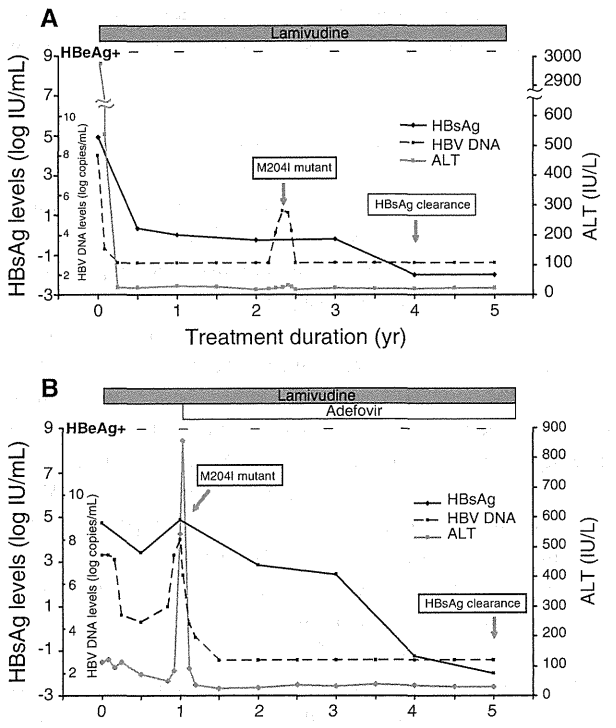

Fig. 4 Case presentation of the typical clinical and virological courses of two representative patients who achieved HBsAg clearance after VBT occurred. **a** Patient 1, a 45-year-old man who was HBeAg+ at baseline and had genotype A. **b** Patient 2, a 38-year-old man who was HBeAg+ at baseline and had genotype A. *VBT* virological breakthrough

HBsAg clearance in both the HBeAg+ and − cohorts, whereas the clearance of HBsAg was associated with previous IFN therapy and the clearance of HBeAg over the first six months only in the HBeAg+ cohort, and baseline HBsAg levels only in the HBeAg− cohort.

HBV genotype was recently reported to influence declines in and the clearance of HBsAg among patients who underwent PEG-IFN therapy [31]. In one study where negativity for serum HBV DNA and seroconversion of HBeAg represented the study end point, genotype was not found to influence response to NA therapy [31]. However, other reports have indicated that genotype does impact on declines in and the clearance of HBsAg [20, 29]. Heathcote et al. [20] reported that 20 HBeAg+ patients (8 %) who were treated with tenofovir achieved HBsAg clearance in three years. Twelve (60 %) of 20 patients were infected with genotype A and the others with genotype D. In this study, cumulative HBsAg clearance rates were 15 % at year 3 in HBeAg+ patients with genotype A. This result seems to be similar regardless of the antiviral potential. Previous studies with more ethnically diverse study populations than ours found that HBsAg clearance rates were highest in patients with genotype A. The similarity between those results and ours implies that the HBV genotype is more influential than ethnicity on HBsAg clearance during NA therapy. Of 28 genotype A patients in our population, the majority (79 %) did not have a family history of infection. Recent work has shown that sexual transmission of acute HBV genotype A infections is increasing in Japan, resulting in chronic HBV infection, especially in young adult patients [32, 33]. Cumulatively, these findings imply that HBsAg clearance is more likely in genotype A patients because they have been infected with HBV for a shorter period of time. Furthermore, Hou et al. [34] demonstrated that genotype A responded better than other HBV genotypes to IFN therapy. They revealed that a lower number of amino acid substitutions at baseline were associated with a better response to IFN therapy, and that this variable was linked with HBV genotype A, which had the lowest number of amino acid substitutions in the core gene among genotypes B, C, or D. Although amino acid substitutions in the core gene were not analyzed in this study, the relation between the core gene and treatment responses of NAs is necessary to be investigated in the future.

Although Gish et al. [19] reported that previous IFN therapy is not associated with HBsAg clearance in patients who are HBeAg+, the opposite was true in our HBeAg+ cohort. These contradictory findings may result from the fact that their patients received NA therapy over a much shorter time period (median duration 23 vs. 75 months, a 3.2-fold difference). We believe that there are two main reasons why HBsAg clearance rates were higher in patients who had previously received IFN therapy: the influence of AST/ALT flares after IFN therapy and changes in host immune response to HBV as a result of the immune-modulating activity of IFN. It has previously been shown that in patients with high baseline ALT levels, HBV DNA and HBeAg are likely to rapidly decrease during NA therapy [35, 36]. In this study, HBsAg clearance was likely to occur in patients who had high ALT levels at baseline, and in patients with previous IFN therapy (Table 2) in the HBeAg+ cohort. High virological responses have been reported in response to robust ALT flares induced by IFN therapy [37, 38]. Moreover, Wursthorn et al. [29] recently indicated that the antiviral potential of NAs and antiviral T cell reactivity are associated with HBsAg clearance in response to telbivudine treatment. These findings may be also associated with the achievement of HBsAg clearance after VBT occurs. Taken together, these results imply that both direct antiviral potential and host immune response are needed to achieve HBsAg clearance, especially in HBeAg+ patients.

We found that the initial HBsAg reduction was a strong predictor of subsequent HBsAg clearance during NA therapy, which supports a similar previous finding [29]. HBsAg reduction over the initial six months is important

for predicting the subsequent HBsAg kinetics in both HBeAg+ and HBeAg− patients. The novel finding in this study was that HBeAg− individuals achieved HBsAg clearance. We found that the median duration to HBsAg clearance was longer in patients with HBeAg− than in those who were HBeAg+ in this study (6.0 vs. 4.4 years). Manesis et al. [28] used modeling to determine that HBeAg− patients receiving LAM treatment would likely require >10 years to achieve HBsAg loss. Furthermore, baseline HBsAg titers were <730 IU/mL in 60 % (12/20) of HBeAg− patients who achieved HBsAg clearance. The only baseline predictive factor of HBsAg clearance was baseline HBsAg levels in HBeAg− patients, except for genotype. There was no difference in HBsAg clearance rates in HBeAg− patients with high- and low-baseline HBV DNA or ALT levels. We hypothesize that HBsAg clearance in these patients may result from long treatment duration and low HBsAg titers.

Our study was limited by the fact that it was a hospital-based retrospective analysis, which means there may be some bias associated with patient type and treatment selection. We were unable to compare HBsAg clearance rates obtained in our study with those of controls untreated with NA. Because all subjects in the study received LAM as an initial NA, and then received rescue therapy when drug-resistant mutations emerged, NA therapy regimens were not uniform across all patients, and there were variations in both treatment dose and duration of previous IFN therapy. We were not able to collect immunological data on our subjects. Finally, our results need to be validated by further studies investigating a large study population receiving long-term ETV or tenofovir with high antiviral potential and a high genetic barrier.

Despite these drawbacks, we were able to determine several factors associated with HBsAg clearance, including HBV genotype and a decline in HBsAg over the initial six months of treatment (HBeAg+ and − cohorts); previous IFN therapy and clearance of HBeAg over the initial six months of treatment (HBeAg+ cohort only); and HBsAg levels (HBeAg− cohort only). It seems that both direct antiviral potential and host immune response are needed to achieve HBsAg clearance by NA therapy. Future studies are needed to validate these findings and to develop treatment regimens for HBsAg clearance in patients with chronic hepatitis B.

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Conflict of interest Dr. Kumada reports having received investigator, lecture, and consulting fees from Bristol–Myers Squibb, Dainippon Sumitomo Pharma Co., MSD K.K., and Toray Co. Dr. Ikeda reports having received investigator, lecture, and consulting fees from Dainippon Sumitomo Pharma Co. No other potential conflicts of interest relevant to this article were reported.

References

1. Ganem D, Prince AM. Hepatitis B virus infection—natural history and clinical consequences. N Engl J Med. 2004;350:1118–29.
2. Lee WM, Hepatitis B. Virus infection. N Engl J Med. 1997;337:1733–45.
3. Chang TT, Gish RG, de Man R, Gadano A, Sollano J, Chao YC, et al. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. N Engl J Med. 2006;354:1001–10.
4. Dienstag JL, Schiff ER, Wright TL, Perrillo RP, Hann HW, Goodman Z, et al. Lamivudine as initial treatment for chronic hepatitis B in the United States. N Engl J Med. 1999;341:1256–63.
5. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M, et al. Adefovir dipivoxil for the treatment of hepatitis B e antigen-negative chronic hepatitis B. N Engl J Med. 2003;348:800–7.
6. Lai CL, Chien RN, Leung NW, Chang TT, Guan R, Tai DI, 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.
7. Lai CL, Gane E, Liaw YF, Hsu CW, Thongsawat S, Wang Y, et al. Telbivudine versus lamivudine in patients with chronic hepatitis B. N Engl J Med. 2007;357:2576–88.
8. Lai CL, Shouval D, Lok AS, Chang TT, Cheinquer H, Goodman Z, et al. Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. N Engl J Med. 2006;354:1011–20.
9. 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.
10. Marcellin P, Heathcote EJ, Buti M, Gane E, de Man RA, Krastev Z, et al. Tenofovir disoproxil fumarate versus adefovir dipivoxil for chronic hepatitis B. N Engl J Med. 2008;359:2442–55.
11. 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.
12. Suzuki Y, Arase Y, Ikeda K, Saitoh S, Tsubota A, Suzuki F, et al. Histological improvements after a three-year lamivudine therapy in patients with chronic hepatitis B in whom YMDD mutants did not or did develop. Intervirology. 2003;46:164–70.
13. Newbold JE, Xin H, Tencza M, Sherman G, Dean J, Bowden S, et al. The covalently closed duplex form of the hepadnavirus genome exists in situ as a heterogeneous population of viral minichromosomes. J Virol. 1995;69:3350–7.
14. Wu TT, Coates L, Aldrich CE, Summers J, Mason WS. In hepatocytes infected with duck hepatitis B virus, the template for viral RNA synthesis is amplified by an intracellular pathway. Virology. 1990;175:255–61.
15. Zoulim F. New insight on hepatitis B virus persistence from the study of intrahepatic viral cccDNA. J Hepatol. 2005;42:302–8.
16. Werle-Lapostolle B, Bowden S, Locarnini S, Wursthorn K, Petersen J, Lau G, et al. Persistence of cccDNA during the natural history of chronic hepatitis B and decline during adefovir dipivoxil therapy. Gastroenterology. 2004;126:1750–8.
17. Wursthorn K, Lutgehetmann M, Dandri M, Volz T, Buggisch P, Zollner B, et al. Peginterferon alpha-2b plus adefovir induce strong cccDNA decline and HBsAg reduction in patients with chronic hepatitis B. Hepatology. 2006;44:675–84.

18. Chang TT, Lai CL, Kew Yoon S, Lee SS, Coelho HS, Carrilho FJ, et al. Entecavir treatment for up to 5 years in patients with hepatitis B e antigen-positive chronic hepatitis B. Hepatology. 2010;51:422–30.
19. Gish RG, Chang TT, Lai CL, de Man R, Gadano A, Poordad F, et al. Loss of HBsAg antigen during treatment with entecavir or lamivudine in nucleoside-naive HBeAg-positive patients with chronic hepatitis B. J Viral Hepat. 2010;17:16–22.
20. Heathcote EJ, Marcellin P, Buti M, Gane E, De Man RA, Krastev Z, et al. Three-year efficacy and safety of tenofovir disoproxil fumarate treatment for chronic hepatitis B. Gastroenterology. 2011;140:132–43.
21. Kobayashi M, Suzuki F, Akuta N, Hosaka T, Sezaki H, Yatsuji H, et al. Loss of hepatitis B surface antigen from the serum of patients with chronic hepatitis treated with lamivudine. J Med Virol. 2007;79:1472–7.
22. Liaw YF, Gane E, Leung N, Zeuzem S, Wang Y, Lai CL, et al. 2-Year GLOBE trial results: telbivudine Is superior to lamivudine in patients with chronic hepatitis B. Gastroenterology. 2009;136:486–95.
23. Reijnders JG, Rijckborst V, Sonneveld MJ, Scherbeijn SM, Boucher CA, Hansen BE, et al. Kinetics of hepatitis B surface antigen differ between treatment with peginterferon and entecavir. J Hepatol. 2011;54:449–54.
24. Buster EH, Flink HJ, Cakaloglu Y, Simon K, Trojan J, Tabak F, et al. Sustained HBeAg and HBsAg loss after long-term follow-up of HBeAg-positive patients treated with peginterferon alpha-2b. Gastroenterology. 2008;135:459–67.
25. Buster EH, Flink HJ, Simsek H, Heathcote EJ, Sharmila S, Kitis GE, et al. Early HBeAg loss during peginterferon alpha-2b therapy predicts HBsAg loss: results of a long-term follow-up study in chronic hepatitis B patients. Am J Gastroenterol. 2009;104:2449–57.
26. Marcellin P, Bonino F, Lau GK, Farci P, Yurdaydin C, Piratvisuth T, et al. Sustained response of hepatitis B e antigen-negative patients 3 years after treatment with peginterferon alpha-2a. Gastroenterology. 2009;136:2169–79, e2161–64.
27. Moucari R, Mackiewicz V, Lada O, Ripault MP, Castelnau C, Martinot-Peignoux M, et al. Early serum HBsAg drop: a strong predictor of sustained virological response to pegylated interferon alfa-2a in HBeAg-negative patients. Hepatology. 2009;49:1151–7.
28. Manesis EK, Hadziyannis ES, Angelopoulou OP, Hadziyannis SJ. Prediction of treatment-related HBsAg loss in HBeAg-negative chronic hepatitis B: a clue from serum HBsAg levels. Antivir Ther. 2007;12:73–82.
29. Wursthorn K, Jung M, Riva A, Goodman ZD, Lopez P, Bao W, et al. Kinetics of hepatitis B surface antigen decline during 3 years of telbivudine treatment in hepatitis B e antigen-positive patients. Hepatology. 2010;52:1611–20.
30. Pocock SJ, Clayton TC, Altman DG. Survival plots of time-to-event outcomes in clinical trials: good practice and pitfalls. Lancet. 2002;359:1686–9.
31. Raimondi S, Maisonneuve P, Bruno S, Mondelli MU. Is response to antiviral treatment influenced by hepatitis B virus genotype? J Hepatol. 2010;52:441–9.
32. Suzuki Y, Kobayashi M, Ikeda K, Suzuki F, Arfase Y, Akuta N, et al. Persistence of acute infection with hepatitis B virus genotype A and treatment in Japan. J Med Virol. 2005;76:33–9.
33. Sugauchi F, Orito E, Ohno T, Tanaka Y, Ozasa A, Kang JH, et al. Spatial and chronological differences in hepatitis B virus genotypes from patients with acute hepatitis B in Japan. Hepatol Res. 2006;36:107–14.
34. Hou J, Schilling R, Janssen HLA, Hansen BE, Heijtink R, Sablon E, et al. Genetic characteristics of hepatitis B virus genotypes as a factor for interferon-induced HBeAg clearance. J Med Virol. 2007;79:1055–63.
35. Akuta N, Tsubota A, Suzuki F, Suzuki Y, Hosaka T, Someya T, et al. Long-term prognosis by lamivudine monotherapy for severe acute exacerbation in chronic hepatitis B infection: emergence of YMDD motif mutant and risk of breakthrough hepatitis—an open-cohort study. J Hepatol. 2003;38:91–7.
36. Liaw YF, Tsai SL, Chien RN, Yeh CT, Chu CM. Prednisolone priming enhances Th1 response and efficacy of subsequent lamivudine therapy in patients with chronic hepatitis B. Hepatology. 2000;32:604–9.
37. Flink HJ, Sprengers D, Hansen BE, van Zonneveld M, de Man RA, Schalm SW, et al. Flares in chronic hepatitis B patients induced by the host or the virus? Relation to treatment response during Peg-interferon {alpha}-2b therapy. Gut. 2005;54:1604–9.
38. Nair S, Perrillo RP. Serum alanine aminotransferase flares during interferon treatment of chronic hepatitis B: is sustained clearance of HBV DNA dependent on levels of pretreatment viremia? Hepatology. 2001;34:1021–6.

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Correlation Between Hepatitis B Virus Surface Antigen Level and Alpha-Fetoprotein in Patients Free of Hepatocellular Carcinoma or Severe Hepatitis

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Alfa-fetoprotein (AFP) is used as a marker of early hepatocarcinogenesis. However, the impact of hepatitis B virus surface antigen (HBsAg) on this relationship in patients with HBV infection is not clear. The present study evaluated the relation between HBsAg and AFP levels at the initial visit in 1,610 untreated HBV patients, free of hepatocellular carcinoma (HCC) or severe hepatitis. The cumulative rate of HCC was significantly lower in patients with a low AFP level (≤10 μg/L; below the upper limit of normal) than in those with a high AFP level (≥11 μg/L) at the initial visit. In patients with HBsAg levels more than 500 IU/ml, HBsAg levels correlated significantly and negatively with AFP levels, and significantly with platelet count. Multivariate analysis of data of patients with HBsAg more than 500 IU/ml identified HBsAg (<7,000 IU/ml), albumin (<3.9 g/dl), platelet count ($<20.0 \times 10^4/mm^3$), gamma-glutamyl transpeptidase (≥50 IU/L), aspartate aminotransferase (≥34 IU/L), HBeAg (positive), and HBV core-related antigen (≥3.0 log U/ml) as determinants of a high AFP. Especially, in patients with HBsAg more than 500 IU/ml and low transaminase levels (below the upper limit of normal), HBsAg was identified as significant determinant of a high AFP. On the other hand, in patients with HBsAg less than 500 IU/ml, multivariate analysis identified albumin, gamma-glutamyl transpeptidase, and HBV core-related antigen as determinants of a high AFP. The results indicated that HBsAg level seems to affect, at least in part, the AFP levels, and that it can be used as a surrogate marker of early hepatocarcinogenesis. ***J. Med. Virol. 86:131–138, 2014.*** © 2013 Wiley Periodicals, Inc.

KEY WORDS: HBV; AFP; HBsAg; HBcrAg; genotype; hepatocellular carcinoma

INTRODUCTION

Hepatitis B virus (HBV) is a small, enveloped DNA virus known to cause chronic hepatitis and often leads to liver cirrhosis and hepatocellular carcinoma (HCC) [Viola et al., 1981; Kobayashi et al., 2002; Yao, 2003]. Evidence suggests that the use of elevated alpha-fetoprotein (AFP) for the prediction of early hepatocarcinogenesis in non-HCC patients could be clinically useful. AFP is a fetal glycoprotein produced by the yolk sac and fetal liver [Bergstrand and Czar, 1956] and has been widely used as a serum marker for the diagnosis of HCC [Sato et al., 1993; Johnson, 2001]. Furthermore, high serum AFP levels are also associated with various chronic liver diseases and hepatic regeneration [Kew et al., 1973; Silver et al., 1974; Elftherious et al., 1977; Alpert and Feller, 1978]. Many patients with chronic hepatitis B who are free of HCC have high AFP levels [Chen and Sung, 1979; Di Bisceglie and Hoofnagle, 1989], and some patients with cirrhosis and concomitant high

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inflammatory activity have very high AFP levels [Yao, 2003; Cheema et al., 2004]. On the other hand, some patients with small HCC lesions have only moderately elevated levels of AFP [Shinagawa et al., 1984; Ebara et al., 1986; Bruix and Sherman, 2005]. At present, however, there are no cutoff levels for serum AFP used to predict HCC in patients with HBV infection.

There is growing interest in the use of hepatitis B surface antigen (HBsAg) level as a prognostic marker in chronic hepatitis B patients [Chan et al., 2010]. The HBsAg levels are useful for identifying the stage of disease [Jaroszewicz et al., 2010; Nguyen et al., 2010], to distinguish true inactive carriers from patients with HBe antigen-negative disease [Brunetto et al., 2010; Martinot-Peignoux et al., 2010; Chan et al., 2011; Liaw, 2011], and to predict the response to interferon therapy [Brunetto et al., 2009; Moucari et al., 2009]. Recent studies has also demonstrated that the HBsAg levels are associated with the risk of progression to HCC, especially in patients with low HBV DNA levels [Chan, 2012; Tseng et al., 2012], and that there is a potential correlation between the HBsAg levels and the stage of liver fibrosis [Seto et al., 2012; Martinot-Peignoux et al., 2013]. However, the impact of viral factors, such as the HBsAg level, on serum AFP level as a predictor of early HCC is not clear at present.

The present study included 1,610 untreated patients with HBV infection, free of HCC or severe hepatitis. The present study was designed to provide answers to the following questions: (1) what is the relation between a high serum AFP level at the initial outpatient visit and subsequent development of hepatocarcinogenesis in antiviral-therapy-naive patients with hepatitis B viral infection? (2) What is the impact of viral factors, such as the HBsAg level, on serum AFP level in such patients, and (3) What is a good surrogate marker for a high serum AFP at the initial visit.

PATIENTS AND METHODS

Patients

Among 6,466 consecutive patients who were diagnosed with HBV infection between March 1972 and December 2012 at Toranomon Hospital, 1,610 were selected in the present study based on the following criteria: (1) They were positive for HBsAg (radioimmunoassay, Dainabot, Tokyo, Japan) and negative for anti-HCV (third-generation enzyme immunoassay, Chiron, CA). (2) They were free of HCC at the initial visit. (3) HBV hepatitis was assessed as less than severe at the initial visit, in order to minimize the potential effects of high inflammatory activity. Severe hepatitis was defined as serum transaminase level of $\geq$300 IU/L, and/or total bilirubin level of $\geq$3.0 mg/dl. (4) They had not received antiviral therapy in the past (e.g., interferon and/or nucleot(s)ide analogs) at the initial visit. (5) They underwent examination of the AFP level (upper limit of normal, 10 μg/L) at the initial visit. Furthermore, the HBsAg level, HBV core-related antigen (HBcrAg) level, and HBV DNA were also assayed using stored frozen sera obtained at the initial visit. (6) They were free of coinfection with human immunodeficiency virus. (7) They were free of other types of chronic liver disease, including hemochromatosis, Wilson disease, primary biliary cirrhosis, alcoholic liver disease, autoimmune liver disease, inherited liver disease including alpha-1 antitrypsin deficiency, and hepatic venous outflow block. (8) They consented to the study.

Table I summarizes the profile and laboratory data at the initial visit of the 1,610 patients included in the present study. They included 1,047 males and 563 females, with a median age of 40 years (range: 18–83 years). The median AFP level was 4 μg/L (range, 1–1,770 μg/L) and the median follow-up time (from the initial visit until the last visit) was 6.0 years (range, 0.0–34.6 years). The study protocol was approved by the Human Ethics Review Committee of Toranomon Hospital.

Laboratory Tests

HBsAg, HBcrAg, and HBV DNA levels were assayed using stored frozen sera obtained at the initial visit. Blood samples were frozen at −80°C within 4 hr of collection and were not thawed until used for testing. Serum HBsAg level was measured using Architect HBsAg QT assay kit (Abbott Laboratories, Tokyo, Japan), which has a lower limit of detection of

TABLE I. Profiles and Laboratory Data at the Initial Visit of 1,610 Patients Infected With HBV

Demographic data	
Number of patients	1,610
Sex (male/female)	1,047/563
Age (years)*	40 (18–83)
Family history of liver disease[a]	836 (51.9%)
Lifetime cumulative alcohol intake (≥500 kg)	112 (7.0%)
Laboratory data*	
Total bilirubin (mg/dl)	0.6 (0.1–2.9)
Aspartate aminotransferase (IU/L)	37 (5–220)
Alanine aminotransferase (IU/L)	48 (5–297)
Albumin (g/dl)	4.2 (1.0–5.6)
Gamma-glutamyl transpeptidase (IU/L)	37 (2–2,370)
Hemoglobin (g/dl)	14.5 (6.9–18.2)
Platelet count (×104/mm^3)	19.1 (2.7–44.7)
Alpha-fetoprotein (μg/L)	4 (1–1,770)
Virological data	
HBeAg (No. of positive)	690 (42.9%)
HBsAg (IU/ml)*	2,845 (0.09 to >125,000)
HBcrAg (log U/ml)*	4.9 (<3.0 to >6.8)
HBV DNA (log copies/ml)*	5.7 (<2.1 to >9.1)
HBV genotype (A/B/C/others/ND)	65/218/1,119/6/202

Data are number and percentages of patients, except those denoted by *, which represent the median (range) values.
[a]Family history of positivity for hepatitis B surface antigen including third-degree relatives.

0.05 IU/ml and upper limit of detection of 250 IU/ml. To expand the upper range from 250 to 125,000 IU/ml, serum samples with the HBsAg levels above the upper range were diluted in a stepwise fashion to 1:20 and 1:500 with Architect diluents using the information supplied by the manufacturer. HBeAg was determined by enzyme-linked immunosorbent assay kit (HBeAg EIA; Institute of Immunology, Tokyo, Japan). Serum HBcrAg level was measured using a Cleia HBcrAg assay kit (Fujirebio, Tokyo, Japan) using a fully automated analyzer system (Lumipulse System; Fujirebio). The cut-off value of HBcrAg was 3.0 log U/ml. HBV DNA was quantified using the Cobas TaqMan HBV v.2.0 (Roche Diagnostics, Tokyo, Japan), which has a dynamic range of 2.1–9.0 log copies/ml.

A commercial kit (HBV Genotype EIA; Institute of Immunology) was used to determine serologically the HBV genotypes using the combination of epitopes expressed on the pre-S2 region product, which is specific to each of the major genotypes.

Follow-Up and Diagnosis of Future Hepatocellular Carcinoma

After the initial visit, patients were followed-up once or three times a month. Imaging studies (ultrasonography, computed tomography, or magnetic resonance imaging) were conducted once or more per year.

Statistical Analysis

Non-parametric tests (Mann–Whitney *U*-test, chi-squared test and Fisher's exact probability test) were used to compare differences between two groups. Correlation analysis was evaluated by the Spearman rank correlation test. The cumulative rate of hepatocarcinogenesis was calculated using the Kaplan–Meier technique; differences between cumulative carcinogenesis curves between groups were tested using the log-rank test. Statistical analyses of the rate of hepatocarcinogenesis according to groups were calculated using the period from the initial visit. Univariate and multivariate logistic regression analyses were used to determine the independent surrogate markers of elevated AFP at the initial visit. The odds ratios (OR) and 95% confidence intervals (95% CI) were also calculated. A two-tailed *P*-value less than 0.05 was considered significant. Variables that achieved statistical significance ($P < 0.05$) on univariate analysis were entered into multiple logistic regression analysis to identify significant independent factors for elevated AFP. Potential surrogate markers of elevated AFP at the initial visit included the following pretreatment variables: age, sex, family history of liver disease, lifetime cumulative alcohol intake, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, gamma-glutamyl transpeptidase (GGT), hemoglobin, platelet count, HBV genotype, HBeAg, HBsAg levels, HBcrAg levels, and HBV DNA levels. Statistical analyses were performed using the Statistical Package for Social Sciences software (SPSS, Inc., Chicago, IL).

RESULTS

Cumulative Rate of Hepatocarcinogenesis According to the AFP Level at the Initial Visit

A total of 1,061 patients naïve to antiviral therapy from the initial visit until the last visit were evaluated for the rate of development of HCC based on the AFP levels at the initial visit. During the follow-up period, HCC was diagnosed in 31 of 905 patients (3.4%) with a low AFP level (≤10 μg/L; below the upper limit of normal) and 37 of 156 patients (23.7%) with a high AFP level (≥11 μg/L) at the initial visit. The cumulative hepatocarcinogenesis rates for patients with low and high AFP levels at the initial visit were 4.7% and 30.2% at the end of 10-year follow-up; 9.1% and 36.5% at the end of 20-year follow-up; and 13.2% and 42.9% at the end of 30-year follow-up, respectively. These results indicate that the rate of hepatocarcinogenesis is significantly higher in patients with HBV infection and high AFP levels than their counterparts with low AFP levels ($P < 0.001$; Log-rank test) (Fig. 1).

HBsAg and AFP Levels at the Initial Visit

Blood samples from all patients were analyzed to determine the relationship between the HBsAg and the AFP levels at the initial visit. The proportions of patients with high AFP levels among those with the HBsAg levels below 500 IU/ml, from 500 to 1,999 IU/ml, from 2,000 to 6,999 IU/ml, from 7,000 to 24,999 IU/ml, and above 25,000 IU/ml were 12.6% (42 of 333 patients), 26.7% (89 of 333), 22.6% (94 of 416), 10.4% (29 of 278), and 6.4% (16 of 250), respectively (Fig. 2A). The relationship between the HBsAg and

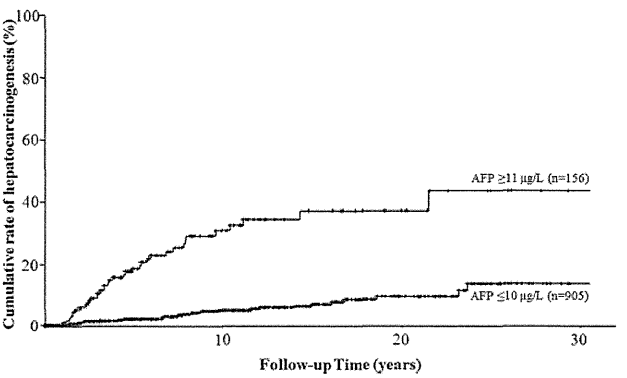

Fig. 1. Cumulative rate of hepatocarcinogenesis according to the AFP level at the initial visit in patients naïve to antiviral therapy from the initial visit until the last visit. The rate of hepatocarcinogenesis was significantly higher in patients with high AFP levels (≥11 μg/L) than in those with low levels (≤10 μg/L) at the initial visit ($P < 0.001$; Log-rank test).

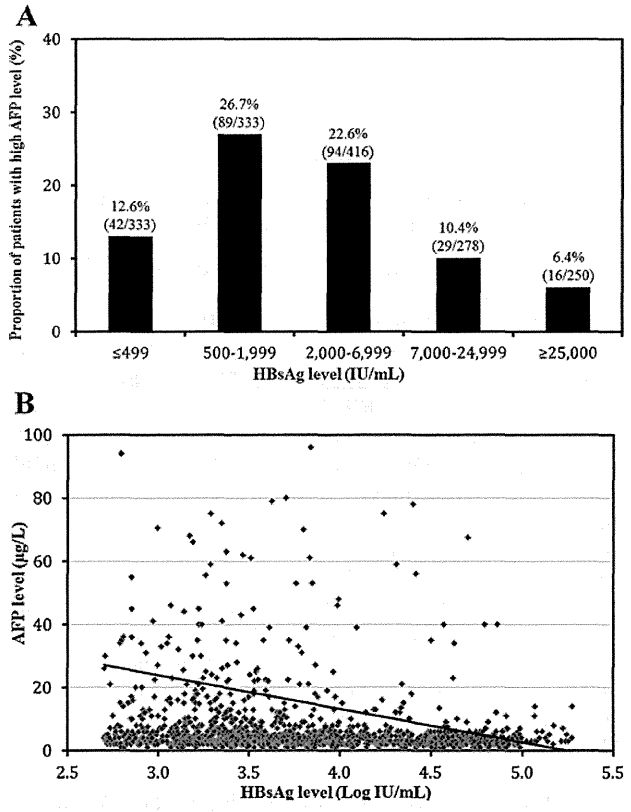

Fig. 2. **A**: Proportions of patients with the high AFP levels ($\geq$11 μg/L) at the initial visit, stratified according to the HBsAg levels. Patients with the HBsAg levels above 500 IU/ml included a significantly lower proportions of patients with the high AFP levels and the HBsAg levels above 7,000 IU/ml (8.5%) than those with the HBsAg levels below 7,000 IU/ml (24.4%) ($P < 0.001$). **B**: Analysis of data of patients with the HBsAg levels above 500 IU/ml at the initial visit, showed a significant negative correlation between logarithmically transformed HBsAg and AFP levels ($r = -0.225$, $P < 0.001$).

the AFP levels at the initial visit suggested the presence of two distinct populations within the study group. Especially, in 1,277 patients with the HBsAg levels above 500 IU/ml, a significantly smaller proportion of patients with high AFP levels were noted among those with HBsAg of more than 7,000 IU/ml (8.5%) than those with the HBsAg levels less than 7,000 IU/ml (24.4%) ($P < 0.001$). Furthermore, the HBsAg levels correlated negatively but significantly with the AFP levels ($r = -0.225$, $P < 0.001$) (Fig. 2B).

The HBsAg Levels and the Platelet Count at the Initial Visit

Blood samples from all patients were analyzed to determine the relationship between the HBsAg levels and the platelet count at the initial visit. The median platelet counts among patients with the HBsAg levels below 500 IU/ml, from 500 to 1,999 IU/ml, from 2,000 to 6,999 IU/ml, from 7,000 to 24,999 IU/ml, and above 25,000 IU/ml were $19.1 \times 10^4/mm^3$, $17.2 \times 10^4/mm^3$, $18.0 \times 10^4/mm^3$, $20.9 \times 10^4/mm^3$, and $21.2 \times 10^4/mm^3$, respectively (Fig. 3A). The relationship between the HBsAg levels and the platelet count at the initial visit also suggested the presence of two distinct populations within the study group. Especially, in 1,277 patients with the HBsAg levels of more than 500 IU/ml, significantly higher platelet counts were noted among those with the HBsAg levels of more than 7,000 IU/ml (the median platelet count; $21.0 \times 10^4/mm^3$) than those with the HBsAg levels less than 7,000 IU/ml (the median platelet count; $17.6 \times 10^4/mm^3$) ($P < 0.001$). Furthermore, the HBsAg

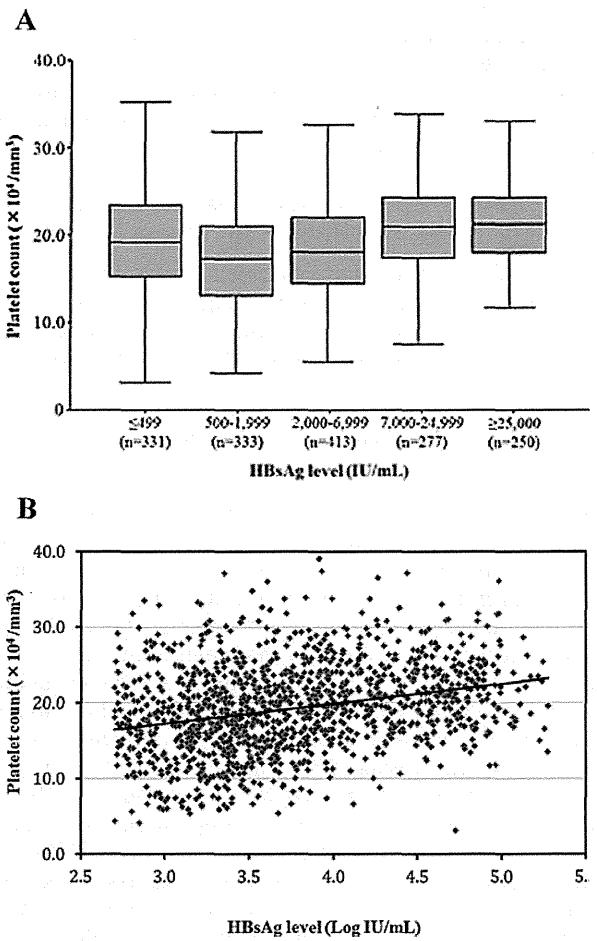

Fig. 3. **A**: The platelet count at the initial visit, stratified according to the HBsAg levels. Bars within the boxes indicate the median platelet count. The boxes denote the 25th to 75th percentiles, the lower and upper bars the 10th and 90th percentiles, respectively. Among patients with the HBsAg levels above 500 IU/ml at the initial visit, those with the HBsAg levels above 7,000 IU/ml had significantly higher platelet count (the median platelet count; $21.0 \times 10^4/mm^3$) compared to those with the HBsAg levels below 7,000 IU/ml (the median platelet count; $17.6 \times 10^4/mm^3$) ($P < 0.001$). **B**: Among patients with the HBsAg levels above 500 IU/ml at the initial visit, logarithmically transformed the HBsAg levels correlated significantly with the platelet count ($r = 0.293$, $P < 0.001$).

levels correlated significantly and positively with the platelet count ($r = 0.293$, $P < 0.001$) (Fig. 3B).

Clinical Profiles and Laboratory Data According to the HBsAg Level at the Initial Visit

Table II summarizes the clinical profiles and laboratory data according to the HBsAg level at the initial visit of 1,610 patients infected with HBV. Patients with the HBsAg levels below 500 IU/ml were significantly older and exhibited lower inflammatory activity (lower levels of AST and ALT), and had lower viral levels (they were HBeAg negative and had lower levels of HBcrAg/HBV DNA), compared to those with the HBsAg levels above 500 IU/ml ($P < 0.001$).

Factors Associated With High AFP Levels at the Initial Visit, Stratified According to the HBsAg Levels

Blood samples from all 1,610 patients were analyzed to determine the factors that affect the AFP level at the initial visit. Among 1,277 patients with the HBsAg levels more than 500 IU/ml at the initial visit, high AFP levels were detected in 228 (17.9%) patients. Univariate analysis identified 12 parameters that correlated significantly with a high AFP level at the initial visit. These included age (≥30 years; $P < 0.001$), AST (≥34 IU/L; $P < 0.001$), ALT (≥43 IU/L; $P < 0.001$), albumin (<3.9 g/dl; $P < 0.001$), GGT (≥50 IU/L; $P < 0.001$), total bilirubin (≥1.0 mg/dl; $P < 0.001$), platelet count ($<20.0 \times 10^4/mm^3$; $P < 0.001$), HBV genotype (C; $P < 0.001$), HBsAg levels (<7,000 IU/ml; $P < 0.001$), HBeAg (positive; $P < 0.001$), HBV DNA (≥5.0 log copies/ml; $P < 0.001$), and HBcrAg (≥3.0 log U/ml; $P < 0.001$). Multivariate analysis that included the above variables identified seven factors that influenced independently the elevated AFP level at the initial visit. These included HBsAg level (<7,000 IU/ml; OR 3.69, $P < 0.001$), albumin (<3.9 g/dl; OR 3.09, $P < 0.001$), platelet count ($<20.0 \times 10^4/mm^3$; OR 2.50, $P = 0.001$), GGT (≥50 IU/L; OR 2.28, $P = 0.001$), AST (≥34 IU/L; OR 2.77, $P = 0.003$), HBeAg (positive; OR 2.07, $P = 0.005$), and HBcrAg (≥3.0 log U/ml; OR 5.10, $P = 0.031$) (Table III).

Among 333 patients with the HBsAg levels less than 500 IU/ml, a high AFP at the initial visit was detected in 42 (12.6%) patients. Univariate analysis identified nine parameters that correlated significantly with a high AFP level at the initial visit. These included AST (≥34 IU/L; $P < 0.001$), ALT (≥43 IU/L; $P = 0.001$), albumin (<3.9 g/dl; $P < 0.001$), GGT (≥50 IU/L; $P < 0.001$), platelet count ($<20.0 \times 10^4/mm^3$; $P = 0.001$), HBV genotype (C; $P < 0.001$), HBeAg (positive; $P < 0.001$), HBV DNA (≥5.0 log copies/ml; $P = 0.001$), and HBcrAg (≥3.0 log U/ml; $P < 0.001$). Multivariate analysis that included the above variables identified three factors that influenced independently the elevated AFP level at the initial visit. These included albumin (<3.9 g/dl; OR 12.8, $P < 0.001$), GGT (≥50 IU/L; OR 6.95, $P = 0.002$), and HBcrAg (≥3.0 log U/ml; OR 5.62, $P = 0.010$) (Table III).

Factors Associated With High AFP Levels at the Initial Visit According to the HBsAg Levels in Patients With Low Transaminase Levels

To minimize the effect of inflammatory activity, we examined the data of 618 (among 1,610 patients) who

TABLE II. Profiles and Laboratory Data of Patients Infected With HBV According to the HBsAg Level at the Initial Visit

	HBsAg <500 IU/L	HBsAg ≥500 IU/L	*P*
Demographic data			
Number of patients	333	1,277	
Sex (male/female)	227/106	820/457	NS
Age (years)*	49 (18–75)	38 (18–83)	<0.001
Family history of liver disease[a]	130 (39.0%)	706 (55.3%)	<0.001
Lifetime cumulative alcohol intake (≥500 kg)	32 (9.6%)	80 (6.3%)	0.037
Laboratory data*			
Total bilirubin (mg/dl)	0.7 (0.2–2.9)	0.6 (0.1–2.9)	0.033
Aspartate aminotransferase (IU/L)	29 (12–175)	40 (5–220)	<0.001
Alanine aminotransferase (IU/L)	32 (7–289)	56 (5–297)	<0.001
Albumin (g/dl)	4.2 (1.1–5.6)	4.2 (1.0–5.5)	NS
Gamma-glutamyl transpeptidase (IU/L)	36 (2–2,370)	38 (4–1,638)	NS
Hemoglobin (g/dl)	14.4 (8.4–17.4)	14.6 (6.9–18.2)	NS
Platelet count ($\times 10^4/mm^3$)	19.1 (2.7–39.6)	19.2 (3.1–44.7)	NS
Alpha-fetoprotein (μg/L)	4 (1–968)	4 (1–1,770)	0.005
Virological data			
HBeAg (No. of positive)	37 (11.1%)	653 (51.1%)	<0.001
HBsAg (IU/ml)*	123 (0.09–498)	4,680 (503 to >125,000)	<0.001
HBcrAg (log U/ml)*	<3.0 (<3.0 to >6.8)	5.9 (<3.0 to >6.8)	<0.001
HBV DNA (log copies/ml)*	3.7 (<2.1 to >9.1)	6.6 (<2.1 to >9.1)	<0.001
HBV genotype (A/B/C/others/ND)	7/104/141/0/81	58/114/978/6/121	<0.001

NS; not significant.
Data are number/percentages of patients, except those denoted by *, which represent the median (range) values.
[a]Family history of positivity for hepatitis B surface antigen including third-degree relatives.

TABLE III. Results of Multivariate Logistic Analysis for Factors Associated With the High AFP Levels at the Initial Visit

Factor	Category	Risk ratio (95%CI)	*P*
Patients with the HBsAg levels above 500 IU/ml (n = 1,277)			
HBsAg (IU/ml)	1: ≥7,000	1	
	2: <7,000	3.69 (2.12–6.41)	<0.001
Albumin (g/dl)	1: ≥3.9	1	
	2: <3.9	3.09 (1.88–5.05)	<0.001
Platelet count ($\times 10^4$/mm^3)	1: ≥20.0	1	
	2: <20.0	2.50 (1.47–4.24)	0.001
Gamma-glutamyl transpeptidase (IU/L)	1: <50	1	
	2: ≥50	2.28 (1.40–3.72)	0.001
Aspartate aminotransferase (IU/L)	1: <34	1	
	2: ≥34	2.77 (1.42–5.39)	0.003
HBeAg	1: Negative	1	
	2: Positive	2.07 (1.24–3.45)	0.005
HBcrAg (log U/ml)	1: <3.0	1	
	2: ≥3.0	5.10 (1.16–22.4)	0.031
Patients with the HBsAg levels below 500 IU/ml (n = 333)			
Albumin (g/dl)	1: ≥3.9	1	
	2: <3.9	12.8 (4.02–41.7)	<0.001
Gamma-glutamyl transpeptidase (IU/L)	1: <50	1	
	2: ≥50	6.95 (2.06–23.5)	0.002
HBcrAg (log U/ml)	1: <3.0	1	
	2: ≥3.0	5.62 (1.51–21.0)	0.010

Low transaminase levels were defined as transaminase levels below the upper limit of normal.

had low transaminase levels (AST ≤33 IU/L and ALT ≤42 IU/L, i.e., below the upper limits of normal) to further determine those factors that determine the high level of AFP at the initial visit. High AFP was detected in 26 (6.1%) patients among 426 with the HBsAg levels above 500 IU/ml and low transaminase levels. Using the data of these patients, univariate analysis identified three parameters that correlated significantly with a high AFP level at the initial visit. These included albumin (<3.9 g/dl; $P = 0.004$), platelet count (<20.0 × 10^4/mm^3; $P = 0.012$), and HBsAg levels (<7,000 IU/ml; $P = 0.004$). Multivariate analysis that included the above variables identified albumin (<3.9 g/dl; OR 3.92, $P = 0.001$) and HBsAg levels (<7,000 IU/ml; OR 4.33, $P = 0.004$) as independent determinants of a high AFP level at the initial visit (Table IV).

Among 192 patients with the HBsAg levels below 500 IU/ml and low transaminase levels, high AFP levels were detected at the initial visit in 12 (6.3%). Univariate analysis identified three parameters that influenced significantly the elevated AFP level at the initial visit. These included albumin (<3.9 g/dl; $P = 0.010$), GGT (≥50 IU/L; $P = 0.011$), and platelet count (<20.0 × 10^4/mm^3; $P = 0.020$). Multivariate analysis that included these variables identified albumin (<3.9 g/dl; OR 7.19, $P = 0.004$) as the only independent determinant of a high AFP level at the initial visit (Table IV).

TABLE IV. Results of Multivariate Analysis for Factors Associated With the High AFP Levels at the Initial Visit

Factor	Category	Risk ratio (95%CI)	*P*
Patients with HBsAg >500 IU/ml and low transaminase levels (n = 426)			
Albumin (g/dl)	1: ≥3.9	1	
	2: <3.9	3.92 (1.71–9.01)	0.001
HBsAg (IU/ml)	1: ≥7,000	1	
	2: <7,000	4.33 (1.58–11.9)	0.004
Patients with HBsAg <500 IU/ml and low transaminase levels (n = 192)			
Albumin (g/dl)	1: ≥3.9	1	
	2: <3.9	7.19 (1.87–27.8)	0.004

Low transaminase levels were defined as transaminase levels below the upper limit of normal.

DISCUSSION

There is little information on the cutoff value of AFP that can be used to predict the future probability of HCC in patients with HBV infection. The present study followed-up patients naïve to antiviral therapy from the initial visit and showed that the rate of hepatocarcinogenesis was significantly higher in those with high AFP levels at the baseline than those with low levels. To our knowledge, the present study is the first to report the hepatocarcinogenesis rate stratified according to the AFP level in patients infected with HBV but free of HCC at the initial visit, based on a large-scale long-term follow-up cohort. The results indicated that patients with high AFP levels at the initial visit are at high risk of HCC, and emphasize the need to determine the factors that could affect the AFP level as surrogate markers of early hepatocarcinogenesis. Previous studies in patients with HCV infection indicated that suppression of the AFP level by treatment with interferon reduced the HCC risk even in those without complete eradication of HCV [Arase et al., 2007; Asahina et al., 2013]. However, there is little

evidence that suppression of the AFP level by antiviral therapy reduces the HCC risk in patients with HBV infection. Further prospective studies are needed to investigate this issue in detail.

In the present study, the relationship between the HBsAg levels and the AFP levels detected at the initial visit suggested the presence of two distinct groups within the study patients. Interestingly, in patients with the HBsAg levels above 500 IU/ml, a significant negative correlation was observed between the HBsAg and the AFP levels, and a significant positive correlation was observed between the HBsAg and the platelet count. Previous studies indicated that high serum AFP levels correlated with liver fibrosis Stage 3 and 4 [Bayati et al., 1998; Chu et al., 2001; Hu et al., 2002, 2004], and that lower thrombocytopenia was closely associated with advanced liver disease [Ikeda et al., 2009; Akuta et al., 2012]. Considered together, these results emphasize the importance of hyper-α-fetoproteinemia and thrombocytopenia in the prediction of severe liver fibrosis, respectively. Based on the present results and the recent reports suggesting the potential correlation between the HBsAg level and the stage of liver fibrosis [Seto et al., 2012; Martinot-Peignoux et al., 2013], it is possible that HBsAg levels could correlate with the stage of fibrosis in patients with the HBsAg levels above 500 IU/ml. Further studies are needed to determine the value of hyper-α-fetoproteinemia in patients with low and high HBsAgemia.

In addition to the HBsAg level, multivariate analysis also identified HBcrAg as another viral factor that influenced independently the AFP level at the baseline. HBcrAg comprises HBcAg, HBeAg and a 22-kDa precore protein coded with the precore/core gene [Kimura et al., 2002, 2005]. Previous studies reported a significant correlation between serum HBcrAg concentrations and intrahepatic levels of covalently closed circular DNA (cccDNA) [Wong et al., 2007; Suzuki et al., 2009]. Other studies indicated that HBcrAg is a useful predictor of HCC during antiviral therapy [Kumada et al., 2013], and post-treatment recurrence of HCC during antiviral therapy [Hosaka et al., 2010]. The present study, based on patients naïve to antiviral therapy showed that high serum HBcrAg concentrations also correlated with high AFP at the initial visit. This is the first report demonstrating the potential usefulness of HBcrAg as a surrogate marker for early hepatocarcinogenesis.

The impact of the HBsAg level on hepatocarcinogenesis is not clear at this stage. In this study, the effect of the HBsAg levels at the initial visit on HCC was assessed in 1,061 consecutive antiviral therapy-naive patients infected with HBV. Analysis of data of 794 patients with the HBsAg levels above 500 IU/ml at the initial visit (after exclusion of patients on antiviral therapy) showed a significantly lower cumulative HCC rate in patients with the HBsAg levels above 7,000 IU/ml than those with levels below 7,000 IU/ml ($P < 0.001$, Log-rank test, Fig. 4). This result suggests that HBsAg levels at the baseline do not only influence AFP, but also play a role in hepatocarcinogenesis. Further studies need to be performed to determine the pathomechanisms of HBsAg in hepatocarcinogenesis.

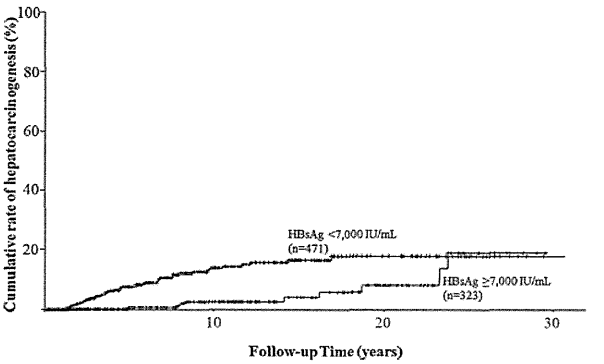

Fig. 4. Cumulative rate of hepatocarcinogenesis stratified according to the HBsAg levels at the initial visit in patients naïve to antiviral therapy from the initial visit until last visit. In a preliminary study based on 794 patients with the HBsAg levels above 500 IU/ml at the initial visit, the cumulative hepatocarcinogenesis rate for patients with the HBsAg levels more than 7,000 IU/ml was significantly lower than for those with levels below 7,000 IU/ml ($P < 0.001$; Log-rank test).

The present study has certain limitations. First, the study did not examine the effects of other genotypes, apart from HBV genotype B or C. Second, the study population was limited to Japanese and did not include other races, and thus generalization of the results to other races cannot be made based on the results. Third, the study did not investigate the effects of antiviral therapy (interferon and/or nucleot(s)ide analogs) on the outcome since such therapy suppressed the AFP levels and thus reduce the risk of HCC in patients with HBV infection.

In conclusion, the present studies demonstrated that the HBsAg level seem to influence the AFP levels and can be used as a surrogate marker for early hepatocarcinogenesis in patients with hepatitis B viral infection.

REFERENCES

Akuta N, Suzuki F, Seko Y, Kawamura Y, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Hara T, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Kumada H. 2012. Complicated relationships of amino acid substitution in hepatitis C virus core region and IL28B genotype influencing hepatocarcinogenesis. Hepatology 56:2134–2141.

Alpert E, Feller ER. 1978. α-fetoprotein (AF) in benign liver disease. Gastroenterology 74:856–858.

Arase Y, Ikeda K, Suzuki F, Suzuki Y, Kobayashi M, Akuta N, Hosaka T, Sezaki H, Yatsuji H, Kawamura Y, Kobayashi M, Kumada H. 2007. Prolonged-interferon therapy reduces hepatocarcinogenesis in aged-patients with chronic hepatitis C. J Med Virol 79:1095–1102.

Asahina Y, Tsuchiya K, Nishimura T, Muraoka M, Suzuki Y, Tamaki N, Yasui Y, Hosokawa T, Ueda K, Nakanishi H, Itakura J, Takahashi Y, Kurosaki M, Enomoto N, Nakagawa M, Kakinuma S, Watanabe M, Izumi N. 2013. α-Fetoprotein levels after interferon therapy and risk of hepatocarcinogenesis in chronic hepatitis C. Hepatology 58:1253–1262.

Bayati N, Silverman Al, Gordon SC. 1998. Serum alpha-fetoprotein levels and liver histology in patients with chronic hepatitis C. Am J Gastroenterol 93:2452–2456.

Bergstrand CG, Czar B. 1956. Demonstration of a new protein fraction in serum from the human fetus. Scand J Clin Lab Invest 8:174.

Bruix J, Sherman M. 2005. Practice guidelines committee, American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma. Hepatology 42:1208–1236.

Brunetto MR, Moriconi F, Bonino F, Lau GK, Farci P, Yurdaydin C, Piratvisuth T, Luo K, Wang Y, Hadziyannis S, Wolf E, McCloud P, Batrla R, Marcellin P. 2009. Hepatitis B virus surface antigen levels: A guide to sustained response to peginterferon alfa-2a in HBeAg-negative chronic hepatitis B. Hepatology 49:1141–1150.

Brunetto MR, Oliveri F, Colombatto P, Moriconi F, Ciccorossi P, Coco B, Romagnoli V, Cherubini B, Moscato G, Maina AM, Cavallone D, Bonino F. 2010. Hepatitis B surface antigen serum levels help to distinguish active from inactive hepatitis B virus genotype D carriers. Gastroenterology 139:483–490.

Chan HL. 2012. Identifying hepatitis B carriers at low risk for hepatocellular carcinoma. Gastroenterology 142:1057–1060.

Chan HL, Wong VW, Wong GL, Tse CH, Chan HY, Sung JJ. 2010. A longitudinal study on the natural history of serum hepatitis B surface antigen changes in chronic hepatitis B. Hepatology 52:1232–1241.

Chan HL, Thompson A, Martinot-Peignoux M, Piratvisuth T, Cornberg M, Brunetto MR, Tillmann HL, Kao JH, Jia JD, Wedemeyer H, Locarnini S, Janssen HL, Marcellin P. 2011. Hepatitis B surface antigen quantification: Why and how to use it in 2011—A core group report. J Hepatol 55:1121–1131.

Cheema AW, Hirschtritt T, Van Thiel DH. 2004. Markedly elevated alpha-fetoprotein levels without hepatocellular carcinoma. Hepatogastroenterology 51:1676–1678.

Chen DS, Sung JL. 1979. Relationship of hepatitis B surface antigen to serum alpha-fetoprotein in nonmalignant diseases of the liver. Cancer 44:984–992.

Chu CW, Hwang SJ, Luo JC, Lai CR, Tsay SH, Li CP, Wu JC, Chang FY, Lee SD. 2001. Clinical, virological, and pathologic significance of elevated serum alpha-fetoprotein levels in patients with chronic hepatitis C. J Clin Gastroenterol 32:240–244.

Di Bisceglie AM, Hoofnagle JH. 1989. Elevations in serum alpha-fetoprotein levels in patients with chronic hepatitis B. Cancer 64:2117–2120.

Ebara M, Ohto M, Shinagawa T, Sugiura N, Kimura K, Matsutani S, Morita M, Saisho H, Tsuchiya Y, Okuda K. 1986. Natural history of minute hepatocellular carcinoma smaller than three centimeters complicating cirrhosis. A study in 22 patients. Gastroenterology 90:289–298.

Elftherious N, Heathcote J, Thomas HC, Sherlock S. 1977. Serum alpha-fetoprotein levels in patients with acute and chronic liver disease. J Clin Pathol 30:704–708.

Hosaka T, Suzuki F, Kobayashi M, Hirakawa M, Kawamura Y, Yatsuji H, Sezaki H, Akuta N, Suzuki Y, Saitoh S, Arase Y, Ikeda K, Kobayashi M, Kumada H. 2010. HBcrAg is a predictor of post-treatment recurrence of hepatocellular carcinoma during antiviral therapy. Liver Int 30:1461–1470.

Hu KQ, Esrailian E, Thompson K, Chase R, Kyulo N, Hassen M, Abdelhalim F, Hillebrand DJ, Runyon BA. 2002. Hepatic steatosis is associated with disease progression of chronic hepatitis C: A large cohort study in the United States. Hepatology 36:349A.

Hu KQ, Kyulo N, Lim N, Elhazin B, Hillebrand DJ, Bock T. 2004. Clinical significance of elevated alpha-fetoprotein (AFP) in patients with chronic hepatitis C, but not hepatocellular carcinoma. Am J Gastroenterol 99:860–865.

Ikeda K, Arase Y, Kawamura Y, Yatsuji H, Sezaki H, Hosaka T, Akuta N, Kobayashi M, Saitoh S, Suzuki F, Suzuki Y, Kumada H. 2009. Necessities of interferon therapy in elderly patients with chronic hepatitis C. Am J Med 122:479–486.

Jaroszewicz J, Calle Serrano B, Wursthorn K, Deterding K, Schlue J, Raupach R, Flisiak R, Bock CT, Manns MP, Wedemeyer H, Cornberg M. 2010. Hepatitis B surface antigen (HBsAg) levels in the natural history of hepatitis B virus (HBV)-infection: A European perspective. J Hepatol 52:514–522.

Johnson PJ. 2001. The role of serum alpha-fetoprotein estimation in the diagnosis and management of hepatocellular carcinoma. Clin Liv Dis 5:145–159.

Kew MC, Purves LR, Bersohn I. 1973. Serum alpha-fetoprotein levels in acute viral hepatitis. Gut 14:939–942.

Kimura T, Rokuhara A, Sakamoto Y, Yagi S, Tanaka E, Kiyosawa K, Maki N. 2002. Sensitive enzyme immunoassay for hepatitis B virus core-related antigens and their correlation to virus load. J Clin Microbiol 40:439–445.

Kimura T, Ohno N, Terada N, Rokuhara A, Matsumoto A, Yagi S, Tanaka E, Kiyosawa K, Ohno S, Maki N. 2005. Hepatitis B virus DNA-negative dane particles lack core protein but contain a 22-kDa precore protein without C-terminal arginine-rich domain. J Biol Chem 280:21713–21719.

Kobayashi M, Arase Y, Ikeda K, Tsubota A, Suzuki Y, Saitoh S, Kobayashi M, Suzuki F, Akuta N, Someya T, Matsuda M, Sato J, Kumada H. 2002. Clinical characteristics of patients infected with hepatitis B virus genotypes A, B, and C. J Gastroenterol 37:35–39.

Kumada T, Toyoda H, Tada T, Kiriyama S, Tanikawa M, Hisanaga Y, Kanamori A, Niinomi T, Yasuda S, Andou Y, Yamamoto K, Tanaka J. 2013. Effect of nucleos(t)ide analogue therapy on hepatocarcinogenesis in chronic hepatitis B patients: A propensity score analysis. J Hepatol 58:427–433.

Liaw YF. 2011. Clinical utility of hepatitis B surface antigen quantitation in patients with chronic hepatitis B: A review. Hepatology 54:E1–E9.

Martinot-Peignoux M, Lada O, Cardoso AC, Lapalus M, Boyer N, Ripault MP, Asselah T, Marcellin P. 2010. Quantitative HBsAg: A new specific marker for the diagnosis of HBsAg inactive carriage. Hepatology 52:992A.

Martinot-Peignoux M, Carvalho-Filho R, Lapalus M, Netto-Cardoso AC, Lada O, Batrla R, Krause F, Asselah T, Marcellin P. 2013. Hepatitis B surface antigen serum level is associated with fibrosis severity in treatment-naïve, E antigen-positive patients. J Hepatol 58:1089–1095.

Moucari R, Mackiewicz V, Lada O, Ripault MP, Castelnau C, Martinot-Peignoux M, Dauvergne A, Asselah T, Boyer N, Bedossa P, Valla D, Vidaud M, Nicolas-Chanoine MH, Marcellin P. 2009. Early serum HBsAg drop: A strong predictor of sustained virological response to pegylated interferon alfa-2a in HBeAg-negative patients. Hepatology 49:1151–1157.

Nguyen T, Thompson AJ, Bowden S, Croagh C, Bell S, Desmond PV, Levy M, Locarnini SA. 2010. Hepatitis B surface antigen levels during the natural history of chronic hepatitis B: A perspective on Asia. J Hepatol 52:508–513.

Sato Y, Nakata K, Kato Y, Shima M, Ishii N, Koji T, Taketa K, Endo Y, Nagataki S. 1993. Early recognition of hepatocellular carcinoma based on altered profiles of alpha-fetoprotein. N Engl J Med 328:1802–1806.

Seto WK, Wong DK, Fung J, Ip PP, Yuen JC, Hung IF, Lai CL, Yuen MF. 2012. High hepatitis B surface antigen levels predict insignificant fibrosis in hepatitis B e antigen positive chronic hepatitis B. PLoS ONE 7:e43087.

Shinagawa T, Ohto M, Kimura K, Tsunetomi S, Morita M, Saisho H, Tsuchiya Y, Saotome N, Karasawa E, Miki M. 1984. Diagnosis and clinical features of small hepatocellular carcinoma with emphasis on the utility of real-time ultrasonography. A study in 51 patients. Gastroenterology 86:495–502.

Silver HK, Gold P, Shuster J, Javitt NB, Freedman SO, Finlayson ND. 1974. Alpha 1-fetoprotein in chronic liver disease. N Engl J Med 291:506–508.

Suzuki F, Miyakoshi H, Kobayashi M, Kumada H. 2009. Correlation between serum hepatitis B virus core-related antigen and intrahepatic covalently closed circular DNA in chronic hepatitis B patients. J Med Virol 81:27–33.

Tseng TC, Liu CJ, Yang HC, Su TH, Wang CC, Chen CL, Kuo SF, Liu CH, Chen PJ, Chen DS, Kao JH. 2012. High levels of hepatitis B surface antigen increase risk of hepatocellular carcinoma in patients with low HBV load. Gastroenterology 142:1140–1149.

Viola LA, Barrison IG, Coleman JC, Paradinas FJ, Fluker JL, Evans BA, Murray-Lyon IM. 1981. Natural history of liver disease in chronic hepatitis B surface antigen carriers. Survey of 100 patients from Great Britain. Lancet 2:1156–1159.

Wong DK, Tanaka Y, Lai CL, Mizokami M, Fung J, Yuen MF. 2007. Hepatitis B virus core-related antigens as markers for monitoring chronic hepatitis B infection. J Clin Microbiol 45:3942–3947.

Yao FY. 2003. Dramatic reduction of the alpha-fetoprotein level after lamivudine treatment of patients with chronic hepatitis B virus infection and cirrhosis. J Clin Gastroenterol 36:440–442.

CASE REPORT

Gut and Liver, Vol. 7, No. 2, March 2013, pp. 246-251

Transcatheter Arterial Chemotherapy with Miriplatin for Hepatocellular Carcinoma Patients with Chronic Renal Failure: Report of Three Cases

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Miriplatin is a novel lipophilic platinum complex that was developed to treat hepatocellular carcinoma (HCC). Although HCC patients frequently have coexisting chronic renal failure, little prospective data are available regarding the clinical toxicity of chemotherapeutic agents used to treat HCC patients with chronic renal failure. In a phase II study, the plasma concentration of total platinum in patients who received miriplatin was very low, and no severe renal toxicity caused by miriplatin injection was reported. Here, we present three cases of HCC with stage 4 chronic renal failure who received transcatheter arterial chemotherapy with miriplatin. All cases were male, ages 72, 84, and 83 years, and had serum creatinine levels of 2.3, 1.6, and 1.9 mg/dL, respectively. Their estimated glomerular filtration rates were 21.9, 20.3, and 22.2 mL/min, respectively. All cases were treated for unresectable HCC with transcatheter arterial chemotherapy with miriplatin. No serious adverse events were observed, and serum creatinine levels did not elevate, even in the patient who experienced renal failure caused by cisplatin administration. These results might suggest that transcatheter arterial chemotherapy with miriplatin can be safely used in HCC patients with chronic renal failure. **(Gut Liver 2013;7:246-251)**

Key Words: Miriplatin; Chronic renal failure; Hepatocellular carcinoma

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant diseases worldwide.[1] Since curative therapies, including resection, liver transplantation, and percutaneous ablation (percutaneous ethanol injection and radiofrequency ablation [RFA]) are applicable in only 30% to 40% of HCC patients, transcatheter arterial chemoembolization (TACE) has been recognized as an effective palliative treatment option for patients with advanced HCC.[2-7] HCC patients frequently have coexisting cirrhosis, which is a predisposing factor for the development of renal dysfunction due to intravascular volume depletion, inadequate renal vasoconstriction, and hyperaldosteronism.[8-13]

Little prospective data are available regarding the clinical toxicity of chemotherapeutic agents used to treat HCC patients with chronic renal failure. Although cisplatin is an effective anticancer drug that is widely used for the treatment of many malignancies, including HCC, it is associated with significant nephrotoxicity, particularly in patients with chronic renal failure.[2,7] Miriplatin is a novel cisplatin derivative containing platinum with a high affinity for the iodized ethyl ester of fatty acids of poppyseed oil (Lipiodol Ultra-fluide; Laboratoire Guerbet, Aulnay-Sous-Bois, France) that is used in TACE. Clinical trials have demonstrated that miriplatin is effective in the treatment of HCC.[14-19]

In a Phase II HCC study, the plasma concentration of total platinum in patients receiving miriplatin was very low, and no severe renal toxicity caused by miriplatin injection was reported.[17] Here we present three cases of HCC with stage 4 chronic renal failure who received transcatheter arterial chemotherapy with miriplatin.[20]

CASE REPORTS

1. Case 1

A 72-year-old man with HCC, liver cirrhosis, and diabetic nephropathy had undergone RFA four times and TACE three times over 5 years. As shown in Fig. 1, a computed tomography (CT) scan of the liver revealed multiple HCCs (tumor size, 15 to 34 mm; tumor number, three; stage, T2N0M0). The serum creati-

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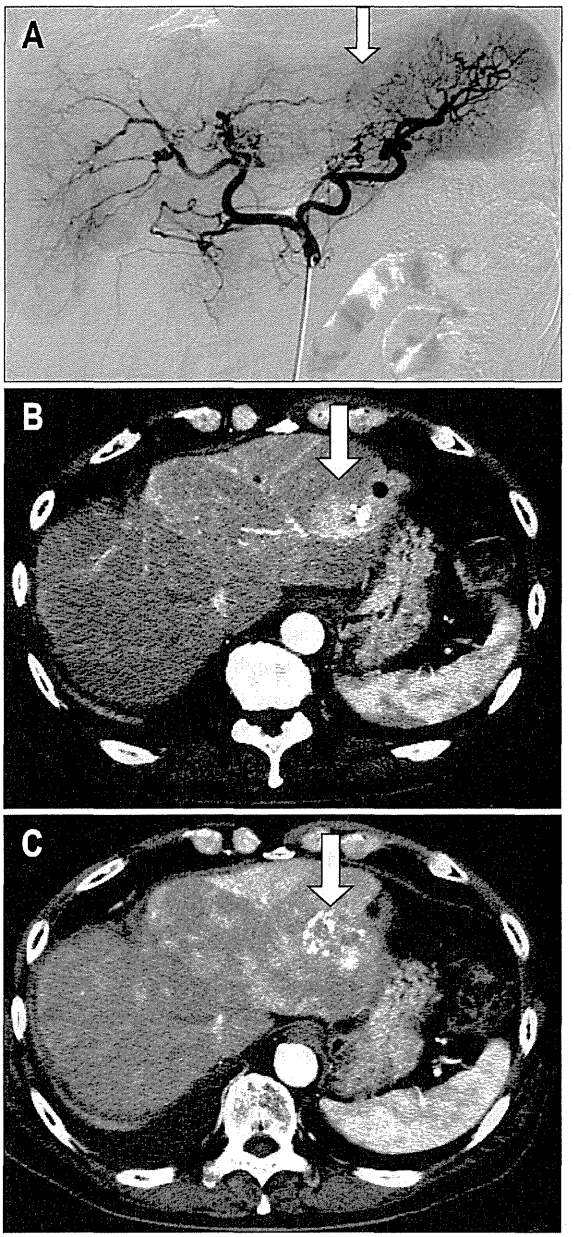

Fig. 1. Case 1. A 72-year-old man with unresectable hepatocellular carcinoma (HCC) who received transcatheter arterial chemoembolization (TACE) with miriplatin. (A) Abdominal angiography showed multiple HCCs (arrow). (B) Computed tomography (CT) showed multiple HCCs (arrow). (C) CT performed 1 month after TACE. The lesions revealed accumulations of lipiodol (arrow). Treatment efficacy was assessed as a partial response.

nine level was 2.3 mg/dL, and the estimated glomerular filtration rate (GFR) was 21.9 mL/min (Table 1).[21]

The patient was hydrated through a peripheral line. The femoral artery was catheterized under local anesthesia, and catheter was inserted superselectively into the hepatic artery that supplied the target tumor, for injection of the miriplatin/lipiodol suspension and 1 mm gelatin particles (1 mm-Gelpart; Nippon Kayaku, Tokyo, Japan). Miriplatin/lipiodol suspension was administrated slowly under careful fluoroscopic guidance. The dose of miriplatin/lipiodol was determined according to tumor size and the degree of liver dysfunction. The patient received TACE with miriplatin (miriplatin 50 mg, lipiodol 2.5 mL, and 1 mm-Gelpart were injected from both the right and left hepatic arteries). Therapy was well tolerated, and the patient's weight and serum creatinine level remained stable after treatment (Fig. 2). Major side effects included grade 1 fever, elevated blood glucose, and grade 1 nausea, which all resolved within 1 week (the National Cancer Institute's Common Terminology Criteria for Adverse Events [CTCAE] version 4.0). Treatment efficacy was assessed 1 month after treatment. Partial response (modified response evaluation criteria in solid tumors, mRECIST) was achieved in all target lesions.[22]

The patient was received two times TACE with miriplatin at intervals of 4 months after the first administration (second and third dosage of miriplatin were 120 mg and dosage of lipiodol were 6 mL). The patient's weight and serum creatinine level still remained stable after repeat injection of miriplatin (serum creatinine level was 2.2 mg/dL after third TACE with miriplatin). Stable disease (mRECIST) was achieved in all target lesions after third TACE with miriplatin.

2. Case 2

An 84-year-old man with HCC, liver cirrhosis, and chronic renal failure had undergone RFA three times and TACE six times over 10 years. As shown in Fig. 3, a CT scan of the liver showed multiple HCCs (tumor size, 12 to 55 mm; tumor number, six; stage, T3N0M0). The serum creatinine level was 1.6 mg/dL, and the estimated GFR was 20.3 mL/min (Table 1).

The patient was hydrated through a peripheral line. The femoral artery was catheterized under local anesthesia, and catheter was inserted superselectively into the hepatic artery that supplied the target tumor, for injection of the miriplatin/lipiodol suspension. Miriplatin/lipiodol suspension was administrated slowly under careful fluoroscopic guidance. The dose of miriplatin/lipiodol was determined according to tumor size and the degree of liver dysfunction.

The patient received transcatheter arterial chemotherapy with miriplatin (miriplatin 50 mg and lipiodol 2.5 mL were injected from both the right and left hepatic arteries). Therapy was well tolerated, and the patient's weight and serum creatinine level remained stable after treatment (Fig. 2). The major side effect of treatment was grade 1 fever, which resolved within 1 week (CTCAE version 4.0). Treatment efficacy was assessed 2 months after therapy. Stable disease (mRECIST) was achieved in all target lesions.

3. Case 3

An 83-year-old man with HCC, liver cirrhosis, hypertension,

Table 1. Patient Characteristics

Characteristic	Case 1	Case 2	Case 3
Age	72	84	83
Gender	Male	Male	Male
Height, cm	159	160	162
Weight, kg	58	47	57
Serum creatinine, mg/dL*	2.3	1.6	1.9
Estimated GFR1, mL/min†	21.9	20.3	22.2
Estimated GFR2, mL/min‡	22.8	32.5	27.0
Etiology	HCV	HCV	HBV
Child-Pugh score	A (6)	A (5)	A (5)
ICG-R15, %	16	13	4
Underlying disease that caused renal failure	Diabetic nephropathy	Chronic glomerulonephritis	Cisplatin induced renal failure
Tumor no.	3	6	40
Maximum tumor size, mm	34	55	39
Cancer stage (TNM)	II (T2N0M0)	III (T3N0M0)	II (T2N0M0)
Dosage of miriplatin, mg	100	100	70
Dosage of lipiodol, mL	5	5	3.5
Use of gelatin sponge particles	Yes	No	Yes
Contrast medium, mL	Iomeprol 60	Iomeprol 50	Iomeprol 190
Use of hydration therapy after miriplatin infusion	Yes	Yes	Yes

GFR, glomerular filtration rate; HCV, hepatitis C virus; HBV, hepatitis B virus; ICG-R15, indocyanine green retention rate at 15 minutes.
*Enzymatic method; †Cockcroft and Gault formula; ‡Japanese equation for estimating GFR.

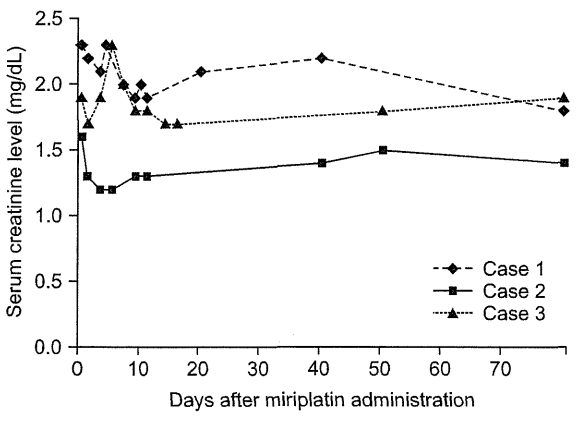

Fig. 2. Serum creatinine level after miriplatin administration in the three cases.

and renal failure that had been caused by cisplatin administration had undergone TACE nine times over 4 years. As shown in Fig. 4, a magnetic resonance imaging scan of the liver revealed multiple HCCs (tumor size, 5 to 39 mm; tumor number, 40; stage, T2N0M0). The patient's serum creatinine level was 1.9 mg/dL, and the estimated GFR was 22.2 mL/min (Table 1).

The patient was hydrated through a peripheral line. The femoral artery was catheterized under local anesthesia, and catheter was inserted superselectively into the hepatic artery that supplied the target tumor, for injection of the miriplatin/lipiodol suspension and 1 mm-Gelpart. Miriplatin/lipiodol suspension was administrated slowly under careful fluoroscopic guidance. The dose of miriplatin/lipiodol was determined according to tumor size and the degree of liver dysfunction.

The patient received TACE with miriplatin (miriplatin 30 mg, lipiodol 1.5 mL, and 1 mm-Gelpart were injected from the right and left hepatic arteries, and miriplatin 10 mg and lipiodol 0.5 mL were injected from the right inferior phrenic artery). Therapy was well tolerated, and the patient's weight and serum creatinine level remained stable after treatment (Fig. 2). Major side effects included grade 1 fever and grade 1 nausea, both of which resolved within 1 week (CTCAE version 4.0). Treatment efficacy was assessed 3 months after therapy. Stable disease (mRECIST) was achieved in all target lesions.

DISCUSSION

Various anticancer drugs, such as doxorubicin hydrochloride, epirubicin hydrochloride, mytomycin C, cisplatin, and neocarzinostatin, have been used at TACE agents for the treatment of HCC. However, the most effective and least toxic TACE protocol for HCC has yet to be identified.

Miriplatin is a novel lipophilic cisplatin derivative that can be suspended in lipiodol and used for transcatheter arterial che-

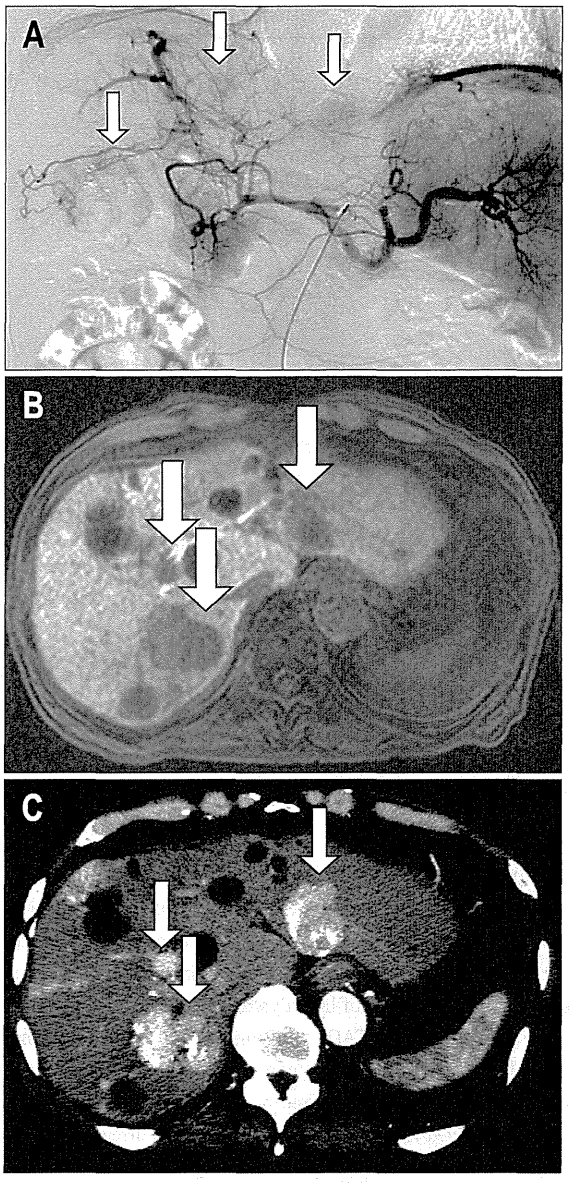

Fig. 3. Case 2. An 84-year-old man with unresectable hepatocellular carcinoma (HCC) who received transcatheter arterial chemotherapy with miriplatin. (A) Abdominal angiography showed multiple HCCs (arrows). (B) Magnetic resonance imaging (hepatobiliary phase) showed multiple HCCs (arrows). (C) Computed tomography performed 2 months after transcatheter arterial chemotherapy with miriplatin. The lesions showed accumulations of lipiodol (arrows). The treatment efficacy was assessed as a stable disease.

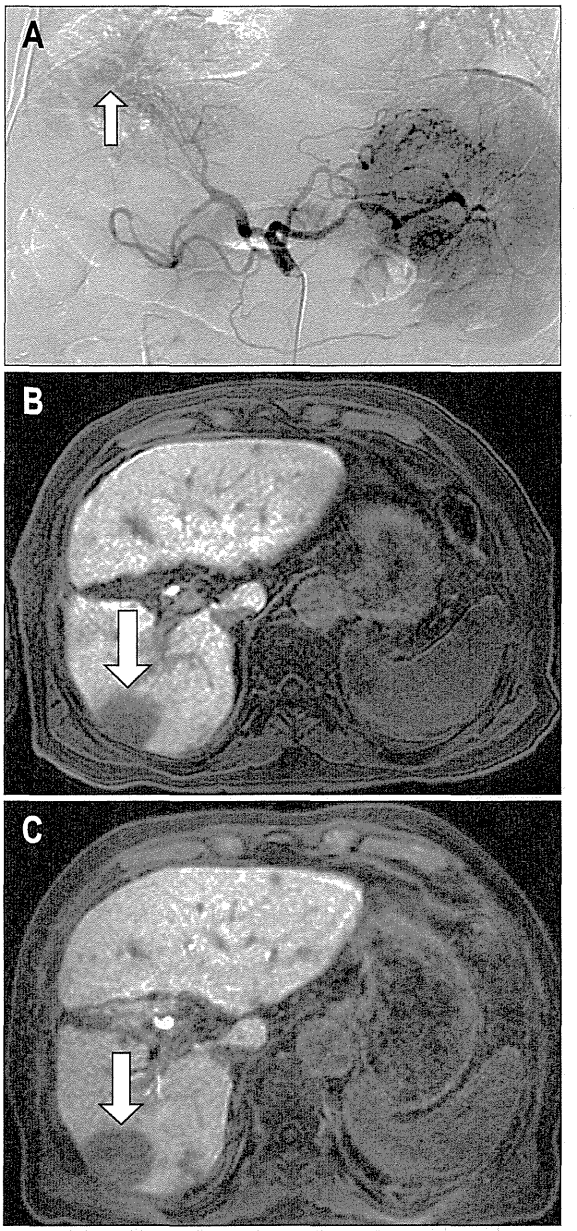

Fig. 4. Case 3. An 83-year-old man with unresectable hepatocellular carcinoma (HCC) who received transcatheter arterial chemoembolization (TACE) with miriplatin. (A) Abdominal angiography showed multiple HCCs (arrow). (B) Gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid (Gd-EOB-DTPA) enhanced magnetic resonance imaging (MRI; hepatobiliary phase) showed multiple HCCs (arrow). (C) Gd-EOB-DTPA enhanced MRI performed 3 months after TACE. The lesions showed accumulations of lipiodol (arrow). The treatment efficacy was assessed as a stable disease.

motherapy of advanced HCC. It is one of the platinum agents, although hydration after administration is not necessary of its weak renal toxicity.

Various types of resistance to therapy can occur during repetition of TACE. Platinum derivatives are frequently administered to patients with advanced HCC that is unresponsive to anthracycline and antibiotic drugs.[23]

Miriplatin was developed as a lipophilic platinum complex in an effort to produce a superior antitumor effect in HCC with lower toxicity compared to cisplatin. Miriplatin-lipiodol suspension is a stable colloidal emulsion that is deposited within HCC tumors, where it gradually releases active derivatives of miripla-

tin.

According to pharmacokinetic studies, the plasma concentration of total platinum in patients treated with miriplatin is much lower than that after administration in patients administered intra-arterial cisplatin: the Cmax is approximately 300-fold lower and the Tmax roughly 500-fold longer than the corresponding values for intra-arterial cisplatin.[17] Theoretically, therefore, it can be administered even in patients of advanced HCC patients with chronic renal failure if visceral angiography can be performed.

Clinical trials have shown that miriplatin is effective for the treatment of HCC, but the safety and efficacy of miriplatin has not been evaluated in HCC patients with chronic renal failure.[16,17] Herein we presented three HCC cases with stage 4 chronic renal failure who received transcatheter arterial chemotherapy with miriplatin. In all three cases, no serious adverse events were observed, and serum creatinine level did not increase, even in the patient who had experienced renal failure due to cisplatin administration (Fig. 2). Repeated injection of miriplatin appears to be also safe in HCC patients with chronic renal failure.

The present results might suggest that transcatheter arterial chemotherapy with miriplatin can be safely used in HCC patients with chronic renal failure. A prospective study is required to assess the most effective, least nephrotoxic anticancer agent among the various platinum derivatives. Miriplatin appears to be a promising agent for HCC patients with chronic renal failure.

CONFLICTS OF INTEREST

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REFERENCES

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74-108.
2. Kamada K, Nakanishi T, Kitamoto M, et al. Long-term prognosis of patients undergoing transcatheter arterial chemoembolization for unresectable hepatocellular carcinoma: comparison of cisplatin lipiodol suspension and doxorubicin hydrochloride emulsion. J Vasc Interv Radiol 2001;12:847-854.
3. Camma C, Schepis F, Orlando A, et al. Transarterial chemoembolization for unresectable hepatocellular carcinoma: meta-analysis of randomized controlled trials. Radiology 2002;224:47-54.
4. Ikeda M, Maeda S, Shibata J, et al. Transcatheter arterial chemotherapy with and without embolization in patients with hepatocellular carcinoma. Oncology 2004;66:24-31.
5. Takayasu K, Arii S, Ikai I, et al. Prospective cohort study of transarterial chemoembolization for unresectable hepatocellular carcinoma in 8510 patients. Gastroenterology 2006;131:461-469.
6. Marelli L, Stigliano R, Triantos C, et al. Transarterial therapy for hepatocellular carcinoma: which technique is more effective? A systematic review of cohort and randomized studies. Cardiovasc Intervent Radiol 2007;30:6-25.
7. Ikeda M, Maeda S, Ashihara H, Nagahama H, Tanaka M, Sasaki Y. Transcatheter arterial infusion chemotherapy with cisplatin-lipiodol suspension in patients with hepatocellular carcinoma. J Gastroenterol 2010;45:60-67.
8. Laragh JH, Cannon PJ, Bentzel CJ, Sicinski AM, Meltzer JI. Angiotensin Ii, Norepinephrine, and renal transport of electrolytes and water in normal man and in cirrhosis with ascites. J Clin Invest 1963;42:1179-1192.
9. Cardenas A, Ginès P, Uriz J, et al. Renal failure after upper gastrointestinal bleeding in cirrhosis: incidence, clinical course, predictive factors, and short-term prognosis. Hepatology 2001;34(4 Pt 1):671-676.
10. Moreau R, Lebrec D. Acute renal failure in patients with cirrhosis: perspectives in the age of MELD. Hepatology 2003;37:233-243.
11. Huo TI, Wu JC, Lee PC, Chang FY, Lee SD. Incidence and risk factors for acute renal failure in patients with hepatocellular carcinoma undergoing transarterial chemoembolization: a prospective study. Liver Int 2004;24:210-215.
12. Huo TI, Wu JC, Huang YH, et al. Acute renal failure after transarterial chemoembolization for hepatocellular carcinoma: a retrospective study of the incidence, risk factors, clinical course and long-term outcome. Aliment Pharmacol Ther 2004;19:999-1007.
13. Hsu CY, Huang YH, Su CW, et al. Renal failure in patients with hepatocellular carcinoma and ascites undergoing transarterial chemoembolization. Liver Int 2010;30:77-84.
14. Maeda M, Uchida NA, Sasaki T. Liposoluble platinum(II) complexes with antitumor activity. Jpn J Cancer Res 1986;77:523-525.
15. Kishimoto S, Ohtani A, Fukuda H, Fukushima S, Takeuchi Y. Relation between intracellular accumulation and cytotoxic activity of cis-[((1R, 2R)-1, 2-cyclohexanediamine-N, N')bis(myristato)] platinum(II) suspended in Lipiodol. Biol Pharm Bull 2003;26:683-686.
16. Fujiyama S, Shibata J, Maeda S, et al. Phase I clinical study of a novel lipophilic platinum complex (SM-11355) in patients with hepatocellular carcinoma refractory to cisplatin/lipiodol. Br J Cancer 2003;89:1614-1619.
17. Okusaka T, Okada S, Nakanishi T, Fujiyama S, Kubo Y. Phase II trial of intra-arterial chemotherapy using a novel lipophilic platinum derivative (SM-11355) in patients with hepatocellular carcinoma. Invest New Drugs 2004;22:169-176.
18. Hanada M, Baba A, Tsutsumishita Y, Noguchi T, Yamaoka T. Intra-hepatic arterial administration with miriplatin suspended in an oily lymphographic agent inhibits the growth of human hepatoma cells orthotopically implanted in nude rats. Cancer Sci 2009;100:189-194.
19. Hanada M, Baba A, Tsutsumishita Y, et al. Intra-hepatic arterial

administration with miriplatin suspended in an oily lymphographic agent inhibits the growth of tumors implanted in rat livers by inducing platinum-DNA adducts to form and massive apoptosis. Cancer Chemother Pharmacol 2009;64:473-483.

20. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Kidney Disease Outcome Quality Initiative. Am J Kidney Dis 2002;39(Suppl 2):S7-S245.
21. Matsuo S, Imai E, Horio M, et al. Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis 2009;53:982-992.
22. Lencioni R, Llovet JM. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. Semin Liver Dis 2010;30:52-60.
23. Kawamura Y, Ikeda K, Hirakawa M, et al. Efficacy of platinum analogue for advanced hepatocellular carcinoma unresponsive to transcatheter arterial chemoembolization with epirubicin. Hepatol Res 2009;39:346-354.

Original Paper

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Efficacy and Anticarcinogenic Activity of Ribavirin Combination Therapy for Hepatitis C Virus-Related Compensated Cirrhosis

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Key Words
Hepatitis C virus · Interferon · Ribavirin · Hepatocellular carcinoma · Cirrhosis · Biochemical response

Abstract
Objective: Anticarcinogenic activity of ribavirin combination therapy for hepatitis C virus (HCV)-related compensated cirrhosis is still unclear. ***Methods:*** In study 1, in 157 consecutive patients with HCV-related compensated cirrhosis, treatment efficacy with interferon plus ribavirin therapy was evaluated for 48 weeks of HCV genotype 1b (HCV-1b) or 24 weeks of HCV-2a/2b. In study 2, in 185 consecutive patients with HCV-related compensated cirrhosis, who showed no sustained virological response following the first course of interferon monotherapy, hepatocarcinogenesis rates were evaluated according to the additional treatment, and they were classified into three groups: no treatment, interferon monotherapy, and ribavirin combination therapy. ***Results:*** In study 1, in HCV-1b, rates of sustained virological response and sustained biochemical response were 21 and 56%, respectively. In HCV-2a/2b, rates of sustained virological response and sustained biochemical response were 70 and 78%, respectively. In HCV-1b, sustained biochemical response rates were significantly higher than those of sustained virological response. In study 2, the hepatocarcinogenesis rates in ribavirin combination therapy were significantly lower than those in interferon monotherapy and no treatment, respectively. ***Conclusion:*** Ribavirin combination therapy for HCV-related compensated cirrhosis reduces the risk of hepatocarcinogenesis in comparison with interferon monotherapy, and higher rates of sustained biochemical response might be associated with lower hepatocarcinogenesis rates.

Introduction

Hepatitis C virus (HCV) usually causes chronic infection, which can result in chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma [1–5]. The life expectancy of patients with HCV-related cirrhosis is largely influenced by the development of hepatocellular carcinoma during the clinical course [3]. Because an effective and curative therapy for hepatocellular carcinoma remains

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Table 1. Profile and laboratory data at the start of ribavirin combination therapy in 157 patients with HCV-related compensated cirrhosis (study 1)

Demographic data	
Patients, n	157[1]
Sex (male/female), n	105/52
Age, years	58 (34–74)
Laboratory data	
Serum aspartate aminotransferase, IU/l	69 (7–235)
Serum alanine aminotransferase, IU/l	70 (14–585)
Leukocytes, /mm^3	4,100 (1,600–8,800)
Hemoglobin, g/dl	14.0 (9.4–17.6)
Platelet count, $\times 10^4$/mm^3	11.3 (6.1–32.2)
HCV genotype (1b/2a/2b), n	120/27/10
Levels of viremia, log IU/ml	6.1 (3.9–7.5)
Treatment	
Past history of interferon-based therapy, n	95 (60.5%)
PEG-IFNα-2b/IFNα-2b, n	110/47
Ribavirin dose, mg/kg	10.7 (2.7–15.1)
Duration of treatment, weeks	
Genotype 1b	48 (1–48)
Genotype 2a or 2b	24 (5–24)

Unless otherwise indicated, values represent median (range).

[1] 24 of the 157 patients with HCV-related compensated cirrhosis in study 1 were also included in study 2. They showed no sustained virological response following the first course of interferon monotherapy (≥24 weeks) and were treated additionally with ribavirin combination therapy (≥24 weeks).

limited at best, primary prevention of hepatocellular carcinoma in patients with chronic liver disease is of great importance at present.

Treatment of HCV-chronic hepatitis with interferon can induce viral clearance and marked biochemical and histological improvement [6, 7]. Furthermore, previous studies showed that interferon monotherapy reduced the risk of hepatocellular carcinoma [8–10]. However, an extended analysis of the Hepatitis C Antiviral Long-Term Treatment against Cirrhosis (HALT-C) cohort recently showed that long-term peginterferon (PEG-IFN) monotherapy could not reduce the incidence of hepatocellular carcinoma among patients with advanced hepatitis C who did not achieve sustained virological response, and patients with cirrhosis who received PEG-IFN monotherapy had a lower risk of hepatocellular carcinoma than controls [11]. Thus, it is controversial whether interferon monotherapy for patients with liver cirrhosis might reduce hepatocarcinogensis. Furthermore, it is still unclear whether ribavirin combination therapy for patients with liver cirrhosis might reduce the risk of hepatocellular carcinoma, and there are also no reports on whether ribavirin combination therapy could reduce the risk in comparison with interferon monotherapy.

The present study investigated the efficacy and anticarcinogenic activity of ribavirin combination therapy for HCV-related compensated cirrhosis, especially in comparison with interferon monotherapy.

Materials and Methods

Study Population

Two retrospective cohort studies were performed to investigate treatment efficacy and anticarcinogenic activity of ribavirin combination therapy for HCV-related compensated cirrhosis.

In the study 1 cohort, 157 consecutive patients of HCV-related compensated cirrhosis were recruited into the study protocol of interferon (PEG-IFNα-2b or IFNα-2b) plus ribavirin combination therapy for 48 weeks of HCV genotype 1b (HCV-1b) or 24 weeks of HCV-2a/2b, from 2001 to 2010 at Toranomon Hospital. In this retrospective study the rates of sustained virological response [HCV-RNA negativity at 24 weeks after the completion of therapy based on the COBAS TaqMan HCV test (Roche Diagnostics)] were evaluated as well as sustained biochemical response [normal level of serum alanine aminotransferase at 24 weeks after the completion of therapy (6–50 IU/l)]. Treatment efficacy was evaluated by intention-to-treat (ITT) analysis classified as treatment failure in patients who could not complete the treatment regimen and per protocol (PP) analysis. Table 1 summarizes the profiles and data of the 157 patients at the commencement of combination therapy with interferon plus ribavirin in study 1. They included 105 men and 52 women aged 34–74 years (median 58 years). 110 (70.1%) patients received PEG-IFNα-2b plus ribavirin, and the remaining 47 (29.9%) patients received IFNα-2b plus ribavirin. They received PEG-IFNα-2b at a median dose of 1.3 μg/kg (range 0.5–1.9 μg/kg) subcutaneously each week or IFNα-2b at a median dose of 6 million units (range 3–6 million units) intramuscularly each day (7 times per week for the initial 2 weeks followed by 3 times per week). They also received oral ribavirin at a median dose of 10.7 mg/kg (range 2.7–15.1 mg/kg) daily. In 56 of the 157 (35.7%) patients, the dose of ribavirin was reduced during treatment due to a fall in hemoglobin concentration. The median total duration of treatment in 120 patients of HCV-1b was 48 weeks (range 1–48 weeks), and that in 37 patients of genotype 2a or 2b was 24 weeks (range 5–24 weeks).

In the study 2 cohort (fig. 1), 185 consecutive patients of HCV-related compensated cirrhosis, who showed no sustained virological response following at the first course of interferon monotherapy (≥24 weeks) from 1987 to 2010 at Toranomon Hospital, were recruited. Hepatocarcinogenesis rates were evaluated according to the additional treatment (second course of treatment), and were classified into three groups: no treatment (106 patients), interferon monotherapy (≥24 weeks; 55 patients), and ribavirin combination therapy (≥24 weeks; 24 patients). 106 patients without treatment did not receive the additional treatment because of concerns about adverse effects, lack of time for treatment, physician recommendation based on the appearance of depression and car-

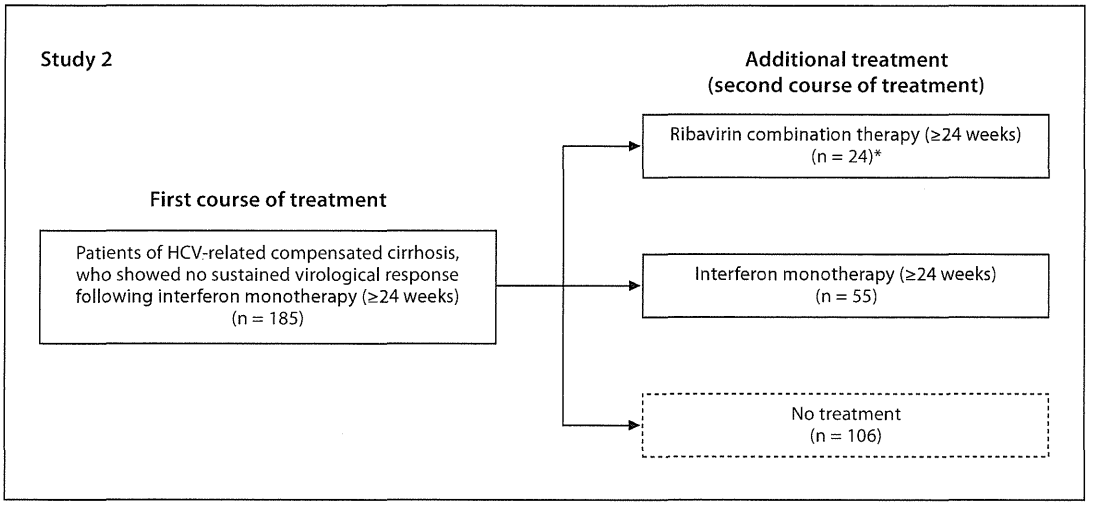

Fig. 1. For study 2, 185 patients with HCV-related compensated cirrhosis, who showed no sustained virological response following the first course of interferon monotherapy (≥24 weeks), were recruited. Hepatocarcinogenesis rates were evaluated according to the additional treatment (second course of treatment), and patients were classified into three groups: no treatment, interferon monotherapy (≥24 weeks), and ribavirin combination therapy (≥24 weeks). * 24 of 157 patients with HCV-related compensated cirrhosis in study 1 were also included in study 2.

diopulmonary disease during and after the first course of interferon monotherapy or the lower levels of serum alanine aminotransferase. The median follow-up time, from the end of the first course of interferon monotherapy until the last visit, was 6.4 years (range 0.0–21.0 years). 24 of the 157 patients in study 1 were also included in study 2; they showed no sustained virological response following the first course of interferon monotherapy (≥24 weeks) and were treated additionally with ribavirin combination therapy (≥24 weeks).

At the additional treatment of interferon monotherapy, 43 patients (78.2%) received IFNα alone, and the remaining 12 patients (21.8%) received IFNβ alone. They received interferon monotherapy including initial aggressive induction therapy (every day for 8 weeks followed by 3 times per week), with a median treatment duration of 44 weeks (range 24–382 weeks) at a median dose of 3 million units (range 3–10 million units) intramuscularly each day.

At the additional treatment of ribavirin combination therapy, 11 patients (45.8%) received PEG-IFNα-2b plus ribavirin, and the remaining 13 patients (54.2%) received IFNα-2b plus ribavirin. They received PEG-IFNα-2b at a median dose of 1.5 μg/kg (range 0.8–1.7 μg/kg) subcutaneously each week or IFNα-2b at a median dose of 6 million units (range 3–6 million units) intramuscularly each day (7 times per week for the initial 2 weeks followed by 3 times per week), with a median treatment duration of 26 weeks (range 24–48 weeks). They also received oral ribavirin at a median dose of 11.0 mg/kg (range 3.0–12.5 mg/kg) daily.

In the present studies, the patients were selected based on the following criteria. (1) Patients had compensated cirrhosis, but no decompensated cirrhosis or hepatocellular carcinoma. The diagnosis of compensated cirrhosis was based on clinical features (absence of signs for decompensation of ascites, encephalopathy, or gastrointestinal bleeding), laboratory tests, and peritoneoscopy or liver biopsy. (2) Patients were negative for hepatitis B surface antigen (radioimmunoassay, Dainabot, Tokyo, Japan), positive for anti-HCV (third-generation enzyme immunoassay, Chiron Corp., Emerville, Calif., USA), and positive for HCV-RNA by qualitative or quantitative analysis. (3) Patients were free of coinfection with human immunodeficiency virus. (4) Lifetime cumulative alcohol intake was <500 kg (mild to moderate alcohol intake). (5) Patients were free of other types of hepatitis, including hemochromatosis, Wilson disease, primary biliary cirrhosis, alcoholic liver disease, and autoimmune liver disease. (6) Each patient signed a consent form of the study protocol that had been approved by the human ethics review committee.

Laboratory Investigations

Blood samples were frozen at –80° within 4 h of collection and were not thawed until used for testing. HCV genotype was determined by PCR using a mixed primer set derived from nucleotide sequences of the NS5 region [12]. HCV-RNA quantitative analysis was measured by branched DNA assay version 2.0 (Chiron Corp., Emeryville, Calif., USA), AMPLICOR GT HCV Monitor version 2.0 using the 10-fold dilution method (Roche Molecular Systems Inc., Pleasanton, Calif., USA), or COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan). High viral load of viremia levels was defined as branched DNA assay ≥1.0 MEq/ml, AMPLICOR GT HCV Monitor ≥100 × 10^3 IU/ml, or COBAS TaqMan HCV test ≥5.0 log IU/ml. Low viral load was defined as branched DNA assay <1.0 MEq/ml, AMPLICOR GT HCV Monitor <100 × 10^3 IU/ml, or COBAS TaqMan HCV test <5.0 log IU/ml. The lower limit of HCV-RNA qualitative analysis (Amplicor, Roche Diagnostics, Mannheim, Germany) was 100 copies/ml, and that of