In conclusion, it is important to monitor the early decline of the Hb concentration after initiation of combination therapy and to reduce the dose of ribavirin at the end of 2 weeks based on the magnitude of the Hb decline. An early reduction of ribavirin before progression to severe anemia can reduce the number of patients who are destined to discontinue ribavirin therapy. This should help improve the patients' quality of life by preventing the progression to severe anemia. Further prospective study is necessary to evaluate the antiviral outcome by ITT analysis using early reduction of ribavirin based on the "2 by 2" standard.

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# Should aged patients with chronic hepatitis C be treated with interferon and ribavirin combination therapy?

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**Abstract**

The aim of this study was to investigate the efficacy and safety of combination therapy of interferon and ribavirin for aged patients with chronic hepatitis C.

*Methods:* This study was conducted at Osaka University Hospital and institutions participating in the Osaka Liver Disease Study Group on 329 patients with chronic hepatitis C receiving interferon and ribavirin combination therapy (group A, under 60 year old, $n = 199$; group B, 60–64 year old, $n = 64$; group C, over 65 year old (mean age, $67.8 \pm 2.2$ year old, $n = 66$)). Of the 293 patients who were tested for HCV serotype and HCV viral loads, 215 had HCV-RNA with serotype 1 and high viral loads (1H) and the other 78 had HCV-RNA with serotype 2 or low viral loads (non-1H).

*Results:* In per-protocol analysis, the overall SVR rate of 1H patients was 28% (51/184). Among the 1H patients, the SVR rate was significantly lower in group C (16%) and group B (17%) than in group A (34%) ($p < 0.05$). The overall SVR rate of non-1H patients was 85% (57/67). No significant difference was found in the SVR rate among group C (79%), group B (100%), and group A (84%). On the other hand, the discontinuance of both drugs due to side effects was 29% (19/66) in group C, 20% (13/64) in group B, and 11% (21/199) in group A, with the discontinuance rates being higher in the older group ($p = 0.002$).

*Conclusions:* In aged chronic hepatitis C patients, interferon and ribavirin combination therapy can be recommended for the non-1H patients who showed a high SVR rate of approximately 65%, but not for the 1H patients.



*Keywords:* Chronic hepatitis C; Aged patient; Interferon and ribavirin combination therapy

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## 1. Introduction

Hepatitis C virus (HCV) is estimated to infect up to 170 million people worldwide [1]. Long persistence of HCV infection can lead to progression of liver fibrosis causing liver cirrhosis and ultimately hepatocellular carcinoma (HCC) [2,3]. In Japan, it is estimated that two million people are infected with HCV, and more than 30,000 patients die of HCC every year, with approximately 80% being caused by HCV infection [4]. It has been reported that HCV carriers in Japan tend to be old [5], and liver fibrosis progresses in aged patients. Moreover, the risk of HCC increases with progression of liver fibrosis and older age, with the occurrence of HCV-related HCC reaching a peak at around the age of 65 years old [3]. Past studies have made clear that interferon (IFN) therapy is effective for eliminating HCV, and IFN therapy significantly reduces the progression of liver fibrosis [6,7] and the risk of HCC, especially among virologic or biochemical responders [8–10]. Furthermore, recently, several groups have reported that IFN therapy, specially the SVR group, improved the survival of patients with HCV [11,12], also in aged patients [13].

The combination therapy with IFN and ribavirin has been reported to be effective for eliminating HCV compared with IFN monotherapy [14–16], but additional side effects of ribavirin, such as hemolytic anemia, which is not found in IFN monotherapy have been reported, leading to discontinuance of the treatment [17]. For aged patients, sufficient informed consent should be obtained before the start of stronger anti-viral therapy with possible severe side effects, because the function of the organs is generally poor, and the adverse effects of IFN therapy have been observed more frequently in older patients [18].

The question arises of whether aged patients with chronic hepatitis C should be treated with the combination therapy of IFN and ribavirin, while IFN monotherapy has been shown to be effective even in aged patients. In this study, we conducted a multi-center, retrospective study of patients with chronic hepatitis C treated by IFN and ribavirin combination therapy, and examined the efficacy and prevalence of side effects to clarify the adaptation of anti-viral treatment for aged patients.

## 2. Patients and methods

### 2.1. Patients

The current study was conducted at Osaka University Hospital and the institutions of the Osaka Liver Disease Study Group. The 329 patients with chronic hepatitis C included in this study were treated with combination IFN-α-2b and ribavirin between January 2001 and April 2004. All patients had HCV RNA detectable in serum by the polymerase chain reaction (PCR) method, had elevated ALT (above the upper limit of the normal) and had been histologically proven to have chronic hepatitis. None of the patients were positive for hepatitis B surface antigen and anti-human immunodeficiency virus antibody or had other forms of liver disease (alcoholic liver disease, hepatotoxic drugs, autoimmune hepatitis). This study protocol was carried out according to the ethical guidelines of the 1975 Declaration of Helsinki and informed consent was obtained from each patient.

### 2.2. Determination of HCV RNA levels

Serum HCV-RNA levels were quantified using branched DNA (bDNA) probe assay (version 2; Chiron, Dai-ichi Kagaku, Tokyo) [19,20] or combined PCR assay (Amplicor-HCV monitor assay) [21]. In this study, a high viral load was designated as the condition of a serum HCV-RNA level of more than $10^6$ equivalents/ml by bDNA assay or more than $10^5$ copies/ml serum by Amplicor-HCV monitor assay [22].

### 2.3. Treatment schedule

The 329 patients were treated with 10 MU ($n = 79$) or 6 MU ($n = 243$) or 3 MU ($n = 7$) IFN-α-2b intramuscularly every day for the first 2 weeks and the three times a week for the following 22 weeks in combination with ribavirin at a daily dose of 600 or 800 mg, depending on body weight (<60 or ≥60 kg, respectively). The starting doses of ribavirin were 800 mg per day for 178 patients, 600 mg per day for 148 patients, and 400 mg per day for three patients. The ribavirin dose was decreased or stopped in 91 patients (28%) due to side effects. The ribavirin dose of 200 mg was reduced if the hemoglobin value was below 10 g/dl. The ribavirin was stopped if Hb fell bellow 8.5 g/dl. One hundred and five patients continued only IFN therapy for 24 weeks after the combination therapy, because the combination therapy of IFN-α-2b and ribavirin for 48 weeks was not covered by medical insurance in Japan at that time. Patients with persistently undetectable HCV RNA 6 months after completion of treatment were considered to have achieved a sustained virological response.

### 2.4. Statistical analysis

Age, histological scores before IFN therapy, serum ALT levels, red blood cell (RBC) count, hemoglobin (Hb), white blood cell (WBC) count and platelet (Plt), and creatinine are expressed as mean ± S.D. Statistical analysis for group comparisons was performed by the $\chi^2$-test. The SVR rate was evaluated using the probability proportional to size analysis (PPS analysis) and the intention-to-treat analysis (ITT analysis). A value of $p < 0.05$ (two-tailed) was considered to indicate significance.

## 3. Results

### 3.1. Clinical characteristics before combination therapy

The baseline clinical features of the 329 patients are shown in Table 1. At the start of the treatment, 130 patients were 60

Table 1
Baseline characteristics of patients according to age

| | Group A ($n=199$) | Group B ($n=64$) | Group C ($n=66$) | $p$-value |
|---|---|---|---|---|
| Age (years old) | 49.0 ± 8.7 | 62.0 ± 1.4 | 67.8 ± 2.2 | |
| Sex (M/F) | 142/54[a] | 36/28 | 43/23 | [a]$p<0.05$ |
| HCV serotype (1/2/unknown) | 142/51/6 | 53/10/1 | 54/12/0 | N.S. |
| HCV-RNA (H/L/unknown) | 173/12/14 | 58/2/4 | 60/5/1 | N.S. |
| 1H/non 1H/unknown | 125/53/21 | 45/8/11 | 45/17/4 | |
| Fibrosis (F 1/F2/F3/F4/unknown) | 75/46/33/6/39 | 26/15/10/2/11 | 19/15/17/4/11 | N.S. |
| ALT (IU/L) | 112 ± 85[b] | 91 ± 49 | 90 ± 57 | $p<0.05$[b] |
| WBC | 5330 ± 1570[b] | 4970 ± 1390 | 4760 ± 1120 | $p<0.05$[b] |
| RBC ($\times 10^4$ μl) | 458 ± 47[b] | 433 ± 45 | 431 ± 47 | $p<0.01$[b] |
| Hb (g/dl) | 14.6 ± 1.5[b] | 14.0 ± 1.2 | 13.7 ± 1.4 | $p<0.01$[b] |
| Plt ($\times 10^4$ μl) | 16.0 ± 7.0[b] | 14.9 ± 5.3 | 14.2 ± 4.9 | $p<0.05$[b] |

*Note*: Data are given as the mean ± S.D. N.S., not significant. Group A, patients under 60 years of age (gender of three patients were unknown); group B, patients older than 60 years but under 65 years of age; group C, patients older than 65 years of age; 1H group, patients with genotype 1 and high viral load; non-1H group, patients other than 1H group.

[a] Significant level was compared with group B.

[b] Significant levels were compared with group B and group C.

years old or older. One hundred ninety-nine patients were under 60 years old (group A), sixty-four patients were 60–64 years old (group B) and sixty-six patients were 65 years old or older (group C). No significant difference was found in serotype, viral load and histological stage among the three groups. In aged patients, ALT, RBC, Hb, WBC, and Plt were less than in young patients (ALT, $p<0.05$; RBC and Hb, $p<0.01$; WBC and Plt, $p<0.05$). Among the patients, 215 had HCV-RNA with genotype 1 and high viral loads (1H group) and 114 had HCV-RNA with genotype 2 or low viral loads (non-1H group).

### 3.2. Initial dosage and treatment duration of interferon

Three kinds of IFN dosage were used in this study. Among group A, 10MU, 6MU, and 3MU were administered for 60 patients, 134 patients, and 5 patients; 12, 52, and none among group B, and 8, 56, and 2 among group C. No significant difference was found in the distribution of IFN dosage among each group. The 24 and 48-week treatments (IFN and ribavirin treatment for 24 weeks followed by IFN monotherapy for 24 weeks) were carried out for 102 patients and 75 patients among group A; 37 and 14 among group B; 32 and 16 among group C. The rates of patients receiving the 48-week treatment were similar for the three groups.

### 3.3. PPS analysis

On PPS analysis, the overall SVR rate of 1H patients was 28% (51/184). The SVR rates were 34% (40/117) for group A, 17% (6/36) for group B, and 16% (5/31) for group C. Among the 1H patients, the SVR rates of group B and C were significantly lower than that for group A ($p<0.05$). The overall SVR rate of non-1H patients was 85% (57/67). No significant difference was found in the SVR rates among group A (84%; 36/43), group B (100%; 5/5), and group C (79%; 11/14) (Fig. 1).

### 3.4. ITT analysis

On ITT analysis, the SVR rate was 24% (51/215) in 1H patients, being 32% (40/125) for group A, 13% (6/45) for group B, and 11% (5/45) for group C. Among the 1H patients, the SVR rates of group B and C were significantly lower than that for group A (A versus B; $p<0.05$, A versus C; $p<0.01$).

On the other hand, in the non-1H group, the SVR rate was 73% (57/78), being 77% (41/53) for group A, 63% (5/8) for group B, and 65% (11/17) for group C. No significant difference was found among the groups (Fig. 2).

### 3.5. Adverse effects

The entire treatment schedule without reduction and discontinuance of both drugs was completed by 174 patients (53%). Sixty-two percent (123/199) of the patients in group A, 42% (27/64) in group B, and 36% (24/66) in group C com-

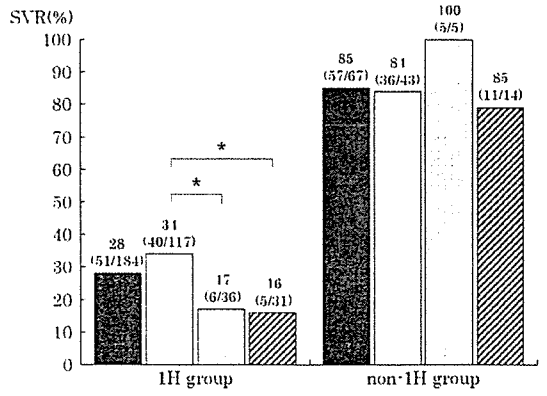


Fig. 1. Efficacy of the combination therapy according to age (PPS analysis). 1H group, patients with genotype 1 and high viral load. Non-1H group, patients not in the 1H group. (■) all patients; (□) group A, patients under 60 years of age; (▩) group B, patients from 60 years and older but under 65 years of age; (▨) group C, patients older than 65 years. Significant levels: *$p<0.05$.

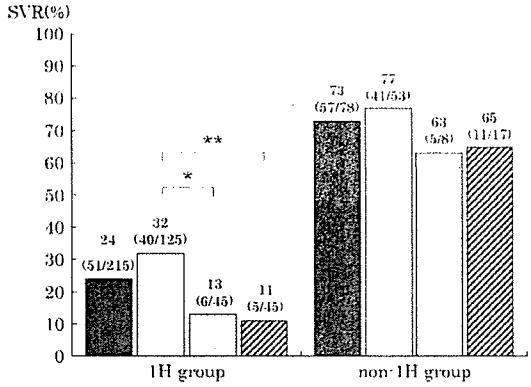


Fig. 2. Efficacy of the combination therapy according to distinction of age (ITT analysis). 1H group, patients with genotype 1 and high viral load. Non-1H group, patients not in the 1H group. (■) all patients; (□) group A, patients under 60 years of age; (▤) group B, patients from 60 years and older but under 65 years of age; (▨) group C, patients older than 65 years. Significant levels: $^{*}p<0.01$; $^{**}p<0.05$.

pleted all treatment schedules (A versus B; $p<0.0001$, A versus C; $p<0.001$). IFN treatment was stopped along with ribavirin in 52 patients (16%), and the IFN dose was decreased in 20 patients (6%). The ribavirin dose was decreased in 72 patients (22%), and stopped without discontinuance of IFN in 20 patients (6%). The discontinuance rate of both drugs was significantly higher in group C (29%, 21/199) and B (20%, 13/64) than group A (11%, 19/66) (Fig. 3).

The reasons for dose reduction and discontinuance of the treatment were anemia, general fatigue, digestive disorder, eczema, neutropenia, and psychological disorder. Among the patients discontinuing both drugs, for those under 60 years old, the major reasons were anemia (32%), general fatigue

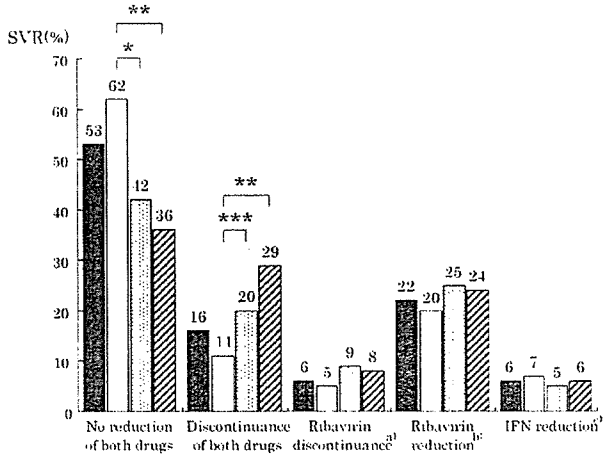


Fig. 3. Dose reduction or discontinuance of IFN and ribavirin. (a) Ribavirin discontinuance without discontinuance of IFN, (b) ribavirin reduction without discontinuance of IFN, and (c) IFN reduction regardless of discontinuance or reduction of ribavirin. (■) all patients; (□) group A, patients under 60 years of age; (▤) group B, patients from 60 years and older but under 65 years of age; (▨) group C, patients older than 65 years. Significant levels: $^{*}p<0.0001$; $^{**}p<0.001$; $^{***}p<0.005$.

(18%), digestive disorder (14%), and psychological disorder (14%). On the other hand, among the patients aged 60 years and older, the discontinuance of therapy due to anemia accounted for approximately 60% (17/28), which was twice as much as those of younger patients, with the difference being significant ($p<0.05$). Other reasons of the discontinuance of therapy among the patients aged 60 years and older were following; digestive disorder (14%), general fatigue (7%), eruption, granulocytopenia, thrombocytopenia, and psychological disorder (4%, respectively). Vascular diseases, such as cerebral bleeding did not appear in this study.

## 4. Discussion

In Japan, randomized control studies have been performed on the combination therapy of IFN and ribavirin for 24 weeks in patients with chronic hepatitis C, and the combination therapy was approved in 2001. However, the patients in these studies were under 60 years of age. Accordingly, the efficacy and adverse effects of combination therapy for aged patients has been still unclear. Since HCV carriers in Japan are older by 10–20 years than those in the United States and the European countries, it is very important to clarify the actual state of affairs for aged patients with chronic hepatitis C receiving the combination therapy, especially in Japan. These findings should be applicable for patients with chronic hepatitis C in other countries in a few decades, because almost the same efficacy and adverse effects are expected in patients treated by pegylated interferon (peg-IFN) and ribavirin combination therapy. In this study, we examined the efficacy and prevalence of the side effects with the focus on patient age.

The aged patients showed higher rates of discontinuance of IFN and ribavirin and lower rates for no reduction of both drugs than younger patients. The most frequent reason for the discontinuance of both drugs was hemolytic anemia which accounted for 60% of the cases in patients 60 years or older. The progress of anemia was frequently noted in aged patients and resulted in the discontinuation of ribavirin. Hemolytic anemia induced by ribavirin administration has been reported to depend on the plasma ribavirin concentration [23], with a high ribavirin concentration leading to it, and the plasma clearance of ribavirin depending on renal function [24]. A major cause for the advance of anemia in aged patients is due to the fact that renal function is poorer than in younger patients, leading to lower ribavirin clearance. As a result, severe hemolytic anemia can be induced by higher ribavirin concentrations. Therefore, the dosage of ribavirin should be reduced at the beginning of treatment in the aged patients with chronic hepatitis C in order to avoid the discontinuance of ribavirin, because the reduction of ribavirin does not decrease the SVR rate of this therapy.

The SVR difference according to age was observed for 1H patients, but not non-1H patients, when only the patients who completed the treatment were examined (PPS analysis).

That is, the SVR rates were still high for the aged patients of the non-1H group, but lower for the aged patients than the young patients in the 1H group. There are two possible reasons for this. First, the number of patients with no reduction of both drugs was significantly fewer for the patients aged 60–64 years and <60 years than for the patients aged ≥65 years, and the older patients tended to require ribavirin reduction or discontinuance (Fig. 3). Second, the liver fibrosis score tended to be higher in aged patients than in young patients, although the significant difference was not seen in this study (Table 1). These factors can decrease the SVR rates in aged patients in the 1H group, from which it is difficult to eliminate the virus, although the aged patients in the non-1H group whose viruses are easily eliminated were not affected. The results on ITT analysis account for the conclusion of the indication for IFN and ribavirin combination therapy of 24 weeks for aged patients; the patients of the 1H group do not have good application whose SVR is approximately 10%. On the other hand, patients of the non-1H group should be given the combination therapy because of the higher SVR rates of about 65%.

Better efficacy of treatments using new drugs, such as peg-IFN and ribavirin combination therapy or NS3/4 protease inhibitor, is greatly anticipated.

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HEPATOLOGY

# Clinical features of hepatocellular carcinoma that occur after sustained virological response to interferon for chronic hepatitis C

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**Abstract**

***Background and Aim***: This study investigated the clinical features of hepatocellular carcinoma in patients with sustained virological response to interferon for hepatitis C viral (HCV) infection.

***Methods***: A total of 7715 patients with HCV infection were treated with interferon and followed up for more than 1 year after withdrawal of interferon in 64 Japanese hospitals and clinics between July 1988 and August 2001. Sustained virological response was obtained in 2515 (32.6%) patients. Of these 2515 patients, clinical data were collected for 38 patients in whom hepatocellular carcinoma developed. Sustained virological response was defined as HCV RNA negativity more than 6 months after the termination of interferon.

***Results***: All patients were HCV RNA negative at the time of diagnosis of hepatocellular carcinoma. The median period until the detection of hepatocellular carcinoma was 4.7 years (range 1.4–9.0 years). There were significant improvements in hepatic function including serum albumin, aspartate aminotransferase, alanine aminotransferase, indocyanine green test, platelet count and histological activity grade in comparison with those before interferon therapy and at the onset of hepatocellular carcinoma. The maximum tumor size in patients without medical follow-up for 1 year or more (median: 60 mm) was significantly larger than in patients who were periodically followed up for 6 months or less (median: 25 mm) ($P = 0.002$).

***Conclusions***: The present findings emphasize the importance of regular medical follow up of patients with HCV infection, as even patients showing a sustained virological response to interferon and in whom hepatic function has improved have the potential to develop hepatocellular carcinoma.



**Key words**: follow-up, hepatitis C virus, hepatocellular carcinoma, interferon, sustained virological response.

## INTRODUCTION

Hepatocellular carcinoma (HCC) is one of most prevalent malignant tumors worldwide, and its incidence is increasing. Most cases are attributable to chronic hepatitis C virus (HCV) or hepatitis B virus (HBV) infection.[1,2] In Japan, epidemiological studies have shown that HCV is more common than HBV as the causative agent of HCC.[3,4] Because HCV infection is related to the development of cirrhosis and HCC, it was assumed that eradication of this infection would provide the most effective means of preventing HCV-related complications, including HCC.

Currently, interferon (IFN), peginterferon or combination therapy with ribavirin, are the available drugs that are effective for terminating HCV infection. IFN

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can induce a long-term favorable response and eradication of HCV RNA from serum after treatment cessation, although the response rate is not fully satisfactory.[5-8] Furthermore, patients with HCV appear to derive a definitive benefit in terms of prevention of progression to cirrhosis and the development of HCC.[7,15] However, even in some patients from whom HCV infection has been eliminated by IFN therapy, HCC can still be detected.[11-27] In these patients, the clinical features of developing HCC have not been fully investigated,[20] although they have been documented in individuals or small numbers of cases.[21-27]

The present study therefore attempted to elucidate the clinical features of HCC, especially the serial changes occurring in the period from before IFN therapy to the detection of HCC. A multicenter, collective study was conducted in the setting of hospitals and clinics belonging to the Japanese Society of Gastroenterology, Kyushu Division, in Japan, as it was felt that a study conducted in a single institution would provide inadequate numbers of sustained responders who developed HCC.

## METHODS

### Patients

This study was conducted at major hospitals and clinics belonging to the Japanese Society of Gastroenterology, Kyushu Division, Japan. A patient cohort in whom HCC had been detected among sustained responders to IFN therapy for chronic hepatitis C was collected by means of data collection instruments. All of the patients included had tested positive for HCV RNA before IFN therapy, and were followed up after withdrawal of IFN therapy for more than 1 year prior to the end of August 2001. Sustained virological response (SVR) was defined as HCV RNA negativity for more than 6 months after termination of IFN therapy. Diagnosis of HCC was based either on histological examination or on typical computed tomographic and/or angiographic findings at each institution. Patients were excluded if HCC was detected within 1 year after the termination of IFN therapy, because in such cases it was highly likely that the cancer had been present at the end of IFN treatment.

In Japan at the time of the study, the standard schedule was 6–10 MU IFN-α every day for the first 2–4 weeks and then three times a week for the following 20–22 weeks, or 6 MU IFN-β every day for 6–8 weeks. Patients treated with peginterferon or combination therapy with ribavirin were not included because these therapies had not been approved by the Ministry of Health, Labor and Welfare in Japan at the time of the study.

At the first data collection, hospitals were approached and information on the number of patients who had undergone IFN therapy for chronic hepatitis C and who had been followed up for more than 1 year after the termination of IFN therapy, the number of SVR patients among them, and the number of patients in whom HCC had developed among the SVR patients was requested; 64 hospitals responded, listed in Appendix I.

In the second data collection, carried out on SVR patients in whom HCC had developed, clinical data were requested for each patient from before IFN therapy and at detection of HCC.

### Data collected

To elucidate the clinical features of HCC that developed in SVR patients, host-related, treatment-related and tumor-related variables before IFN therapy and at detection of HCC were investigated (Table 1). Assessments of the staging of liver fibrosis and the grade of inflammatory activity were based on the classification of Desmet *et al.*,[28] where staging is defined as: F0 (no fibrosis), F1 (fibrous portal expansion), F2 (bridging fibrosis), F3 (bridging fibrosis with architectural distortion), and F4 (cirrhosis); and grading is defined as: A0 (no activity), A1 (mild activity), A2 (moderate activity), and A3 (severe activity).

Follow-up ended with the last recorded visit before 31 August 2001. The period until the detection of HCC was measured from the day of termination of IFN therapy to the day when HCC was first diagnosed by imaging modalities such as ultrasonography or computed tomography. The medical follow-up period for the detection of HCC after SVR was defined as the interval

**Table 1** Clinical features of 38 patients with chronic hepatitis C in whom hepatocellular carcinoma (HCC) developed after sustained response to interferon

| Clinical feature | Before interferon | At detection of HCC | *P*-value |
|---|---|---|---|
| **Host-related variables** | | | |
| Age (years) [median (range)] | 60 (36–71) | 64 (38–77) | <0.0001 |
| <60 [*n* (%)] | 16 (42%) | 4 (11%) | |
| >60 [*n* (%)] | 22 (58%) | 34 (89%) | |
| Sex [*n* (%)] | | | |
| Men | 34 (89%) | — | — |
| Alcohol abuse [*n* (%)]† | | | |
| Positive | 2 (5%) | — | — |
| Viral load before interferon (copies/mL) [*n* (%)] | | | |
| $>10^6$ | 2 (13%) | — | — |

**Table 1** *Continued*

| Clinical feature | Before interferon | At detection of HCC | *P*-value |
|---|---|---|---|
| Serological group before interferon [*n* (%)] | | | |
| Group 1 | 6 (33%) | — | — |
| Group 2 | 12 (67%) | — | — |
| Hepatic function [median (range)] | | | |
| Platelet ($\times 10^4$/mm$^3$) | 11.6 (6.6–31.0) | 16.5 (7.3–31.0) | <0.0001 |
| Total bilirubin (mg/dL) | 0.7 (0.3–1.5) | 0.7 (0.3–16.8) | 0.32 |
| Albumin (g/dL) | 4.2 (3.3–5.0) | 4.4 (3.2–5.2) | 0.10 |
| Aspartate aminotransferase (IU/L) | 78 (29–288) | 29 (14–159) | <0.0001 |
| Alanine aminotransferase (IU/L) | 109 (24–295) | 23 (8–178) | <0.0001 |
| Prothrombin time | 81 (49–124) | 89 (68–137) | 0.03 |
| Indocyanine green $R_{15}$ (%) | 15.0 (5.0–45.0) | 10.6 (3.1–27.4) | 0.0009 |
| Histologic fibrosis staging [*n* (%)] | | | |
| F0 | 0 (0%) | 1 (6%) | |
| F1 | 9 (26%) | 3 (19%) | |
| F2 | 10 (29%) | 8 (50%) | |
| F3 | 10 (29%) | 2 (13%) | |
| F4 | 6 (17%) | 2 (13%) | 0.11 |
| Histologic activity grade [*n* (%)] | | | |
| A0 | 0 (0%) | 6 (38%) | |
| A1 | 7 (23%) | 8 (50%) | |
| A2 | 17 (57%) | 2 (13%) | |
| A3 | 6 (20%) | 0 (0%) | 0.001 |
| **Treatment-related variables** | | | |
| Treatment periods (weeks) [median (range)] | 24 (2–31) | — | — |
| Interferon type [*n* (%)] | | | |
| α | 36 (95%) | — | — |
| β | 2 (5%) | — | — |
| Total amount of interferon [median (range)] | 480 (126–846) | — | — |
| Prior interferon therapy [*n* (%)] | | | |
| Positive | 2 (5%) | — | — |
| **Tumor-related variables** | | | |
| Number of tumors [*n* (%)] | | | |
| Solitary | — | 31 (82%) | — |
| Multiple (range) | — | 7 (18%) | — |
| Maximum tumor size (mm) | | | |
| Median | | 30 (12–150) | |
| ≤30 [*n* (%)] | — | 21 (57%) | — |
| >30 [*n* (%)] | — | 16 (43%) | — |
| Alpha-fetoprotein (ng/mL) [*n* (%)] | | | |
| >20 | 4 (16%) | 15 (41%) | 0.07 |
| PIVKA-II (AU/mL) [*n* (%)] | | | |
| >0.063 | 0 (0%) | 13 (43%) | 0.01 |
| Differentiation of HCC [*n* (%)] | | | |
| Well-differentiated | — | 11 (44%) | — |
| Moderately differentiated | — | 11 (44%) | — |
| Poorly differentiated | — | 2 (8%) | — |
| Combined type | — | 1 (4%) | |
| Period until development of HCC (years) [median (range)] | — | 4.7 (1.4–9.0) | — |
| Period of medical follow-up (months) [median (range)] | — | 3 (0.5–57) | — |
| First treatment for HCC[‡] [*n* (%)] | | | |
| Resection | — | 16 (43%) | — |
| Local ablation | — | 10 (27%) | — |
| Transarterial treatment | — | 11 (30%) | — |

PIVKA-II, protein induced by vitamin K absence or antagonist-II; $R_{15}$, indocyanine green retention rate at 15 min.
[†]Ethanol intake ≥80 g/day for ≥5 years. [‡]One patient has not yet undergone treatment for HCC.

during which checks for HCC were performed using tumor markers and/or imaging modalities.

Differences between data obtained before IFN therapy and at detection of HCC were evaluated using the Wilcoxon signed-rank test. All *P*-values presented in this report are of the two-tailed type. Differences at $P < 0.05$ were considered statistically significant. All analyses were conducted using SPSS 8.0 J (SPSS Inc. Chicago, IL, USA).

## RESULTS

In the first data collection, a total of 7715 patients with chronic hepatitis C were identified who had been treated with IFN and followed up for more than 1 year after the termination of IFN therapy from July 1988 to August 2001 in 64 hospitals and clinics. A SVR was obtained in 2515 patients (32.6%), among whom HCC was detected in 42 (1.7%) from 24 hospitals (38%).

In the second data collection, clinical data were received for 41 patients from 23 hospitals. Of these patients, three were excluded from the analysis because of detection of HCC within 1 year after IFN therapy (one patient), concomitant hepatitis B virus infection (one patient), and a history of treatment for HCC before IFN therapy (one patient). Accordingly, the study subjects comprised 38 patients who had developed HCC after SVR to IFN therapy for chronic hepatitis C. The profiles of the patients are shown in Fig. 1.

Table 1 summarizes the clinical features of the 38 HCV patients in whom HCC developed after SVR to IFN therapy. All of the patients were HCV RNA negative at the time of HCC detection, when their median age was 64 (range 38–77) years, and 34 of the patients (89%) were ≥60 years of age. Thirty-four patients (89%) were men (sex ratio 8.5:1). When data from before IFN therapy and at the detection of HCC were compared, there were significant improvements in platelet count, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and indocyanine green retention rate at 15 min (ICG $R_{15}$). In the 16 patients who underwent liver biopsy before IFN therapy and at the time of HCC detection, serial changes in histological fibrosis staging and activity grade were observed (Fig. 2). Histological activity grade improved significantly after IFN therapy ($P = 0.004$). However, there was no significant improvement of histological fibrosis staging after IFN therapy ($P = 0.10$).

With regard to the HCC that developed, 31 patients (82%) had a solitary tumor and 22 patients (57%) had a tumor <3 cm in diameter. The median period until the detection of HCC was 4.7 years (range 1.4–9.0 years), and there were nine patients in whom HCC less than 3 cm in size developed more than 5 years after IFN therapy (Fig. 3). The median period of medical follow-up after the termination of IFN therapy was 3 months (range 0.5–57 months), and eight patients were not followed up for 1 year or more. The maximum tumor size in these patients (median 60 mm; range 40–150 mm) was significantly larger than in patients who were periodically followed up for 6 months or less (median

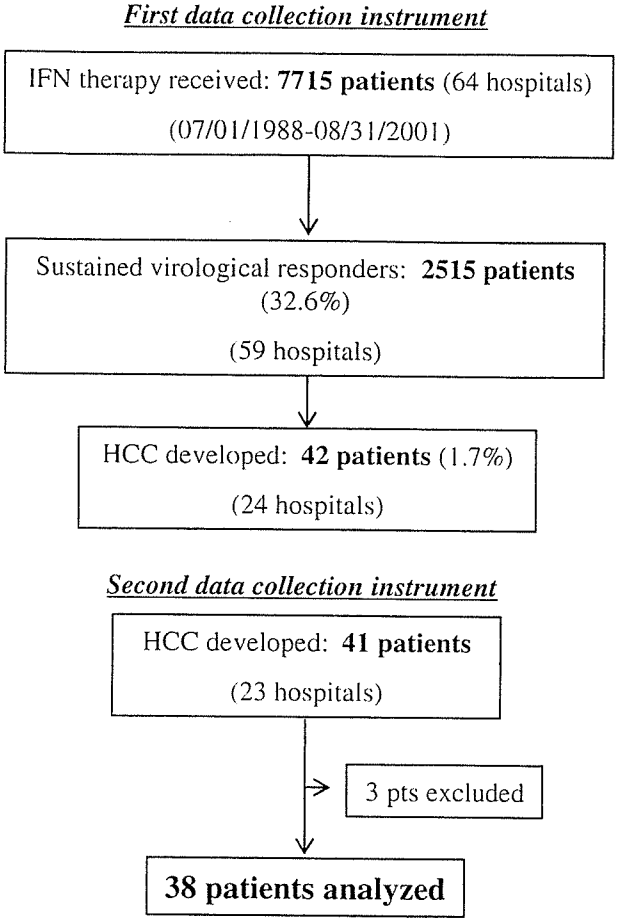


**Figure 1** Profile of patients and data collection. One hospital did not respond to second data collection request. IFN, interferon; HCC, hepatocellular carcinoma.

25 mm; range 12–51 mm) ($P = 0.002$). Of the 38 patients, 16 underwent hepatic resection for HCC.

## DISCUSSION

Chronic hepatitis C is a progressive disease that is related to the development of cirrhosis and HCC. IFN, peginterferon, or combination therapy with ribavirin are widely used as standard treatments for chronic hepatitis C, the therapeutic scope being viral clearance and resolution of hepatic inflammation.[5–8] In theory, if successful in this respect, these treatments should have the additional effect of preventing HCC. Sustained eradication of HCV by IFN therapy has been shown to improve hepatic fibrosis as well as hepatic inflammation, and to suppress the occurrence of HCC.[5–15] However, there have been several reported cases of HCC that developed after successful IFN therapy.[11–27] The clinical features of HCC and the mechanisms of carcinogenesis have not yet been fully elucidated because development of HCC is very rare in sustained responders to IFN therapy.[20–27] Therefore, a multicenter study was set up to collect and analyze the clinical data for

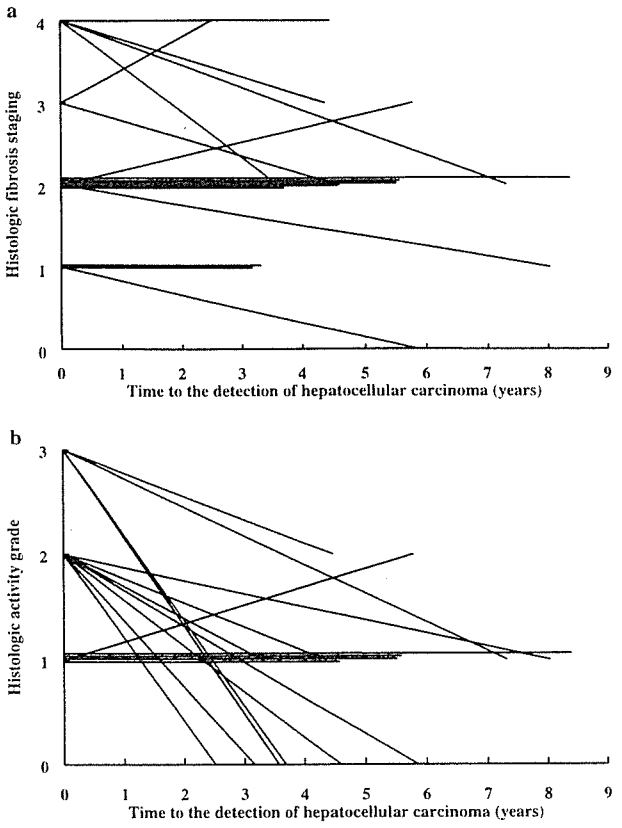


**Figure 2** Serial changes in (a) histological fibrosis staging and (b) histological activity grading for each patient when compared before interferon therapy and at detection of hepatocellular carcinoma.

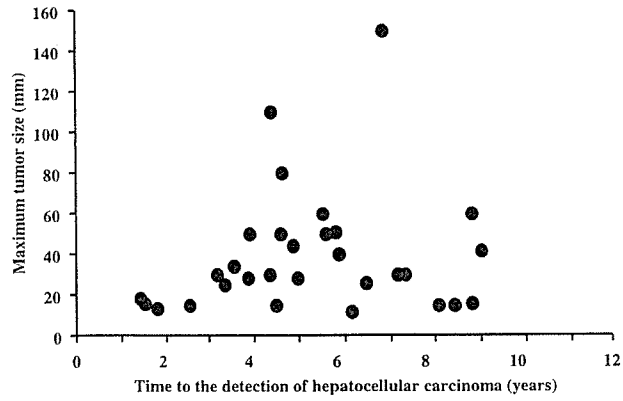


**Figure 3** Maximum tumor size and time until detection of hepatocellular carcinoma.

patients who showed a SVR to IFN therapy for chronic hepatitis C and in whom HCC subsequently developed.

In this study, a total of 7715 patients with chronic hepatitis C received IFN therapy, and among them, a SVR was obtained in 2515 (32.6%). Among the patients with SVR who developed HCC, clinical data were collected for 38 patients. In regards to the clinical features of the HCC that developed in these patients, the percentage of those who were ≥60 years of age at the time of HCC detection (89%), and the percentage of men (89%) (sex ratio 8.5:1) were both high. In these patients, platelet count, albumin, AST, ALT, indocyanine green $R_{15}$ and histological activity grade also improved significantly after IFN therapy ($P < 0.05$), although there was no significant improvement of histological fibrosis staging after IFN therapy ($P = 0.10$). Therefore, it was obvious that IFN therapy improved hepatic inflammation and hepatic function, as suggested by the results of other studies.[7-15] However, the other clinical features could not be clarified in this study, because we had no data from controls with which to compare the clinical variables of HCC that developed in patients showing SVR to IFN therapy. Potential control groups might include HCV patients with HCC who did not receive IFN therapy, or HCV patients with HCC who received IFN therapy but did not show a sustained response.[20-23] Additional comparative studies will be required in order to sufficiently elucidate the clinical features of HCC developing after SVR to IFN.

In the present study, there were 38 patients who developed HCC after successful IFN therapy, with a median period of 4.7 years (range 1.4–9.0 years) until detection of HCC. Moreover, the maximum tumor size in patients without medical follow-up for 1 year or more (median 60 mm) was significantly larger than in patients who were periodically followed up for 6 months or less (median 25 mm) ($P = 0.002$). As other studies have also indicated,[20,21] these findings suggest that the risk of HCC in sustained responders is not completely eliminated and that careful medical follow-up is important even after successful IFN therapy, which makes it difficult to determine the optimal follow-up period after SVR. If HCC had been detected at an earlier stage by regular follow-up, these patients could have been offered potentially curative treatment such as hepatic resection; such patients generally have good hepatic function after elimination of HCV. Moreover, it has also been reported that recurrence after curative treatment of HCC in SVR patients is less frequent than in non-SVR patients.[22,23] However, the enormous health care costs associated with screening all SVR patients for many years should be borne in mind. Therefore, it is also essential to identify the risk factors for development of HCC[20] and to establish the follow-up strategies in SVR patients.

Why does HCC develop even in patients showing a SVR to IFN therapy? HCV is a positive, single-stranded RNA virus without a DNA intermediate in its replicative cycle, so that integration of HCV nucleic acid sequences into the host genome, like that occurring in HBV infection, seems unlikely.[29] Therefore, HCV itself is probably not the causative factor of HCC after SVR. One assumption is that preexisting microscopic tumor foci that are not detected by diagnostic imaging are responsible for the appearance of HCC after SVR to IFN therapy, although in this study patients were excluded if HCC was detected within 1 year after the termination of IFN therapy. However, in the present series, there were nine patients in whom HCC less than 3 cm in size developed more than 5 years after IFN therapy. Although the rapidity of tumor growth may depend on individual tumor characteristics, considering

the late onset of small HCC in these patients, de novo HCC development after eradication of HCV should not be ignored. This has also been reported by Toyoda *et al.* on the basis of analysis that calculated the doubling time of HCC that occurred after SVR[24] and a long-term follow-up study of SVR patients.[21] It is conceivable that long-standing chronic liver inflammation and liver regeneration may provide the basis for tumor development. Carcinogenesis may not be a single-step event, but a complex, multi-step process, although the mechanisms are still unknown. Future studies should be aimed at defining the basic oncogenic mechanisms by which SVR patients develop HCC. Moreover, exploring the underlying mechanisms for the development of HCC in SVR patients may help identify new strategies for prevention of HCC.

In conclusion, even patients showing a SVR to IFN treatment of chronic hepatitis C and in whom hepatic function improves have the potential to develop HCC. The results of this study underline the importance of periodic medical follow-up for these patients.

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## APPENDIX I

### Participating hospitals and clinics

In addition to the hospitals of the study authors, data were supplied by the following hospitals and clinics in the Kyushu Division of the Japanese Society of Gastroenterology: Nippon Steel Yawata Memorial Hospital; Yame General Hospital; 1st Department of Internal Medicine, Ryukyu University School of Medicine; 2nd Department of Internal Medicine, Kagoshima University School of Medicine; Hayato Town Medical Association Medical Center; Department of Internal Medicine, Saga University School of Medicine; Department of Medicine and Biosystemic Science, Kyushu University School of Medicine; Nishinihon Hospital; Kagoshima Kouseiren Hospital; Miyata Memorial Hospital; 2nd Department of Internal Medicine, Nagasaki University School of Medicine; Yonabaru Central Hospital; 2nd Department of Internal Medicine, Miyazaki University School of Medicine; Fukuoka Red Cross Hospital; Kita-kyushu Medical Center Hospital; Munakata Medical Association Hospital; Naika Yamaguchi Urban Clinic; Yamaga Municipal General Hospital; Shin Kokura Hospital; Iizuka Hospital; Oita Prefectural Hospital; 3rd Department of Internal Medicine, University of Occupational and Environmental Health School of Medicine; Kokura National Hospital; Kumamoto Regional Medical Center; Shin Beppu Hospital; Oita National Hospital; Kami Goto Hospital; 1st Department of Internal Medicine, Oita University School of Medicine; National Kyushu Medical Center; 1st Department of Internal Medicine, Nagasaki University School of Medicine; Hirahara Naika Clinic; Kumamoto National Hospital; Saiseikai Fukuoka General Hospital; Arita Ichou Hospital; Labour Welfare Corporation Moji Rosai Hospital; 1st Department of Internal Medicine, Kumamoto University; Kumamoto Red Cross Hospital; National Kyushu Cancer Center Hospital; Kagoshima Kyousaikai Nanpuh Hospital; Iwao Hospital; Saga Social Insurance Hospital; Labour Welfare Corporation Nagasaki Rosai Hospital; Oita Red Cross Hospital; Koebaru Central Hospital; National Sanatorium Kumamoto South Hospital; 2nd Department of Internal Medicine, Oita Medical University; Labour Welfare Corporation Kumamoto Rosai Hospital; Amakusa Regional Medical Center; Miyuki Hospital; Saiseikai Futsukaichi Hospital; Hakujyuji Hospital; Hakuaikai Hospital.

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# Increasing hepatitis C virus-associated hepatocellular carcinoma mortality and aging: Long term trends in Japan

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**Abstract**

*Background:* The incidence of hepatocellular carcinoma (HCC) in Japan has been increasing. The aim of the study was to determine the epidemiological trends in HCC mortality in Japan.
*Methods:* We reviewed the medical records of all patients whose death was caused by liver disease between 1981 and 2000 at two hospitals. The courses of death were separated based on presence or absence of HCC when death ensued. Additionally, cohorts of patients with HCC were analyzed in 5-year time periods.
*Results:* The number of deaths from hepatitis C virus (HCV)-associated HCC steadily increased 2.6 times from 49 to 128 during observation period. The mean age at death from HCV-associated HCC from 1996 to 2000 was significantly higher than that in the period from 1981 to 1985 ($p < 0.0001$).
*Interpretation:* Deaths from HCV-associated HCC increased from 1981 to 2000, consistent with the aging of the population in Japan.


*Keywords:* Hepatitis C virus; Hepatocellular carcinoma; Epidemiological

## 1. Introduction

Hepatocellular carcinoma (HCC) affects approximately half a million people each year worldwide, making it the fifth most common malignancy in men and the ninth most common in women [1–7]. Recently, a trend of increasing rates of HCC has been reported from several developed countries in North America, Europe and Asia [1–9], and the incidence of primary liver cancer in Japan has been increasing over the past four decades [10,11]. HCC often develops in patients with liver cirrhosis caused by hepatitis C virus (HCV), hepatitis B virus (HBV) or excessive alcohol consumption.

Of the hepatitis viruses that cause HCC, HCV is more common than HBV in Japan [12–15]. Although the age-adjusted incidence rates of HCC have been increasing during the period of rising HCC mortality, the temporal and demographic features of survival for HCC patients in Japan are unknown. Hence, we have analyzed these trends over time, using information from two independent databases that deal with HCC in Japan.

## 2. Patients and methods

We reviewed the medical records of all patients who died from liver disease and received medical care between 1981 and 2000 at the Liver Disease Center, National Nagasaki Medical Center and at The First Department of Internal

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Medicine, Nagasaki University School of Medicine. A total of 1001 patients were studied. All the patients were followed-up after diagnosis until death in one of the two hospitals and we were able to confirm their date of death and that death had occurred after severe liver disease.

All patients were entered into this study because sera were stored at −80 °C. These sera were used to assay HBV or HCV infection. A diagnosis of chronic HCV infection was based on the presence of anti-HCV antibody and HCV-RNA detected by polymerase chain reaction (PCR), whereas diagnosis of chronic HBV infection was based on the presence of hepatitis B surface antigen (HBsAg) or anti-hepatitis B core antigen (anti-HBc) reactivity. Diagnosis of HCC was based on histological findings or on characteristic images in dynamic computed tomography, dynamic magnetic resonance imaging and hepatic angiography. Demographic information, including age at death, sex and year of death, was collected from the patients' chart. Excessive alcohol consumers (an alcohol consumption of >50 g/day for 5 years) were not including in this study.

The courses of death were separated into those occurring with or without HCC when death ensued. Additionally, the patients with HCC were analyzed in 5 yearly intervals (1981–1985, 1986–1990, 1991–1995 and 1996–2000). Patients were classified according to 5-year age groups, and by HBV or HCV infection, and the number of patients in each age group with HBV- or HCV-associated HCC was calculated in each time period.

The SAS computer program for Windows was used to perform statistical analysis of the data, using analysis of variance (ANOVA).

## 3. Results

A total of 1001 patients died at the Liver Disease Center, National Nagasaki Medical Center and at The First Department of Internal Medicine, Nagasaki University School of Medicine from 1981 to 2000. The patients with HBV-associated HCC were 73.7% (210 of 285) in HBV-related disease and the patients with HCV-associated HCC were 84.5% (381 of 451) in HCV-related disease. There were 653 patients with HCC died. The mean time during followed-up were 2.5 years. The proportion of patients diagnosed with HBV-associated HCC was 32% (210 of 653), whereas 58% (381 of 653) had HCV-associated HCC, and an additional 2% (12 of 653) had HCC associated with both viruses (Table 1).

Table 1
Course of death from 1981 to 2000

| | HBV | HCV | Overlap | Others | Total |
|---|---|---|---|---|---|
| HCC (%) | 210 (32) | 381 (58) | 12 (2) | 50 (8) | 653 (100) |
| Chronic liver failure | 47 | 35 | 1 | 36 | 119 |
| GI bleeding | 8 | 17 | 1 | 13 | 39 |
| Other disease | 3 | 5 | 0 | 16 | 24 |
| Acute liver failure | 10 | 1 | 3 | 19 | 33 |
| Other cancer | 7 | 12 | 0 | 114 | 133 |
| Total (%) | 285 (28) | 451 (45) | 17 (2) | 248 (25) | 1001 (100) |

HCC, hepatocellular carcinoma; GI bleeding, gastrointestinal bleeding; HBV, hepatitis B virus; HCV, hepatitis C virus; overlap, both HBV and HCV positive; other, both HBV and HCV negative.

From 1981 to 2000, 210 patients died of HBV-associated HCC, whereas 381 died of HCV-associated HCC. Table 2 shows the number and the mean age at death from HBV- or HCV-associated HCC during the 5-year periods 1981–1985, 1986–1990, 1991–1995 and 1996–2000. The number of deaths from HBV-associated HCC was not changed within the range from 49 to 58 during the four 5-year periods: 54 (1981–1986), 49 (1986–1990), 49 (1991–1995) and 58 (1996–2000), and the mean age at death was not also statistically significantly different among the periods: 55.4 ± 9.9 (1981–1985), 55.6 ± 10.3 (1986–1990), 55.5 ± 10.6 (1991–1995) and 59.3 ± 10.2 (1996–2000). In contrast, the number of deaths from HCV-associated HCC steadily increased 2.6 times from 49 to 128 during same observation period: 49 (1981–1986), 90 (1986–1990), 114

Table 2
Mean age of KBV associated HCC deaths

| Year | 1981 – 1985 | 1986 – 1990 | 1991 – 1995 | 1996 – 2000 | total |
|---|---|---|---|---|---|
| Number | 54 | 49 | 49 | 58 | 210 |
| Mean age (y.o.) | 55.4 | 55.6 | 55.5 | 59.3 | 56.8 |
| SD | 9.9 | 10.3 | 10.6 | 10.2 | 10.3 |
| | NS | NS | NS | | |
| | NS | NS | | | |
| | NS | | | | |
| | NS | | | | |

Mean age of HCV-associated HCC deaths

| Year | 1981 – 1985 | 1986 – 1990 | 1991 – 1995 | 1996 – 2000 | total |
|---|---|---|---|---|---|
| Number | 49 | 90 | 114 | 128 | 381 |
| Mean age (y.o.) | 60.0 | 63.0 | 64.1 | 67.0 | 64.3 |
| SD | 8.1 | 7.0 | 7.2 | 7.9 | 7.8 |
| | NS | NS | 0.0267 | | |
| | 0.0176 | 0.0016 | | | |
| | < 0.0001 | | | | |

HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; S.D., standard deviation; NS, not significant.

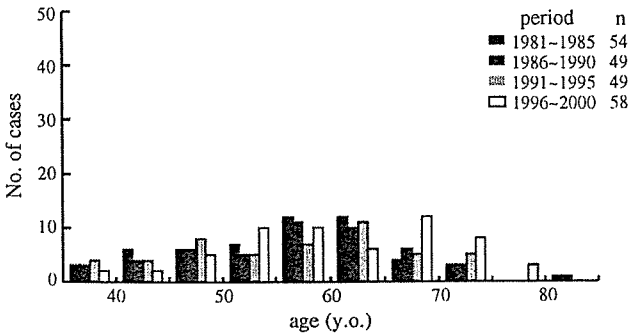


Fig. 1. Age distribution of the total number of deaths from hepatitis B virus-associated hepatocellular carcinoma from 1981 to 2000. There was no change of number of patients and age distribution of patients who died from hepatitis B virus-associated hepatocellular carcinoma during the four time periods.

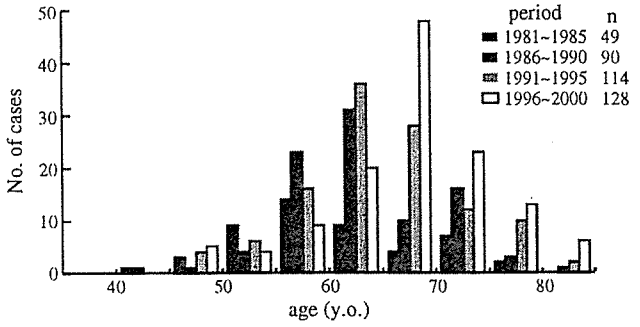


Fig. 2. Age distribution of the total number of deaths from hepatitis C virus-associated hepatocellular carcinoma from 1981 to 2000. The number of death from HCV associated HCC has increased 2.6 times during recent 20 years and this increase was provided by a close association with older shift of age distribution.

(1991–1995) and 128 (1996–2000). In addition, the mean age at death from HCV-associated HCC also increased over time. The mean age at death from 1996 to 2000 (67.0 ± 7.9 years old) was significantly higher than that from 1981 to 1985 (60.0 ± 8.1) ($p < 0.0001$), 1986 to 1990 (63.0 ± 7.0) ($p = 0.0016$) and 1991 to 1995 (64.1 ± 7.2) ($p = 0.0267$), respectively.

Fig. 1 shows the age distribution for deaths from HBV-associated HCC during the four 5-year periods. There was no change of number of patients and age distribution for deaths from HBV-associated HCC during these periods. In contrast, Fig. 2 shows the age distribution for deaths from HCV-associated HCC during the four 5-year periods. The number of patients with HCV-associated HCC aged more than 60 years in 1981–1985, 1986–1990, 1991–1995 and 1996–2000 were 22, 61, 88 and 110 patients, respectively. Fig. 2 indicated that the number of death from HCV associated HCC has increased during recent 20 years and this increase was provided by a close association with older shift of age distribution.

Table 3 shows the age distribution of HCC deaths in 5-year period (1981–1985, 1986–1990, 1991–1995 and 1996–2000). The number of patients with HCV-associated HCC obviously had an increase in the ratio of patients aged more than 60 years ($p < 0.0001$): 18.6% (1981–1985), 37.9% (1986–1990), 51.2% (1991–1995) and 54.4% (1996–2000). There was a significant difference of age distribution in the patients with HCV-associated HCC between aged more than and less than 60 years old in each 5-year period ($p < 0.0001$). In contrast, there was no difference in the age distribution of patients with other types of during these periods.

Fig. 3 shows the ratio between HCV-associated deaths and HBV-associated HCC deaths in 5-year period (1981–1985, 1986–1990, 1991–1995 and 1996–2000). The ratio between HCV-associated HCC and HBV-associated HCC increased and reached a plateau during the observation period: 0.9 (1981–1985), 1.8 (1986–1990), 2.3 (1991–1995) and 2.2 (1996–2000) (1981–1985 versus 1991–1995, $p = 0.0030$; 1981–1985 versus 1996–2000, $p = 0.0042$). Above all, the ratio of patients aged more than 60 years old increased during the observation period: 1.1 (1981–1985), 3.0 (1986–1990), 4.2 (1991–1995) and 3.8 (1996–2000) (1981–1985 versus

Table 3
Age distribution of HCC deaths in 5-year period

| Age (y.o.) | 1981–1985, no. (%) | 1986–1990, no. (%) | 1991–1995, no. (%) | 1996–2000, no. (%) | | p-Value |
|---|---|---|---|---|---|---|
| HBV | | | | | | |
| <60 | 34 (28.8) | 29 (18.0) | 28 (16.3) | 29 (14.4) | ] | NS |
| >60 | 20 (17.0) | 20 (12.5) | 21 (12.2) | 29 (14.4) | | |
| HCV | | | | | | |
| <60 | 27 (22.9) | 29 (18.0) | 26 (15.1) | 18 (8.9) | ] | <0.0001 |
| >60 | 22 (18.6) | 61 (37.9) | 88 (51.2) | 110 (54.4) | | |
| Overlap | | | | | | |
| <60 | 1 (0.9) | 3 (1.9) | 2 (1.2) | 1 (0.5) | ] | NS |
| >60 | 0 | 2 (1.2) | 0 | 3 (1.5) | | |
| Other | | | | | | |
| <60 | 5 (4.2) | 2 (1.2) | 4 (2.3) | 2 (1.0) | ] | NS |
| >60 | 9 (7.6) | 15 (9.3) | 3 (1.7) | 10 (4.9) | | |
| Total | 118 (100) | 161 (100) | 172 (100) | 202 (100) | | |

HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; overlap, both HBV and HCV positive; other, both HBV and HCV negative.

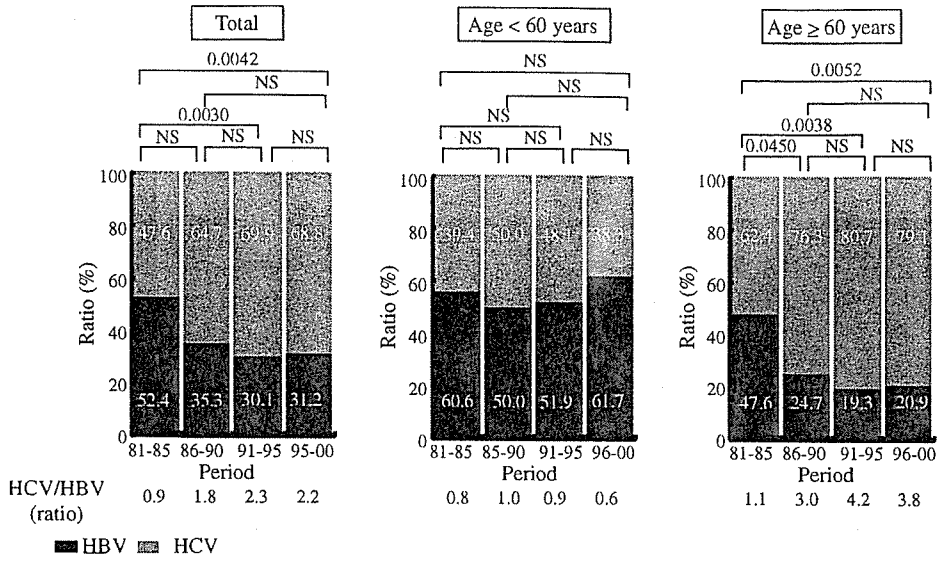


Fig. 3. Ratio between hepatitis C virus-associated hepatocellular carcinoma deaths and hepatitis B virus-associated hepatocellular carcinoma from 1981 to 2000. The ratio between HCV-associated HCC and HBV-associated HCC increased and reached a plateau during the observation period.

1986–1990, $p=0.0450$; 1981–1985 versus 1991–1995, $p=0.0038$; 1981–1985 versus 1996–2000, $p=0.0052$). In contrast, there was no difference in the ratio of patients aged more than 60 years old of during these periods.

## 4. Discussion

HCC accounts for approximately 6% of all human cancers. It is estimated that half a million cases occur annually worldwide, making HCC the fifth most common malignancy in men and the ninth in women [1–7,9]. The age-adjusted mortality rate from HCC has increased over the past decades in Japan [16], and in the current study more than 90% of deaths from HCC were HBV- and/or HCV-related and the number of deaths from HCV-associated HCC apparently increased 2.6 times from 1981 to 2000, and the mean age of deaths from HCV-associated HCC also significantly rose. During the same period, the number and the age distribution of deaths from HBV-associated HCC remained unchanged. The increase in the number of deaths from HCV-associated HCC seemed to be closely associated with the shift of age distribution of HCV infected population between 1981 and 2000. Although our data had the limitations of applying the findings from two hospitals to a general population, Kiyosawa described that deaths due to HCC in Japan have continued to increase in males, particularly in those older than 60 years of age between 1982 and 2003. This also suggests that the average age of diagnosis of HBV-related HCC was similar in all three time periods. In contrast, the average age of patients with HCV-related HCC rose from 61.6 years in 1982 to 63.1 years in 1990 and 67.8 years in 2003 [11]. The research group for population-based cancer registration in Japan described that incidence of HCC in Japan have continued to increase and reached a plateau in males and female from 1975 to 1999. Above all, the age distribution incidence and incident rate of HCC reached a peak older than 65 years old in males and female [17]. And, this study suggested that the ratio between HCV-associated HCC and HBV-associated HCC increased and reached a plateau from 1981 to 2000, especially more than 60 years old. Where did these findings and difference of HCC development between HCV and HBV, which were considered to be both oncogenic virus after long-term persistent infection with inflammation and fibrotic change in the liver but popular hepatitis virus infections in Japan, come from?

The simple reason may be explained as follows. From 1981 to 2000, mortality from a variceal hemorrhage in cirrhotic patients has declined [9,18]. Long term nutritional supplementation with oral branched-chain amino acids has been useful in the prevention of progressive hepatic failure, and improvement of surrogate markers and perceived health status in advanced cirrhosis has occurred [19,20]. Additionally, many new treatments and techniques have been introduced for HCC, including transcatheter arterial embolization, percutaneous ethanol injection therapy, microwave coagulation therapy, radiofrequency ablation, systemic chemotherapy and advance surgical techniques. However, these advances of medical treatment cannot explain the difference between HBV-associated HCC and HCV-associated HCC.

Alternatively, well considered reasons of the recent rapid increase of the number of patients who died from HCV-associated HCC in Japan, were shown in the current two studies. First, Hamada et al. recently reported that the majority of HCC patients develop HCC when they are aged over 60 years old, regardless of the timing of HCV infection. This result was obtained by the long-term observation of the patients infected by post-transfused HCV infection [21]. This also suggests that HCC has increased among patients over 60 years old with HCV infection and such phenomenon has never been observed nor reported till now in patients with HBV infection.

Second, the chronically HCV-infected population is aging in Japan. Yoshizawa et al. reported that age-specific prevalence rates for the presence of anti-HCV antibody among ~300,000 voluntary blood donors from Hiroshima in 1999 clearly increased with the age, reaching the highest rate of 7% in individuals who were more than 70 years old [11,22]. In a word, HCV infected people become older with years in Japan and they were regarded as a high risk for HCC. Then, the number of deaths from HCV-associated HCC has been increased recent 20 years in Japan.

El-Serag et al. reported that an increase in the number of cases of HCC affecting mainly younger age groups has occurred in the United States (U.S.) over the past two decades [23,24]. HCV infection accounts for most of the increase in the number of cases of primary liver cancer [4,6,7,9,25], while the rates of primary liver cancer associated with alcoholic cirrhosis and HBV infection have remained unchanged [4,6,9]. Tanaka et al. reported that HCV was introduced into the U.S. population around 100 years ago and was widely disseminated between 1954 and 1978 [26]. Most HCV-infected patients in the U.S. were born between 1940 and 1965 [27,28], and are therefore younger than HCV-infected Japanese patients. Hence, the burden of disease associated with HCV infection will probably increase in the U.S. during the next 10–20 years, as has occurred in Japan, as this cohort reaches an age at which complications of chronic liver disease typically occur [1–7,26]. The current study suggests that increased HCV-associated HCC will occur in the U.S. over the next two to three decades.

In conclusion, we found that the number of patients with HCV-associated HCC in Japan has increased, consistent with aging of the population, but the number of patients with HBV-associated HCC has remained unchanged over the last 20 years.

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# Influence of Genotypes and Precore Mutations on Fulminant or Chronic Outcome of Acute Hepatitis B Virus Infection

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**The outcome of acute hepatitis B virus (HBV) infection is variable, influenced by host and viral factors. From 1982 through 2004, 301 patients with acute HBV infection entered a multi-center cross-sectional study in Japan. Patients with fulminant hepatitis (n = 40) were older (44.7 ± 16.3 vs. 36.0 ± 14.3 years, $P < .0017$), less predominantly male (43% vs. 71%, $P = .0005$), less positive for hepatitis B e antigen (HBeAg) (23% vs. 60%, $P < .0001$), less infected with subgenotype Ae (0% vs. 13%, $P < .05$), and more frequently with Bj (30% vs. 4%, $P < .0001$) than those with acute self-limited hepatitis (n = 261). Precore (G1896A) and core-promoter (A1762T/G1764A) mutations were more frequent in patients with fulminant than acute self-limited hepatitis (53% vs. 9% and 50% vs. 17%, $P < .0001$ for both). HBV infection persisted in only three (1%) patients, and they represented 2 of the 23 infected with Ae and 1 of the 187 with the other subgenotypes (9% vs. 0.5%, $P = .032$); none of them received antiviral therapy. In multivariate analysis, age 34 years or older, Bj, HBeAg-negative, total bilirubin 10.0 mg/dL or greater, and G1896A mutation were independently associated with the fulminant outcome. In *in vitro* transfection experiments, the replication of Bj clone was markedly enhanced by introducing either G1896A or A1762T/G1764A mutation. In conclusion, persistence of HBV was rare (1%) and associated with Ae, whereas fulminant hepatitis was frequent (13%) and associated with Bj and lack of HBeAg as well as high replication due to precore mutation in patients with acute HBV infection. *Supplementary material for this article can be found on the HEPATOLOGY website (http://interscience.wiley.com/jpages/0270-9139/suppmat/index.html).* (HEPATOLOGY 2006; 44:326-334.)**

*Abbreviations: HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; HBc, hepatitis B core antigen; HBsAg, hepatitis B surface antigen; EIA, enzyme immunoassay; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; ALT, alanine aminotransferase.*

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