.1 ± 5.6
Aspartate aminotransferase I (U/L)	54 ± 36
Alanine aminotransferase I (U/L)	60 ± 50
Gamma-glutamyl-transpeptidase I (U/L)	59 ± 68
Albumin (g/dL)	4.2 ± 0.3
Total cholesterol (mg/dL)	173 ± 31
Low-density lipoprotein cholesterol (mg/dL)	103 ± 28
Alpha-fetoprotein (ng/mL)	10.9 ± 20.7
HCV RNA ($\log_{10}$IU/mL)	6.4 ± 0.9
Initial dose of PEG-IFN (μg/kg)	1.5 ± 0.2
Initial dose of ribavirin (mg/kg)	11.2 ± 1.6
Initial daily dose of telaprevir (1500/2250 mg)	84/72
Administration intervals of telaprevir (q8/q12 h)	96/60

Data are expressed as numbers or mean ± standard deviation

ISDR interferon sensitivity-determining region, *HCV* hepatitis C virus, *PEG-IFN* PEG-interferon

[30]. For the remaining 42 patients, the presence or absence of cirrhosis was evaluated using ultrasonography and/or computed tomography findings.

Patient profiles are shown in Table 1. In this study, all treatment-experienced patients were treated with PEG-IFN and ribavirin combination therapy. Patients in this study were categorized as relapsers (HCV RNA undetectable at the end of treatment and then positive in follow-up), partial responders (≥2 $\log_{10}$IU/mL reduction in HCV RNA at week 12 but never undetectable), or null responders (< 2 $\log_{10}$IU/mL reduction in HCV RNA at week 12). In this study, partial responders and null responders were analyzed as non-responders.

All patients were treated with PEG-IFN-α-2b, RBV, and TVR triple therapy. TVR (Telavic; Mitsubishi Tanabe Pharma, Osaka, Japan) was administered every 8 h after meals (q8 h) at 500 or 750 mg, or every 12 h after meals (q12 h) at 750 or 1125 mg. The initial daily dose of TVR (1500 or 2250 mg per day) and administration intervals (q8 or q12 h) were determined by each attending physician according to age, sex, body weight, and hemoglobin level. PEG-IFN-α-2b (PEG-Intron, MSD, Tokyo, Japan) was injected subcutaneously at a median dose of 1.5 μg/kg per week. The RBV (Rebetol, MSD, Tokyo, Japan) dose was adjusted by body weight (600 mg for < 60 kg; 800 mg for ≥60 to <80 kg; and 1000 mg for ≥80 kg; in the case of hemoglobin < 13 g/dL at start of therapy, the RBV dose was reduced by 200 mg), based on the guidelines of the Ministry of Health, Labor and Welfare of Japan, and the drug was administered orally after breakfast and dinner. Triple therapy was given for 12 weeks, followed by an additional 12 weeks of PEG-IFN-α-2b and RBV combination therapy (T12PR24). Administration of each drug was appropriately reduced or withdrawn when a serious adverse event was suspected to be developing or if a serious adverse event occurred during the course of treatment. Regardless of adverse events, treatment was stopped for patients who had HCV RNA > 3 $\log_{10}$IU/mL at week 4 or detectable HCV RNA at week 12, or those showing a > 2 $\log_{10}$IU/mL increase in HCV RNA levels from the lowest level during therapy, because of the low likelihood of achieving SVR and the high risk of developing antiviral resistance.

Adherence to PEG-IFN was calculated based on the initial weekly dose, and that to RBV was calculated based on the initial daily dose. Adherence to TVR was defined as 100 % when 2250 mg was given each day for 12 weeks, which is the recommended daily dose.

The virological response was analyzed on an intent-to-treat basis. The successful endpoint of treatment was SVR for patients showing undetectable HCV RNA for 24 weeks after cessation of treatment. Patients were defined as relapse when HCV RNA levels became undetectable until the end of treatment, but became positive during the follow-up period. Patients were defined as at viral breakthrough when HCV RNA became undetectable during the treatment period, but then became positive again before the end of the treatment period. Patients were defined as non-response when HCV RNA was detectable throughout the treatment period. Furthermore, RVR was defined as undetectable HCV RNA at week 4 after starting treatment.

All patients provided written informed consent. This study protocol was prepared following ethics guidelines established in conformity with the 2008 Declaration of Helsinki, and was approved by the Ethics Committee of each participating institution.

Measurement of HCV RNA, and amino acid substitution in the core and NS5A regions of HCV genotype 1b

HCV genotype was determined by direct sequencing followed by phylogenic analysis of the NS5B region [31]. The

antiviral effects of the therapy on HCV were assessed by measuring serum HCV RNA levels. In this study, HCV RNA levels were evaluated at baseline; weeks 1, 4, 8, 12, 16, 20, and 24 during treatment; and once every 4 weeks after cessation of treatment. HCV RNA levels were determined using the COBAS AmpliPrep/CABAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan). The linear dynamic range of the assay was 1.2–7.8 $\log_{10}$IU/mL, and undetectable samples were defined as negative.

Core amino acid substitution at position 70 was determined according to a previously described method [32, 33]. Core amino acid substitution at position 70 was defined as wild-type (arginine) or mutant-type (glutamine or histidine). Additionally, substitutions at amino acids 2290–2248 of the NS5A region [interferon-sensitivity determining region (ISDR)] were determined using a previously described method [34]. Amino acid substitutions in ISDR were defined as wild-type (0 or 1) or non-wild-type ($\geq$2).

Single-nucleotide polymorphism genotyping

Genomic DNA was extracted from whole blood using the MagNA Pure LC and a DNA Isolation Kit (Roche Diagnostics). The genetic polymorphism rs8099917, near the *IL28B* gene [17, 18], was genotyped by real-time detection PCR using the TaqMan SNP Genotyping Assays and the 7500Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The rs8099917 genotypes were classified into 2 categories, including TT (major genotype) and non-TT genotype (minor genotype: TG or GG).

Statistical analysis

Continuous variables are expressed as the mean and standard deviation. Categorical data were analyzed using the Chi-squared test and Fisher's exact test, while continuous data were analyzed using the non-parametric Mann–Whitney *U* test. Univariate and multiple logistic regression analyses were used to identify factors that significantly contributed to SVR. The odds ratios (OR) and 95 % confidence intervals (95 % CI) were also calculated. All *P* values for statistical tests were 2-tailed, and values of < 0.05 were considered statistically significant. Variables that achieved statistical significance ($P < 0.05$) according to univariate analysis were entered into multiple logistic regression analyses to identify significant independent predictive factors of SVR.

Receiver-operating characteristics (ROC) analyses were performed to determine cut-off values for sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy for predicting SVR. Statistical analysis was performed using SPSS version 17.0 (IBM-SPSS, Chicago, IL, USA).

Results

Characteristics of patients and treatment outcome

Table 1 summarizes the characteristics of the patients. In total, 78 patients (50.0 %) were treatment-naïve, and 78 patients (50.0 %) were treatment-experienced with PEG-IFN and RBV. The *IL28B* TT genotype was present in 67.9 % (106 of 156) of the patients. The proportion of patients with cirrhosis was 23.1 % (36 of 156). In total, 72 patients (46.1 %) were treated with TVR at 2250 mg/day, and 84 patients (53.9 %) were treated with TVR at 1500 mg/day. In terms of dosing schedule, 96 patients (61.5 %) were treated q8 h, and 60 patients (38.5 %) were treated q12 h.

Regarding treatment outcomes, 125 patients (80.1 %) achieved SVR; 14 patients (9.0 %) relapsed. 12 patients (7.7 %) showed viral breakthrough, and the remaining five patients (3.2 %) showed non-response. For the *IL28B* SNP genotypes, among the 106 patients with the TT genotype, 102 (96.2 %) achieved an SVR, and one (0.9 %) relapsed; two (1.9 %) showed viral breakthrough, and one (0.9 %) showed non-response Among the 50 patients with the non-TT genotype, 23 (46.0 %) achieved an SVR; 13 (26.0 %) relapsed. Ten (20.0 %) showed viral breakthrough, and four (8.0 %) showed non-response. Thus, the SVR rate was significantly higher in patients with the TT genotype than in those with the non-TT genotype [102 of 106 patients (96.2 %) vs. 23 of 50 (46.0 %), $P < 0.0001$] (Fig. 1). According to previous treatment response, among the 78 treatment-naïve patients, 66 (84.6 %) achieved an SVR; five (6.4 %) relapsed. Five (6.4 %) showed viral breakthrough, and two (2.6 %) showed non-response. Among the 50 relapsers, 48 (96.0 %) achieved an SVR; one (2.0 %) relapsed, and one (2.0 %) showed viral breakthrough. Among the 14 partial responders, eight (57.1 %)

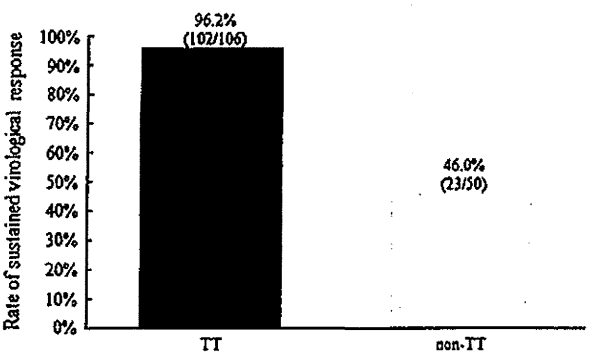

Fig. 1 Rate of sustained virological response according to the *IL28B* (rs8099917) genotype. The rate of sustained virological response was significantly higher in patients with the TT genotype than in those with the non-TT genotype ($P < 0.0001$)

achieved an SVR; four (28.6 %) relapsed, and two (14.3 %) showed viral breakthrough. Among the 14 null responders, three (21.4 %) achieved an SVR; four (28.6 %) relapsed. Four (28.6 %) showed viral breakthrough, and three (21.4 %) showed non-response. The SVR rate was significantly different across the four categories of previous treatment response ($P < 0.0001$). In particular, the SVR rate was significantly lower in non-responders than in treatment-naïve patients or relapsers [114 of 128 patients (89.1 %) vs. 11 of 28 patients (39.3 %), $P < 0.0001$].

Six patients stopped triple therapy before 12 weeks. The reasons were loss of appetite in three patients, severe anemia in one patient, systemic skin flare in one patient, and viral breakthrough in one patient. Among the six patients, five (83.3 %) with the IL28B TT genotype achieved an SVR, and one (16.7 %) with the non-TT genotype who showed viral breakthrough did not achieve an SVR.

Association between reduced serum HCV RNA levels at week 1 after starting therapy and SVR

ROC curve analysis was performed in 156 patients, to evaluate the association between reduced serum HCV RNA levels at week 1 after starting therapy and SVR. The area under the ROC curve was 0.754, and the best cut-off value was calculated as 4.7 $\log_{10}$IU/mL (Fig. 2). The SVR rate was significantly higher in patients with a reduction of $\geq$4.7 $\log_{10}$IU/mL at week 1 than in those with a reduction of <4.7 $\log_{10}$IU/mL [65 of 68 patients (95.6 %) with $\geq$4.7 $\log_{10}$IU/mL vs. 60 of 88 patients (68.2 %) with <4.7 $\log_{10}$IU/mL, $P < 0.0001$]. All four patients with the TT genotype who failed to show an SVR had a reduction of

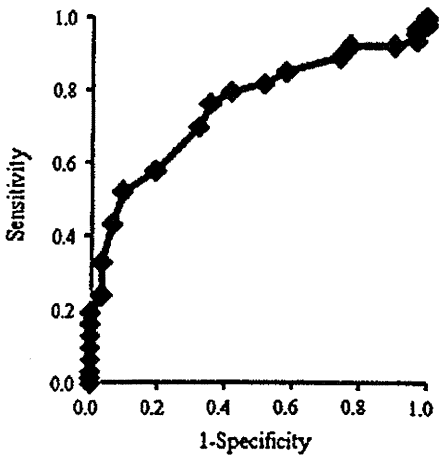

Fig. 2 Receiver operating characteristic (ROC) analysis for prediction of a sustained virological response according to the reduction in serum HCV RNA levels at week 1 after the start of therapy. The area under the ROC curve was 0.754

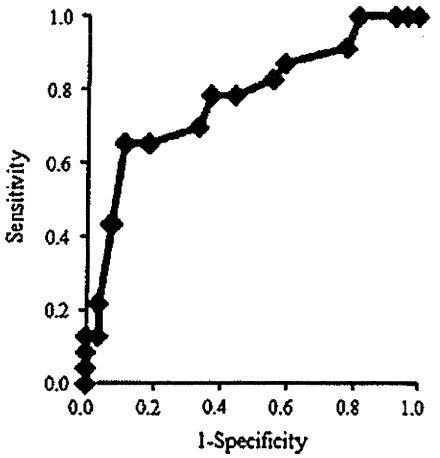

Fig. 3 Receiver operating characteristics (ROC) analysis for prediction of a sustained virological response in the *IL28B* (rs8099917) non-TT genotype according to the reduction in serum HCV RNA levels at week 1 after the start of therapy. The area under the ROC curve was 0.777

<4.7 $\log_{10}$IU/mL at week 1 (4.1 $\log_{10}$IU/mL in treatment-naïve patient, 3.8 $\log_{10}$IU/mL in partial responder, 3.7 $\log_{10}$IU/mL in null responder, and 4.6 $\log_{10}$IU/mL in null responder, respectively).

Patients with the *IL28B* TT genotype presented an extremely high SVR rate. Therefore, the ROC analysis focused on 50 patients with the *IL28B* non-TT genotype. The area under the ROC curve was 0.777, and the best cut-off value was calculated as 4.7 $\log_{10}$IU/mL, which was similar to the value calculated for all patients (Fig. 3). The SVR rate was significantly higher in patients with a reduction of $\geq$4.7 $\log_{10}$IU/mL at week 1 than in those with a reduction of <4.7 $\log_{10}$IU/mL [15 of 18 patients (83.3 %) with a reduction of $\geq$4.7 $\log_{10}$IU/mL vs. 8 of 32 patients (25.0 %) with a reduction of <4.7 $\log_{10}$IU/mL, $P = 0.0001$].

Predictive factors associated with SVR

According to the univariate analysis, the following factors were associated with SVR: treatment-naïve patients or relapsers ($P < 0.0001$); *IL28B* TT genotype ($P < 0.0001$); higher white blood cell count ($P = 0.0098$), platelet count ($P = 0.0299$), total cholesterol level ($P = 0.0467$), and low-density lipoprotein cholesterol level ($P = 0.0080$); lower gamma glutamyl transpeptidase level ($P = 0.0014$) and alpha-fetoprotein level ($P = 0.0175$); core amino acid substitution at position 70 of the wild-type ($P = 0.0010$); achievement of RVR ($P < 0.0001$); and reduction of $\geq$4.7 $\log_{10}$IU/mL in HCV RNA levels at week 1 ($P = 0.0003$). Multiple logistic regression analysis identified the following four independent factors: *IL28B* TT

Table 2 Factors associated with sustained virological response

Variable	Simple			Multiple		
	OR	95 % CI	*P* value	OR	95 % CI	*P* value
Host-related factor						
Age (year)	1.00	0.96–1.04	0.9488			
Sex male vs. female	1.23	0.56–2.72	0.6019			
Body weight (kg)	0.99	0.97–1.02	0.7195			
Body mass index (kg/m^2)	0.97	0.87–1.08	0.5494			
Cirrhosis absence vs. presence	2.20	0.93–5.18	0.0711			
Treatment-naïve or relapsers vs. non-responders	12.58	4.92–32.21	< 0.0001	5.58	1.28–24.38	0.0224
rs8099917 TT vs. non-TT	29.93	9.54–93.92	< 0.0001	73.65	11.28–480.93	< 0.0001
White blood cells (/μL)	1.00	1.00–1.00	0.0098			
Hemoglobin (g/dL)	1.16	0.87–1.55	0.3196			
Platelets (×10^4/μL)	1.09	1.01–1.18	0.0299			
Aspartate aminotransferase I(U/L)	0.99	0.98–1.00	0.1034			
Alanine aminotransferase I(U/L)	1.00	0.99–1.00	0.3574			
Gamma-glutamyl-transpeptidase I(U/L)	0.99	0.99–1.00	0.0014			
Albumin (g/dL)	3.14	0.65–15.22	0.1548			
Total cholesterol (mg/dL)	1.01	1.00–1.03	0.0467			
Low-density lipoprotein-cholesterol (mg/dL)	1.03	1.01–1.05	0.0080			
Alpha-fetoprotein (ng/mL)	0.97	0.95–1.00	0.0175			
Virus-related factor						
HCV RNA ($\log_{10}$IU/mL)	1.01	0.64–1.60	0.9695			
Core amino acid substitution 70 wild-type vs. mutant-type	4.01	1.75–9.17	0.0010			
ISDR of NS5A non-wild-type vs. wild type	2.13	0.46–9.79	0.3319			
Treatment-response factor						
Rapid virological response + vs. −	9.43	3.89–22.87	< 0.0001	12.59	2.33–69.97	0.0032
Reduction in HCV RNA level at week 1 ≥4.7 $\log_{10}$/mL vs. <4.7 $\log_{10}$IU/mL	10.11	2.92–34.99	0.0003	18.99	2.74–131.63	0.0029
Treatment-related factor						
Administration intervals of telaprevir q8 vs. q12 h	1.20	0.54–2.67	0.6572			
Initial daily dose of telaprevir 2250 vs. 1500 mg	1.46	0.65–3.26	0.3545			
Duration of therapy (weeks)	0.66	0.92–1.13	1.0226			
Adherence of PEG-IFN (%)	1.00	0.98–1.01	0.5762			
Adherence of ribavirin (%)	1.00	1.00–1.00	0.8539			
Adherence of telaprevir (%)	1.01	0.99–1.03	0.4877			

HCV hepatitis C virus, *ISDR* interferon sensitivity-determining region, *Peg-IFN* PEG-interferon

genotype ($P < 0.0001$, OR = 73.65, 95 % CI = 11.28–480.93), reduction of ≥4.7 $\log_{10}$IU/mL in HCV RNA at week 1 ($P = 0.0029$, OR = 18.99, 95 % CI = 2.74–131.63), achievement of RVR ($P = 0.0032$, OR = 12.59, 95 % CI = 2.33–69.97), and treatment-naïve patients or relapsers ($P = 0.0224$, OR = 5.58, 95 % CI = 1.28–24.38) (Table 2).

When analyses focused on patients with the *IL28B* non-TT genotype alone, previous relapsers ($P = 0.0020$), higher white blood cell count ($P = 0.0255$) and platelet count ($P = 0.0161$), lower body mass index ($P = 0.0400$), aspartate aminotransferase level ($P = 0.0303$), alpha-fetoprotein level ($P = 0.0304$), achievement of RVR ($P = 0.0011$), and reduction of ≥4.7 $\log_{10}$IU/mL in HCV RNA levels at week 1 ($P = 0.0003$) were identified as factors associated with SVR by univariate analysis. The multiple logistic regression analysis identified the following three independent factors: a reduction of ≥4.7 $\log_{10}$IU/mL in HCV RNA at week 1 ($P = 0.0043$, OR = 29.35, 95 % CI = 2.88–299.22), achievement of RVR