

population attributable fraction of 62.0% for hepatocellular carcinoma cases with HCV infection was highest, and hepatocellular carcinoma cases with HBV infection, alcohol consumption of  $\geq 40$  g of ethanol per day, or obesity had population attributable fractions in the range of 13.4% to 20.1%. These are only approximate estimates of the potential for reducing hepatocellular carcinoma occurrence, as we do not know what effect removal of one risk factor would have on the distribution of the other risk factors.

Multivariate analysis after adjusting for severity of liver fibrosis indicated that hepatocellular carcinoma risk for HBV and HCV infections significantly decreased, which is consistent with the existing notion that hepatocellular carcinoma risk increases with progression from chronic hepatitis B and C to liver cirrhosis. A large-scale meta-analysis (34) and a case-control study (35) showed a combined effect of HBV and HCV infections on hepatocellular carcinoma risk, whereas our study did not detect similar effects among those with HBV+/HCV+ status. This difference may be partly attributable to the extremely limited number of coinfecting subjects with HBV and HCV among our study population. It may be also partly because most past epidemiologic studies have defined chronic HCV infection by either anti-HCV antibody positivity or by HCV RNA positivity in serum (34, 35).

Several epidemiologic studies and clinical trials revealed an association between obesity and hepatocellular carcinoma risk (9-12), but few population-based cohort studies have been conducted with precise adjustment for HBV and HCV infection status, the major risk factors for hepatocellular carcinoma. Obesity was recently found to be one of the etiologic factors for non-alcoholic steatohepatitis, which is considered a non-B, non-C liver disease, and it has been shown to be a risk factor for hepatocellular carcinoma (12, 16). Although many clinical studies showed that, among chronic hepatitis C patients, obesity was associated with progression of inflammation, insulin resistance, hepatic steatosis, and liver fibrosis (17, 36), a study by Nair et al. (12) reported that obesity was not an independent risk factor for hepatocellular carcinoma among liver cirrhosis patients with HBV and HCV. On the other hand, a recent Western cohort study showed that being overweight (BMI, 25 to  $<30$  kg/m<sup>2</sup>) or obese (BMI,  $\geq 30$  kg/m<sup>2</sup>) was an independent risk factor for hepatocellular carcinoma (37).

In the present study, we adjusted for potentially confounding factors including hepatitis virus infection and also found that being obese 10 years before hepatocellular carcinoma diagnosis was associated with a 4.57-fold increase in hepatocellular carcinoma risk. Furthermore, we observed a statistically significant, positive, multiplicative interaction between HCV infection and increased BMI on the risk for hepatocellular carcinoma, which indicates decisively that the joint effect of the two factors is greater than additive.

Obesity contributes to a high rate of visceral fat storage, accelerating production of tumor necrosis factor- $\alpha$ , interleukin 6, resistin, and leptin, and decreasing production of adiponectin (16). These cytokines presumably foster insulin resistance (16), cause hepatic steatosis and oxidative stress, and eventually promote hepatocellular carcinoma occurrence. A large number of studies pointed out association between progression of

**Table 5. Interaction between hepatitis virus infection status and increase of BMI on hepatocellular carcinoma risk (joint hepatitis virus/BMI)**

Viral etiology	BMI (kg/m <sup>2</sup> )	RR* (95% CI) <sup>†</sup>	Likelihood ratio P <sup>†</sup>
HBV-/HCV-	+1	1.05 (0.95-1.17)	0.33
HBV+/HCV-	+1	0.89 (0.64-1.23)	0.50
HBV-/HCV+	+1	1.39 (1.11-1.83)	0.003 <sup>‡</sup>
HBV+/HCV+	+1	— <sup>§</sup>	— <sup>§</sup>

\*Adjusted for continuous alcohol consumption, smoking habit, coffee drinking, diabetes mellitus, and radiation dose to the liver.

<sup>†</sup>Likelihood bounds and P values for relative risks estimated separately within each BMI/hepatitis virus category.

<sup>‡</sup>A quadratic term was also significant for HBV-/HCV+ individuals (P = 0.013). However, only the relative risk for the linear model in continuous BMI is shown because it is not possible to express the risk as a single value with a two-parameter linear-quadratic model.

<sup>§</sup>Neither could the joint effect of obesity and simultaneous HBV+/HCV+ status be estimated because of small numbers of jointly affected cases and controls.

liver fibrosis and insulin resistance or hepatic steatosis (15-17), but authenticity of any connection is now being questioned (8, 36). Interestingly, in this study, obesity remained an independent risk factor for hepatocellular carcinoma even after adjusting for all confounding factors including severity of liver fibrosis. The following are the possible reasons why obesity increases hepatocellular carcinoma risk irrespective of severity of liver fibrosis: Several animal experiments showed that liver tumors were not always accompanied by advanced fibrosis among a variety of genetically engineered mouse models with steatohepatitis (38), and some reports indicated several nonalcoholic steatohepatitis-derived human cancer cases without significant liver fibrosis (39). The findings suggest that significant liver fibrosis is not essential for the carcinogenic process, but that steatohepatitis itself is a state conferring a risk for high carcinogenicity. With regard to the proven relationship between obesity and such malignant tumors as colon, breast, and ovarian cancers (10), the cell proliferation activity of insulin due to hyperinsulinemia is believed to play a role in a common carcinogenic mechanism (5).

It is well documented that obesity induces insulin resistance, with a tendency to cause diabetes mellitus. In the case of hepatic cirrhosis accompanied by highly advanced liver fibrosis, glucose intolerance tends to lead to diabetes mellitus. A recent animal experiment showed that HCV contributed to progression of insulin resistance, resulting in diabetes mellitus (40). The present study failed to show that diabetes mellitus 10 years before hepatocellular carcinoma diagnosis was an independent risk factor for hepatocellular carcinoma, but an adjustment for all factors, except alcohol consumption and BMI, brought about a 30% increase in the effect of diabetes on hepatocellular carcinoma risk (data not shown). Such findings suggest a relationship between diabetes mellitus and alcohol consumption, as well as BMI. Therefore, by taking into account the proven association between alcohol consumption, obesity, and increased risk for hepatocellular carcinoma, our results will not likely refute an association between diabetes mellitus and hepatocellular carcinoma risk.

A large number of epidemiologic studies showed that heavy alcohol consumption was an independent risk factor for hepatocellular carcinoma and that there was

correlation between increased risk for hepatocellular carcinoma and amount of alcohol consumed (3, 9, 13, 14). In addition, in some case-control studies of hepatocellular carcinoma risk, synergistic interactions between alcohol consumption and hepatitis virus infection, or between obesity and diabetes mellitus, have been observed (9, 13, 14). In the present study, after adjusting for other factors such as hepatitis virus infection and BMI, alcohol consumption of  $\geq 40$  g of ethanol produced a 4.36-fold increase in hepatocellular carcinoma risk. A few recent case-control studies suggested that ethanol consumption of  $< 50$  to  $60$  g/d (41, 42) or alcohol exposure  $< 1,500$  gram-years (9) had protective effects on the progression of liver fibrosis and risk for developing hepatocellular carcinoma. Reasons for such discrepancy between our result and former reports are unclear, but factors such as gender, age, race (43), hereditary predisposition, and etiology of liver disease presumably affect the severity of alcohol-related liver diseases. Our study also showed that effects of alcohol consumption of  $\geq 40$  g of ethanol per day on hepatocellular carcinoma risk were reduced after adjusting for all confounding factors including severity of liver fibrosis. The finding suggests that alcohol consumption may contribute to hepatic carcinogenesis by enhancing oxidative stress and aggravating liver fibrosis.

As a result of recent assessments by the IARC, hepatocellular carcinoma has been positioned as a smoking-related malignant disease (44). However, it has yet to be determined whether smoking itself has direct hepatic carcinogenic effects or whether smoking contributes to hepatic carcinogenesis by way of progression of liver fibrosis. A case-control study showed that 4-aminobiphenyl DNA adducts contained in tobacco smoke are a liver carcinogen (45). In the present study, we adjusted for potential confounding factors including hepatitis virus infection and failed to detect significant smoking effects on hepatocellular carcinoma risk; however, a multivariate analysis that excluded hepatitis virus infection showed significant effects of smoking (data not shown). With adjustment for all factors including severity of liver fibrosis, effects of smoking on hepatocellular carcinoma risk were found to be marginally significant. These findings suggest the possibility that smoking, in conjunction with hepatitis virus infection, further enhances the risk for hepatocellular carcinoma and might directly contribute to the mechanism of liver carcinogenesis.

Several epidemiologic studies indicated the involvement of coffee in decreased alanine aminotransferase activity and  $\gamma$ -glutamyltransferase level, suppression of progression to liver cirrhosis, and inhibited development of hepatocellular carcinoma (18, 19). Such oxidation inhibitors as caffeine, coffee diterpenes, and chlorogenic acid are among candidate substances in coffee that potentially reduce the risk for hepatocellular carcinoma, and several animal experiments have shown that such substances have direct inhibitory effects on hepatic carcinogenesis (46). Adjusting for all potential confounding factors including hepatitis virus infection rendered the effects of coffee drinking on hepatocellular carcinoma risk marginally significant, whereas adjusting for all factors, except hepatitis virus infection, revealed significant effects of coffee drinking (data not shown). Furthermore, adjusting for all factors including severity

of liver fibrosis erased the effects of coffee drinking on hepatocellular carcinoma risk. These findings suggest that coffee drinking may somehow suppress liver fibrosis and thereby indirectly reduce hepatocellular carcinoma risk.

The main strengths of our study are its prospective cohort-based, nested case-control design, which minimized selection bias and provided for the use of stored sera and a wealth of epidemiologic information obtained before hepatocellular carcinoma diagnosis. Indeed, the distributions of HBV and HCV infection status among hepatocellular carcinoma cases and controls and mean age at diagnosis among hepatocellular carcinoma cases were similar to those in previous reports on Japanese populations (2, 4). Another major strength of our study is that it incorporated, in a strict and in-depth manner, HBV and HCV infection status and showed the interrelationship between these and numerous other epidemiologic factors. It is difficult and expensive to perform full cohort serum analyses, whereas the nested case-control design used here can provide substantial reductions in cost and effort with little loss of statistical efficiency.

The main limitation of our study is that the severity of liver fibrosis could not be classified into fibrosis stage of F0 to F4 based on liver specimens. We used platelet counts and type IV collagen concentrations as surrogate, but independent, markers of liver fibrosis. Previous reports showed a strong correlation between platelet count and fibrosis stage in the presence of chronic hepatitis C (47) and a close association between levels of type IV collagen, a basic component of the hepatic basal membrane, and severity of liver fibrosis. Another limitation of our study is the usage of sera that had been stored for long periods of time. Proteins and HCV RNA tend to degrade during prolonged storage of either frozen or freeze-dried sera. However, we minimized this degradative effect by the selection of matched controls relative to time and method of serum storage. Furthermore, we have previously shown that the freeze-dried sera are interchangeable with frozen sera in serologic and molecular biological detection of HBV and HCV (22, 25). Finally, some hepatocellular carcinoma cases had to be excluded because of nonavailability of stored sera. We did not detect any differences between included and excluded cases in terms of demographic variables or BMI.

In conclusion, HBV and HCV infection and obesity were independent risk factors for hepatocellular carcinoma, even after taking into account the severity of liver fibrosis. Moreover, the combination of HCV infection and increased BMI exerted a synergistic effect on the risk for hepatocellular carcinoma. Alcohol consumption of  $\geq 40$  g of ethanol per day was also an independent risk factor for hepatocellular carcinoma, likely contributing to the development of hepatocellular carcinoma through liver fibrosis. The radiation effect on hepatocellular carcinoma risk was shown to be marginally significant in univariate analysis; whether the radiation effect is confounded with other factors will be closely examined in a separate report. A precise understanding of the mechanism by which obesity contributes to development of hepatocellular carcinoma should lead to better therapeutic strategies, public health policies, and cost-effectiveness.

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## HEPATOLOGY

## Prospective study of short-term peginterferon- $\alpha$ -2a monotherapy in patients who had a virological response at 2 weeks after initiation of interferon therapy

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### Key words

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### Abstract

**Background and Aims:** Long-term interferon (IFN) therapy is effective in eliminating hepatitis C virus (HCV). However, it carries the risk of adverse effects and reduced quality of life. To assess whether short-term IFN therapy effectively eliminates HCV, we performed a prospective pilot study of pegylated (peg)IFN- $\alpha$ -2a therapy for 8 or 24 weeks.

**Methods:** After excluding patients with high titers of genotype-1, 55 HCV patients received pegIFN- $\alpha$ -2a. Patients who became negative for HCV-RNA at week 2 were allocated to either an 8-week ( $n = 19$ ) or 24-week ( $n = 15$ ) course of IFN. We evaluated the efficacy of and tolerance to IFN therapy.

**Results:** The sustained virological response rate was excellent in the two groups (8 weeks, 89.5% [17/19]; 24 weeks, 100% [15/15], respectively). IFN dose reduction was required in one patient of the 8-week group, but in six patients of the 24-week group ( $P = 0.028$ ). Treatment was completed by all patients of the 8-week group, but discontinued in five patients of the 24-week group ( $P = 0.011$ ).

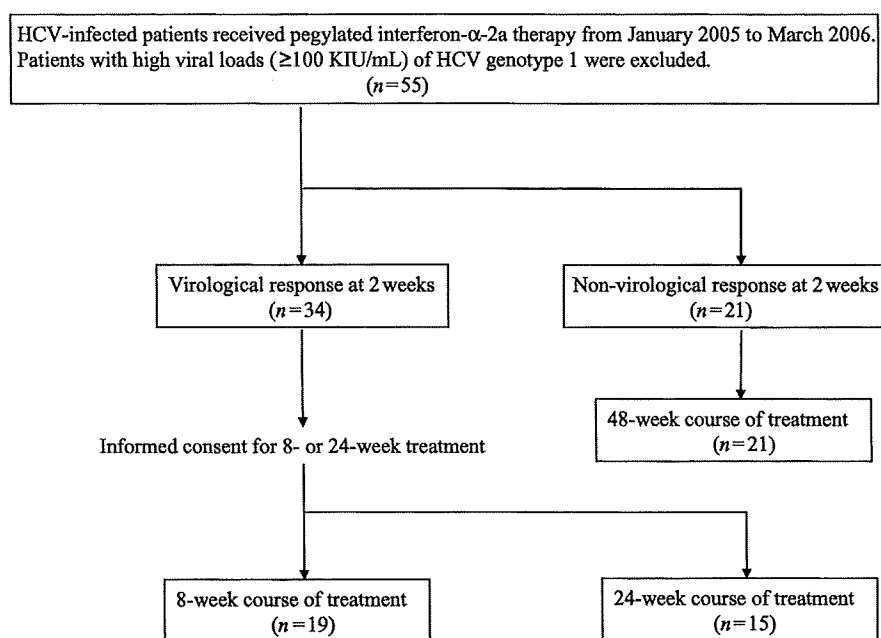
**Conclusions:** The 8-week IFN therapy is more tolerable than the 24-week therapy and had similar outcomes. Excluding the patients with high titers of genotype-1, we recommend switching to an 8-week course of pegIFN- $\alpha$  monotherapy once patients show an ultra rapid virological response at week 2 from the start of IFN therapy.

### Introduction

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease with an estimated 170 million chronic carriers worldwide.<sup>1</sup> Chronic HCV infection is usually associated with liver cirrhosis (LC) and hepatocellular carcinoma (HCC).<sup>2-6</sup> In Japan, 60–70% of patients with HCC or LC are HCV carriers.<sup>7</sup> Antiviral therapy of interferon (IFN) is widely used for the treatment of chronic HCV infection and is assumed to prevent progression to LC and HCC, especially in patients who show a sustained virological response (SVR).

The reported total HCV-RNA elimination rate is approximately 30–40% in patients treated with conventional IFN monotherapy.<sup>8-10</sup> However, better results have been reported when pegylated (peg)IFN- $\alpha$  is used in both naive patients and in those who fail to respond to or relapse after conventional IFN- $\alpha$  monotherapy. In Japan, two kinds of pegIFN are available: pegIFN- $\alpha$ -2a and pegIFN- $\alpha$ -2b. PegIFN- $\alpha$ -2b can be used with ribavirin, a purine nucleoside analog, in naive patients with genotypes 1 and 2 with a

high viral load (>100 KIU/mL of HCV-RNA) or patients with any viral load in whom previous IFN treatment did not eliminate HCV-RNA. PegIFN- $\alpha$ -2a has been used in Japan without ribavirin only since December 2003 because of health insurance restrictions. However, ribavirin combination therapy has been covered by public health insurance since March 2007 in Japan. The HCV elimination rate with pegIFN- $\alpha$ -2b plus ribavirin combination therapy is up to 54% in patients with genotype 1.<sup>11</sup> Several investigators have reported that pegIFN and ribavirin combination therapy for a period of 24 or 48 weeks ensures a viral clearance in most patients with HCV genotypes 2 or 3 infection.<sup>12,13</sup> However, ribavirin combination therapy frequently causes anemia and should be carefully used in the elderly, anemic, or pregnant young patients, and in those who require long-term treatment.<sup>14</sup> Apart from patients with a high viral load of genotype 1, IFN monotherapy is also effective in HCV elimination even when used without ribavirin. Previous studies suggest that the SVR achieved with pegIFN- $\alpha$ -2a is similar to that observed with pegIFN- $\alpha$ -2a combined with ribavirin in patients with hepatitis C.<sup>15,16</sup>



**Figure 1** Flow diagram of the clinical trial. HCV, hepatitis C virus.

Although the tolerability of pegIFN is similar to that of the conventional IFN,<sup>15</sup> the 180  $\mu$ g dose of pegIFN- $\alpha$ -2a therapy for 48 weeks is sometimes not tolerated by some patients. With the exception of those with high viral loads of genotype 1, the above regimen is expected to produce a high viral clearance rate, especially in patients with an early virological response. Several studies report the effectiveness of short-course IFN therapy (<24 weeks) for patients with an early virological response.<sup>17–20</sup> Therefore, a treatment duration of 48 weeks may be too long or more than sufficient for some patients, especially when one considers the undesirable adverse effects or the cost of treatment.

In the present study, we conducted a prospective controlled trial to compare the efficacy of an 8-week versus a 24-week course of pegIFN- $\alpha$ -2a (180  $\mu$ g/time/week) for patients negative for HCV-RNA at 2 weeks after the initiation of therapy.

## Methods

### Patients

Between January 2005 and March 2006, a total of 55 HCV-infected patients received pegIFN- $\alpha$ -2a therapy at Hiroshima University Hospital (Hiroshima, Japan) and its associated hospitals in Japan. Patients with high viral loads ( $\geq 100$  KIU/mL) of HCV genotype 1 were excluded from this study because of their low SVR rate. Among the 55 patients, 34 consecutive patients who showed a rapid virological response at 2 weeks were enrolled in this study (Fig. 1). Eligible patients had antibodies to HCV, were positive for HCV-RNA at study entry, and had not received previous IFN therapy. They included 21 men and 13 women, with a mean age of 53 years (range, 21–71 years). Their HCV genotypes were 1b, 2a, and 2b with variant HCV-RNA (5.1–400 KIU/mL by a reverse transcriptase-polymerase chain reaction [RT-PCR]). All patients underwent liver biopsies within 12 weeks before the start of IFN therapy and were confirmed to have chronic hepatitis by

histopathological examination. Patients with any other cause of liver disease including coinfection with hepatitis-B virus or HIV, alcoholic hepatitis, fatty liver, autoimmune hepatitis, or previous organ transplantation were excluded from this study.

### Study design

This multicenter prospective controlled study compared the efficacy and safety of 8 weeks versus 24 weeks of pegIFN- $\alpha$ -2a monotherapy in previously untreated patients with chronic hepatitis C who had a virological response at 2 weeks after the start of IFN. Patients with a virological response at 2 weeks were invited to sign a consent form accepting treatment with IFN for 8 weeks only. Those patients who refused consent received a 24-week course of treatment. The primary measure of efficacy was SVR, which was defined as undetectable HCV-RNA in the serum at 24 weeks after the cessation of treatment. All patients agreed to participate in the research protocol, which was approved by the hospital research ethics board, and gave written informed consent. The eligible patients received pegIFN- $\alpha$ -2a (Pegasys, F. Hoffmann-LaRoche, Basel, Switzerland) at 180  $\mu$ g once per week subcutaneously, either for 8 weeks or 24 weeks, without ribavirin. Other patients who showed no rapid virological response at 2 weeks after the start of pegIFN- $\alpha$ -2a were treated for 24–48 weeks.

All patients were evaluated in an outpatient setting for safety, tolerance, and efficacy every week during the IFN treatment. Blood count was checked just before the IFN injection every week. The qualitative detection of HCV-RNA was performed by a standardized qualitative RT-PCR assay (Amplicor HCV monitor v2.0; Roche diagnostics Co., Tokyo, Japan) at the first 2 weeks and every 4 weeks during and after IFN treatment. The primary efficacy end point for this study was defined as a disappearance of detectable serum HCV-RNA at week 24 after the completion of the IFN treatment.

**Table 1** Patients' characteristics

	8-week group ( <i>n</i> = 19)	24-week group ( <i>n</i> = 15)
Age (years)	51 <sup>†</sup> (21–71)	47 <sup>†</sup> (25–58)
Sex (male/female)	14/5	7/8
Height (cm)	169 <sup>†</sup> (147–178)	161 <sup>†</sup> (139–178)
Weight (kg)	64.6 <sup>†</sup> (40.6–85)	59 <sup>†</sup> (47–92.4)
Body mass index (kg/m <sup>2</sup> )	23.4 <sup>†</sup> (18.0–27.8)	21.5 <sup>†</sup> (18.6–30.5)
Platelet count (x10 <sup>4</sup> / $\mu$ L)	19.5 <sup>†</sup> (9.6–30.7)	18.1 <sup>†</sup> (8.9–31.7)
Alanine aminotransferase (IU/L)	55 <sup>†</sup> (22–152)	60 <sup>†</sup> (21–184)
$\gamma$ -Glutamyl transpeptidase (IU/L)	25 <sup>†</sup> (9–155)	47 <sup>†</sup> (14–137)
Creatinine (mg/dL)	0.76 <sup>†</sup> (0.6–0.9)	0.68 <sup>†</sup> (0.38–0.85)
Total cholesterol (mg/dL)	160 <sup>†</sup> (116–219)	154 <sup>†</sup> (125–201)
Fasting blood glucose (mg/dL)	90 <sup>†</sup> (72–104)	96 <sup>†</sup> (84–115)
Diabetes mellitus	0	1
Hyaluronic acid (ng/mL)	24 <sup>†</sup> (13–72)	78 <sup>†</sup> (16–191)
HCV genotype (1b/2a/2b)	2/15/2	2/10/3
HCV-RNA (KIU/mL)	45 <sup>†</sup> (5.1–370)	43 <sup>†</sup> (5.3–400)
Fibrosis (F1/F2/F3/F4)	6/8/5/0	5/6/4/0

<sup>†</sup>Median. HCV, hepatitis C virus.

### Statistical analysis

We compared the response to an 8-week course of pegIFN- $\alpha$ -2a with that to a 24-week course of pegIFN- $\alpha$ -2a. The  $\chi^2$ -test and Fisher's exact test were used for comparisons of categorical variables between groups, while Student's *t*-test and the Wilcoxon test were used for continuous and ordinal variables as appropriate. *P*-values less than 0.05 were considered to indicate statistical significance. The JMP version 5.1 statistical software package (SAS Institute, Cary, NC, USA) was used for the statistical analysis of data.

## Results

### Baseline characteristics

Thirty-four patients who became HCV-RNA-negative at week 2 subsequently received either an 8-week course (*n* = 19) or 24-week (*n* = 15) course of 180  $\mu$ g pegIFN- $\alpha$ -2a. The baseline characteristics of the two groups at the start of the IFN therapy are summarized in Table 1. None of the patients had LC, based on clinical, laboratory, and histopathological findings. Table 2 also shows the data of 21 patients with a non-rapid virological response at 2 weeks after the start of pegIFN- $\alpha$ -2a. The pretreatment viral loads of non-rapid virological responders were significantly higher than those of rapid virological responders (*P* < 0.0001).

### Tolerance of IFN therapy and adverse events

Among the 19 patients of the 8-week group, the dose was reduced by 50% (to 90  $\mu$ g of pegIFN- $\alpha$ -2a) in one patient with SVR at 3 weeks due to a fall in platelet count. However, all other patients were able to complete the full 8-week course without discontinuation. In 15 patients of the 24-week course, the dose was reduced

**Table 2** Characteristics of 21 patients who did not show a rapid virological response

Age (years)	51 <sup>†</sup> (22–76)
Sex (male/female)	11/8
Height (cm)	164.5 <sup>†</sup> (148–175.5)
Weight (kg)	58.5 <sup>†</sup> (42.5–75)
Body mass index (kg/m <sup>2</sup> )	22.5 <sup>†</sup> (16.9–27.3)
Platelet count (x10 <sup>4</sup> /L)	20.5 <sup>†</sup> (12–28.6)
Alanine aminotransferase (IU/L)	93 <sup>†</sup> (17–157)
$\gamma$ -Glutamyl transpeptidase (IU/L)	39 <sup>†</sup> (10–145)
Creatinine (mg/dL)	0.58 <sup>†</sup> (0.5–0.96)
Total cholesterol (mg/dL)	158 <sup>†</sup> (111–214)
Fasting blood glucose (mg/dL)	87 <sup>†</sup> (68–119)
Diabetes mellitus	0
Hyaluronic acid (ng/mL)	45.6 <sup>†</sup> (10–100)
HCV genotype (1b/2a/2b)	0/15/6
HCV-RNA (KIU/mL)	660 <sup>†</sup> (40–830)
Fibrosis (F1/F2/F3/F4)	12/6/3/0

<sup>†</sup>Median. HCV, hepatitis C virus.

to half (90  $\mu$ g of pegIFN- $\alpha$ -2a) in six patients due to neutropenia (*n* = 2; one patient at 8 weeks and one patient at 10 weeks), thrombocytopenia (*n* = 3; two patients at 9 weeks and one patient at 10 weeks) and epigastralgia (*n* = 1; at 14 weeks). Furthermore, IFN therapy was withdrawn in another five patients, including two patients at 8 weeks due to thrombocytopenia, two patients at 12 weeks due to generalized fatigue, and one patient at 18 weeks due to various neurological symptoms, such as hand numbness. Thus the proportion of patients who required a dose reduction was lower in the 8-week group than in the 24-week group (*P* = 0.028). Furthermore, the proportion of patients who completed the treatment was significantly higher in the 8-week group than the 24-week group (*P* = 0.011). We concluded that our patients with HCV could tolerate 8 weeks of IFN therapy better than 24 weeks.

### Biochemical and virological responses to therapy

With regard to the alanine aminotransferase (ALT) response to IFN therapy, all patients of both groups showed biochemical normalization at the end of treatment and at 6 months after the end of treatment. There was no difference in the sustained ALT response between the 8-week group and 24-week group. With regard to the virological response to IFN therapy, all patients of both groups exhibited a rapid decrease in HCV-RNA, reaching undetectable levels (HCV-RNA  $\leq$  100 copies/mL) by week 2. All patients had negative HCV-RNA levels at the end of treatment and none showed a null response. There was no significant difference in the rate of fall of the virological load between patients who had a sustained response and those who had a relapse, as discussed later. The proportions of patients who showed a SVR in the 8-week group and 24-week group were not significantly different (89.5% [17/19] and 100% [15/15], respectively [*P* = 0.195]). Two patients of the 8-week group had viral relapse after the end of treatment; one who had HCV genotype 2a with 50 KIU/mL pretreatment viral load relapsed at 12 weeks after the end of the treatment while the other had genotype 2b with 230 KIU/mL pretreatment viral load and relapsed at 8 weeks after the end of the treatment. The non-



rapid virological responders had a lower SVR rate. Eight (38%) patients showed SVR, 11 (52%) patients developed relapse after discontinuation of IFN, and two (10%) patients had no virological response.

## Discussion

In Japan, pegIFN- $\alpha$ -2a monotherapy has been covered by public health insurance since December 2003. The standard duration of treatment with pegIFN- $\alpha$ -2a is 24 weeks for patients with low viral loads of genotype HCV-1 and any viral loads of genotype HCV-2 infection. Recent studies have reported that a treatment duration of more than 24 weeks in such cases does not increase the SVR rate.<sup>11,13,21,22</sup> Moreover, patients with early virological response seem to have a high rate of SVR.<sup>23–25</sup> In those patients, to reduce unnecessary exposure to treatment and its potential side-effects and to reduce costs, short-term IFN therapy has been used by several groups.<sup>17–21</sup> However, details of the IFN regimen differ from those of others and there are no studies that use short-term of pegIFN- $\alpha$ -2a treatment. We therefore conducted a prospective pilot study on the efficacies of an 8-week and 24-week pegIFN- $\alpha$ -2a regimen for patients with low viral titers of genotype HCV-1 and any viral titers of genotype HCV-2 who exhibited a virological response at 2 weeks after the initiation of IFN. In our study, patients with a relatively low viral load before the start of the IFN therapy tended to have a very early virological response.

Our results demonstrated that the virological response to the 8-week treatment (89.5% [17/19]) was excellent and was similar to the 24-week course (100% [15/15]). This high SVR rate of 8-week pegIFN- $\alpha$ -2a monotherapy seems as high as that reported in another short course study of 14-week pegIFN plus ribavirin combination treatment for patients with HCV genotype HCV-2 or HCV-3.<sup>26</sup> This high SVR rate of the 8-week course of pegIFN- $\alpha$ -2a may be associated with a rapid viral disappearance. Several studies have indicated that negative HCV-RNA at week 2 after the commencement of IFN is a predictor of SVR.<sup>19,20,27,28</sup> Therefore, for patients with a low HCV-1 viral load or those with HCV-2 infection with any viral load, we recommend switching to an 8-week course of pegIFN- $\alpha$ -2a monotherapy once they show an ultra rapid virological response, that is, negative HCV-RNA at week 2 from the start of IFN therapy. Furthermore, a longer course of IFN therapy with or without ribavirin can be prescribed when HCV-RNA becomes positive after discontinuation of the 8-week course of IFN therapy.

Although in our study all of the patients in the 24-week course showed SVR, it seems that 24 weeks is a long treatment period for those patients who become negative for HCV-RNA by week 2 of treatment to ascertain SVR. Our results showed that all patients in the 8-week course completed the course to the end of treatment. However, 33% of the patients of the 24-week course did not continue their treatment to the end of the course. Because patients tend to adhere to shorter regimens, which are also better tolerated than longer treatment regimens, a shorter exposure will probably translate into a better benefit–risk ratio in patients with early virological response.

Our study identified two relapsers among patients of the 8-week course after discontinuation of pegIFN- $\alpha$ -2a therapy. These two patients had a negative history of exposure to new HCV infection. One patient who had genotype HCV-2b and a high pretreatment

viral load (HCV-RNA: 230KIU/mL) relapsed at 8 weeks after the discontinuation of IFN therapy, while the other who had genotype HCV-2a and a low pretreatment viral load (HCV-RNA: 50KIU/mL) relapsed at 12 weeks after the discontinuation of IFN therapy. These two patients could have SVR after additional IFN therapy for 24 weeks (one patient; pegIFN- $\alpha$ -2a monotherapy, one patient; pegIFN- $\alpha$ -2b and ribavirin combination therapy). We could not identify a definite factor associated with SVR or relapse. Although pretreatment factors, like genotype, viral load, and grade of fibrosis can be used to predict the mean treatment outcome for study cohorts, they are often of limited value in individual patients.<sup>29,30</sup>

As mentioned earlier, a short course of pegIFN- $\alpha$ -2a therapy for 8 weeks could be recommended in those patients who show an ultra rapid virological response at week 2 after the initiation of IFN therapy. Although the study by Shiffman *et al.*<sup>31</sup> demonstrates the inferiority of a shorter regimen in a large-scale, randomized, controlled study, the characteristics of their patients were largely different from those of our study, including racial difference (mostly Caucasian patients versus Japanese, a heavier body weight for the Caucasians versus Japanese patients) and differences in pretreatment viral load (variable and higher HCV-RNA level versus relatively low viral load in our patients). Patients' selection was also different between the two studies; our study was carried out only in cases negative for HCV-RNA at 2 weeks after the start of IFN compared to their randomized study, irrespective of a rapid virological response.

In conclusion, patients chronically infected with low titers of HCV-1 and those with HCV-2, regardless of their viral loads, who achieve an ultra rapid virological response, that is, HCV-RNA negativity at week 2, can receive only 8 weeks of pegIFN- $\alpha$ -2a monotherapy without compromising the chance of SVR. The results of our prospective study are encouraging, although the study population was small and was based on non-randomized methodology. The data of the present study are not conclusive for patients with very high pretreatment viremia who might achieve a rapid virological response or for those patients who do not achieve a rapid virological response. Further clinical trials are required to optimize the treatment duration in these patients.

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## Clinicopathological features of elderly patients with hepatitis C virus-related hepatocellular carcinoma

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**Background.** It is well known that the incidence of hepatocellular carcinoma (HCC) in patients with hepatitis C virus (HCV) correlates with progression of liver fibrosis. However, there is little information on the impact of aging on hepatocarcinogenesis. The aim of this study was to elucidate the clinicopathological features of elderly patients with HCV-related HCC. **Methods.** The study subjects were 693 consecutive patients newly diagnosed with HCC with anti-HCV. First, we divided them into a younger group (<70 years) and an elderly group (≥70 years) and compared clinicopathological features between the two groups. Next, we selected pure HCV-related HCC patients by excluding the patients with other probable factors for hepatocarcinogenesis (anti-HBc, interferon therapy, and alcohol) and compared the two groups again. **Results.** Higher platelet count, lower male/female ratio, lower rate of habitual alcohol consumption, and better Child-Pugh class were recognized in the elderly group than the younger group, statistically. In 133 cases of hepatic resection, fibrosis stage was lower in the elderly than the younger group. After selection of pure HCV-related HCC patients, in a stepwise multivariate analysis, male sex and platelet count  $<10 \times 10^4/\text{mm}^3$  were significant variables associated with age <70. Regarding the latency period to HCC development, the patients who received a blood transfusion at an older age developed HCC sooner despite their lower grade of fibrosis. **Conclusions.** The elderly patients developed HCC more often, despite their lower grade of fibrosis, compared with the younger patients. In addition to fibrosis, aging could be a factor affecting HCV-related hepatocarcinogenesis.

**Key words:** hepatocellular carcinoma, hepatitis C virus, elderly patients, aging, fibrosis

### Introduction

Since the discovery of the hepatitis C virus (HCV) RNA in 1989,<sup>1,2</sup> there has been no doubt that HCV is associated with hepatocarcinogenesis based on the high prevalence of HCV infection among hepatocellular carcinoma (HCC) patients who are otherwise negative for hepatitis B virus (HBV) markers.<sup>3–14</sup> In Japan, HCV is considered one of the main causes of HCC.<sup>15,16</sup> Several studies have examined patients with HCV and identified age, the extent of liver fibrosis, male sex, alcohol consumption, positivity for hepatitis B surface antigen (HBsAg), and high alpha-fetoprotein (AFP) level as risk factors for hepatocarcinogenesis.<sup>15–20</sup> The annual incidence of HCC correlates with the severity of liver fibrosis, from 0.5% among patients with stage F0 or F1 fibrosis to 7.9% among patients with stage F4 fibrosis.<sup>21</sup> In patients with chronic hepatitis C, age at infection, alcohol consumption, and male sex are independent factors associated with increased rate of progression of liver fibrosis.<sup>22</sup> Progression of fibrosis is dependent mainly on age and duration of infection. Moreover, the highest rate of progression of liver fibrosis occurs in patients aged ≥50 years.<sup>23</sup> In Japan, we and other groups have reported a recent increase in the number of elderly HCC patients with HCV,<sup>24–26</sup> but the impact of aging on the incidence of HCC has not been investigated carefully.

Thus, we started analyzing HCV-related HCC patients who were positive for anti-HCV but negative for HBsAg to determine the correlation between aging and liver fibrosis in relation to the incidence of HCC with HCV. To date, several factors for affecting hepatocarcinogenesis have been reported, such as antibody to hepatitis B core antigen (anti-HBc), previous interferon therapy, and alcohol consumption. It was reported that previous HBV infection increased the risk for development of HCC in HCV-related liver cirrhosis.<sup>33,27</sup> Also, alcohol consumption also considered a sig-

nificant risk factor for development of HCC with HCV.<sup>15,16,18</sup> Conversely, interferon therapy reduces the risk for development of HCC.<sup>19,21</sup> Therefore, we also analyzed patients without these factors to study them as the nearest condition to the natural history of HCV-related HCC patients and considered them as pure HCV-related HCC patients. In this study, we elucidated the clinicopathological features of elderly patients with HCV-related HCC and examined the impact of aging on hepatocarcinogenesis.

## Methods

### *Patients*

A total of 1038 consecutive patients newly diagnosed with HCC were consulted or admitted to Hiroshima University Hospital from January 1988 to December 2005. Of these patients, 693 patients (474 men, 41–92 years of age; 219 women, 41–88 years of age), positive for anti-HCV but negative for HBsAg, were enrolled in the present study. The 693 patients were divided into two groups according to age at initial diagnosis: 440 patients (325 men, 115 women) were <70 years old (younger group), and 253 patients (149 men, 104 women) were ≥70 years old (elderly group). We recorded epidemiological data (age, sex, history of blood transfusion, history of habitual alcohol consumption) and chemical data (serum bilirubin, aspartate aminotransferase, alanine aminotransferase, serum albumin, and prothrombin time) for each patient. A detailed epidemiological questionnaire was administered during face-to-face interviews. Then, we compared the clinical and pathological features of these groups. All patients submitted informed consent for collection and subsequent use of data for research purposes, and the study was carried out in accordance with the Helsinki Declaration.

### *Selection of pure HCV-related HCC*

As already mentioned, we first studied anti-HCV-positive and HBsAg-negative patients with HCC. However, this study included HBsAg-negative and anti-HBc-positive patients. It is recognized that previous HBV infection may be a cofactor for the development of HCC in HCV-related HCC patients.<sup>27</sup> Therefore, we excluded 167 patients (116 males, 51 females) who were positive for anti-HBc, the remainder being 526 patients (358 males, 168 females). Next, we excluded 57 patients (40 males, 17 females) who were negative for HCV-RNA. These patients probably became virus negative as a result of interferon therapy or spontaneously. The remainder included 469 patients (318 males, 151

females). There are some data that interferon reduces HCC development even in patients who do not achieve sustained eradication of HCV<sup>19,21,28</sup>; therefore, we also excluded 84 patients (67 males, 17 females) with history of interferon therapy. The remainder comprised 385 patients (251 males, 134 females). Habitual alcohol consumption is recognized as a risk factor of HCC development,<sup>15,16,18</sup> and in our study the proportion of patients with habitual alcohol consumption was greater in the younger group than in the elderly group. Thus, we excluded 48 patients (46 males, 2 females) with excessive alcohol consumption; the remainder included 337 patients (205 males, 132 females). We decided that 337 patients were appropriate to be the next subjects of our study as pure HCV-related HCC patients. We wanted to elucidate the relationship between the impact of aging and HCV-induced fibrosis for HCC development as the nearest condition to the natural history, so we removed those patients with other probable factors affecting hepatocarcinogenesis.

### *HCC diagnosis*

The diagnosis of HCC was based on hypervascularity, confirmed by dynamic computed tomography (CT), magnetic resonance imaging, angiography, or CT angiography, when the serum levels of HCC-related tumor markers, such as alpha-fetoprotein or protein induced in the absence of vitamin K or antagonist II (PIVKA-II), were increased or a mass lesion was detected by ultrasonography. When a nodule was not proven to be hypervascular, percutaneous biopsy under ultrasonography was performed for confirmation of the diagnosis of HCC. Staging adopted in this study was the revised version of the Liver Cancer Study Group of Japan in 2000.<sup>29</sup>

### *Blood biochemical tests and viral markers*

Routine serum biochemical tests were carried out with automated techniques. Anti-HCV in sera was assayed using the Anti-HCV-EIA Cobas Core Test (Hoffmann La Roche, Basel, Switzerland). All serum samples collected were analyzed with second- or third generation anti-HCV tests. If the index was ≥1.0, anti-HCV was considered positive. HBsAg and anti-HBc in sera were assayed using the HBsAg-EIA Cobas Core Test (Hoffmann La Roche) and the Anti-HBc-EIA Cobas Core Test (Hoffmann La Roche), respectively. HCV-RNA in sera was detected by qualitative reverse transcription-polymerase chain reaction (RT-PCR) (Hoffmann La Roche). For cases before March 1990, serum samples frozen at -20°C were retrieved from storage and tested for anti-HCV using the aforementioned method.

### Alcohol consumption

The average quantity of alcohol consumed per day was evaluated regardless of type of alcohol beverage. Habitual alcohol consumption was defined as regular consumption of at least one alcohol drink per week over 10 years. In particular, excessive habitual drinkers were defined as persons consuming more than 86 g ethanol per day.

### Platelet count

We evaluated the platelet count, which is known to correlate significantly with the stage of liver fibrosis in patients with chronic hepatitis C; also, platelet count  $<10 \times 10^4/\text{mm}^3$  has been used as a marker for liver cirrhosis.<sup>30-35</sup>

### Stage of liver fibrosis and activity grade of chronic hepatitis

Of 693 patients, 133 patients underwent hepatic resection within 6 months from first diagnosis. The resected specimens were fixed in 10% formalin neutral buffered solution, embedded in paraffin, and processed for staining with hematoxylin and eosin. Staging of fibrosis and grading of liver disease activity was assessed by a pathologist on a blinded basis according to the criteria of Desmet et al.<sup>36</sup>

### Statistical analysis

Values are expressed as median and 75th and 25th percentiles. Statistical analysis was performed using the Mann-Whitney *U* test and Spearman's nonparametric test. Univariate and multivariate logistic regression

using the Statistical Package for Social Science (SPSS) version 6.1 for Windows (SPSS Japan, Tokyo, Japan) was performed to evaluate the values of the patients' clinical and laboratory features associated with age at initial diagnosis of HCC. *P* values less than 0.05 were considered to indicate statistical significance.

## Results

### Characteristics of patients with HCV-related HCC

Table 1 summarizes the characteristics of HCC patients with HCV, according to age at initial diagnosis. Male/female ratio was significantly higher in the younger group than the elderly group ( $P < 0.001$ ). The median age of male patients (66 years old) was lower than that of female patients (69 years old) ( $P < 0.001$ ). The median of platelet count was 8.6 (25<sup>th</sup>, 6.1; 75<sup>th</sup>, 13.3) and 10.0 (25<sup>th</sup>, 7.3; 75<sup>th</sup>, 13.4)  $\times 10^4/\text{mm}^3$  in the younger and the elderly groups, respectively. Platelet count of the elderly group was significantly higher than that of the younger group ( $P = 0.005$ ). The proportion of patients whose platelet count was  $\geq 10 \times 10^4/\text{mm}^3$ , considered as having a noncirrhotic liver, was greater in the elderly group than the younger group ( $P = 0.003$ ). When we compared the platelet count of the younger group and that of the elderly group according to sex, the median of platelet count was 9.2 (25<sup>th</sup>, 6.2; 75<sup>th</sup>, 14.2) and 10.5 (25<sup>th</sup>, 7.8; 75<sup>th</sup>, 13.7)  $\times 10^4/\text{mm}^3$  in the younger and the elderly male groups, respectively. The median of platelet count was 7.9 (25<sup>th</sup>, 5.9; 75<sup>th</sup>, 10.6) and 8.8 (25<sup>th</sup>, 7.0; 75<sup>th</sup>, 12.4)  $\times 10^4/\text{mm}^3$  in the younger and the elderly female groups, respectively. Platelet count of the elderly group was significantly higher than that of the younger group for both sexes (males,  $P = 0.023$ ; females,  $P = 0.017$ ). The

**Table 1.** Characteristics of all patients with hepatitis C virus (HCV)-related hepatocellular carcinoma (HCC), according to age at diagnosis

Age at diagnosis	Younger group (<70 years)	Elderly group ( $\geq 70$ years)	<i>P</i> <sup>a</sup>
Sex (male/female)	325/115	149/104	<0.001
Platelet count ( $\times 10^4/\text{mm}^3$ ) <sup>b</sup>	8.6	10.0	0.005
( $<10/\geq 10$ ) $\times 10^4/\text{mm}^3$	266/174	124/129	0.003
Child-Pugh classification	271/149/20	184/69/0	0.001
(A or non-cirrhosis/B/C)			
Stage (I/II/III/IV)	88/170/116/66	49/109/73/22	NS
Alpha-fetoprotein (ng/ml) <sup>b</sup>	44.6	46.8	NS
History of blood transfusion (+/-)	100/340	55/198	NS
History of habitual alcohol consumption	64/193/183	23/95/135	0.001
(Excessive/habitual/none)			
Anti-HBc (+/-)	100/340	67/186	NS
Diabetes mellitus (with/without)	96/344	60/193	NS

NS, not significant

<sup>a</sup> Comparison between patients less than 70 years of age and those older than 70 years

<sup>b</sup> Values are median

**Table 2.** Significant variables in association with development of HCV-related HCC under 70 years old using univariate logistic analysis

Variables	Odds ratio	95% confidence interval	P value
Habitual alcohol consumption <sup>a</sup>	2.363	1.351–4.133	0.003
Male <sup>a</sup>	1.973	1.420–2.740	<0.001
Child-Pugh class B or C <sup>a</sup>	1.663	1.187–2.329	0.003
Platelet count <10 × 10 <sup>4</sup> /mm <sup>3</sup> <sup>f</sup>	1.575	1.153–2.152	0.004

<sup>a</sup>Significant variables in multivariate analysis

Child-Pugh class was A or non-cirrhosis, B and C in 62%, 34%, and 4% of the younger group, and 73%, 27%, and 0% of the elderly group, respectively. Child Pugh class were significantly better in the elderly group than the younger group ( $P = 0.001$ ). No significant difference was observed in stage grouping of HCC between the two groups. The median AFP level was 44.6 and 46.8 ng/ml for the younger and the elderly group, respectively. No significant difference was observed in serum AFP level between the two groups. History of blood transfusion was reported by 23% and 22% of the younger and the elderly group, respectively; it was not a significant difference. With regard to alcohol consumption, 14%, 44%, and 42% of the younger group and 9%, 38%, and 53% of the elderly group had history of excessive, habitual, and no drinking, respectively. Significant differences were observed in history of habitual alcohol consumption between the younger and the elderly group ( $P = 0.001$ ). No significant difference was observed in positive rate of anti-HBc and the rate of diabetes mellitus.

#### Variables associated with age <70 years at diagnosis of HCC

Sex, platelet count, Child-Pugh class, history of blood transfusion, history of habitual alcohol consumption, anti-HBc and diabetes mellitus were selected as independent variables in logistic regression analyses, with the age <70 years at diagnosis as dependent variables. In a univariate analysis, habitual alcohol consumption, male sex, Child-Pugh class B or C, and platelet count <10 × 10<sup>4</sup>/mm<sup>3</sup> were significantly correlated with age <70 years at diagnosis (Table 2). In a stepwise multivariate analysis, habitual alcohol consumption (odds ratio, 1.968, 95% confidence interval, 1.107–3.500;  $P = 0.021$ ), male sex (odds ratio, 1.933, 95% confidence interval, 1.373–2.722;  $P < 0.001$ ), platelet count <10 × 10<sup>4</sup>/mm<sup>3</sup> (odds ratio, 1.587, 95% confidence interval, 1.144–2.200;  $P = 0.006$ ) and Child-Pugh class B or C (odds ratio, 1.532, 95% confidence interval, 1.080–2.174,  $P = 0.017$ ) were significant variables associated with age <70 years at diagnosis in HCV-related HCC patients.

**Table 3.** Fibrosis stage and activity grade of liver histology

	<70 years	≥70 years	P <sup>a</sup>
Male/female	67/23	29/14	
Fibrosis stage			0.009
F0–1	4 (4%)	2 (5%)	
F2	13 (14%)	14 (38%)	
F3	26 (27%)	8 (22%)	
F4	53 (55%)	13 (35%)	
Activity grade			0.047
A0–1	6 (6%)	5 (13%)	
A2	79 (82%)	31 (84%)	
A3	11 (12%)	1 (3%)	

Data are numbers and percentages (in parentheses) of patients

<sup>a</sup>Comparison between patients less than 70 years of age and those older than 70 years

#### Fibrosis stage and activity grade

Of the 693 patients, 133 underwent hepatic resection. Their fibrosis staging and activity grading of the liver were evaluated (Table 3). Fibrosis stage F0–1, F2, F3, and F4 were identified in 4%, 14%, 27%, and 55% of the younger group, and in 5%, 38%, 22%, and 35% of the elderly group, respectively. The fibrosis stage of liver histology was significantly lower in the elderly group ( $P = 0.009$ ). The activity grades A0–1, A2, and A3 were recognized in 6%, 82%, and 12% of the younger group, and 13%, 84%, and 3% of the elderly group, respectively. The activity grade of liver histology was significantly lower in the elderly group ( $P = 0.047$ ). Among 133 patients who underwent hepatic resection, both fibrosis stage and activity grade were significantly lower in the elderly patients.

#### Characteristics of pure HCV-related HCC patients

Table 4 summarizes the characteristics of pure HCV-related HCC patients. Male/female ratio was significantly higher in the younger group than the elderly group ( $P = 0.004$ ). The median age of male patients (67 years) was lower than that of female patients (69 years) ( $P = 0.001$ ). The median platelet count was 8.7 (25<sup>th</sup>, 6.1; 75<sup>th</sup>, 12.4) and 9.9 (25<sup>th</sup>, 7.0; 75<sup>th</sup>, 13.1) × 10<sup>4</sup>/mm<sup>3</sup> in the younger and the elderly group, respectively. The pro-

**Table 4.** Characteristics of patients with pure HCV-related HCC, according to age at diagnosis

Age at diagnosis	Younger group (<70 years)	Elderly group (≥70 years)	P <sup>a</sup>
Sex (male/female)	138/68	67/64	0.004
Platelet count ( $\times 10^4/\text{mm}^3$ ) <sup>b</sup>	8.7	9.9	NS
( $<10/\geq 10$ ) $\times 10^4/\text{mm}^3$	134/72	69/62	0.024
Child-Pugh classification (A or non-cirrhosis/B/C)	123/72/11	83/48/0	NS
Stage (I/II/III/IV)	48/69/55/34	23/63/33/12	NS
Alpha-fetoprotein (ng/ml) <sup>b</sup>	45.2	60.2	NS
Genotype (1b/2a or 2b or 3a) <sup>c</sup>	127/18	83/24	0.035
History of blood transfusion (+/-)	47/159	32/99	NS
Diabetes mellitus (with/without)	43/163	35/96	NS

NS, not significant

<sup>a</sup>Comparison between patients less than 70 years of age and those older than 70 years<sup>b</sup>Values are median<sup>c</sup>Of the patients, 75 were not tested (51 were <70 years, 24 were ≥70 years)**Table 5.** Significant variables in association with development of pure HCV-related HCC under 70 years old using univariate logistic analysis

Variables	Odds ratio	95% confidence interval	P value
Male <sup>a</sup>	1.924	1.195–3.098	0.007
Platelet count $<10 \times 10^4/\text{mm}^3$ <sup>a</sup>	1.672	1.070–2.614	0.024
Genotype 1	2.040	1.043–3.990	0.037

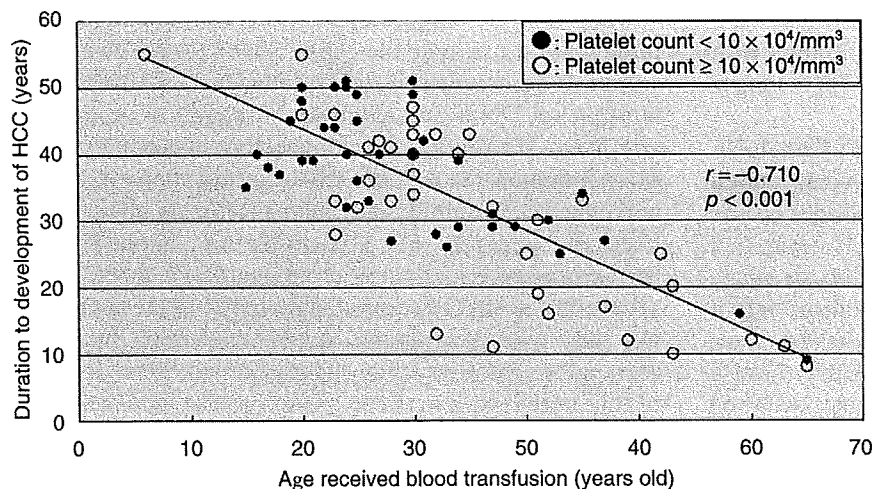
<sup>a</sup>Significant variables in multivariate analysis

portion of patients whose platelet count was  $\geq 10 \times 10^4/\text{mm}^3$  was greater in the elderly group than the younger group ( $P=0.024$ ). Child-Pugh class also seemed to indicate better liver function in the elderly group than that of the younger group, but it was not statistically significant. Stage grouping of HCC, serum AFP level, history of blood transfusion, and rate of diabetes mellitus showed no significant difference between the younger and the elderly group. The proportion of patients with HCV genotype 1 was greater in the younger group than the elderly group ( $P=0.035$ ). Next, we selected sex, platelet count, Child-Pugh class, genotype, history of blood transfusion, and diabetes mellitus as independent variables in logistic regression analyses, with the age <70 years at diagnosis as dependent variable. In a univariate analysis, male sex, platelet count  $<10 \times 10^4/\text{mm}^3$ , and genotype 1 were significantly correlated with age <70 years at diagnosis (Table 5). In a stepwise multivariate analysis, male (odds ratio, 2.035, 95% confidence interval, 1.208–3.427;  $P=0.008$ ) and platelet count  $<10 \times 10^4/\text{mm}^3$  (odds ratio, 2.105, 95% confidence interval, 1.242–3.570;  $P=0.006$ ) were significant variables associated with age <70 years at diagnosis in pure HCV-related HCC patients.

#### Latency period to development of HCC

Of the 337 patients, 79 patients received blood transfusion (BT) and none of these transfusions was pro-

vided after the diagnosis of chronic liver disease. These patients were presumed infected with HCV by BT, and thus we considered the interval between BT and diagnosis of HCC as the latency period to development of HCC. Figure 1 shows that the duration to development of HCC was shorter in patients received BT at an older age than those transfused at a younger age. Interestingly, platelet counts of many patients who received BT at an older age were  $\geq 10 \times 10^4/\text{mm}^3$ , which meant that many patients who received BT at an older age develop HCC more rapidly when they had a noncirrhotic liver, in other words, elderly patients might tend to develop HCC despite their liver fibrosis stage and activity grade. Then, we selected sex, platelet count, Child-Pugh class, genotype, diabetes mellitus, and age at BT as independent variables in logistic regression analyses, with the duration to development of HCC <25 years as dependent variable. In a univariate analysis, age when received BT  $\geq 35$  years, platelet count  $\geq 10 \times 10^4/\text{mm}^3$ , and Child-Pugh class A were significantly correlated with duration to development of HCC <25 years (Table 6). In a stepwise multivariate analysis, age when received BT  $\geq 35$  years (odds ratio, 43.666, 95% confidence interval, 4.950–385.231;  $P=0.001$ ) and platelet count  $\geq 10 \times 10^4/\text{mm}^3$  (odds ratio, 6.962, 95% confidence interval, 1.156–41.931;  $P=0.034$ ) were significant variables associated with the duration to development of HCC <25 years.



**Fig. 1.** Interval between age at blood transfusion and age at initial diagnosis in 79 patients with pure hepatitis C virus (HCV)-related hepatocellular carcinoma (HCC). Closed circles, patients with platelet count  $<10 \times 10^4/\text{mm}^3$ ; open circles, patients with platelet count  $\geq 10 \times 10^4/\text{mm}^3$ . Statistical analysis by Spearman's nonparametric test

**Table 6.** Significant variables in association with duration to development of HCC within 25 years using univariate logistic analysis

Variables	Odds ratio	95% confidence interval	P value
Age received BT $\geq 35$ years <sup>a</sup>	48.831	5.823–409.514	<0.001
Platelet count $\geq 10 \times 10^4/\text{mm}^3$ <sup>a</sup>	8.462	1.733–41.306	0.008
Child-Pugh class A	5.173	1.064–25.160	0.042

BT, blood transfusion

<sup>a</sup>Significant variables in multivariate analysis

**Discussion**

In the present study, we demonstrated the clinicopathological features of elderly patients with HCV-related HCC compared with younger patients. The elderly patients were characterized as lower male/female ratio, better liver function, and less alcohol consumption than the younger patients. At first, we examined all 693 patients with anti-HCV and without HBsAg. Next, we examined 337 patients after excluding patients with factors affecting development of hepatocarcinogenesis. Results of these two examinations were almost the same, as already mentioned. Ultimately, we wanted to emphasize that elderly patients with HCV-related HCC tend to develop HCC despite their low-grade fibrosis stage, and they should be followed more closely.

Several risk factors for the development of HCV-related HCC have been identified to date, such as age, degree of liver fibrosis, male sex, alcohol consumption, positivity for HBsAg, and high AFP level.<sup>15-20</sup> Recently, elderly HCC patients with HCV have increased in number in Japan.<sup>24-26</sup> To improve the survival of elderly cirrhotic patients by increasing the percentage of cancers amenable to effective treatment, it is important to clarify the characteristics of elderly HCC patients with HCV. In the present study, we divided patients with HCV-related HCC into two groups, the younger and the elderly group, according to age at first diagnosis. In

previous studies,<sup>16,18,20</sup> the authors divided HCC patients into two groups by age 50, 60, or 70. As we reported previously, elderly patients with HCV-related HCC have recently increased in number,<sup>24</sup> and in this study the median ages of patients were 66 and 69 for men and women, respectively. Because we want to clarify the characteristics of elderly patients in detail, they were divided by age less than or more than 70 years.

The incidence of HCC in HCV-related liver cirrhosis is significantly higher in patients with HBV antibody, such as anti-HBs and anti-HBc, than in those without.<sup>27</sup> First, we analyzed the patients who were positive for anti-HCV but negative for HBsAg. Furthermore, we also excluded anti-HBc-positive patients to reduce the influence of HBV on such analysis, and then we performed the next examination.

Several studies have shown that high AFP value is one of the risk factors of HCC.<sup>15-20</sup> In the present study, there was no significant difference in AFP between the elderly and the younger group.

Alcohol consumption is considered a significant risk factor for HCV-related HCC.<sup>15,16,18</sup> Our results showed significant differences between the elderly and the younger group, which might suggest that habitual alcohol drinkers develop HCC earlier than non-drinkers.

Silini et al.<sup>37</sup> reported a close association between HCV 1b and HCC, but recent studies have failed to

establish a correlation between HCV genotype and HCC.<sup>28,38</sup> In our study, the proportion of patients with genotype 1b was significantly greater in the younger group than the elderly group.

The Child-Pugh class correlates well with liver function. Our results showed that the elderly group tended to have better Child-Pugh class than the younger group. Platelet counts also indicated that the elderly group had better liver function and lower grade of fibrosis stage. The results of the pathological examination might support a tendency for the elderly group to have a lower grade of fibrosis stage, although this result may have a bias for selecting patients with good liver function when elderly patients underwent hepatic resection. Several studies reported that fibrosis stage and aging have major impacts on the incidence of HCC.<sup>15-21</sup> Yoshida et al.<sup>21</sup> suggested that the risk of HCC is strongly associated with the stage of liver fibrosis and that the annual incidence of HCC increased with the degree of liver fibrosis in Japan. In addition, progression of liver fibrosis is also strongly associated with aging.<sup>22,23</sup> As we recently reported, the time interval between blood transfusion and diagnosis of HCC was significantly shorter when patients received blood transfusions at an older age than at a younger age.<sup>24</sup> Therefore, we assumed that fibrosis stage could correlate strongly with aging for hepatocarcinogenesis and that elderly HCC patients should have a higher grade of fibrosis stage than younger patients. However, we found many cases with HCC and low-grade fibrosis stage among the elderly group. Furthermore, patients who received blood transfusions at an older age developed HCC within a shorter period of time than those at a younger age. It should be noted, however, that the former included more patients with a noncirrhotic liver than the latter. Elderly patients seemed to have a tendency to develop HCC earlier despite their low-grade fibrosis stage. Why is aging an independent risk factor for hepatocarcinogenesis? Chiramonte et al.<sup>39</sup> suggested that increasing age is a risk factor, probably because it reflects a longer duration of cirrhosis, but in our study the majority of elderly patients developed HCC from a noncirrhotic liver. These findings suggest that the shorter duration to development of HCC was not only the result of early progression of liver fibrosis stage but caused by other reasons, e.g., aging. Surprisingly, fibrosis stage seemed to be inversely proportional to age at diagnosis. This finding could mean that the impact of aging is stronger than the degree of liver fibrosis for development of HCV-related HCC. However, the present study is a retrospective study and included only HCC patients while excluding non-HCC patients with HCV. Further studies are needed to determine the correlation between HCV patients with HCC and those without.

Benson et al.<sup>40</sup> suggested that the increase in cancer rates that occurs after the age of 30 is the result of activation of quiescent cells with damaged DNA, or deactivation of DNA surveillance or repair, or impaired apoptosis. We reported previously that in chronic hepatitis C patients, the rate of DNA synthesis becomes high and genomic instability may increase during alternating necrosis and regeneration, resulting in malignant transformation and progression to an aggressive state.<sup>41</sup> We also reported that telomere length in the liver shortened not only with progression of fibrosis staging but also with aging. Another study suggested that the reduction of telomere length in chronic liver disease increased the risk of HCC development.<sup>42</sup> Considered together, the foregoing results suggest that elderly patients might have sufficiently short telomeres for developing HCC even if they had a noncirrhotic liver because the telomeres have shortened in length with aging.

In conclusion, we demonstrated in the present study that elderly patients with HCV developed HCC despite low-grade fibrosis stage. Further studies will be required about the impact of aging on HCV-related hepatocarcinogenesis.

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ORIGINAL ARTICLE

## Combination therapy of lamivudine and adefovir in Japanese patients with chronic hepatitis B

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### Abstract

**Purpose** This study aimed to clarify the long-term efficacy of the lamivudine treatment in Japanese patients with chronic hepatitis B either with or without lamivudine resistance or with or without adefovir add-on treatment.

**Methods** We followed 110 patients who received lamivudine for more than 12 months, including 67 hepatitis B e antigen (HBeAg)-positive and 43 HBeAg-negative patients.

**Results** The median follow-up after the onset of lamivudine was 48 (range = 12–86) months. In all the patients with or without lamivudine resistance, the level of alanine aminotransferase (ALT) normalization decreased from 70.0% at 1 year to 36.4% at 5 years and the loss of serum

HBV DNA level decreased from 72.7% at 1 year to 31.8% at 5 years. Sixty patients (54.6%) developed a lamivudine-resistant mutation, and this occurrence was more frequently observed in those who were HBeAg-positive ( $P < 0.01$ ), those with a low level of ALT ( $P < 0.05$ ), and those with a high level of serum HBV DNA ( $P < 0.01$ ). Thirty-six of 60 patients received adefovir in addition to lamivudine to treat breakthrough hepatitis. A Cox proportional hazards model analysis revealed the level of baseline HBV DNA to be the best predictive factor for the virus recrudescence (risk ratio = 0.466, 95% confidence interval [CI]: 0.246–0.842,  $P = 0.011$ ) and the breakthrough hepatitis (risk ratio = 0.444, 95% CI: 0.218–0.879,  $P = 0.019$ ). We carefully monitored the efficacy of this treatment both in patients

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who received adefovir and in those who did not since the beginning of the lamivudine treatment. The normalization level of ALT was 61.4% at 5 years and the loss of serum HBV DNA was 61.4% at 5 years since lamivudine was started. A histologic improvement was observed in patients with ALT levels less than two times the upper limit of normal at the time of a second liver biopsy.

**Conclusions** Although the efficacy of lamivudine is limited because of breakthrough hepatitis, adefovir was used as a salvage treatment of patients with lamivudine-resistant chronic hepatitis B. In addition, lamivudine was used for the treatment of Japanese patients with chronic hepatitis B with or without lamivudine resistance, and was found to be useful regarding the long-term virologic and biochemical responses.

**Keywords** Chronic hepatitis B Lamivudine Adefovir

## Introduction

The number of patients chronically infected with hepatitis B virus (HBV) is reported to be more than 350 million worldwide [1, 2]. These patients are at an increased risk to develop cirrhosis, hepatic decompensation, and hepatocellular carcinoma [3, 4]. The aims of treatment of chronic hepatitis B are to achieve a sustained suppression in HBV replication and remission in liver disease. The endpoints used to assess the treatment response include the normalization of the alanine aminotransferase (ALT) level, the loss of serum HBV DNA, the loss of hepatitis B e antigen (HBeAg) with or without the detection of antibody to HBeAg (HBeAb), and an improvement in the liver histology.

Interferon, which has been shown to have an antiproliferative effect on the virus, has been used for the treatment of chronic hepatitis B; however, its efficacy has been limited to only a small percentage of preselected patients [5, 6]. Lamivudine is the first nucleoside analog to be approved for the treatment of the patients with chronic hepatitis B. Although the short-term efficacy of lamivudine therapy has been well documented, the occurrence of lamivudine-resistant mutations has also been reported to increase with extended use [7–9]. Adefovir dipivoxil is a nucleotide analogue of adenosine monophosphate and has been shown to be effective in suppressing not only wild-type HBV but also lamivudine-resistant HBV [10, 11].

Lamivudine has been used in Japan since November 2000 for the treatment of patients with chronic hepatitis B. Since December 2004, adefovir, which decreases the incidence of lamivudine-resistant mutations, has been administered to patients demonstrating a flare-up of

hepatitis. The aims of this study were to clarify (1) the long-term efficacy of the lamivudine treatment of Japanese patients with chronic hepatitis B with or without adefovir add-on treatment of breakthrough hepatitis due to lamivudine resistance, (2) the rate of both occurrence of the lamivudine-resistant mutant virus and recurrence of hepatitis related to the lamivudine-resistant virus, and (3) the long-term consequences of the lamivudine and adefovir combination therapy for chronic hepatitis B.

## Patients and methods

Eligible patients were enrolled at Fukuoka University Hospital. The key inclusion criteria were seropositivity for hepatitis B surface antigen and serum HBV DNA. Both HBeAg-positive and HBeAg-negative patients were included. Lamivudine was administered orally at a dose of 100 mg daily for the treatment of the patients who had elevated ALT levels, namely, more than 1.5 times the upper limit of normal. Exclusion criteria included decompensated liver disease, a coexisting serious medical or psychiatric illness, a history of alcohol or drug abuse within 1 year before entry, and coinfection with hepatitis C virus or human immunodeficiency virus, and advanced hepatocellular carcinoma. We examined the effects of the lamivudine treatment on the normalization of the ALT levels, the loss of HBeAg, HBeAg seroconversion, and the loss of serum HBV DNA at 1, 2, 3, 4, and 5 years, respectively. To determine the factors that influenced the occurrence of lamivudine-resistant mutation in HBV, we also examined the pre-treatment clinical factors.

Among the patients who developed a lamivudine-resistant virus, the patients who showed an elevated ALT levels of more than two times the upper limit of normal and that continued for longer than 2 months received adefovir at an oral dose of 10 mg daily in addition to lamivudine. In those patients who had adefovir in addition to lamivudine for the treatment of recurrence hepatitis, we examined the effect of the adefovir treatment on the normalization of the ALT levels and the loss of serum HBV DNA since the time the adefovir treatment was started. Furthermore, we studied the efficacy of this combination therapy on the normalization of the ALT levels and the loss of serum HBV DNA at 1, 2, 3, 4, and 5 years, respectively, since the time the lamivudine treatment was started.

The histologic improvement was analyzed in 15 patients who underwent a second liver biopsy. The patients were subjected to a liver biopsy using a Menghini needle guided by ultrasonography. Formalin-fixed liver specimens were stained with hematoxylin and eosin for morphologic evaluations, with Masson's trichrome staining for the assessment of fibrosis.

HBeAg and HBeAb were detected via a chemiluminescent enzyme immunoassay (ARCHITECT HBeAg and ARCHITECT HBeAb, Abbott Japan, Tokyo, Japan). The serum HBV DNA levels in all patients before and during lamivudine treatment were measured via Transcription Mediated Amplification (DNA probe [FR]-HBV; Fujirebio Inc, Tokyo, Japan). The HBV levels were expressed as the log genome equivalent per milliliter (LGE/ml) and the upper and lower detection limits of the assay were 8.7 and 3.7 log copies/ml, respectively. The incidence of lamivudine mutations in the tyrosine-methionine-aspartate-aspartate (YMDD) motif was measured by means of a polymerase chain reaction-enzyme linked mini-sequence assay (PCR-ELMA; SMITEST HBV-YMDD Mutation Detection Kit, Medical and Biological Laboratories, Nagano, Japan).

#### Statistical analysis

Fisher's exact test was used for sex and HBeAg positivity for comparisons between groups. Comparisons of the means  $\pm$  standard deviations were performed using Student's *t*-test for age, the ALT level, and the serum HBV DNA level. A *P*-value of less than 0.05 was considered to be statistically significant. For multivariate analyses, the Cox proportional hazards model analysis was used. Ninety-five CIs were used throughout.

#### Results

Patients (*n* = 327) who were seropositive for hepatitis B surface antigen were from our hospital and all were Japanese patients. Among these cases, 141 patients received lamivudine as the therapy for chronic hepatitis B and 110

eligible patients have been followed for more than 1 year (67 patients who were HBeAg-positive and 43 patients who were HBeAg-negative) (Table 1). This study reviewed the cumulative outcome of the patients with chronic hepatitis B who were treated with lamivudine, and the patients who had been followed for more than 2, 3, 4, and 5 years numbered 94, 79, 63, and 44, respectively. The median follow-up after the start of lamivudine was 48 (range = 12–86) months. In all patients, including those who were either HBeAg-positive or HBeAg-negative, the normalization of ALT levels decreased with the duration of lamivudine treatment from 70.0% at 1 year to 56.4%, 48.3%, 49.2%, and 36.4% at 2, 3, 4, and 5 years and the loss of serum HBV DNA decreased from 72.7% at 1 year to 53.2%, 41.8%, 39.7%, and 31.8% at 2, 3, 4, and 5 years, respectively (Table 2). These results included all the patients with or without lamivudine resistance. In our intention-to-treat analysis, the patients who had an efficacy of adefovir salvage treatment of breakthrough hepatitis were excluded from the efficacy group of lamivudine treatment. In the HBeAg-positive patients, the normalization of the ALT levels decreased during the lamivudine treatment from 71.6% at 1 year to 55.7%, 42.6%, 41.9%, and 22.6% at 2, 3, 4, and 5 years, respectively (Table 2). Their loss of serum HBV DNA was 22.6% and HBeAg seroconversion was 22.6% during the 5-year period of lamivudine treatment. In the HBeAg-negative patients, the normalization of the ALT levels was 67.4% at 1 year and 57.6%, 60.0%, 65.0%, and 69.2% at 2, 3, 4, and 5 years and the loss of serum HBV DNA was 76.7% at 1 year and 69.7%, 66.0%, 53.0%, and 53.8% at 2, 3, 4, and 5 years, respectively.

The occurrence of a lamivudine-resistant mutation was seen in 60 patients. The mutation proportion increased from 27.3% at 1 year to 43.6%, 50.1%, 53.6%, and 54.6%

**Table 1** Patients baseline demographics and disease characteristics (*n* = 110)

	HBeAg-positive <i>n</i> = 67	HBeAg-negative <i>n</i> = 43	All patients <i>n</i> = 110
Gender, male:female (%male)	43:24 (64.2%)	29:14 (67.4%)	72:38 (65.6%)
Age (years)			
Mean $\pm$ SD	41.0 $\pm$ 12.5	47.9 $\pm$ 9.4	43.7 $\pm$ 11.9
Median	42	47	45
Range	18–68	27–69	18–69
Alanine aminotransferase (IU/l) <sup>a</sup>			
Mean $\pm$ SD	208.5 $\pm$ 246.9	185.4 $\pm$ 238	199.3 $\pm$ 244.8
Median	110	89	92
Range	45–1148	45–907	45–1148
HBV DNA (LGE/ml)			
Mean $\pm$ SD	7.52 $\pm$ 1.06	6.16 $\pm$ 1.95	6.99 $\pm$ 1.62
Median	7.6	6.9	7.3
Range	4.3–8.7	3.7–8.4	3.7–8.7

HBeAg: Hepatitis B e antigen

<sup>a</sup> The upper limit of the normal range is 30 IU per liter

**Table 2** The efficacy of lamivudine treatment in the patients with or without lamivudine-resistance ( $n = 110$ )

	At 1 year ( $n = 110$ )	At 2 years ( $n = 94$ )	At 3 years ( $n = 79$ )	At 4 years ( $n = 63$ )	At 5 years ( $n = 44$ )
All patients ( $n = 110$ )					
ALT normalization	77 (70.0%)	53 (56.4%)	38 (48.3%)	31 (49.2%)	16 (36.4%)
Loss of HBV DNA	80 (72.7%)	50 (53.2%)	33 (41.8%)	25 (39.7%)	14 (31.8%)
	At 1 year ( $n = 67$ )	At 2 years ( $n = 61$ )	At 3 years ( $n = 54$ )	At 4 years ( $n = 43$ )	At 5 years ( $n = 31$ )
HBeAg-positive patients ( $n = 67$ )					
ALT normalization	48 (71.6%)	34 (55.7%)	23 (42.6%)	18 (41.9%)	7 (22.6%)
Loss of HBV DNA	47 (70.1%)	27 (44.3%)	19 (35.2%)	14 (32.6%)	7 (22.6%)
Loss of HBeAg	20 (29.9%)	20 (32.8%)	19 (35.2%)	13 (30.2%)	7 (22.6%)
HBeAg seroconversion	16 (23.9%)	9 (31.1%)	18 (33.3%)	13 (30.2%)	7 (22.6%)
	At 1 year ( $n = 43$ )	At 2 years ( $n = 33$ )	At 3 years ( $n = 25$ )	At 4 years ( $n = 20$ )	At 5 years ( $n = 13$ )
HBeAg-negative patients ( $n = 43$ )					
ALT normalization	29 (67.4%)	19 (57.6%)	15 (60.0%)	13 (65.0%)	9 (69.2%)
Loss of HBV DNA	33 (76.7%)	23 (69.7%)	14 (66.0%)	11 (53.0%)	7 (53.8%)

ALT: Alanine aminotransferase

HBeAg: Hepatitis B e antigen

at 2, 3, 4, and 5 years of treatment, respectively. Table 3 shows the number of patients who developed virus recrudescence at each year. This occurrence was more frequent in those who were HBeAg-positive ( $P < 0.01$ ), those with a low ALT level ( $P < 0.05$ ), and those with a high level of serum HBV DNA ( $P < 0.01$ ) at the time the lamivudine treatment was started (Table 4). Thirty-six (60.0%) of the

**Table 3** The occurrence of lamivudine-resistant mutation during 5 years

	Number of patients	Baseline HBV DNA levels Mean $\pm$ SD (LGE/ml)
At 1 year	30	7.46 $\pm$ 1.15
At 2 years	18	7.56 $\pm$ 1.07
At 3 years	8	6.98 $\pm$ 2.35
At 4 years	3	7.46 $\pm$ 0.70
At 5 years	1	6.3

**Table 4** Comparison between those patients who had virus recrudescence and those who did not

	Virus recrudescence ( $n = 60$ )	Non-virus recrudescence ( $n = 50$ )	<i>P</i>
Age (years) mean $\pm$ SD	43.5 $\pm$ 11.4	44.4 $\pm$ 12.3	NS
Gender: male:female (%male)	44:16 (73.3%)	28:22 (56.0%)	NS
HBeAg-positive patients	44 (73.3%)	23 (46.0%)	<0.01
Alanine aminotransferase (IU/l)			
Mean $\pm$ SD	157.9 $\pm$ 218.5	246.6 $\pm$ 261.6	<0.05
Serum HBV DNA (LGE/ml)			
Mean $\pm$ SD	7.40 $\pm$ 1.36	6.49 $\pm$ 1.75	<0.01

HBeAg: Hepatitis B e antigen  
NS: Not significant

60 patients received adefovir for the treatment of breakthrough hepatitis due to a lamivudine-resistant mutation, whereas the other 24 patients with a slight elevation in the ALT level did not receive adefovir. The patients who received adefovir had significantly higher levels of serum HBV DNA at the time the lamivudine treatment was started than those who did not receive adefovir (Table 5). Cox proportional hazards model analysis was used to determine the factors predicting viral recrudescence and breakthrough hepatitis. The patients who had more than 7.0 LGE/ml of serum HBV DNA level at the time the lamivudine treatment was started showed a significantly frequent occurrence of a lamivudine-resistant mutation (risk ratio = 0.466, 95% CI: 0.246–0.842,  $P = 0.011$ ; Table 6). The patients who had more than 7.7 LGE/ml of serum HBV DNA at the time the lamivudine treatment was started more frequently received adefovir add-on therapy for breakthrough hepatitis (risk ratio = 0.444, 95% CI: 0.218–0.879,  $P = 0.019$ ; Table 6).