to be an independent and positive predictive factor for ALT normalization. Considering that patients who did achieve ALT normalization had lower Alb levels than patients with elevated ALT at the final follow up (4.4 vs 4.6 g/dL, $P < 0.01$), and Alb levels are significantly higher in non-alcoholic fatty liver disease,[25] we speculate that fatty liver disease is related to the abnormal ALT. To clarify this, further studies by liver biopsy and/or ultrasonography will be needed.

In conclusion, long-term ADV treatment was highly effective in LAM-resistant CHB patients in terms of virological and biochemical response. In addition, the emergence of resistance to the add-on ADV therapy appears to be delayed and infrequent, in contrast to LAM. Furthermore, lower HBV DNA level and lower Alb level were significant predictive factors for better outcomes. Even though add-on ADV therapy in LAM-resistant CHB patients was highly effective in the long term, CHB patients with LAM or entecavir monotherapy need to be carefully followed-up and the optimal timing of ADV intervention should be determined on the basis of HBV DNA level and progression of liver disease.

ACKNOWLEDGMENTS

THIS STUDY WAS supported in part by a Grant-in-Aid from the NHO (no. H21-NHO(Kan)-01). We express our deepest appreciation to the members of the Liver Disease Network Group of the NHO and to their institutions. For complete details, please see Appendix I.

REFERENCES

1 Lok ASF. Chronic hepatitis B. *N Engl J Med* 2002; 346: 1682–3.
2 Iloeje UH, Yang H-I, Su J, Jen C-L, You S-L, Chen C-J. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. *Gastroenterology* 2006; 130: 678–86.
3 Chen C-J, Yang H-I, Su J *et al.* Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA* 2006; 295: 65–73.
4 Conjeevaram HS, Lok AS-F. Management of chronic hepatitis B. *J Hepatol* 2003; 38 (Suppl 1): S90–103.
5 Lai CL, Chien RN, Leung NW *et al.* A one-year trial of lamivudine for chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. *N Engl J Med* 1998; 339: 61–8.
6 Lai C-L, Dienstag J, Schiff E *et al.* Prevalence and clinical correlates of YMDD variants during lamivudine therapy for patients with chronic hepatitis B. *Clin Infect Dis* 2003; 36: 687–96.
7 Lok ASF, Lai C-L, Leung N *et al.* Long-term safety of lamivudine treatment in patients with chronic hepatitis B. *Gastroenterology* 2003; 125: 1714–22.
8 Dienstag JL, Schiff ER, Wright TL *et al.* Lamivudine as initial treatment for chronic hepatitis B in the United States. *N Engl J Med* 1999; 341: 1256–63.
9 Perrillo R, Hann H-W, Mutimer D *et al.* Adefovir dipivoxil added to ongoing lamivudine in chronic hepatitis B with YMDD mutant hepatitis B virus. *Gastroenterology* 2004; 126: 81–90.
10 Peters MG, Hann Hw H, Martin P *et al.* Adefovir dipivoxil alone or in combination with lamivudine in patients with lamivudine-resistant chronic hepatitis B. *Gastroenterology* 2004; 126: 91–101.
11 Lampertico P, Viganò M, Manenti E, Iavarone M, Lunghi G, Colombo M. Adefovir rapidly suppresses hepatitis B in HBeAg-negative patients developing genotypic resistance to lamivudine. *Hepatology* 2005; 42: 1414–19.
12 Papatheodoridis GV, Dimou E, Dimakopoulos K *et al.* Outcome of hepatitis B e antigen-negative chronic hepatitis B on long-term nucleos(t)ide analog therapy starting with lamivudine. *Hepatology* 2005; 42: 121–9.
13 Rapti I, Dimou E, Mitsoula P, Hadziyannis SJ. Adding-on versus switching-to adefovir therapy in lamivudine-resistant HBeAg-negative chronic hepatitis B. *Hepatology* 2007; 45: 307–13.
14 Kurashige N, Hiramatsu N, Ohkawa K *et al.* Factors contributing to antiviral effect of adefovir dipivoxil therapy added to ongoing lamivudine treatment in patients with lamivudine-resistant chronic hepatitis B. *J Gastroenterol* 2009; 44: 601–7.
15 Shin SR, Koh KC, Gwak G-Y *et al.* A low viral load predicts a higher initial virologic response to adefovir in patients with Lamivudine-resistant chronic hepatitis B. *Gut Liver* 2010; 4: 530–6.
16 Mitsui F, Tsuge M, Kimura T *et al.* Importance of serum concentration of adefovir for Lamivudine-adefovir combination therapy in patients with lamivudine-resistant chronic hepatitis B. *Antimicrob Agents Chemother* 2010; 54: 3205–11.
17 Aizawa M, Tsubota A, Fujise K *et al.* Clinical course and predictive factors of virological response in long-term lamivudine plus adefovir dipivoxil combination therapy for lamivudine-resistant chronic hepatitis B patients. *J Med Virol* 2011; 83: 953–61.
18 Buti M, Elefsiniotis I, Jardi R *et al.* Viral genotype and baseline load predict the response to adefovir treatment in lamivudine-resistant chronic hepatitis B patients. *J Hepatol* 2007; 47: 366–72.
19 Lok AS, Chung HT, Liu VW, Ma OC. Long-term follow-up of chronic hepatitis B patients treated with interferon alfa. *Gastroenterology* 1993; 105: 1833–8.
20 van Zonneveld M, Honkoop P, Hansen BE *et al.* Long-term follow-up of alpha-interferon treatment of patients with chronic hepatitis B. *Hepatology* 2004; 39: 804–10.
21 Niederau C, Heintges T, Lange S *et al.* Long-term follow-up of HBeAg-positive patients treated with interferon alfa for chronic hepatitis B. *N Engl J Med* 1996; 334: 1422–7.

22 Lok AS, Lai CL, Wu PC, Leung EK. Long-term follow-up in a randomised controlled trial of recombinant alpha 2-interferon in Chinese patients with chronic hepatitis B infection. *Lancet* 1988; 2: 298–302.
23 Perrillo RP, Lai C-L, Liaw Y-F *et al.* Predictors of HBeAg loss after lamivudine treatment for chronic hepatitis B. *Hepatology* 2002; 36: 186–94.
24 Schalm SW, Heathcote J, Cianciara J *et al.* Lamivudine and alpha interferon combination treatment of patients with chronic hepatitis B infection: a randomised trial. *Gut* 2000; 46: 562–8.
25 Jimba S, Nakagami T, Takahashi M *et al.* Prevalence of non-alcoholic fatty liver disease and its association with impaired glucose metabolism in Japanese adults. *Diabetic Med* 2005; 22: 1141–5.

SUPPORTING INFORMATION

ADDITIONAL SUPPORTING INFORMATION may be found in the online version of this article:

Appendix S1 Relationship of liver cirrhosis with virological response on the basis of fibrosis, using 60 out of 158 patients liver biopsy had been performed. Fibrosis was related with platelet counts but neither with albumin levels nor with the virological response.

APPENDIX I

THE LIVER DISEASE Network Group of the National Hospital Organization consists of the following physicians and their institutions: Hiromi Ishibashi, Hiroshi Yatsuhashi, Department of Clinical Research Center, Nagasaki Medical Center; Makoto Nakamuta, Department of Gastroenterology, Kyushu Medical Center; Michiyasu Yagura, Department of Gastroenterology, Tokyo National Hospital; Hirotsugu Takano, Department of Gastroenterology, Kure Medical Center; Takeaki Satoh, Center for Liver Disease, Kokura Medical Center; Toyokichi Muro, Department of Gastroenterology, Oita Medical Center; Hideo Nishimura, Department of Gastroenterology, Asahikawa Medical Center; Hiroshi Mano, Department of Gastroenterology, Sendai Medical Center; Fujio Makita, Department of Gastroenterology, Nishigunma National Hospital; Akira Saitou, Department of Gastroenterology, Nishisaitama-chuo National Hospital; Masahiko Takahashi, Department of Gastroenterology, Tokyo Medical Center; Shigeo Hayashi, Department of Gastroenterology, National Disaster Medical Center; Tatsuji Komatsu, Department of Gastroenterology, Yokohama Medical Center; Yukio Watanabe, Department of Gastroenterology, Sagamihara National Hospital; Masakazu Kobayashi, Department of Gastroenterology, Matsumoto Medical Center; Hideo Morimoto, Hajime Ohta, Department of Gastroenterology, Kanazawa Medical Center; Masaaki Shimada, Department of Gastroenterology, Nagoya Medical Center. Toshiki Komeda, Department of Gastroenterology, Kyoto Medical Center; Taizo Hijioka, Department of Gastroenterology, Osaka Minami Medical Center; Haruhiro Yamashita, Department of Gastroenterology, Okayama Medical Center; Eiichi Takesaki, Department of Gastroenterology, Higashi Hirosima Medical Center; Toru Hayashi, Department of Gastroenterology, Zentsuji National Hospitail; Mitsuaki Koga, Department of Gastroenterology, Ureshino Medical Center; Kazuhiro Sugi, Departoment of Gastroenterology, Kumamoto Medical Center; Hironori Sakai, Department of Gastroenterology, Beppu Medical Center; Tetsuo Yamamoto, Department of Gastroenterology, Yonago Medical Center; Yukio Oohara, Department of Gastroenterology, Hokkaidou Medical Center; Michio Kato, Department of Hepatology, Minamiwakayama Medical Center; Naohiko Masaki, Department of Gastroenterology, Kohnodai Hospital.

J Gastroenterol (2012) 47:577–585
DOI 10.1007/s00535-011-0522-7

ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Long-term effect of lamivudine treatment on the incidence of hepatocellular carcinoma in patients with hepatitis B virus infection

Mika Kurokawa · Naoki Hiramatsu · Tsugiko Oze · Takayuki Yakushijin · Masanori Miyazaki · Atsushi Hosui · Takuya Miyagi · Yuichi Yoshida · Hisashi Ishida · Tomohide Tatsumi · Shinichi Kiso · Tatsuya Kanto · Akinori Kasahara · Sadaharu Iio · Yoshinori Doi · Akira Yamada · Masahide Oshita · Akira Kaneko · Kiyoshi Mochizuki · Hideki Hagiwara · Eiji Mita · Toshifumi Ito · Yoshiaki Inui · Kazuhiro Katayama · Harumasa Yoshihara · Yasuharu Imai · Eijirou Hayashi · Norio Hayashi · Tetsuo Takehara

Received: 30 August 2011 / Accepted: 29 November 2011 / Published online: 11 January 2012

Abstract
Background Nucleotide analogues have recently been approved for the treatment of patients with hepatitis B virus (HBV) infection. However, it is still controversial whether the decrease of HBV-DNA amount induced by treatment with nucleotide analogues can reduce the risk of hepatocellular carcinoma (HCC) development in HBV patients.

Methods A total of 293 HBV patients without HCC who were treated with lamivudine (LAM) were enrolled in a multicenter trial. The incidence of HCC was examined after the start of LAM therapy, and the risk factors for liver carcinogenesis were analyzed. The mean follow-up period was 67.6 ± 27.4 months.

Results On multivariate analysis for HCC development in all patients, age ≥50 years, platelet count $<14.0 \times 10^4/mm^3$, cirrhosis, and median HBV-DNA levels of ≥4.0 log copies/ml during LAM treatment were significant risk factors. The cumulative carcinogenesis rate at 5 years was

Electronic supplementary material The online version of this article (doi:10.1007/s00535-011-0522-7) contains supplementary material, which is available to authorized users.

M. Kurokawa · N. Hiramatsu (✉) · T. Oze · T. Yakushijin · M. Miyazaki · A. Hosui · T. Miyagi · Y. Yoshida · H. Ishida · T. Tatsumi · S. Kiso · T. Kanto · A. Kasahara · T. Takehara
Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine 2-2, Yamadaoka, Suita, Osaka 565-0871, Japan
e-mail: hiramatsu@gh.med.osaka-u.ac.jp

S. Iio
Higashiosaka City General Hospital, Higashiosaka, Japan

Y. Doi
Otemae Hospital, Osaka, Japan

A. Yamada
Sumitomo Hospital, Osaka, Japan

M. Oshita
Osaka Police Hospital, Osaka, Japan

A. Kaneko
NTT West Osaka Hospital, Osaka, Japan

K. Mochizuki · H. Hagiwara · N. Hayashi
Kansai Rosai Hospital, Amagasaki, Japan

E. Mita
National Hospital Organization Osaka National Hospital, Osaka, Japan

T. Ito
Osaka Kouseinenkin Hospital, Osaka, Japan

Y. Inui
Hyogo Prefectural Nishinomiya Hospital, Nishinomiya, Japan

K. Katayama
Osaka Medical Center for Cancer and Cardiovascular Disease, Osaka, Japan

H. Yoshihara
Osaka Rousai Hospital, Sakai, Japan

Y. Imai
Ikeda Municipal Hospital, Ikeda, Japan

E. Hayashi
Kinki Central Hospital of Mutual Aid Association of Public School Teachers, Itami, Japan

3% in patients with chronic hepatitis and 30% in those with cirrhosis. For the chronic hepatitis patients, the log-rank test showed the significant risk factors related to HCC development to be age ≥50 years, platelet count $<14.0 \times 10^4/mm^3$, and hepatitis B e antigen negativity, but median HBV-DNA levels of <4.0 log copies/ml (maintained viral response, MVR) did not significantly suppress the development of HCC. In cirrhosis patients, however, the attainment of MVR during LAM treatment was revealed to reduce the risk of HCC development.

Conclusions These results suggest that the incidence of HCC in HBV patients with cirrhosis can be reduced in those with an MVR induced by consecutive LAM treatment.

Keywords Lamivudine · Chronic hepatitis B · Cirrhosis · Hepatocellular carcinoma · HBV-DNA level

Abbreviations

HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
LAM	Lamivudine
ADV	Adefovir
ETV	Entecavir
Hbs Ag	Hepatitis B surface antigen
PCR	Polymerase chain reaction
TMA	Transcription-mediated amplification
IVR	Initial viral response
MVR	Maintained viral response
HBe Ag	Hepatitis B e antigen
CT	Computed tomography
MRI	Magnetic resonance imaging
ALT	Alanine aminotransferase

Introduction

More than 350 million people worldwide suffer from chronic infection with hepatitis B virus (HBV) [1–3]. Chronic HBV infection eventually leads to the development of cirrhosis and hepatocellular carcinoma (HCC), and raises the risk of hepatic disease-related death [4–6]. In Japan, up to 15% of HCC patients are diagnosed with HBV-related liver disease [7].

HCC is one of the most common malignancies in Japan and its incidence has been increasing over the past 30 years. Recently, various treatments such as transcatheter arterial embolization/chemoembolization, radio-frequency ablation, and hepatic resection have been reported to yield significant improvements in overall patient survival [8–11]. However, HCC relapse has thus far been observed in a majority of treated patients due to its highly malignant potential. In this regard, successful treatment of chronic HBV infection should prevent the patient's liver from progressing to cirrhosis and reduce the risk of HCC development. In recent years, the treatment of chronic hepatitis has changed greatly with the development of various antiviral therapies with nucleoside/nucleotide analogues such lamivudine (LAM), adefovir (ADV), and entecavir (ETV) [12–15]. LAM has long been used against chronic hepatitis, and many reports have demonstrated that LAM is effective in stabilizing inflammatory activity, suppressing HBV-DNA replication, and improving liver histological findings in chronic hepatitis patients [16, 17] and in HBV-related cirrhosis patients [18]. Furthermore, LAM has been reported to reduce the incidence of HCC in patients with chronic hepatitis B [19]. However, it is still controversial whether or not treatment using nucleotide analogues can reduce the risk of HCC development in HBV-infected patients [20, 21], and the relationship between the effect of HBV suppression and HCC development during LAM treatment has not yet been discussed in detail. Also, the risk factors for HCC development in HBV-infected patients who have been treated with LAM have not been sufficiently evaluated. In this study, we aimed to clarify whether the decrease of HBV-DNA amount induced by LAM therapy could reduce the incidence of HCC in HBV-infected patients.

Patients and methods

Patient selection and study design

This study was conducted at Osaka University Hospital and other institutions participating in the Osaka Liver Forum in Japan. The subjects were 293 consecutive patients with HBV infection who underwent continuous LAM therapy for more than 24 weeks from September 2000 to September 2006. All patients tested positive for hepatitis B surface antigen (HBs Ag) or had detectable levels of HBV DNA in their sera according to findings from a polymerase chain reaction (PCR)-based method or a transcription-mediated amplification (TMA) method. Exclusion criteria were patients with anti-hepatitis C antibody, anti-human immunodeficiency virus antibody, and other liver diseases (alcoholic liver disease, drug-induced liver disease, and autoimmune hepatitis). Also excluded were patients with a history of HCC and those who developed HCC within the first 24 weeks of the follow-up period after the initiation of LAM therapy (because of the possibility that microscopic HCC had been present before the initiation of treatment).

All patients were treated with 100 mg of LAM daily. Of the 293 patients, 129 underwent ADV (10 mg/day) therapy in addition to receiving ongoing LAM treatment. For 43 patients who started ETV administration in lieu of LAM, the observation period was terminated when they started

ETV. LAM resistance was confirmed by virological breakthrough and was defined as an increase in serum HBV-DNA by >1 $\log_{10}$ greater than the nadir [22]. If virological breakthrough developed and alanine aminotransferase (ALT) was elevated over the upper normal limit, the patients received add-on ADV at 10 mg/day.

In this study, all patients were examined for serum HBV-DNA level just before therapy initiation and every 6 months during treatment. The initial viral response (IVR) was defined as HBV-DNA <4.0 log copies/ml in the first 24 weeks of the follow-up period after the initiation of LAM therapy, and the maintained viral response (MVR) was defined as median HBV-DNA levels of less than 4.0 log copies/ml measured every 6 months during therapy.

This study protocol followed the ethical guidelines of the Declaration of Helsinki amended in 2008, and informed consent was obtained from each patient.

HBV testing

HBs Ag, hepatitis B e antigen (HBe Ag) and anti-hepatitis B e antibody (anti-HBe) levels were examined by chemiluminescence immunoassay or enzyme immunoassay. HBV DNA was measured by a PCR-based method (Amplicor HBV monitor; Roche Diagnostics, Tokyo, Japan) or a TMA method (TMA-HPA; Fujirebio, Tokyo, Japan), which have lower detection limits of 2.6 and 3.7 log copies/ml, respectively. The LAM-resistant YMDD mutant virus was examined by a PCR-ELMA method. Serum samples were stored frozen at −80°C.

Diagnosis of HCC and cirrhosis

Ultrasonography was carried out before LAM therapy and every 3–6 months during the follow-up period. New space-occupying lesions detected or suspected at the time of ultrasonography were further examined by computed tomography (CT), magnetic resonance imaging (MRI), or hepatic angiography. HCC was diagnosed by the presence of typical hypervascular characteristics on angiography, in addition to the findings from CT or MRI. If no typical image of HCC was observed, fine-needle aspiration biopsy was carried out with the patient's consent or the patient was carefully followed until a diagnosis was possible with definite observation by CT, MRI, or hepatic angiography. Cirrhosis was diagnosed by liver biopsy or laparoscopy, and for patients without this information, by clinical data, imaging modalities, and portal hypertension.

Statistical analysis

Quantitative variables were expressed as means ± SD. Quantitative variables at the baseline were compared among two groups, the chronic hepatitis and cirrhosis groups, using the Mann–Whitney *U*-test. Categorical data, such as gender and status of HBe Ag, were compared using Fisher's exact test. The cumulative incidence of HCC was evaluated with a Kaplan–Meiercurve and the differences between groups were analyzed by the log-rank test. For multivariate analysis to investigate factors affecting the cumulative incidence of HCC, Cox's regression analysis was carried out. A value of $p < 0.05$ (two-tailed) was considered to be statistically significant. All calculations were performed with SPSS version 15.0J (SPSS, Chicago, IL, USA).

Results

Baseline characteristics of patients

The baseline clinical features of the enrolled patients before LAM administration are shown in Table 1. The mean age of the patients was 48.0 ± 10.7 years, 214 (73%) of the entire group were male, and 163 (56%) tested positive for HBe Ag. Of the 293 patients, 205 (70%) were diagnosed as having chronic hepatitis and 88 (30%) as having cirrhosis. The median HBV-DNA level was 7.0 (range 3.0 to 8.5<) log copies/ml. At baseline, the aspartate aminotransferase (AST) level was 131 ± 151 IU/l, the ALT level was 203 ± 252 IU/l, the total bilirubin level was 1.2 ± 1.6 mg/dl, the albumin (Alb) level was 3.8 ± 0.5 g/dl, and the platelet count was 13.7 ± 5.4 × 10^4/mm^3. The mean follow-up period for all patients was 67.6 ± 27.4 months, with a range of 12–110 months from the start of LAM treatment. There were significant differences between patients with chronic hepatitis and those with liver cirrhosis in age, AST, ALT, total bilirubin, Alb, and platelet counts.

Cumulative incidence of development of HCC

Figure 1a shows the Kaplan–Meier curve of the cumulative HCC incidence for all HBV patients treated with LAM or LAM plus ADV. Of the 293 patients with HBV infection, 32 (10.9%) developed HCC and the cumulative carcinogenesis rate was 6% at 3 years, 12% at 5 years, and 15% at 7 years.

Figure 1b shows the Kaplan–Meier curve of the cumulative HCC incidence according to initial diagnosis (chronic hepatitis vs. cirrhosis). Eight (4%) of the 205 enrolled chronic hepatitis patients developed HCC and the cumulative carcinogenesis rate was 2% at 3 years, 3% at 5 years, and 5% at 7 years. On the other hand, 24 (27%) of the 88 enrolled cirrhosis patients developed HCC and the cumulative carcinogenesis rate was 15% at 3 years, 30% at 5 years, and 35% at 7 years.

Table 1 Patient characteristics

Factor	All	Chronic hepatitis	Cirrhosis	*p* value
Number of patients	293	205	88	
Age (years)	48.0 ± 10.7	46.3 ± 10.7	51.9 ± 9.8	<0.001**
Sex (male/female)	214/79	147/58	67/21	0.475
HBe Ag (positive	163 (56%)	121 (59%)	42 (48%)	0.068
HBV DNA (log copies/ml)[a]	7.0 (3.0 to 8.5<)	6.8±1.1	6.6 ± 1.1	0.162
AST (IU/l)	131 ± 151	143 ± 162	104 ± 120	0.045*
ALT (IU/l)	203 ± 252	235 ± 269	129 ± 189	<0.001**
Total bilirubin (mg/dl)	1.2 ± 1.6	0.9 ± 0.6	1.8 ± 2.7	<0.001**
Alb (g/dl)	3.8 ± 0.5	3.9 ± 0.4	3.5 ± 0.6	<0.001**
Platelets ($\times 10^4$/mm^3)	13.7 ± 5.4	15.6 ± 9.3	9.3 ± 3.8	<0.001**
Follow-up period (months)	67.6 ± 27.4	68.5 ± 26.5	65.5 ± 29.5	0.393

HBe Ag Hepatitis B e antigen, *HBV* hepatitis B virus, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *Alb* albumin

[a] Values are expressed as medians

* $p < 0.05$, ** $p < 0.001$, comparing patients with chronic hepatitis and those with liver cirrhosis using the Mann–Whitney *U*-test for quantitative variables and Fisher's exact test for categorical variables

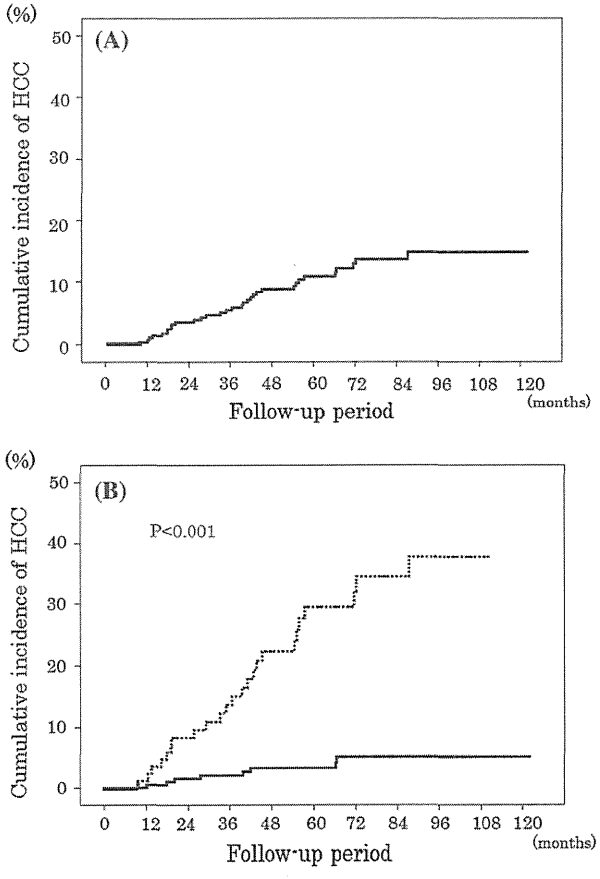


Fig. 1 Cumulative incidence of development of hepatocellular carcinoma (*HCC*) in patients with hepatitis B virus infection treated with lamivudine (LAM). **a** All cases; **b** chronic hepatitis or cirrhosis. *Solid line* Chronic hepatitis, *dotted line* cirrhosis

Risk factors for cumulative incidence of HCC development in all HBV-infected patients

Univariate analysis with the log-rank test was performed for all HBV-infected patients treated with LAM, with the results shown in Table 2. Univariate analysis with the log-rank test showed that the following were significant risk factors for the development of HCC: older age (≥50 years) ($p < 0.001$), cirrhosis ($p < 0.001$), high total bilirubin level (>1.2 g/dl) ($p = 0.004$), low Alb level (<3.8 g/dl) ($p = 0.019$), low platelet count (<14 × 10^4/mm^3) ($p < 0.001$), and non-MVR ($p = 0.035$).

Stepwise multivariate analyses of four of these variables were performed by Cox's regression analysis for all patients treated with LAM with the results shown in Table 3. The analysis indicated the following factors as independent significant risk factors related to the development of HCC: age ≥50 years [hazard ratio (HR) 3.20, 95% confidence interval [CI] 1.08–9.53, $p = 0.036$], platelet count <14.0 × 10^4/mm^3 (HR 4.76, 95% CI 0.05–0.96, $p = 0.045$), cirrhosis (HR 4.64, 95% CI 1.75–12.4, $p = 0.002$), and non-MVR (HR 2.70, 95% CI 1.09–6.56, $p = 0.032$).

Cumulative incidence of and risk factors for HCC development in patients with chronic hepatitis and cirrhosis

The results of univariate analysis with the log-rank test for the development of HCC in chronic hepatitis patients treated with LAM are shown in Table 4, and the following were significant risk factors: older age (≥50 years) ($p = 0.002$), HBe Ag negativity ($p = 0.005$), and low platelet count (<14 × 10^4/mm^3) ($p = 0.004$). Suppression of median HBV-DNA levels to <4.0 log copies/ml by LAM treatment was not associated with the development of HCC in the chronic hepatitis patients. Only non-MVR (median HBV-DNA amount ≥4.0 log copies/ml) was shown to be a significant risk factor for the development of HCC in the cirrhosis patients ($p = 0.029$), while the factors of age, HBe Ag status, and platelet count were not significant in these patients (Table 4).

Table 2 Risk factors for HCC development in all HBV-infected patients by univariate analysis

Factor	95% CI	*p* value
Age (years) (<50/≥50)	2.15–14.5	<0.001
Sex (male/female)	0.33–1.76	0.520
Initial diagnosis (chronic hepatitis/cirrhosis)	3.75–1.176	<0.001
HBe Ag (positive/negative)	0.31–1.29	0.209
HBV DNA (log copies/ml) (<7.0/>7.0)	0.33–1.35	0.262
AST (IU/l) (<40/≥40)	0.33–2.22	0.742
ALT (IU/l) (<40/≥40)	0.17–1.16	0.188
Total bilirubin (mg/dl) (<1.2/>1.2)	1.43–6.72	0.004
Alb (g/dl) (<3.8/≥3.8)	0.19–0.86	0.019
Platelets ($\times 10^4$/mm^3) (<14/≥14)	0.02–0.31	<0.001
Emergence of LAM-resistant viruses (positive/negative)	0.51–2.03	0.968
IVR (positive/negative)	0.52–3.25	0.575
MVR (positive/negative)	1.04–5.95	0.035

HCC Hepatocellular carcinoma, *HBV* hepatitis B virus, *CI* confidence interval, *HBe Ag* hepatitis B e antigen, *HBV* hepatitis B virus, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *Alb* albumin, *IVR* initial viral response, *MVR* maintained viral response, *LAM* lamivudine

Table 3 Risk factors for HCC development in all HBV-infected patients by multivariate analysis

Factor	Category	Risk ratio	95% CI	*p* value
Age (years)	<50	1	1.08–9.53	0.036
	≥50	3.20		
Initial diagnosis	Chronic hepatitis	1	1.75–12.4	0.002
	Cirrhosis	4.64		
Platelets ($\times 10^4$/mm^8) (<14/≥14)	≥14	1	0.05–0.96	0.045
	<14	4.76		
MVR	Negative	1	1.09–6.56	0.032
	Positive	0.37		

HCC Hepatocellular carcinoma, *HBV* hepatitis B virus, *CI* confidence interval, *MVR* maintained viral response

Cumulative incidence of HCC development according to effectiveness of treatment (MVR vs. non-MVR)

Figure 2a shows the Kaplan–Meier curve of cumulative HCC incidence in all HBV-infected patients treated with LAM according to the effectiveness of treatment (MVR vs. non-MVR). The cumulative carcinogenesis rate for MVR-positive patients was 2% at 3 years, 4% at 5 years, and 6% at 7 years. On the other hand, the cumulative carcinogenesis rate for MVR-negative patients was 5% at 3 years, 13% at 5 years, and 16% at 7 years. MVR during LAM significantly suppressed the cumulative HCC incidence compared with non-MVR in all HBV-infected patients ($p = 0.035$).

Table 4 Risk factors for HCC development by univariate analysis (chronic hepatitis/cirrhosis)

	95% CI	*p* value
Chronic hepatitis		
Age (years) (<50/≥50)	0.26–8.38	0.002
Sex (male/female)	0.37–6.42	0.556
HBe Ag (positive/negative)	0.01–0.74	0.005
HBV DNA (log copies/ml) (<7.0/≥7.0)	0.11–1.99	0.296
AST (IU/l) (<40/≥40)	0.11–2.64	0.482
ALT (IU/l) (<40/≥40)	0.06–1.41	0.101
Total bilirubin (mg/dl) (<1.2/≥1.2)	0.67–6.67	0.574
Alb (g/dl) (<3.8/≥3.8)	0.13–8.58	0.960
Platelets ($\times 10^4$/mm^3) (<14/≥14)	0.01–0.72	0.004
Emergence of LAM-resistant viruses (positive/negative)	0.27–4.28	0.927
IVR (positive/negative)	0.29–8.67	0.590
MVR (positive/negative)	0.51–37.10	0.144
Cirrhosis		
Age (years) (<50/≥50)	0.86–6.17	0.089
Sex (male/female)	0.21–1.82	0.380
HBe Ag (positive/negative)	0.80–4.17	0.149
HBV DNA (log copies/ml) (<7.0/≥7.0)	0.40–2.01	0.795
AST (IU/l) (<40/≥40)	0.27–3.07	0.873
ALT (IU/l) (<40/≥40)	0.13–1.47	0.167
Total bilirubin (mg/dl) (<1.2/≥1.2)	0.82–4.80	0.126
Alb (g/dl) (<3.8/≥3.8)	0.28–1.58	0.354
Platelets ($\times 10^4$/mm^3) (<14/≥14)	0.03–1.51	0.084
Emergence of LAM-resistant viruses (positive/negative)	0.44–2.18	0.948
IVR (positive/negative)	0.90–8.32	0.063
MVR (positive/negative)	1.07–0.029	

HCC Hepatocellular carcinoma, *HBV* hepatitis B virus, *CI* confidence interval, *HBe Ag* hepatitis B e antigen, *HBV* hepatitis B virus, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *Alb* albumin, *IVR* initial viral response, *MVR* maintained viral response

Figure 2b shows the Kaplan–Meier curve of the cumulative HCC incidence in chronic hepatitis patients according to the effectiveness of treatment (MVR vs. non-MVR). The cumulative carcinogenesis rate for MVR-positive patients was 0% at 3 years, 0% at 5 years, and 2% at 7 years. On the other hand, the cumulative carcinogenesis rate for MVR-negative patients was 2% at 3 years, 4% at 5 years, and 6% at 7 years. MVR during LAM did not significantly suppress the cumulative HCC incidence compared with non-MVR in the chronic hepatitis patients ($p = 0.144$).

Figure 2c shows the Kaplan–Meier curve of the cumulative HCC incidence in cirrhosis patients according to the effectiveness of treatment (MVR vs. non-MVR).

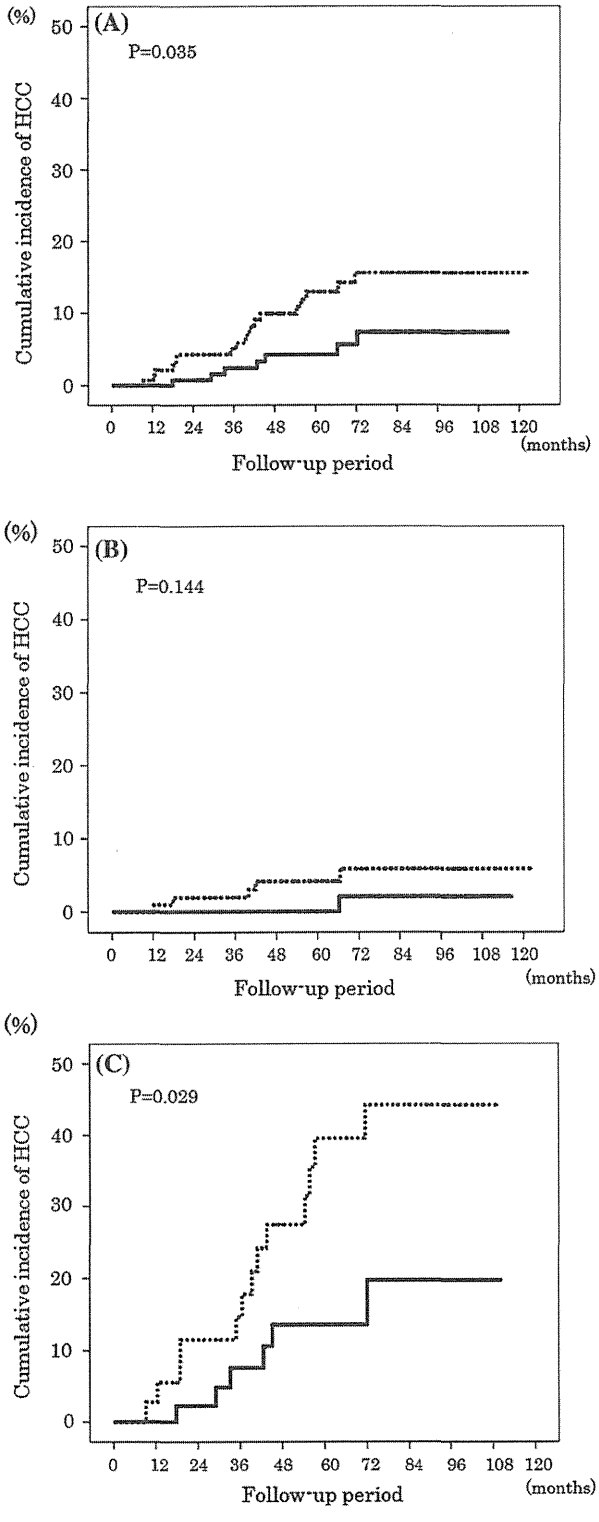


Fig. 2 Cumulative incidence of development of HCC according to the effectiveness of treatment (MVR vs. non-MVR). **a** All cases; **b** chronic hepatitis; **c** cirrhosis. *Solid lines* MVR, *dotted lines* non-MVR. *MVR* Maintained viral response

The cumulative carcinogenesis rate for MVR-positive patients was 8% at 3 years, 14% at 5 years, and 14% at 7 years. On the other hand, the cumulative carcinogenesis rate for MVR-negative patients was 18% at 3 years, 40% at 5 years, and 44% at 7 years. MVR during LAM significantly suppressed the cumulative HCC incidence compared with non-MVR in the cirrhosis patients ($p = 0.029$).

Relationship between IVR and MVR

Maintained viral response (MVR) was achieved by 142 (48%) of the 293 patients enrolled in this study. IVR was achieved by 204 (79%) of the 259 patients who were examined for IVR. The relationship between IVR and MVR is shown in Fig. 3; 60% (122/204) of the IVR-positive patients achieved an MVR, while only 16% (9/55) of the IVR-negative patients achieved an MVR ($p < 0.001$). The LAM-resistant YMDD mutant virus was found in 149 (51%) of all patients during follow-up, and in 52% (107/204) of the IVR-positive patients, a finding which was nearly equal to that for the IVR-negative patients (51%, 28/55). Among the IVR-positive patients, the MVR rate was lower in patients with the YMDD mutation, compared with that in those without the YMDD mutation (44%, 47/107 vs. 77%, 75/97, $p = 0.016$), while the MVR rates were low in the IVR-negative patients, irrespective of their YMDD mutation status (with and without the mutation, 15 vs. 18%, respectively). ADV was added to LAM treatment for 73 (68%) of the 107 IVR-positive patients with the YMDD mutation and 20 (36%) of the 55 IVR-negative patients with the YMDD mutation. However, MVR was only achieved at the low rates of 33% (24/73) for the former patients and 20% (4/20) for the latter.

Discussion

Lamivudine treatment has been shown to improve the liver histological findings in patients with HBV-infected liver disease by reducing the HBV load and stabilizing inflammatory activity [16–18]. One report has shown that LAM effectively reduced the incidence of HCC in patients with chronic hepatitis B, but the study only compared LAM-treated patients with non-treated patients in a matched case-controlled study [19]. However, there have been few detailed reports about the relationship between virological response and HCC development in HBV-infected patients during LAM treatment. In the present study, we retrospectively examined the incidence of HCC to clarify the indicators of LAM therapy, including median HBV-DNA levels, for reducing the risk of HCC in HBV-infected patients.

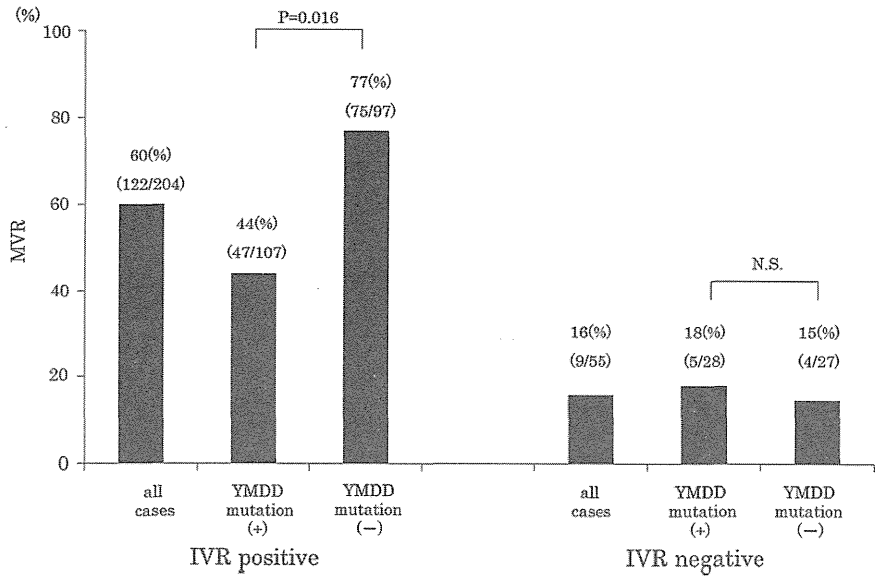


Fig. 3 Relationship between IVR and MVR. *IVR* Initial viral response, *MVR* maintained viral response, *N.S.* not significant

Many investigators have reported that serum HBV DNA levels higher than 4.0–4.5 log copies/ml before HBV treatment serve as a strong risk predictor of HCC [23–25]. Di Marco et al. [26] have reported that the incidence of HCC was higher in patients with serum HBV levels of more than 5.0 log copies/ml, at least once, during LAM therapy than in those in whom serum HBV levels were maintained at 5.0 log copies/ml or less. However, the add-on ADV therapy had not been adopted when the study of Di Marco et al. was reported. When the use of ADV is possible, an evaluation method is needed to measure the antiviral effects of nucleoside/nucleotide analogues against HBV-related liver disease. In the present study, we set the cut-off value for HBV-DNA at 4.0 log copies/ml. The basis of this cut-off value is that a serum HBV DNA level higher than 4.0 log copies/ml before HBV treatment was reported to serve as a strong risk predictor of HCC [23]. MVR, defined as a median HBV-DNA level of less than 4.0 log copies/ml measured every 6 months during therapy, was adopted as an indicator of viral replication, and non-MVR (median HBV-DNA >4.0 log copies/ml) during LAM therapy was shown to be significantly associated with the development of HCC in HBV-infected patients. We also found that a median HBV-DNA level of >4.0 log copies/ml during LAM therapy was a risk factor for HCC development. On the other hand, IVR, defined as HBV-DNA of <4.0 log copies/ml in the first 6 months of the follow-up period after the initiation of therapy, was not associated with the development of HCC in HBV patients in this study. As shown in Fig. 3, 84% of the IVR-negative patients could not achieve an MVR, suggesting that it is crucial to achieve an IVR in order to achieve an MVR. The reason why IVR was not a significant factor for MVR seemed to be the appearance of the YMDD mutation, which reduced the antiviral effect of LAM for HBV in IVR-positive patients. The LAM-resistant YMDD mutant virus was found in 52% of the IVR-positive patients. Although ADV was added to LAM treatment for 73 patients, only 33% of these patients could achieve an MVR. We speculate that the antiviral effect of ADV is not very strong [27] and it takes time to reduce the YMDD mutant virus, which may explain the low MVR rate (33%) in patients with the add-on ADV therapy. The immediate administration of ADV when the LAM-resistant YMDD mutant virus appears can be important [28]. A switch to ETV, which induces resistant virus less frequently, could also raise MVR rates among IVR-positive patients without the YMDD mutant virus.

As the duration of the add-on ADV therapy was included in this study, we compared the cumulative incidence of HCC in patients receiving LAM monotherapy with that in patients who also received the add-on ADV therapy. Sixteen (10%) of the 164 patients who received the LAM monotherapy developed HCC and the cumulative carcinogenesis rate was 6% at 3 years, 10% at 5 years, and 15% at 7 years. On the other hand, 16 (12%) of the 129 patients who received LAM plus ADV developed HCC and the cumulative carcinogenesis rate was 6% at 3 years, 12% at 5 years, and 14% at 7 years. No significant difference was found between these two groups ($p = 0.986$). In addition, we examined the cumulative incidence of HCC development according to the effectiveness of treatment (MVR vs. non-MVR) in patients for whom the observation period was terminated when ADV was added, and the same results were obtained (data not shown).

Older age (≥50 years), cirrhosis, and low platelet count ($<14 \times 10^4/mm^3$) were shown to be significantly associated with the development of HCC in patients with HBV infection. These results were consistent with those of

previous reports [29–31], suggesting that patients of older age with advanced fibrosis should be followed up carefully for longer periods in order to detect early stages of HCC even if LAM therapy does effectively suppress HBV. Of note, in the present study we estimated the cumulative HCC incidence according to the initial diagnosis of chronic hepatitis or cirrhosis. In the chronic hepatitis patients, older age (≥50 years), HBe Ag negativity, and low platelet count ($<14 \times 10^4/mm^3$) were significant risk factors for the development of HCC, but this was not the case in the cirrhosis patients. Because liver biopsies had not been performed, the liver fibrosis stage could not be evaluated with respect to the risk factors for HCC in this study. Instead, the factors of age, HBe Ag status, and platelet count may reflect the degree of liver fibrosis in chronic hepatitis patients. In fact, cirrhotic patients, in comparison with chronic hepatitis patients, were of older age (chronic hepatitis vs. cirrhosis: 46.3 ± 10.7 vs. 51.9 ± 9.8 years, $p < 0.001$), had higher rates of HBe Ag negativity (chronic hepatitis vs. cirrhosis: 39 vs. 51%, $p = 0.065$), and had lower platelet counts (chronic hepatitis vs. cirrhosis: 15.6 ± 4.9 vs. $9.3 \pm 3.8 \times 10^4/mm^3$, $p < 0.001$). This seems to explain why none of these factors were significant risk factors for HCC in cirrhotic patients. On the other hand, in the chronic hepatitis patients, MVR was not a significant factor for HCC development, while MVR was a significant factor for HCC development in the cirrhotic patients. We speculate that HBV suppression induced by LAM therapy could reduce the incidence of HCC in patients infected with HBV, especially those with cirrhosis, who displayed higher malignant potential. Investigation over a longer period is needed to clarify the effect of HBV suppression on the development of HCC in chronic hepatitis patients.

In conclusion, the present study shows that the attainment of an MVR induced by LAM therapy has a significant beneficial effect on the clinical course of HBV-infected patients by decreasing the incidence of HCC. The newer nucleotide analogues, such as ETV and tenofovir, should be able to further reduce the incidence of HCC, given their greater potency.

Acknowledgments This work was supported by Grants-in-Aid for Research on Hepatitis and BSE from the Ministry of Health Labour and Welfare of Japan and for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology. 2007;45:507–39.
2. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral Hepat. 2004;11:97–107.
3. Lee WM. Hepatitis B virus infection. N Engl J Med. 1997;337:1733–45.
4. Beasley RP. Hepatitis B virus: the major etiology of hepatocellular carcinoma. Cancer. 1988;61:1942–56.
5. Lok SF. Chronic hepatitis B. N Engl J Med. 2002;346:1682–3.
6. Tang B, Kruger WD, Chen G, Shen F, Lin WY, Mboup S, et al. Hepatitis B viremia is associated with increased risk of hepatocellular carcinoma in chronic careers. J Med Virol. 2004;72:35–40.
7. Kim SR, Kudo M, Hino O, Han KH, Chung YH, Lee HS. Epidemiology of hepatocellular carcinoma in Japan and Korea. A review. Oncology. 2008;75:13–6.
8. Marcellin P, Chang TT, Lim SG, Tong MJ, Sievert W, Shiffman ML, et al. Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. N Engl J Med. 2003;348:808–16.
9. Taura K, Ikai I, Hatano E, Fujii H, Uyama N, Shimahara Y. Implication of frequent local ablation therapy for intrahepatic recurrence in prolonged survival of patients with hepatocellular carcinoma undergoing hepatic resection: an analysis of 610 patients over 16 years old. Ann Surg. 2006;244:265–73.
10. Shimada K, Sano T, Sakamoto Y, Kosuge T. A long-term follow-up and management study of hepatocellular carcinoma patients surviving for 10 years or longer after curative hepatectomy. Cancer. 2005;104:1939–47.
11. Takayasu K, Arii S, Ikai I, Omata M, Okita K, Ichida T, et al. Prospective cohort study of transarterial chemoembolization for unresectable hepatocellular carcinoma in 8510 patients. Gastroenterology. 2006;131:461–9.
12. Dienstag JL, Perrillo RP, Schiff ER, Bartholomew M, Vicary C, Rubin M. A preliminary trial of lamivudine for chronic hepatitis B infection. N Engl J Med. 1995;333:1657–61.
13. Nevens F, Main J, Honkoop P, Tyrrell DL, Barber J, Sullivan MT, et al. Lamivudine therapy for chronic hepatitis B: a six-month randomized dose-ranging study. Gastroenterology. 1997;113:1258–63.
14. Rapti I, Dimou E, Mitsouka P, Hadziyannis SJ. Adding-on versus switching-to adefovir therapy in lamivudine-resistant HBVAg-negative chronic hepatitis B. Hepatology. 2007;45:307–13.
15. Chang TT, Lai CL, Kew Yoon S, Lee SS, Coelho HS, Carriho FJ, et al. Entecavir treatment for 5 years in patients with hepatitis Be antigen-positive chronic hepatitis B. Hepatology. 2010;51:422–30.
16. Kiyosawa K, Tanaka E, Sodeyama T. Hepatitis C virus and hepatocellular carcinoma. Curr Stud Hematol Blood Transfus. 1998;62:161–80.
17. Ohkoshi S, Norio O, Ichida T. The long-term clinical outcome of 1-year treatment of chronic hepatitis B with lamivudine-5 years observation. Hepatol Res. 2003;27:13–7.
18. Chu CM, Liaw YF. Hepatitis B virus-related cirrhosis: natural history and treatment. Semin Liver Dis. 2006;26:142–52.
19. Matsumoto A, Tanaka E, Rokuhara A, Kiyosawa K, Kumada H, Omata M, et al. Efficacy of lamivudine for preventing hepatocellular carcinoma in chronic hepatitis B: a multicenter retrospective study of 2795 patients. Hepatol Res. 2005;32:173–84.
20. Shamliyan TA, MacDonald R, Shaukat A, Taylor BC, Yuan JM, Johnson JR, et al. Antiviral therapy for adults with chronic hepatitis B: a systematic review for a National Institutes of Health Consensus Development Conference. Ann Intern Med. 2009;150(2):111–24.
21. Liaw YF, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H, et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. N Engl J Med. 2004;351(15):1521–31.

22. Perrillo R, Hann HW, Mutimer D, Willems B, Leung N, Lee WM, et al. Adefovir dipivoxil added to ongoing lamivudine in chronic hepatitis B with YMDD mutant hepatitis B virus. Gastroenterology. 2004;126:81–90.
23. Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA. 2006;295:65–73.
24. Chan HL, Hui AY, Wong ML, Tse AM, Hung LC, Wong VW, et al. Genotype C hepatitis B virus infection is associated with increased risk of hepatocellular carcinoma. Gut. 2004;53:1494–8.
25. Chan HL, Tse CH, Koh FM, Wong VW, Wong GL, Chan SL, et al. High viral load and hepatitis B virus subgenotype Ce are associated with increased risk of hepatocellular carcinoma. J Clin Oncol. 2008;26:177–82.
26. Di Marco V, Marzano A, Lampertico P, Andreone P, Santantonio T, Almasio PL, et al. Clinical outcome of HBeAg-negative chronic hepatitis B in relation to virological response to lamivudine. Hepatology. 2004;40:883–91.
27. Marcellin P, Chang TT, Lim SG, Tong MJ, Sievert W, Shiffman ML, et al. Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. N Engl J Med. 2003;348:808–16.
28. Kurashige N, Hiramatsu N, Ohkawa K, Yakushijin T, Kiso S, Kanto T, et al. Factors contributing to antiviral effect of adefovir dipivoxil therapy added to ongoing lamivudine treatment in patients with lamivudine-resistant chronic hepatitis B. J Gastroenterol. 2009;44(6):601–7.
29. Chen CJ, Yu MW, Liaw YF. Epidemiological characteristics and risk factors of hepatocellular carcinoma. J Gastroenterol Hepatol. 1997;12:5294–308.
30. Yun LF. Natural history of chronic hepatitis B virus infection and long-term outcome under treatment. Liver Int. 2009;29:100–7.
31. Sung JJ, Tsoi KK, Wong VW, Li KC, Chan LL. Meta-analysis: treatment of hepatitis B infection reduces risk of hepatocellular carcinoma. Aliment Pharmacol Ther. 2008;28:1067–77.

肝臓　53巻7号　434-435（2012）

＜速　報＞

Telaprevir/Peginterferon-alfa2b/Ribavirin併用療法導入直後の腎機能低下機序に関する検討

坂根　貞嗣　榊原　祐子　由雄　敏之　中水流正一
外山　隆　石田　永　三田　英治*

緒言：Telaprevir（TVR）併用療法で報告された有害事象の一つに，血中クレアチニン(Cr)上昇がある．我々は治療開始直後に見られる腎機能低下について検討した．

対象と方法：対象は当院でTVR併用療法を導入した17例．開始時，4日目，2週目の血清Cr値を測定し，推定糸球体濾過量（eGFR）を比較した．また，4日目の早朝第一尿の尿中Na濃度(UNa)を測定し，ナトリウム排泄率(FENa%)も算出した．開始時から比べた4日目のeGFRの低下量をΔeGFRと定義し，ΔeGFRとUNa，ΔeGFRとFENa%のそれぞれの相関をPearson相関係数で検討した．本研究は十分に説明と同意を得て行った．

結果：開始前のeGFRは平均77.5 ml/min/1.73 m²であったが，4日目に平均14.6 ml/min/1.73 m²低下した．ΔeGFRとUNaは有意に負の相関を示し(r＝－0.726，p＝0.0010)，ΔeGFRとFENa%についても有意に負の相関を示した(r＝－0.541，p＝0.0249)(Fig.)．開始時のTVR投与量が2250 mg/日であったのは14例で，そのうち経過中にeGFRの20%以上の低下を認めた7例については1500 mg/日への減量を行った．TVRを1500 mg/日で開始した3例については減量を行わなかった．また，全例に2000 ml/日以上の飲水を奨励し，飲水量が不充分と判断した5例については輸液を行った．その結果，eGFRは2週目に平均68.9 ml/min/1.73 m²まで改善した．

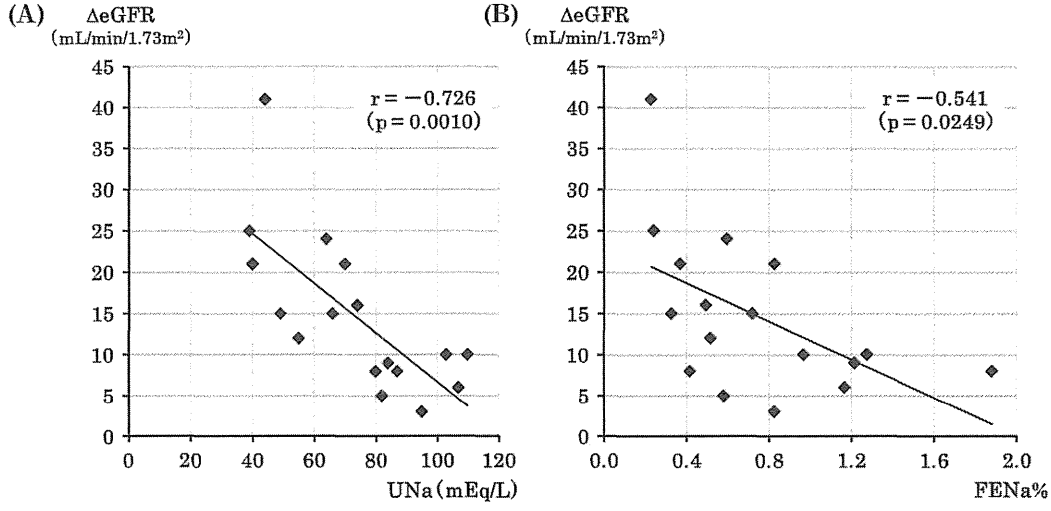


Fig. Relation of the eGFR decrease to (A) UNa and (B) FENa%

$$\text{FENa\%} = \frac{\text{UNa}}{\text{serum Na}} \div \frac{\text{urine Cr}}{\text{serum Cr}} \times 100\%$$

独立行政法人国立病院機構大阪医療センター消化器科
*Corresponding author: eijim67@onh.go.jp

$利益相反申告　寄付金（MSD（株））
＜受付日2012年5月17日＞＜採択日2012年6月19日＞

考察：腎機能障害は血中リバビリン濃度に影響し，貧血増悪の危険因子になる重要な有害事象である[1)-3)]．今回の検討で，4日目にUNaもしくはFENa%が低い症例ほど，有意にeGFRの低下が大きかった．UNaやFENa%の低下は腎前性腎不全に特徴的で，4日目に認められるこの腎機能低下は腎性ではなく腎前性が機序として考えられた．

結語：TVR併用療法開始後早期の腎機能低下は腎前性の機序であり，腎機能低下症例にはTVR減量や輸液など適切な対処が望ましい．

謝辞：腎障害機序に関し御助言をいただきました大阪医療センター腎臓内科科長，伊藤孝仁先生にこの場を借りて深謝いたします．

索引用語：テラプレビル，有害事象，腎機能低下

文献：1）Sulkowski MS, Wasserman R, Brooks L, et al. J Viral Hepat 2004; 11: 243—250　2）Maeda Y, Kiribayashi Y, Moriya T, et al. Ther Drug Monit 2004; 26: 9—15　3）Toyoda H, Kumada T, Kiriyama S, et al. J Viral Hepat 2008; 15: 651—658

英文要旨

Mechanism of renal dysfunction in the early phase of telaprevir therapy in combination with peginterferon-alfa2b and ribavirin

Sadatsugu Sakane, Yuko Sakakibara, Toshiyuki Yoshio, Shoichi Nakazuru, Takashi Toyama, Hisashi Ishida, Eiji Mita*

Seventeen patients with chronic hepatitis C undergoing telaprevir therapy showed a decrease in the estimated glomerular filtration rate (eGFR), which averaged 77.5 ml/min/1.73 m^2 before treatment, by 14.6 ml/min/1.73 m^2 on average at day 4. To distinguish between the two main causes of acute renal dysfunction, prerenal mechanism and acute tubular necrosis, we measured the sodium concentration in urine (UNa) and fractional excretion of sodium (FENa). The eGFR decrease showed a significant correlation with UNa and also with FENa. These findings indicate that the prerenal mechanism is responsible for the eGFR decline. As patients with an eGFR drop in the early phase of therapy may have an additional risk of adverse events, renal function should be closely monitored.

Key words: telaprevir, adverse event, renal dysfunction

Kanzo 2012; 53: 434—435

Department of Gastroenterology and Hepatology, National Hospital Organization, Osaka National Hospital
*Corresponding author: eijim67@onh.go.jp

Hepatology Research

Hepatology Research 2012 doi: 10.1111/j.1872-034X.2012.01097.x

Original Article

Effects of branched-chain amino acid granules on serum albumin level and prognosis are dependent on treatment adherence in patients with liver cirrhosis

Koichi Takaguchi,[1] Hisataka Moriwaki,[2] Hisashi Doyama,[3] Masayuki Iida,[4] Michiyasu Yagura,[5] Noritomo Shimada,[8] Masahiro Kang,[9] Haruki Yamada[6] and Hiromitsu Kumada[7]

[1]*Department of Hepatology, Kagawa Prefectural Central Hospital, Kagawa,* [2]*Department of Medicine, Gifu University Graduate School of Medicine, Gifu,* [3]*Department of Gastroenterology, Ishikawa Prefectural Central Hospital, Kanazawa,* [4]*Department of Internal Medicine, Nagoya Midori Municipal Hospital, Nagoya,* [5]*Department of Gastroenterology, National Hospital Organization Tokyo National Hospital,* [6]*Department of Internal Medicine, Social Insurance Chuo General Hospital,* [7]*Department of Hepatology, Toranomon Hospital, Tokyo,* [8]*Department of Internal Medicine, Medical Plaza Heiwadai Hospital, Chiba, and* [9]*Department of Internal Medicine, Sato Daiichi Hospital, Oita, Japan*

Aim: To test if the treatment adherence to branched-chain amino acid (BCAA) granules influences the serum albumin level and prognosis in prospective 2984 patients with decompensated liver cirrhosis who were prescribed BCAA granules containing 952 mg of L-isoleucine, 1904 mg of L-leucine and 1144 mg of L-valine at 4.15 g/sachet three times a day after meals.

Methods: The primary end-point was the time to the event defined as "hospital admission due to progression of hepatic failure", and factors affecting this outcome were explored. Changes in serum albumin level were evaluated as the secondary end-point.

Results: Patients were divided into the good adherence group (those who reported to have taken "nearly all" prescribed doses) and the poor adherence group (those who reported to have taken "approximately half" or "less" doses), because such stratification was validated by treatment responses in plasma BCAA/tyrosine ratio. Factors related to the primary end-point were age, drug adherence during 6 months of study treatment, previous hepatic cancer, current clinical manifestations, previous clinical manifestations, baseline serum albumin level, platelet count and total bilirubin level. The cumulative event-free survival was significantly higher in the good adherence group. Increase in the serum albumin level was also greater in the good adherence group.

Conclusion: Higher BCAA treatment adherence better raised the serum albumin level, leading to improvement of event-free survival. These results indicate the importance of patient instruction for the adequate use of BCAA granules.

Key words: branched-chain amino acids, hepatic failure, liver cirrhosis, prognosis, serum albumin, treatment adherence

INTRODUCTION

ALTHOUGH LIVER CIRRHOSIS is caused by any of a wide variety of etiologies,[1] clinical features of the disease share complications such as ascites, edema, hepatic encephalopathy and esophageal varices.[2] Some of these complications are attributable to decreased serum concentrations of albumin and other proteins,[3–5] and oral supplemental branched-chain amino acid (BCAA) therapy with BCAA granules or BCAA-enriched nutrients is recommended, in addition to dietary treatment with adequate protein and energy intake, for the management of these complications.[6–8]

Branched-chain amino acid granules are used for the improvement of hypoalbuminemia in patients with decompensated liver cirrhosis,[4,9–11] and several studies have demonstrated their efficacy in reducing complications of liver cirrhosis.[4,12–14] Furthermore, a reduction in

Correspondence: Professor Hisataka Moriwaki, Department of Medicine, Gifu University Graduate School of Medicine, 1-1 Yanagido, Gifu 501-1194, Japan. Email: hmori@gifu-u.ac.jp
Received 13 July 2012; revision 26 August 2012; accepted 27 August 2012.

the risk of hepatic cancer is also reported in patients taking BCAA granules.[12,15–17]

On the other hand, the patients' treatment adherence was not so favorable owing to the size of individual doses and unpleasant taste, causing interruption of treatment[13] or reduction of doses.[4] Although serum albumin level has been shown to improve in a dose-dependent manner based on the prescribed BCAA doses,[10] no studies have investigated exactly how treatment adherence may influence the serum albumin level and prognosis of patients with liver cirrhosis.

We conducted the present analysis to evaluate how treatment adherence may affect the serum albumin level and prognosis in a prospective cohort of 5042 patients with liver cirrhosis who had started BCAA treatment at a fixed dose of three sachets/day in a preceding study.[18]

METHODS

Study design and protocol

THIS WAS A multicenter prospective observational study to determine the incidence of adverse events, including hepatocellular carcinoma (HCC) and cirrhosis-related events, under the actual condition of treatment in patients with decompensated liver cirrhosis who were prescribed BCAA granules between June 2003 and December 2006,[18] and were further followed up thereafter.

A total of 5042 patients with decompensated liver cirrhosis, who presented hypoalbuminemia despite adequate dietary intake, were enrolled in this study at 929 medical institutions in Japan. These patients were p.o. administrated BCAA granules containing 952 mg of L-isoleucine, 1904 mg of L-leucine and 1144 mg of L-valine (Livact Granules, Ajinomoto Pharmaceutical, Tokyo, Japan) at 4.15 g/sachet three times a day after meals.

Patient flow is shown in Figure 1. Of the 5042 patients enrolled, the medical records were not available for 222 patients, and 123 patients were lost to follow up after the initial hospital visit. Thus, the remaining 4697 patients constituted the prospective cohort. Patients meeting any of the following criteria were then excluded, and the remaining 2984 patients were subjected to the analysis: (i) a baseline serum albumin level higher than 3.5 g/dL; (ii) a baseline serum total bilirubin level of 3.0 mg/dL or higher; (iii) unknown duration of study observation; (iv) baseline dosage of prescribed BCAA granules other than three sachets/day; or (v) unknown BCAA treatment adherence for 6 months after the start of study observation.

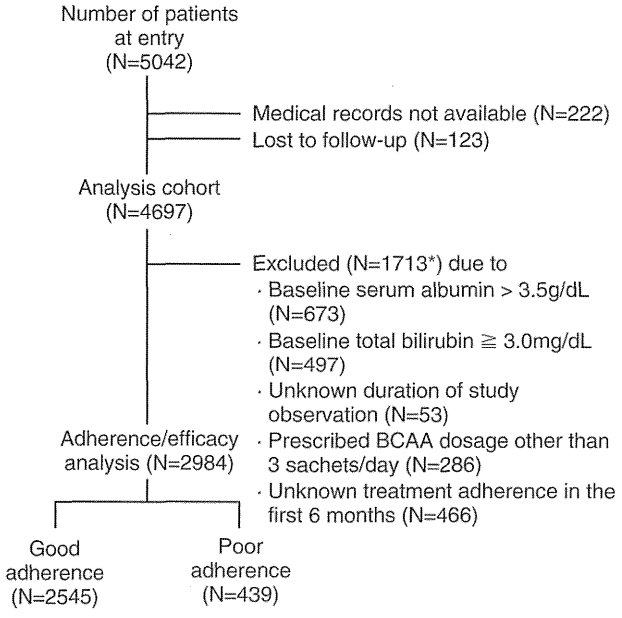


Figure 1 Patient flow. *Among the 1713 patients, 262 were excluded by meeting two or more conditions of the exclusion criteria. BCAA, branched-chain amino acid.

The patients' treatment adherence was evaluated by a questionnaire analysis at the end of the 6-month surveillance period. The questionnaire provided three answer arms who took "nearly all", "approximately half" and "less" of the prescribed dose of BCAA granules at three sachets/day. Each patient was instructed to select one of the above three answer arms that best reflected his/her drug adherence status in the preceding study period.

The primary end-point was the time to onset of the event, defined as hospital admission due to progression of hepatic failure, including ascites, edema, jaundice and hepatic encephalopathy. Changes in liver function during the 6 months were evaluated as the secondary end-point.

This study was conducted in accordance with the Japanese Good Post-Marketing Surveillance Practice.

Statistical analysis

Continuous data were expressed as mean ± standard deviation, and differences in mean values were statistically tested using paired or unpaired Student's *t*-test as appropriate. Categorical variables were compared by Wilcoxon signed rank test, Wilcoxon rank sum test or χ^2-test as required. The cumulative event-free survival rates were estimated using the Kaplan–Meier method

and compared by log–rank test. Any risk factors contributing to the primary end-point were investigated by univariate and multivariate analyses using a Cox proportional hazards model. Data analysis was performed using JMP ver. 9.02 and SAS ver. 9.2 (both SAS Institute, Cary, NC, USA). The level of significance was assessed as two-sided $P < 0.05$.

RESULTS

Patients' characteristics and flow

OF THE PROSPECTIVE cohort consisting of 4697 patients, 1713 were excluded by meeting the exclusion criteria (Fig. 1). Among them, 673 patients had a baseline serum albumin level higher than 3.5 g/dL, 497 patients had a baseline serum total bilirubin level of 3.0 mg/dL or higher, 53 patients had an unknown duration of study observation, 286 patients were prescribed BCAA granules of a dosage other than three sachets/day, and 466 patients reported unknown treatment adherence during the 6 months of study observation. Two hundred and sixty-two patients were excluded by fulfilling two or more conditions of the exclusion criteria. Thus, the remaining 2984 patients were subjected to the adherence/efficacy analysis (Fig. 1). Clinical characteristics of these patients are shown in Table 1. The observation period ranged 6.0–47.9 months, with a median of 21.6 months.

Risk factors for the primary end-point

For the primary end-point, univariate and multivariate analyses using a Cox proportional hazards model identified the following independent factors to influence the development of the event: age, treatment adherence for the 6 months of study observation, previous hepatic cancer, current clinical manifestations, previous clinical manifestations, baseline serum albumin level, platelet count and serum total bilirubin level (Table 2).

Treatment adherence and plasma BCAA/tyrosine ratio

All these variables except treatment adherence have already been documented as risk factors in patients with liver cirrhosis.[19,20] Taking notice of treatment adherence, therefore, 2545 patients who reported to have taken "nearly all" the prescribed doses during the 6-month period comprised the good adherence group and 439 patients who reported to have taken "approximately half" or "less" of the prescribed doses during that period comprised the poor adherence group for further analysis.

Table 1 Clinical characteristics of patients

Characteristics		$n = 2984$
Sex	Male	1584 (53.1%)
	Female	1400 (46.9%)
Age (years)	20–29	1 (0.0%)
	30–39	24 (0.8%)
	40–49	165 (5.5%)
	50–59	530 (17.8%)
	60–69	1038 (34.8%)
	70–79	1024 (34.3%)
	80–89	195 (6.5%)
	>90	7 (0.2%)
	Mean ± SD	66.1 ± 10.1
Cause of liver cirrhosis	HBV	217 (7.3%)
	HCV	1755 (58.8%)
	Alcohol	487 (16.3%)
	PBC	74 (2.5%)
	AIH	63 (2.1%)
	HBV + HCV	16 (0.5%)
	HBV + alcohol	29 (1.0%)
	HCV + alcohol	92 (3.1%)
	HBV + HCV + alcohol	2 (0.1%)
	Other	57 (1.9%)
	Unknown	192 (6.4%)
Treatment adherence (during 6 months)	All	2545 (85.3%)
	Half or less	439 (14.7%)
Previous hepatic cancer	Yes	504 (16.9%)
	No	2454 (82.2%)
	Unknown	26 (0.9%)
Current clinical manifestations	Yes	1568 (52.6%)
	No	1410 (47.3%)
	Unknown	6 (0.2%)
Previous clinical manifestations	Yes	1291 (43.3%)
	No	1670 (56.0%)
	Unknown	23 (0.8%)
Diabetes	Yes	536 (18.0%)
	No	2448 (82.0%)
Serum albumin (g/dL)		3.04 ± 0.36
Platelet (×10 000/μL)		9.73 ± 6.15
AST (IU/L)		67.1 ± 62.8
ALT (IU/L)		47.8 ± 40.9
Serum total bilirubin (mg/dL)		1.30 ± 0.62
BTR		2.95 ± 1.37

For categorical variables, the number of patients and percentage are shown. For continuous variables, the mean ± SD is presented. AIH, autoimmune hepatitis; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BTR, branched-chain amino acid/tyrosine ratio; HBV, hepatitis B virus; HCV, hepatitis C virus; PBC, primary biliary cirrhosis; SD, standard deviation.

Table 2 Risk factors for the event

Explanatory variable		Univariate				Multivariate			
		Hazard ratio	*P*-value	95% CI		Hazard ratio	*P*-value	95% CI	
				Lower limit	Upper limit			Lower limit	Upper limit
Sex	Male/female	1.18	0.0625	0.99	1.40	1.18	0.0685	0.99	1.42
Age (years)		1.01	0.0190	1.00	1.02	1.02	<0.0001	1.01	1.03
Cause of liver cirrhosis	HBV (yes/no)	1.01	0.9673	0.74	1.34				
	HCV (yes/no)	0.86	0.0928	0.72	1.03				
	Alcohol (yes/no)	1.16	0.1775	0.93	1.42				
Treatment adherence (during 6 months)	Half or less/all	1.74	<0.0001	1.39	2.15	1.94	<0.0001	1.54	2.42
Previous hepatic cancer	Yes/no	1.53	<0.0001	1.25	1.86	1.76	<0.0001	1.42	2.16
Current clinical manifestations	Yes/no	2.21	<0.0001	1.84	2.65	1.66	<0.0001	1.36	2.04
Previous clinical manifestations	Yes/no	1.88	<0.0001	1.59	2.24	1.45	<0.0001	1.20	1.74
Diabetes	Yes/no	1.24	0.0488	1.00	1.52				
Serum albumin (g/dL)	Lower level	2.51	<0.0001	2.02	3.10	2.00	<0.0001	1.57	2.54
Platelet (×10 000/μL)	Lower level	1.04	<0.0001	1.02	1.06	1.03	0.0010	1.01	1.05
AST (IU/L)	Higher level	1.00	0.8840	1.00	1.00				
ALT (IU/L)	Higher level	1.00	0.0156	0.99	1.00				
Serum total bilirubin (mg/dL)	Higher level	1.68	<0.0001	1.47	1.92	1.49	<0.0001	1.29	1.72
BTR	Lower level	1.22	0.0839	0.98	1.59				

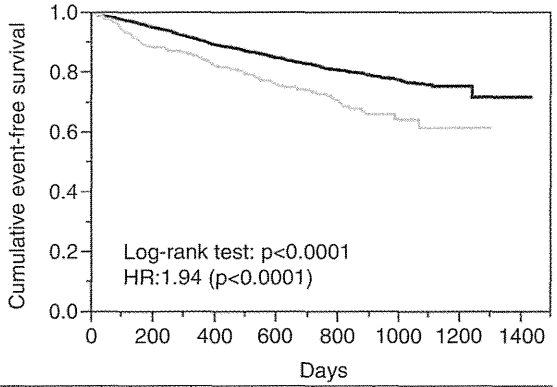
Univariate and multivariate analyses were performed using a Cox proportional hazards model, and hazard ratios, *P*-values and 95% CI of the hazard ratios are shown. For the multivariate analysis, variables were selected and determined by backwards selection ($P = 0.2$) using a model incorporating all factors except BTR. BTR was excluded from the multivariate analysis because a considerable proportion of patients lacked BTR data.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BTR, branched-chain amino acid/tyrosine ratio; CI, confidence interval; HBV, hepatitis B virus; HCV, hepatitis C virus.

As treatment adherence was judged based on patients' self-reports, we further attempted to validate the treatment adherence by changes in the BCAA/tyrosine ratio (BTR) as an indicator reflecting true BCAA treatment adherence. Although the number of patients with BTR data was limited ($n = 185$ and 19, respectively), both absolute BTR and relative increase in BTR (increase in BTR/baseline BTR) were higher in the good adherence group (absolute BTR, 4.26 ± 0.65 for the good adherence group and 3.79 ± 0.52 for the poor adherence group; and relative increase in BTR, 0.53 ± 0.8 for the good adherence group and 0.30 ± 0.68 for the poor adherence group; $P < 0.1$ for both) at 6 months of treatment, while there was no significant difference in baseline BTR between the two groups (2.94 ± 0.49 and 2.86 ± 0.46). A comparison between the two groups was thus considered to be feasible.

Treatment adherence and event-free survival

Regarding the primary end-point, Kaplan–Meier analysis and log–rank test showed a significantly higher cumulative event-free survival rate for the good adherence group as compared with the poor adherence group (Fig. 2).



	0	200	400	600	800	1000	1200	1400
Good adherence	2545	2201	1856	1545	981	345	30	2
Poor adherence	439	301	231	182	100	32	4	0

Figure 2 Comparison of cumulative event-free survival rate by treatment adherence status. Cumulative event-free survival rates were estimated for the good adherence and poor adherence groups using the Kaplan–Meier method, and are shown along with the number of patients at risk. Two curves were compared by log–rank test, and hazard ratio (HR) was calculated by Cox proportional hazards model. (—) Good adherence; (---) poor adherence.

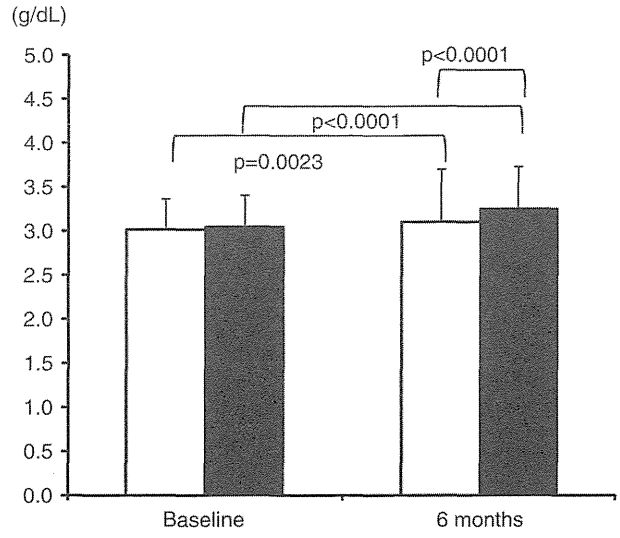


Figure 3 Comparison of serum albumin levels by treatment adherence status. Columns and bars indicate mean and standard deviation of serum albumin levels obtained at baseline and at 6 months of study treatment, respectively. Statistical assessment within each adherence group was carried out by paired Student's *t*-test. For differences between the groups at baseline and at 6 months, Student's *t*-test was conducted. (□) Poor adherence ($n = 366$); (■) good adherence ($n = 2378$).

Treatment adherence and blood biochemistry

Changes in liver function-related parameters during 6 months of the study treatment were examined for each of the good adherence and poor adherence groups. No significant difference was noted in platelet count, aspartate aminotransferase or alanine aminotransferase (ALT) between these groups. At 6 months of study treatment, serum total bilirubin level significantly increased in the poor adherence group but not in the good adherence group. Serum albumin level rose significantly in both of these groups at 6 months of study treatment, and the increase was significantly greater for the good adherence group (Fig. 3).

Comparison of clinical characteristics between good adherence group and poor adherence group

Baseline clinical characteristics were compared between the good adherence group and poor adherence group as shown in Table 3. Patients of the poor adherence group showed a significantly younger age, lower proportion of

Table 3 Clinical characteristics of patients by adherence status

Characteristics		Good adherence, $n = 2545$	Poor adherence, $n = 439$	*P*-value
Sex	Male	1334 (52.4%)	250 (56.9%)	$P = 0.0789$
	Female	1211 (47.6%)	189 (43.1%)	
Age (years)	20–29	1 (0.0%)	0 (0.0%)	
	30–39	16 (0.6%)	8 (1.8%)	
	40–49	135 (5.3%)	30 (6.8%)	
	50–59	445 (17.5%)	85 (19.4%)	
	60–69	894 (35.1%)	144 (32.8%)	
	70–79	888 (34.9%)	136 (31.0%)	
	80–89	161 (6.3%)	34 (7.7%)	
	>90	5 (0.2%)	2 (0.5%)	
	Mean ± SD	66.3 ± 9.9	65.2 ± 11.1	$P = 0.0344$
Cause of liver cirrhosis	HBV	184 (7.2%)	33 (7.5%)	$P = 0.0111$
	HCV	1539 (60.5%)	216 (49.2%)	
	Alcohol	393 (15.4%)	94 (21.4%)	
	PBC	59 (2.3%)	15 (3.4%)	
	AIH	52 (2.0%)	11 (2.5%)	
	HBV + HCV	13 (0.5%)	3 (0.7%)	
	HBV + alcohol	23 (0.9%)	6 (1.4%)	
	HCV + alcohol	77 (3.0%)	15 (3.4%)	
	HBV + HCV + alcohol	2 (0.1%)	0 (0.0%)	
	Other	46 (1.8%)	11 (2.5%)	
	Unknown	157 (6.2%)	35 (8.0%)	
Previous hepatic cancer	Yes	448 (17.6%)	56 (12.8%)	$P = 0.0110$
	No	2078 (81.7%)	376 (85.6%)	
	Unknown	19 (0.7%)	7 (1.6%)	
Current clinical manifestations	Yes	1321 (51.9%)	247 (56.3%)	$P = 0.1545$
	No	1218 (47.9%)	192 (43.7%)	
	Unknown	6 (0.2%)	0 (0.0%)	
Previous clinical manifestations	Yes	1094 (43.0%)	197 (44.9%)	$P = 0.6969$
	No	1432 (56.3%)	238 (54.2%)	
	Unknown	19 (0.7%)	4 (0.9%)	
Diabetes	Yes	457 (18.0%)	79 (18.0%)	$P = 0.9844$
	No	2088 (82.0%)	360 (82.0%)	
Serum albumin (g/dL)		3.04 ± 0.36	3.01 ± 0.35	$P = 0.1519$
Platelet (×10 000/μL)		9.56 ± 5.85	10.76 ± 7.63	$P = 0.0002$
AST (IU/L)		67.2 ± 66.1	66.2 ± 38.1	$P = 0.7578$
ALT (IU/L)		48.4 ± 42.7	44.2 ± 28.7	$P = 0.0518$
Serum total bilirubin (mg/dL)		1.29 ± 0.61	1.33 ± 0.66	$P = 0.2430$
BTR		2.98 ± 1.42	2.82 ± 1.07	$P = 0.4400$

For categorical variables, the number of patients and percentage are shown. For continuous variables, the mean ± SD is presented. Statistical analysis was conducted by χ^2-test or by Student's *t*-test.
AIH, autoimmune hepatitis; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BTR, branched-chain amino acid/tyrosine ratio; HBV, hepatitis B virus; HCV, hepatitis C virus; PBC, primary biliary cirrhosis; SD, standard deviation.

hepatitis C virus positivity and higher proportion of alcoholic cirrhosis, lower incidence of previous hepatic cancer, and higher platelet count (Table 3). Also, they tended to be male patients with lower serum ALT activity (Table 3).

DISCUSSION

THE LOTUS STUDY demonstrated that the outcome of patients with advanced liver cirrhosis was improved by the treatment with BCAA granules at three

sachets/day, compared with the dietary treatment.[4] As utilized in that study, the recommended dosage of BCAA granules is one sachet three times a day p.o. after meals; however, some patients may not take all three sachets in a day due to problems such as treatment adherence. We therefore conducted the present prospective cohort study to examine how differences in the actual intake of BCAA granules may influence the prognosis of patients with liver cirrhosis.

Assessment of clinical characteristics of the patients included in the present study indicated that these patients shared average clinical features of liver cirrhosis in Japanese patients such as accountable etiologies.[1] Logistic analysis revealed that none of these causes was an independent risk factor for patients' outcome. Indeed, the prognosis of patients with liver cirrhosis was determined by eight factors including treatment adherence, regardless of the cause of liver cirrhosis (Table 2).

We focused on the treatment adherence among the eight independent risk factors in the present study, because the clinical significance of the other seven factors has already been described.[19,20] For this concern, patients were divided into the good adherence group (those who reported to have taken "nearly all" prescribed doses) and the poor adherence group (those who reported to have taken "approximately half" or "less" doses), because such stratification was validated by treatment responses in plasma BCAA/tyrosine ratio. Actually, 85.3% of patients reported to have taken "nearly all" three sachets of BCAA granules/day as prescribed. This result was comparable to the 86% adherence in the patients of the LOTUS study.[4] In the present study, treatment adherence was monitored longer after the first 6 months continuously, and remained similar: 81.1% for 7–12 months, 80.6% for 13–18 months and 79.7% for 19–24 months. These data indicate that treatment adherence observed for the first 6-month period was kept over longer treatment periods and, therefore, suggest that it is reasonable to monitor the treatment adherence of the first 6-month period for the long-term prognosis.

Improvement of hypoalbuminemia was reported to depend on the prescribed daily BCAA doses (8, 12 or 16 g),[10] but the present study first showed that, at the fixed prescribed dose (three sachets or 12 g/day), serum albumin level rose sufficiently only when the patient had good adherence (Fig. 3). Thus, good treatment adherence resulted in an improved serum albumin level (Fig. 3), and, consequently brought about a higher event-free survival (Fig. 2), as a decreased serum albumin level was also an independent risk factor for the patients (Table 2).

As to possible clinical factors that affect patients' BCAA adherence, we detected male sex, younger age, distribution of etiologies of liver cirrhosis, lower incidence of previous hepatic cancer, higher platelet count and lower serum ALT activities in the poor adherence group (Table 3). Among these factors, only male sex was also a possible unfavorable outcome marker (Table 2), but other factors were rather favorable or had no significance (e.g. cause of liver cirrhosis) for patients' outcome (Table 2). Such observation suggests that particular caution should be paid for drug adherence in male cirrhotics.

The limitation of such studies on advanced liver cirrhosis is the possibility that earlier development of events shortly after the start of the study influenced treatment adherence. To address this concern, we additionally performed analysis after excluding the patients who developed any event within 6 months of the study, and the cumulative event-free survival rate was still significantly higher for the good adherence group than that for the poor adherence group (hazard ratio = 1.57, $P = 0.0043$), as was the case with the analysis on the whole analysis set.

In conclusion, higher treatment adherence for BCAA is considered to be associated with an improved serum albumin level, thereby leading to improved patient outcome. These results indicate the importance of patient instruction for the adequate use of BCAA granules.

REFERENCES

1 Aoyagi Y, Nishiguchi S, Michitaka K. *Cause of Liver Cirrhosis 2008*. Tokyo: Chugai-Igakusha, 2008; 1–10. (in Japanese.)
2 Kuntz E, Kuntz H-D. *Hepatology, Principles and Practice: History, Morphology, Biochemistry, Diagnostics, Clinic, Therapy*. Heidelberg: Springer, 2002; 192–202.
3 Merli M, Riggio O, Dally L. Does malnutrition affect survival in cirrhosis? PINC (Policentrica Italiana Nutrizione Cirrosi). *Hepatology* 1996; **23** (5): 1041–6.
4 Muto Y, Sato S, Watanabe A *et al.* Effects of oral branched-chain amino acid granules on event-free survival in patients with liver cirrhosis. *Clin Gastroenterol Hepatol* 2005; **3**: 705–13.
5 Tajika M, Kato M, Mohri H *et al.* Prognostic value of energy metabolism in patients with viral liver cirrhosis. *Nutrition* 2002; **18** (3): 229–34.
6 Plauth M, Cabré E, Riggio O *et al.* ESPEN guidelines on enteral nutrition: liver disease. *Clin Nutr* 2006; **25** (2): 285–94.