INTRODUCTION

Hepatitis C virus (HCV) infection rarely resolves spontaneously once it becomes chronic [1]. Consequently, most patients in Japan with chronic HCV infection are likely to progress steadily to liver cirrhosis and hepatocellular carcinoma (HCC), which develops approximately 30 years after blood transfusion [2–4]. HCC is one of the most common malignancies, especially in Southeast Asia, and a major cause of death for patients with chronic HCV infection. In the early 1990s, interferon was introduced worldwide as a therapy for patients with chronic hepatitis C and was effective in inducing normalization of serum alanine aminotransferase (ALT) [5,6], eliminating HCV RNA [7,8], and improving liver histological findings [9–11] in patients with chronic hepatitis C.

To evaluate the effect of interferon therapy on the incidence of HCC and the risk of mortality for chronic hepatitis C patients, a randomized controlled trial is needed. However, a prospective randomized trial with untreated control patients is ethically impossible, because interferon therapy has already been established as a standard treatment for patients with chronic hepatitis C. Therefore, almost all chronic hepatitis C patients, except for cases with medical conditions such as depression, autoimmune disease and severe diabetes mellitus, have been treated with interferon in Japan. Recently, several investigators have reported this therapy as being effective for reducing the incidence of HCC among patients who showed normalization of ALT during and after interferon therapy, as well as among those in whom HCV was eradicated [12–17]. However, a reduced risk of HCC does not necessarily lead to improvement in survival. Indeed, little is known about the effects of interferon therapy on the mortality of patients with chronic hepatitis C. Several investigators [14, 18–23] have tried to evaluate the impact of interferon therapy on mortality. Four of these studies indicated that interferon therapy significantly reduced the mortality of compensated HCV-related cirrhotic patients [18,20] or of chronic hepatitis C patients including patients with compensated cirrhosis [21,23]. However, lack of analysis on response to interferon [18,20–23] or lack of information on disease-specific mortality [20,21] has made it difficult to evaluate the benefits of interferon for survival. Recently, Yoshida *et al.* [24] demonstrated that interferon therapy improved survival by preventing liver-related deaths of chronic hepatitis C patients showing a sustained virological response. However, whether a biochemical response to interferon therapy results in a reduced risk of mortality has not been investigated.

We conducted a multi-centre, large-scale, retrospective cohort study of patients with chronic hepatitis C, who had been enrolled at the end of 1997 at participating hospitals in order to analyse the effect of interferon therapy on the incidence of HCC. The aim of the present study was to examine the effect of interferon therapy on the mortality and causes of death among chronic hepatitis C patients.

PATIENTS AND METHODS

Patients

We recruited chronic hepatitis C patients from four previous studies which were conducted to assess the effect of interferon therapy on the incidence of HCC [12,14,15,17]. All patients meeting the following criteria were included in this study: (i) histological diagnosis of chronic hepatitis or cirrhosis; (ii) no history of clinical signs at entry into the study of complications of cirrhosis, i.e. ascites, jaundice, encephalopathy, or variceal bleeding; (iii) no evidence of HCC at entry into the study as assessed by ultrasonography and/or computed tomography; (iv) absence of serum hepatitis B surface antigen; (v) absence of co-existing liver diseases such as autoimmune hepatitis or primary biliary cirrhosis; (vi) absence of excessive alcohol consumption (>80 g/day); and (vii) absence of human immunodeficiency virus antibodies, as described previously [12,14,15,17]. A total of 3025 patients who met these criteria and whose initial sera tested positive for anti-HCV as determined by either first- or second-generation ELISA (Ortho Diagnostics, Tokyo, Japan) and HCV RNA were included in the study. The sera of patients who had been diagnosed as non-A, non-B hepatitis before anti-HCV testing became available (i.e. before 1989) had been frozen at −80 °C and were retrospectively assayed.

Of the 3025 chronic hepatitis C patients, 2762 had received interferon after 1987, when interferon became available in Japan. Interferon-treated patients received a 4–12-month course of interferon therapy, which was initiated within 1 month of liver biopsy. The remaining 263 patients did not undergo interferon therapy or any other antiviral therapy, including almost all patients with biopsy-proven chronic hepatitis who had refused interferon treatment due to adverse effects, lack of time for therapy, or their inability to undergo treatment as a consequence of depression, severe diabetes mellitus or other medical conditions.

Criteria for biochemical and virological responses to interferon therapy

The biochemical response during the follow-up up to 6 months after the completion of interferon therapy was defined according to previously described criteria with minor modifications [8,9]. In the sustained response group, ALT levels decreased to the normal range during therapy and remained within that range up to 24 weeks after therapy without any abnormal elevation. In the transient response group, ALT levels decreased to the normal range by the end of therapy, remained normal during therapy but returned to abnormal levels during the 24 weeks following interferon therapy. In the no-response group, ALT levels did not decrease to the normal range, or fluctuated during therapy and the subsequent 24 weeks. Both biochemical transient

and nonresponders were designated as nonsustained biochemical responders.

A sustained virological response was defined as HCV RNA negativity at more than 6 months after the cessation of interferon therapy. Patients showing positive HCV RNA at the same time were designated as nonsustained virological responders.

Histological evaluation

Liver biopsy was carried out before interferon therapy in all cases. Specimens were fixed in formaldehyde and embedded in paraffin. The sections were stained with haematoxylin-eosin and Azan-Mallory and analysed by two pathologists without any knowledge of the clinical and laboratory data. Histological findings were scored according to the classification of Desmet *et al.* [25].

Follow-up

The starting date of the follow-up for both the interferon-treated and untreated groups was defined as the date of liver biopsy. Biochemical examinations including α-fetoprotein and abdominal ultrasonography were carried out before interferon therapy and every 3–6 months thereafter at the outpatient clinic of the respective hospitals. The end of the follow-up was the date of death or the latest confirmation of survival. Follow-up data on the patients were obtained from the participating hospitals. Follow-up data that were not available from the hospitals were collected from the resident registry of the local municipal office. Death from liver-related disease was defined as death from HCC, liver failure determined by the presence of one or more of ascites, jaundice and hepatic encephalopathy, or variceal bleeding diagnosed on the basis of endoscopic findings of patients presenting with upper gastrointestinal haemorrhage.

Five untreated patients were observed for over 162 months, which corresponded to the longest period of observation of those treated with interferon. In these subjects, only the follow-up data up to 162 months were considered. Seventy-one patients whose follow-up period was shorter than 12 months were excluded from the study. The final numbers of study subjects were 2698 for the interferon-treated group and 256 for the untreated group.

Informed consent was obtained from each patient included in the study. The study protocol was in accordance with the Helsinki Declaration of 1975 (revised in 1983) and approved by the Ethical Committee of the Osaka University Graduate School of Medicine.

Statistical analysis

The chi-square test was used to compare the frequency of gender between the interferon-treated and untreated groups. The difference in age at liver biopsy and ALT between the two groups, expressed as median, was assessed for significance with the Student's *t*-test. The Wilcoxon rank-sum test was used to compare the distribution of age at liver biopsy and histological staging. Cumulative survival curves were determined with the Kaplan–Meier method, and the log-rank test was used to compare the cumulative survival rates.

The observed number of deaths was compared with the expected number, which was calculated by applying sex, 5-year age, 5-year calendar time, and cause-specific mortality rates for the general population in Japan, as prepared by the Statistics and Information Department, Japan Ministry of Health and Welfare [26]. The standardized mortality ratio (SMR) was expressed by dividing the observed number of deaths by the expected number of deaths. The standard error and the 95% CI of SMR were estimated by assuming Poisson's distribution, and differences in mortality between the study cohort and the general population were considered to be significant if the CI did not include unity.

Survival was also analysed by using Cox proportional hazards regression controlling for age (continuous variable), gender, stages of liver fibrosis (stage: 0/1/2/3/4) and time at liver biopsy (1991/1992). Risk ratios attributable to biochemical sustained, transient and no responses and to virological sustained and nonsustained responses were calculated in comparison with no treatment by using dummy variables.

Data analysis was performed with the SAS/PC statistical package (SAS Institute, Cary, NC, USA). All reported *P*-values were two-sided and $P < 0.05$ was considered to be significant.

RESULTS

Patient characteristics at entry

Of the 2698 patients treated with interferon, 901 (33.3%) had a sustained biochemical response, 701 (26.0%) a transient biochemical response and the remaining 1096 patients (40.6%) were classified as biochemical nonresponders. Serum HCV RNA remained negative at more than 6 months after cessation of interferon therapy in 738 (81.9%) of the sustained biochemical responders, designated as sustained virological responders, whereas serum HCV RNA remained positive in 133 (14.8%). Serum HCV RNA was not examined after the termination of interferon therapy in 30 sustained biochemical responders, who were excluded from the analysis according to virological responses to interferon. Positive HCV RNA after interferon therapy was detected in all of the biochemical transient and nonresponders.

The demographic and clinical features of interferon-treated patients according to virological and biochemical responses to interferon and of untreated patients at the time of enrolment are summarized in Table 1. Untreated patients were significantly older than interferon-treated patients ($P = 0.04$), but frequency distribution of age at liver biopsy

Table 1 Characteristics of interferon-treated patients according to virological and biochemical responses to interferon and of untreated patients

	Interferon-treated							
	Virological response		Biochemical response					
	SVR ($n = 738$)	non-SVR ($n = 1930$)	SBR ($n = 901$)	TBR ($n = 701$)	BNR ($n = 1096$)	Total ($n = 2698$)	Untreated ($n = 256$)	*P*-value
Median age (range)	51 (20–72)	54 (20–76)	52 (20–73)	53 (20–75)	54 (20–76)	53 (20–76)	54 (21–72)	0.04
Age at biopsy (%)								
≤49	337 (45.7)	687 (35.6)	392 (43.5)	277 (39.5)	369 (33.7)	1038 (38.5)	75 (29.3)	0.12
50–59	240 (32.5)	759 (39.3)	303 (33.6)	280 (39.9)	428 (39.1)	1011 (37.5)	123 (48.9)	
≥60	161 (21.8)	484 (25.1)	206 (22.9)	144 (20.5)	299 (27.3)	649 (24.1)	58 (22.7)	
Sex (M/F)	507/231	1210/720	595/306	440/261	703/393	1738/960	157/99	0.32
Median ALT (U/L), SD	91	92	87	79	103	92	98	0.57
(range)	(7–1110)	(11–1195)	(7–1110)	(13–1195)	(13–828)	(7–1195)	(9–563)	
Stage of fibrosis (%)								
0	5 (0.7)	11 (0.6)	7 (0.8)	4 (0.6)	5 (0.9)	16 (0.6)	9 (3.5)	0.34
1	259 (35.1)	476 (24.7)	337 (37.4)	228 (32.5)	190 (17.3)	755 (28.0)	84 (32.8)	
2	263 (35.6)	614 (31.8)	297 (33.0)	238 (34.0)	349 (31.8)	884 (32.8)	40 (15.6)	
3	189 (25.6)	725 (37.6)	235 (26.1)	209 (29.8)	471 (43.0)	915 (33.9)	93 (36.3)	
4	22 (3.0)	104 (5.4)	25 (2.8)	22 (3.1)	81 (7.4)	128 (4.7)	30 (11.7)	

SVR, sustained virological responders; SBR, sustained biochemical responders; TBR, transient biochemical responders; BNR, biochemical nonresponders; ALT, alanine aminotransferase.

and the stages of liver fibrosis, gender and ALT did not differ significantly. In sustained biochemical responders, the ratio of male patients and median ALT levels were significantly higher for patients with HCV eradication than for those without it ($P < 0.001$, each), whereas median age and the frequency distribution of the stages of liver fibrosis were not significantly different between the two groups.

Follow-up data

The mean period of observation (total cases: 6.0 ± 2.2 years) of the interferon-treated and untreated patients was 5.8 and 8.0 years, respectively, with the former being significantly shorter than the latter ($P = 0.0001$) because interferon therapy was not introduced in Japan until 1987.

Table 2 Follow-up data for interferon-treated patients according to virological and biochemical responses to interferon and for untreated patients

	Interferon-treated						
	Virological response		Biochemical response				
	SVR ($n = 738$)	non-SVR ($n = 1930$)	SBR ($n = 901$)	TBR ($n = 701$)	BNR ($n = 1096$)	Total ($n = 2698$)	Untreated ($n = 256$)
Mean period of observation, year (SD)	5.7 (2.0)	5.8 (1.9)	5.6 (2.0)	5.7 (1.8)	5.9 (1.9)	5.8 (1.9)	8.0 (3.4)
No. of deaths	7	94	10	10	81	101	52
Liver-related deaths	1	68	1	5	63	69	42
Death from HCC	1	57	1	4	53	58	31
Death from other liver diseases	0	11	0	1	10	11	11
Liver-unrelated deaths	9	26	9	5	18	32	10

SVR, sustained virological responders; SBR, sustained biochemical responders; TBR, transient biochemical responders; BNR, biochemical nonresponders; HCC, hepatocellular carcinoma.

The sustained virological responders, nonsustained virological responders, sustained biochemical responders, transient biochemical responders and biochemical nonresponders were observed for a mean of 5.7, 5.8, 5.6, 5.7 and 5.9 years, respectively (Table 2).

We identified 153 deaths from all causes during the follow-up. The 153 patients who died consisted of 10 sustained biochemical responders (seven of whom were sustained virological responders and three of whom were sustained biochemical responders without HCV eradication), 10 transient biochemical responders, 81 biochemical nonresponders and 52 cases without interferon treatment. Death from all causes did not occur in 30 sustained biochemical responders whose serum HCV RNA was not examined after cessation of interferon therapy. Death from liver-related disease was identified in 111 (73%) of the 153 patients who died: only one death (10%) from liver-related disease (death from HCC) was found among sustained responders with HCV eradication, five (50%) among transient biochemical responders (death from HCC in four cases), 63 (78%) among biochemical nonresponders (death from HCC in 53 cases) and 42 (81%) among untreated patients (death from HCC in 31 cases) (Table 2).

Cumulative survival

The cumulative survival rates from all causes of death were found to be significantly higher for interferon-treated than for untreated patients ($P < 0.001$) (Fig. 1a) The respective 5-year survival rates of interferon-treated and untreated groups were 97.8 and 95.3%, and the 10-year survival rates 87.2 and 77.1%. The cumulative survival rates for sustained virological responders were significantly higher than for nonsustained virological responders ($P < 0.001$) (Fig. 1b), with 5-year survival rates of 99.5 and 97.1%, and 10-year survival rates of 97.8 and 81.9%, respectively. The cumulative survival rates for sustained biochemical responders were significantly higher than for nonsustained biochemical responders ($P < 0.001$). When nonsustained biochemical responders were divided into transient biochemical responders and biochemical nonresponders, the cumulative survival rates for the transient biochemical responders were significantly higher than for the biochemical nonresponders ($P < 0.001$) (Fig. 1c). The respective cumulative survival rates for sustained biochemical responders, transient biochemical responders and biochemical nonresponders were 99.2, 99.1 and 95.8% at the end of the fifth year and 97.8, 97.6 and 72.6% at the end of the 10th year. Among sustained biochemical responders, the cumulative survival rates for sustained virological responders and sustained biochemical responders without HCV eradication were 99.5 and 99.2% at the end of fifth year and 97.8 and 99.2% at the end of the 10th year, showing no statistical significance ($P = 0.18$).

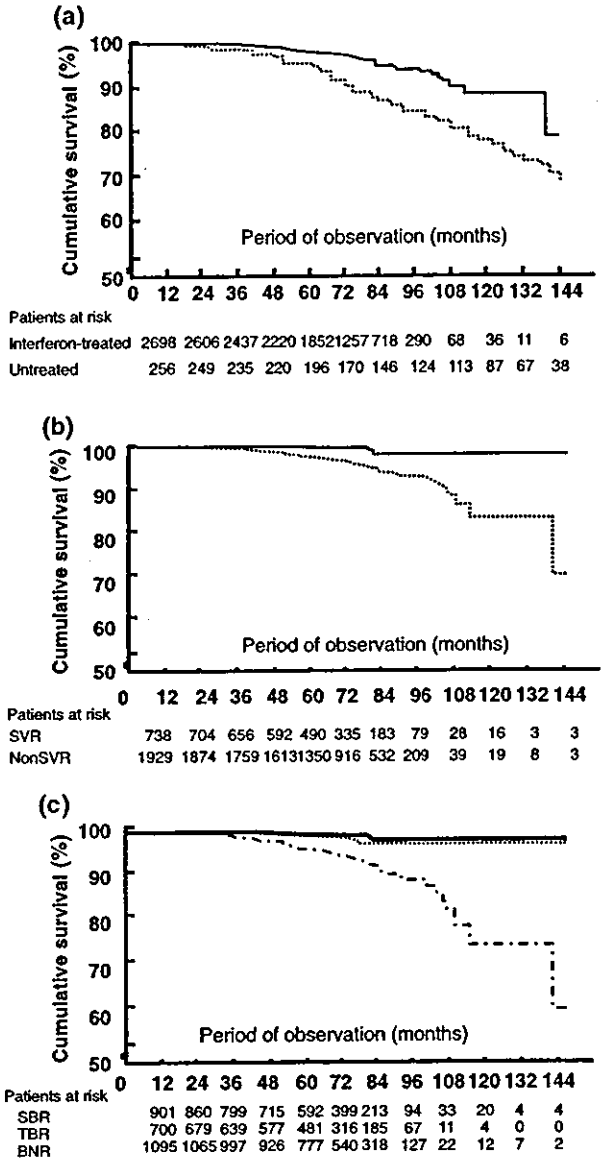

Fig. 1 Cumulative survival rates from all causes of death for patients with chronic hepatitis C. (a) For interferon-treated patients (solid line) and untreated patients (dotted line). (b) According to the virological response to interferon therapy; sustained virological responders (SVR) (solid line) and nonsustained virological responders (non-SVR) (dotted line). (c) In terms of the biochemical responses to interferon, sustained biochemical responders (SBR) (solid line), transient biochemical responders (TBR) (dotted line) and biochemical nonresponders (BNR) (dash-and-dot line).

Standardized mortality ratio

Differences in mortality among interferon-treated and untreated patients from the general population were further assessed by calculating SMR, the ratio of the observed number of deaths to the expected number. Overall mortality

Table 3 Standardized mortality ratios (SMR) in patients with chronic hepatitis C according to virological and biochemical responses to interferon

	Overall deaths			Liver-related deaths			Liver-unrelated deaths		
	Observed	Expected	SMR (95% CI)	Observed	Expected	SMR (95% CI)	Observed	Expected	SMR (95% CI)
Untreated	52	19.2	2.7 (2.0–3.6)	42	1.9	22.2 (16.0–30.0)	10	17.3	0.6 (0.3–1.1)
Interferon-treated	101	112.7	0.9 (0.7–1.1)	69	12.6	5.5 (4.3–6.9)	32	100.0	0.3 (0.2–0.5)
Virological response									
Sustained (HCV RNA negative)	7	29.8	0.2 (0.1–0.5)	1	3.3	0.3 (0.0–1.7)	6	26.5	0.2 (0.1–0.5)
Nonsustained (HCV RNA positive)	94	82.2	1.1 (0.9–1.4)	68	9.2	7.4 (5.8–9.4)	26	73.0	0.4 (0.2–0.5)
Biochemical response									
Sustained response	10	36.5	0.3 (0.1–0.5)	1	4.0	0.3 (0.0–1.4)	9	32.5	0.3 (0.1–0.5)
Transient response	10	27.5	0.4 (0.2–0.7)	5	3.2	1.6 (0.5–3.7)	5	24.3	0.2 (0.1–0.5)
No response	81	48.8	1.7 (1.3–2.1)	63	5.4	11.6 (8.9–14.9)	18	43.3	0.4 (0.3–0.7)

Difference from the expected number of deaths was considered significant if 95% CI of SMR did not include unity.

for untreated patients (SMR: 2.7; 95% CI: 2.0–3.6) but not for the interferon-treated patients (SMR: 0.9; 95% CI: 0.7–1.1) was significantly higher than for the general population. Liver-related mortality was high for untreated patients (SMR: 22.2; 95% CI: 16.0–30.0) and also for interferon-treated patients, although to a lesser degree (SMR: 5.5; 95% CI: 4.3–6.9) (Table 3). For sustained virological responders overall mortality was low (SMR: 0.2; 95% CI: 0.1–0.5), and liver-related mortality (SMR: 0.3; 95% CI: 0.0–1.7) was equivalent to that for the general population. In contrast, liver-related mortality was high for nonsustained virological responders (SMR: 7.4; 95% CI: 5.8–9.4).

Sustained and transient biochemical responders showed a low overall mortality compared with that for the general population (SMR: 0.3; 95% CI: 0.1–0.5, and SMR: 0.4; 95% CI: 0.2–0.7, respectively), whereas overall mortality was high for biochemical nonresponders (SMR: 1.7; 95% CI: 1.3–2.1). Liver-related mortality was not high for sustained and transient biochemical responders (SMR: 0.3; 95% CI: 0.0–1.4, and SMR: 1.6; 95% CI: 0.5–3.7, respectively) compared with that for the general population, but it was high for biochemical nonresponders (SMR: 11.6; 95% CI: 8.9–14.9) (Table 3). Overall and liver-related mortality for sustained biochemical responders without HCV eradication was equivalent to that for the general population (SMR: 0.5; 95% CI: 0.1–1.5, and SMR: 0.0; 95% CI: 0.0–6.1, respectively).

Interferon-treated patients had a statistically lower risk of liver-unrelated death than the general population (SMR: 0.3; 95% CI: 0.2–0.5), whereas untreated patients did not (SMR: 0.6; 95% CI: 0.3–1.1).

Multivariate analysis

The effect of interferon on the risk of death was assessed by Cox proportional hazards regression controlling for age, gender, score of liver fibrosis and time at liver biopsy. Interferon therapy significantly reduced the risk of overall death to a ratio of only 0.47, in comparison with no treatment. When patients were classified according to virological responses to interferon, sustained virological responders showed reduced risks of overall death (risk ratio: 0.14; 95% CI: 0.056–0.352; $P < 0.001$) and liver-related death (risk ratio: 0.04; 95% CI: 0.005–0.301; $P = 0.002$) compared with untreated patients, whereas nonsustained virological responders did not. Similarly, sustained biochemical responders showed a lower risk of death from all causes (risk ratio: 0.16; 95% CI: 0.069–0.354; $P < 0.001$) and liver-related diseases (risk ratio: 0.03; 95% CI: 0.004–0.230; $P < 0.001$). Transient biochemical responders had a high, but still significantly reduced risk of overall death (risk ratio: 0.19; 95% CI: 0.083–0.445; $P < 0.001$) and liver-related death (risk ratio: 0.18; 95% CI: 0.063–0.532; $P = 0.002$), whereas the risk for nonresponders and untreated patients did not

18 Benvegnu L, Chemello L, Noventa F, Fattovich G, Pontisso P, Alberti A. Retrospective analysis of the effect of interferon therapy on the clinical outcome of patients with viral cirrhosis. *Cancer* 1998; 83: 901–909.
19 Valla DC, Chevallier M, Marcellin P *et al.* Treatment of hepatitis C virus-related cirrhosis: a randomized, controlled trial of interferon alfa-2b versus no treatment. *Hepatology* 1999; 29: 1870–1875.
20 Serfaty L, Aumaitre H, Chazouilleres O *et al.* Determinants of outcome of compensated hepatitis C virus-related cirrhosis. *Hepatology* 1998; 27: 1435–1440.
21 Nishiguchi S, Shiomi S, Nakatani S *et al.* Prevention of hepatocellular carcinoma in patients with chronic active hepatitis C and cirrhosis. *Lancet* 2001; 357: 196–197.
22 Fattovich G, Giustina G, Degos F *et al.* Morbidity and mortality in compensated cirrhosis type C: a retrospective follow-up study of 384 patients. *Gastroenterology* 1997; 112: 463–472.
23 Niederau C, Lange S, Heintges T *et al.* Prognosis of chronic hepatitis C: results of a large prospective cohort study. *Hepatology* 1998; 28: 1687–1695.
24 Yoshida H, Arakawa Y, Sata M *et al.* Interferon therapy prolonged life expectancy among chronic hepatitis C patients. *Gastroenterology* 2002; 123: 483–491.
25 Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Sheuer PJ. Classification of chronic hepatitis: grading and staging. *Hepatology* 1994; 19: 1513–1520.
26 Statistics and Information Department, Japan Ministry of Health and Welfare. *Vital Statistics in Japan* (in Japanese). Tokyo: Health and Welfare Statistics Association, 2002.

GASTROENTEROLOGY 2004;127:S17–S26

Hepatocellular Carcinoma: Recent Trends in Japan

KENDO KIYOSAWA, TAKEJI UMEMURA, TETSUYA ICHIJO, AKIHIRO MATSUMOTO, KANAME YOSHIZAWA, AMAL GAD, and EIJI TANAKA

Department of Internal Medicine, Gastroenterology, and Hepatology, Shinshu University School of Medicine, Matsumoto, Japan

During the past 20 years, primary liver cancer, 95% of which is hepatocellular carcinoma (HCC), has ranked third in men and fifth in women as a cause of death from malignant neoplasm in Japan. The numbers of deaths and death rate from HCC showed a sharp increase beginning in 1975. Although both hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are important causes, HCV-related HCC has accounted for most of the recent increase and now represents 75% of all HCC in Japan. Geographically, HCC is more frequent in western than eastern Japan, and the death rate of HCC in each prefecture correlates with prevalence of anti-HCV. Among patients with HCV-related HCC, a history of blood transfusion was a relatively important source of infection in the 1990s, whereas community-acquired infections increased after 2000. There was a negative correlation between the duration from onset of infection to development of HCC and the age at onset. Interferon therapy for chronic hepatitis C has reduced the risk for HCC, indicating that early detection of HCV carriers and better treatment will contribute to improved outcomes. Nationwide screening for HCV and HBV began in 2002 in Japan, and reduction of HCC is anticipated. Further research should focus on mechanisms of carcinogenesis by HCV and HBV, development of more effective treatments, and establishment of early detection and treatment approaches. Better understanding of HCC unrelated to HCV and HBV and possibly because of steatohepatitis and diabetes should also be a major concern in future studies.

On a global scale, the Japanese have one of the longest average life expectancies, and the size of the aged population has been rising rapidly. These trends have led to a rising demand for improved health and quality of life in the elderly population. The 3 leading causes of death in Japan are malignant neoplasms, cardiovascular diseases, and cerebrovascular diseases. Death rates because of malignant neoplasms and cardiovascular disease have been increasing rapidly in recent decades. The age-adjusted death rate because of maligant neoplasms in 1960 was 100.4 per 100,000 people, and the total number of deaths was 93,773. The death rates from malignant neoplasms subsequently increased to 116.3 in 1970, 139.1 in 1980, 177.2 in 1990, and 235.6 per 100,000 people in 2000 (greater than a 2-fold increase). In 2001, the death rate because of malignant neoplasms was 238.8 per 100,000 people, and total deaths were 300,658. Since 1981, malignant neoplasms have been the leading cause of death in Japan. Among causes of death from malignant neoplasms, primary liver cancer, 95% of which is hepatocellular carcinoma (HCC), 4% cholangiocarcinoma, and 1% other have been particularly prominent. For the last 30 years, liver cancer has been the third leading cause of death from malignant neoplasms in men (following stomach and lung cancer). In women, liver cancer has ranked fifth during the past decade, following stomach, colon, lung, and breast cancer. In 2001, the death rates per 100,000 people for the leading causes of cancer mortality in men were 45.6 for lung, 37.1 for stomach, and 27.3 for primary liver cancer; rates in women have been 14.6 for stomach, 13.6 for colon, 12.2 for lung, 11.1 for breast, and 8.8 for primary liver cancer.

There are 2 major causes of HCC in Japan: hepatitis B virus (HBV) and hepatitis C virus (HCV) infection. The increase in incidence of HCC in Japan, however, has largely been attributable to HCV infection and the increase of this disease in the general population during the last 50 to 60 years.[1] Using a molecular clock analysis based on sequencing of HCV isolates, it has been hypothesized that genotype 1 HCV first appeared in Japan in the 1880s and that a major spread of infection in the population began in the 1930s and peaked between 1940 and 1970.[2] Thus, an understanding of recent trends in HCC in Japan depend on an understanding of the epidemiology and natural history of HCV infection.

Abbreviations used in this paper: HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; ICD, international classification of disease; SVR, sustained virologic response.

0016-5085/04/$30.00
doi:10.1053/j.gastro.2004.09.012

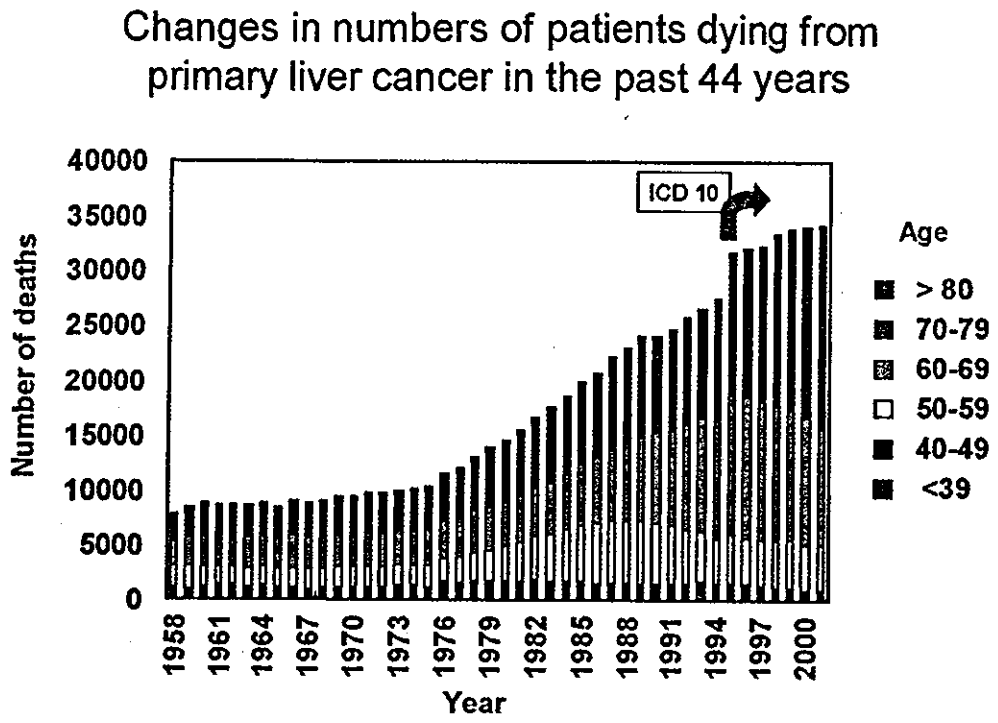

Figure 1. Secular trends in numbers of deaths because of primary liver cancer in Japan from 1958 to 2001. Totals from the Vital Statistics of Japan to which statistics are reported every year by the Ministry of Health, Labor, and Welfare.

Changes in Deaths and the Death Rate of Primary Liver Cancer in the Past 44 Years

Changes in annual death totals of primary liver cancer in different age groups between 1958 and 2001 are shown in Figure 1. The total number of deaths from HCC was stable and less than 10,000 people per year until 1975 when numbers increased sharply. The increase in 1995 was probably due to the change in International Classification of Disease (ICD) codes from ICD9 to ICD10. Peak numbers of deaths from HCC were in patients below the age of 69 years until 1999, when the peak age rose to over 70 years.

Death rates from liver cancer by gender are shown in Figure 2. Rates were consistently higher in men than women. Total age-adjusted death rates per 100,000 people were 9.4 in 1960, 8.7 in 1965, 9.2 in 1970, 9.3 in 1975, 12.0 in 1980, 15.8 in 1985, 19.7 in 1990, 25.5 in 1995, and 27.1 in 2000. A sharp rise in death rates for primary liver cancer in men began in 1975 and a more gradual rise in women in 1980. Since 1995, the rate of rise in men has slowed, although total numbers are still increasing.

Changes in Prevalence of HBV-Related and HCV-Related HCC in the Past 14 Years

A nationwide survey of primary liver cancer has been conducted every 2 years since 1968 by the Liver Cancer Study Group of Japan. The results of survey up to 1999 have been reported in several publications.[3–5] A total of 99,196 patients have been entered in this registry between 1980 and 1999. Of the total, 95% (93,901) were diagnosed as HCC histologically and clinically, indicating that the majority of primary liver cancer in Japan is HCC.

Five serologic surveys performed between 1990 and 1999 have documented that most patients with HCC are positive for either hepatitis B surface antigen (HBsAg) or antibody to hepatitis C virus (anti-HCV). Tests for HBsAg became available in 1975 and those for anti-HCV in 1990. HBsAg-positive cases of HCC constituted 42% of cases in 1977–1978, but only 16% of cases in

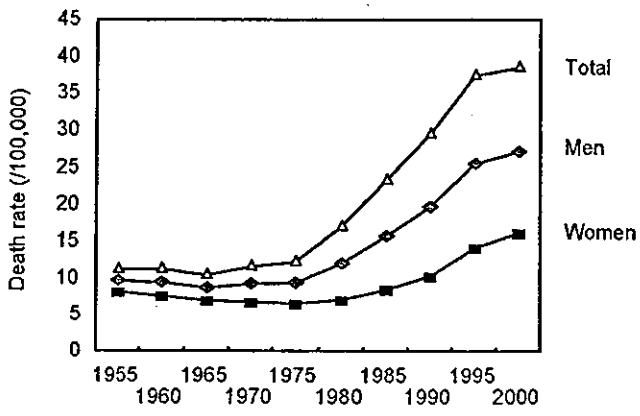

Figure 2. Changes in the age-adjusted death rate of primary liver cancer overall and in men and women separately for the years 1958 to 2001.

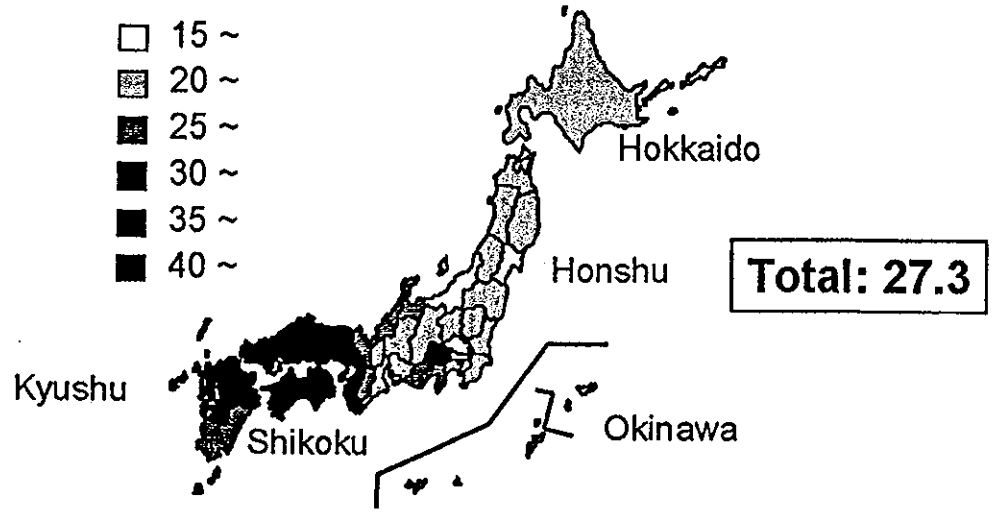

Figure 3. Death rates of primary liver cancer in 48 prefectures of Japan in 2001, extracted from the Vital Statistics of Japan 2001.

1998–1999. In contrast, anti-HCV-positive cases of HCC have accounted for more that 70% of cases diagnosed in the last 10 years. In cross-sectional studies, HBV-related HCC accounted for 51% of cases in 1982, 23% of cases in 1990, and 14% of cases in 2003.[6,7] Although anti-HCV was not available in 1982, non-HBV-related HCC or anti-HCV-positive cases represented 49% of cases in 1982, 61% in 1990, and 81% in 2003. Thus, HCV-related HCC has become an increasing majority of cases. Thus, the increased rates of death because of primary liver cancer in Japan appear to reflect the increase in numbers of HCV-related HCC.

Geographic Variation in Liver Cancer and HBV/HCV Infection

Although Japan is a relatively small country with a homogenous population, the incidence of HCC varies greatly among different regions. The Vital Statistics of Japan for 2001 published in 2003 by the Japanese Ministry of Health, Labor, and Welfare on the incidence of deaths as a result of HCC in its 48 prefectures shows a gradient increase of death rates for HCC along the axis of Japan from east to west (Figure 3).[8] The average age-adjusted death rate of HCC among the 48 prefectures was 27.3 per 100,000 people. Prefectures with death rates greater than 30 per 100,000 people were found chiefly on the Kyushu Islands, the Shikoku Islands, and the western area of Honshu Island. Nationwide health screening for HBsAg and anti-HCV in the over 40 years of age population has been performed since 2002, and prevalence rates for these markers have been analyzed by prefecture in Japan. The average HBsAg prevalence was 1.2% in the total Japanese population. Although high prevalence rates of HBsAg are found in a few areas, such as Okinawa (3.1%), Hokkaido (2.4%), and Shimane (2.1%), rates of HBsAg are generally evenly distributed throughout most of Japan. In contrast, there is considerable variation in the prevalence of anti-HCV in different geographic areas. Highest rates are found in Kyushu Island, Shikoku Island, and on the western side of Honshu Island (~1.5%). There were highly significant associations between death rates from HCiC in each prefecture and prevalence of anti-HCV (Figure 4*B*, correlation coefficient = 0.616; $P = .01$, $Y = 18.1 + 8.752X$), but no correlation with the prevalence of HBsAg (Figure 4*A*, correlation coefficient = 0.093; $P = .533$). For instance, although Hokkaido and Okinawa have high prevalence rates of HBsAg (2.4% and 3.1%, respectively), death rates from HCC in Hokkaido and Okinawa were 22.7 and 10.6 per 100,000 people, respectively, both of which are below the national average (23.7). In contrast, areas with high rates of anti-HCV, such as Saga (3.9%), Hiroshima (1.8%), Fukuoka (1.7%), and Kagawa (1.7%), had high death rates for primary liver cancer—43.1, 39.6, 39.8, and 31.9 per 100,000 people, respectively—which were higher than the national average. Thus, although rates of HBV infection in the population correlates best with rates of HCC in most countries of the world, in Japan, this is not the case, and HCV appears to be the major contributor to rates of primary liver cancer.

Changes in Clinical Characteristics of HBV- and HCV-Related HCC

Results of cross-sectional studies of HCC conducted at Shinshu University are shown in Table 1.[6,7] Men accounted for the majority of cases of both HBV- and HCV-related HCC for all age groups. The propor-

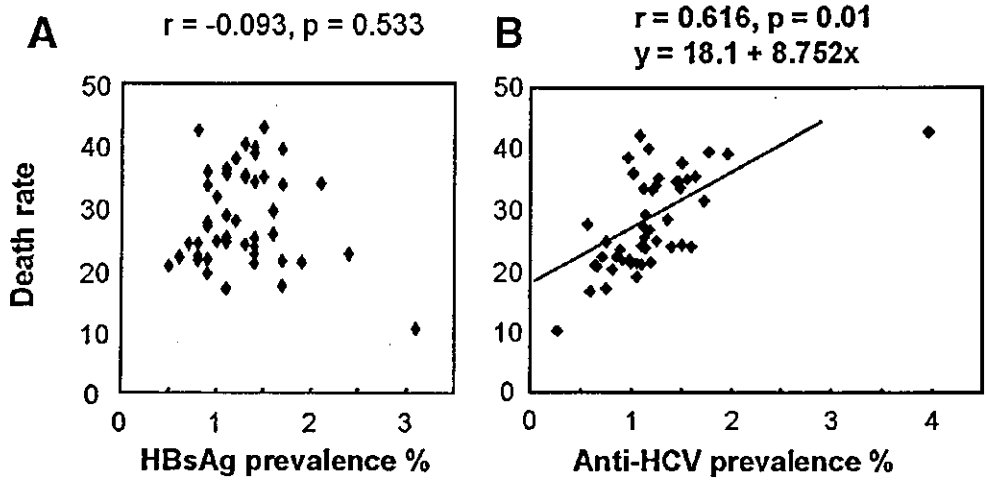

Figure 4. Relationship between age-adjusted annual death rates from primary liver cancer and prevalence of HBsAg (*A*) and anti-HCV (*B*) among the general population over 40 years of age in 2002.

tion of cases of HCC in men was 81% in 1982, 90% in 1990, but only 72% in 2003, indicating a recent relative increase in cases among women in recent years. The average age of diagnosis of HBV-related HCC was similar in all 3 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. The proportion of cases reporting a history of blood transfusion was 13% in 1982, 42% in 1990, and 36% in 2003 in non-HBV (HBsAg-negative) and anti-HCV positive patients with HCC. For these time periods, the intervals between date of blood transfusion and diagnosis of HCC were 23.4, 29.0, and 36.6 years, respectively. This estimated incubation period from onset of infection to clinical diagnosis of HCC has also been found by Tong et al in Los Angeles, who reported the average interval between blood transfusion and diagnosis of chronic hepatitis as 13.7 years, diagnosis of cirrhosis as 20.6 years, and HCC as 28.3 years.[9] The reason for the increasing interval between transfusion and diagnosis of HCC in recent years is not known, but a possible explanation is the recent introduction of therapy of hepatitis C, which may prolong survival or time to HCC. Another explanation is a delay in diagnosis of HCV infection and/or HCC. The correlation of age at the time of blood transfusion and interval from transfusion to diagnosis of HCC is shown in Figure 5. These results demonstrate an inverse relationship between age of exposure to HCV and appearance of liver cancer (correlation coefficient = 0.753; $P < .001$; $Y = 59.0 - 0.728X$). Such results suggest that progression to cirrhosis and HCC is more rapid with older age of onset of hepatitis C. Similar findings have been reported by others. Tanaka et al analyzed the interval between the age of blood transfusion and age of diagnosis of HCC in 115 Japanese patients.[10] The average interval was 30.6 years, and it decreased with increasing ages at the time of transfusion: 34.8 years for patients transfused before the age of 30 years, 30.4 years for those transfused between the ages of 30 and 40 years, and 24.7 years for those transfused above the age of 40 years. Hamada et al reported a significant inverse correlation between the interval from HCV infection to the development of HCC and the age at time of infection (correlation coefficient = 0.702; $P <$

Table 1. Comparison of Clinical Features of HBV- and HCV-Related Hepatocellular Carcinoma in 1982, 1990, and 2003 in Shinshu University Hospital

	HBV related			HCV related		
	1982 (n = 55)	1990 (n = 29)	2003 (n = 27)	1982 (n = 53)	1990 (n = 50)	2003 (n = 108)
Male	78%	83%	81%	81%	90%	72%
Age (*y*)	55.4 ± 11.1	54.8 ± 9.8	53.8 ± 9.0	61.6 ± 10.1	63.1 ± 7.3	67.8 ± 7.8
Family clustering	33%	45%	44%	1.8%	4%	2.7%
History of BT	8.3%	6.9%	3.7%	13%	42%	26%
Interval between BT and DX				23.4 ± 7.0	29.0 ± 13.6	36.6 ± 10.1
Heavy drinker[a]	3.6%	6.9%	3.7%	7.5%	10%	7.4%

BT, blood transfusion; Dx, diagnosis of hepatocellular carcinoma.
[a]Heavy drinker: more than 80 g ethanol daily, 1982,[6] 1990.[7]

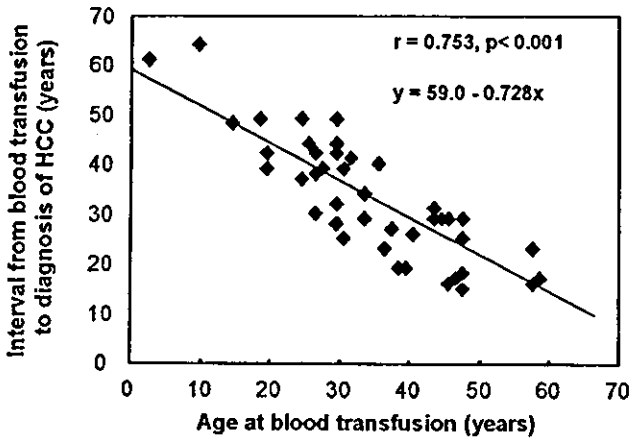

Figure 5. Relationship between age at the time of blood transfusion and interval from blood transfusion to diagnosis of HCC.

.0001; Y = 61.1 − 0.82X), indicating that the age of patients, rather than the duration of HCV infection, was more significant for development of HCC in patients with posttransfusion hepatitis C.[11] In a similar fashion, Poynard et al found a large difference among the probabilities of fibrosis progression by age of onset of HCV infection.[12] Among patients infected before the age of 20 years, there were few if any events during the first 10 years of infection. Among patients aged 20 to 30 years and 30 to 40 years, there was an increase in progression of fibrosis after 30 years of infection. In patients infected between ages 40 and 50 years, there was a clear increase in progression of fibrosis after 10 years of infection. Finally, among patients infected after 50 years of age, there were rapid rates of progression for all stages of fibrosis. These findings indicate that age has a major effect on rate of progression of hepatitis C, development of cirrhosis, and HCC.

Risk Factors for HCC

There are 3 major categories of risk factors that appear to influence the incidence of HCC: host, viral, and environmental factors. Host factors include gender, age, ethnicity, stage of liver disease, diabetes mellitus, and hepatic steatosis. Viral factors include genotype, viral levels, DNA integration, rates of mutation, and coinfection with other agents. Environmental factors include toxins such as aflatoxin B1, medications and hormones, and nicotine or smoking.

Host Factors

Gender and age. Men are 2 to 3 times more likely to develop HCC than women, although this proportion appears to be decreasing recently in patients with HCV-related HCC in Japan. The average age of diagnosis of HBV-related HCC in Japan is approximately 53 years, an average that has not changed in the last 20 years. In contrast, the average age of diagnosis of HCC has increased progressively in recent years among persons with HCV infection, as discussed previously.

Ethnicity. There are marked differences in rates of HCC in different ethnic and racial groups worldwide.[13] Persons of Asian and African decent have higher age-adjusted death rates than the white population, although this may relate largely to higher rates of underlying hepatitis B or C. Importantly, the incidence of HCC among white patients with HCV-related cirrhosis has been reported to be 1.2% in the United States, 3% to 4% in France, and 1.6% to 3% in Italy,[14] whereas the reported incidence in Japanese patients with cirrhosis because of HCV is 6% to 7%.[15] Ethnic and racial variation in rates of HCC may be due to differences in distribution of HBV and HCV infections and particularly the age of onset and duration of infection. Nevertheless, the real reason for these discrepancies and the possible role of racial differences remains unclear.

Stage of liver disease. Advanced hepatic fibrosis is an important risk factor for developing HCC. Yoshida et al reported that male sex, older age, and advanced fibrosis were associated with a higher risk of HCC, both in the interferon-untreated patients and in the patients who showed nonsustained response to interferon therapy.[16]

Alcohol consumption. Several of studies have shown that heavy alcohol intake increases the risk of cirrhosis and HCC in patients with hepatitis C in Japan. Whether this is due to a higher rate of cirrhosis in patients with HCV who drink alcohol or whether alcohol per se has a carcinogenic effect has not been adequately assessed.[17–19]

Diabetes and hepatic steatosis. Type 2 diabetes and hepatic steatosis have been found to be increased in frequency in patients with hepatitis C and advanced liver disease in case series and epidemiologic studies. El-Serag et al analyzed a large cohort study of United States veterans admitted to Veterans Administration Hospitals from 1985 to 2000 and found that diabetes was an independent risk factor for chronic liver disease and HCC.[20] There have been no reports on the relationship between diabetes, hepatitis C, and HCC from Japan. However, studies using HCV core gene transgenic mice have shown that hepatic steatosis related to presence of the transgene and direct evidence of insulin resistance have been found in human HCV infection.[21,22] Although hepatic steatosis has been recognized as a preconditioning status of HCC in HCV core transgenic mouse, the

causal association between type 2 diabetes and HCC has not yet been clarified.

Oncogenes. Oncogenes are believed to play a role in development of HCC. Recently, a complementary DNA (cDNA) microarray approach was used to identify unique gene sets that are abnormally expressed in HCC[23] and preneoplastic chronic liver diseases.[24] However, no specific host genes or mutations were found to be associated for HCC.

Viral Factors: Hepatitis B Virus

HBV genotype. In southern Asia and Japan, the most common genotypes of HBV are B and C, which are found rarely in the United States and Western Europe outside of patients of Asian descent. In large surveys conducted by Orito et al, the proportion of cases of chronic hepatitis B because of genotype B was 12% and the proportion because of genotype C was 85%. These proportions are similar to those among patients with HBV-related HCC, 13% because of genotype B and 86% because of genotype C.[25] In the patients with genotype B, the mean age of diagnosis of HCC was 70 years, which was higher than that of genotype C patients, which was 55 years. These findings suggested that both genotypes of HBV were capable of leading to HCC but that the process was more rapid in patients with genotype C. In Taiwan, where genotype B is more common than genotype C (61% vs. 31%, respectively) in patients with HBV-related HCC, the mean age of those with genotype B was 50 years and younger than those with genotype C (59 years).[26] These differences may be explained by a distinct distribution of subtypes of genotype B between the 2 countries. In Taiwan as well as in most Asian countries, only the subtype Ba is found, whereas the majority of Japanese patients have the subtype Bj.[27]

Mutations in either the precore or the core promoter regions of HBV account for the finding of HBeAg-negative chronic hepatitis B, a common form of chronic HBV infection in Asia and Japan. Orito et al found that precore mutations (at nucleotide [nt] 1896) were responsible for the majority of patients with genotype B who had HBeAg-negative chronic hepatitis B and HCC. In contrast, the frequency of the core promoter double mutation at nt 1762 and nt 1764, which may regulate the transcriptional activity of precore/core RNA and pregenomic RNA, was significantly more frequent in patients with HBV genotype C than B and was independent of HBeAg status. The double mutation in the core promoter was also found frequently in patients with HCC, thereby suggesting its association with hepatocarcinogenesis. Natural history studies suggest that patients infected with HBV genotype B are often negative for HBeAg and have lower average ALT levels and a better prognosis those patients with genotype C. Patients with genotype C tend to remain HBeAg positive for a longer time and are more likely to have elevated ALT levels and more advanced liver disease than patients with genotype B. These findings indicate that HBV genotype, along with associated clinical features of disease, may be an important factor in development of HBV-related HCC.

Integration of HBV DNA. HBV DNA is known to become integrated into host DNA during chronic infection. There does not appear to be a specific or prominent site in host DNA at which HBV DNA is integrated at a high rate, and the link of these integration sites to HCC is not well established.[28] Nevertheless, many HBV-related HCCs have clonal HBV DNA integrations, some of which have been associated with mutations in important growth factors and oncogenes.

Occult HBV Infection. Occult HBV infection is characterized by persistent presence of HBV DNA in serum or tissue of patients who are HBsAg negative.[29] Occult HBV infection has been reported in HCV-related and -unrelated HCC by several groups of Japanese researchers.[30–32] Recently, Pollicino et al[33] tested tumor tissues from 107 patients and the corresponding nontumor liver tissue from 72 of these patients with HBsAg-negative HCC for HBV DNA and compared results with those from 192 patients with HBsAg-negative chronic hepatitis B. HBV DNA was detected in 68 of 107 cases of HCC (63.5%) and in 63 of 192 cases of chronic hepatitis (32.8%) ($P < .0001$; odds ratio, 3.6; 95% confidence interval: 2.2–5.9). The significant association of occult HBV with HCC was found irrespective of age, gender, and evidence of HCV infection. Both integrated viral DNA and covalently closed circular HBV genomes were detected in patients with occult HBV. Thus, they concluded that occult HBV is a risk factor for development of HCC.

Viral Factors: Hepatitis C Virus

HCV genotype. Although HCV is classified into at least 6 different genotypes, HCV genotypes 1 and 2 account for most cases of infection in Japan. Tanaka et al[10] conducted epidemiologic studies of the HCV genotype in Japanese patients with chronic liver disease from 16 medical institutions in Japan in 1993. Of 4176 patients with chronic HCV infection collected from the 4 main islands of Japan, 2794 had chronic hepatitis, 727 had cirrhosis, and 655 had HCC. The prevalence of HCV genotypes 1 and 2 was similar on the 4 islands. HCV genotype 1 predominated in each disease category, ranging from 69% to 76%. HCV genotype 1 was relatively more common in patients with more advanced disease

and HCC (7% greater), whereas genotype 2 was less common (7% less) ($P < .001$). Patients with HCV genotype 1 or 2 had similar demographic features with regard to age, gender, and history of blood transfusion. A similar association of genotype 1 with more advanced disease was reported from our group at Shinshu University,[34] but the association of HCV genotype 1 and disease progression has not been demonstrated in prospective studies and is still a subject of controversy.

Viral load and viral quasispecies. There is no evidence that either serum viral levels or viral quasispecies are associated with development of HCC. There was no evidence that the HCV genome is reverse transcribed into DNA or can become integrated into host DNA.

Coinfection with other viruses. Occult infection with HBV has been associated with non-HBV-related HCC, and Japanese patients with HCV-related HCC have a relatively high prevalence of antibody to hepatitis B core antigen (anti-HBc), indicating previous or ongoing HBV infection. However, thus far, no definitive mechanism of synergistic action of HCV and HBV in causing HCC has been identified other than the possibility that the 2 infections together are more likely to lead to cirrhosis than either alone. Future research is necessary. Coinfection with human immunodeficiency virus (HIV) in HCV-related HCC cases in Japan is rare, and there is no evidence that HIV-HCV coinfection is a significant cause of this cancer in Japan.

Environmental Factors

Environmental carcinogens such as aflatoxin B1 increase liver cancer risk 3-fold and have been linked to a specific mutation on codon 249 of the p53 tumor-suppressor gene.[35] Fortunately, aflatoxin exposure is rare in Japan, and there is no evidence of aflatoxin B1 or other chemical agents linked to the increasing rates of HCC in Japan.

Treatment of HCC and Survival Between 1988 and 1999

The report of the 15th follow-up survey of primary liver cancer conducted by the Liver Society Study Group of Japan provided information on the survival rates of patients who underwent hepatic resection (N = 21,711), ethanol injection therapy (N = 12,876), transcatheter arterial embolization (TAE) (N = 22,869), and microwave coagulation therapy (N = 1751).[3] All patients were registered between 1988 and 1999, excepting those undergoing microwave coagulation who were registered only after 1992. The survival rates of the respective treatments are shown in Figure 6. Surgical procedures used for HCC were 6.6% extensive hepatic lobectomy or trisegmentectomy, 15.2% hepatic lobectomy, 23.7% segmentectomy, 18.7% subsegmentectomy (resection of Couinaud's segment), and 35.8% wedge resection. The cumulative survival rates at 5 and 10 years were 52.3% and 27.3%, respectively. These percentages were higher than those of other treatments. Good survival rates have been found to correspond with small tumor diameter (<5 cm), fewer numbers of tumors (<2), lack of portal vein invasion, and early clinical stage (I). Radiofrequency ablation therapy had not yet become popular during this period in Japan. Living donor liver transplantation for HCC with decompensated cirrhosis has been allowed by medical insurance since January 2004. Thus, modalities for HCC have been expanded.

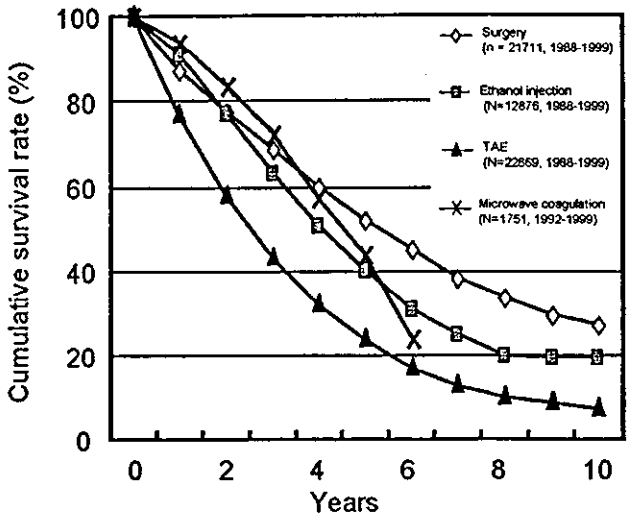

Figure 6. Cumulative survival rate relative to each therapy for HCC. Report of the 15th follow-up survey by the Liver Cancer Study Group of Japan. TAE, transcatheter arterial embolization therapy.

Measures to Reduce the Occurrence of HCC

In 1999, the Japan Society of Hepatology published the "Liver Cancer White Paper" aimed at promoting a national effort to eradicate liver cancer. The White Paper proposed 4 steps: (1) improve sanitary conditions and better patient information on means to prevent hepatitis virus infection; (2) routine screening for HBsAg and anti-HCV of the general population as well as in high-risk groups, including those having a history of blood transfusion, major surgery, tattooing, and injection drug use to identify carriers of HCV and HBV; (3) introduction and greater availability of newer treatments (combination therapy with interferon and ribavirin for hepatitis C and lamivudine therapy for hepatitis B) against chronic hepatitis aiming at inhibiting progres-

Table 2. Summary of the Findings in Representative Studies on the Incidence of HCC Among Patients With Cirrhosis and Chronic Hepatitis Treated With Interferon in Japan

			Treated					
	Untreated		Non-SVR		SVR		Total	
Author	No. HCC/ no. cases	%	No. HCC/ no. cases	%	No. HCC/ no. cases	%	No. HCC/ no. cases	%
Nishiguchi[40]	17/45	38					2/45	4.4
Kasahara[41]			19/709	16.8	13/313	4.3	32/1022	3.1
Imai[42]	19/140	13					18/419	4.3
Ikeda[43]	67/452	15	23/730	3.2	5/461	1.1	28/1191	2.4
Yoshida[44]	13/459	2.8	49/1613	3.0	7/817	0.9	56/2340	2.4
Okanoue[45]	22/55	40					7/40	18

HCC, hepatocellular carcinoma; SVR, sustained virologic response.

sion of disease to cirrhosis and HCC; and (4) construction of a communication network between physicians and hepatologists to detect and treat HCC earlier.[36]

The nationwide campaign instituted by the Japan Society of Hepatology to eradicate HCC was initiated in 1999. Members of the Society engaged in a grass roots movement in the last week of each May in each prefecture throughout Japan. Nationwide screening for anti-HCV and HBsAg at 5-year intervals for those over 40 years of age in the general population started in 2002. In 2002, 31,393 new HCV carriers and 24,430 new HBsAg carriers were identified.[37] The course and outcome of these newly identified cases need further follow-up evaluation. However, the long-term outcome of these HCV carriers may not be as pessimistic as has been reported for chronically infected persons in the United States,[38] where routine screening of the general population has not been recommended.[39,40]

Interferon alfa (or peginterferon) and rivabirin combination therapy for hepatitis C and lamivudine therapy for hepatitis B were introduced in Japan using public health insurance. In addition, living donor liver transplantation for liver failure and HCC in adults has been available since January 2004, and radiofrequency ablation therapy for HCC since April 2004. The Japan Society for Hepatology conducted a consensus meeting on hepatitis C, hepatitis B, and HCC and established guidelines for these diseases in 2001 and 2003. A network system for communication between physicians and hepatologists concerning liver disease has been constructed in each area.

Antiviral Therapy Suppresses the Incidence of HCC

A summary of different studies on the incidence of HCC among patients with HCV-related cirrhosis who were treated with interferon in Japan is noted in Table 2. Nishiguchi et al[41] conducted a prospective, randomized controlled trial that examined the effects of therapy on development of HCC. In that study, 100 patients with compensated cirrhosis were randomized to receive 6 million units (MU) of interferon alfa 3 times weekly for 3 to 6 months or prospective monitoring without treatment. After a 2–7-year period of follow-up evaluation, HCC was significantly less frequent in the treated group (4%) than the untreated group (38%). Other studies from Japan were retrospective and not prospectively controlled. These studies have suggested a moderate decrease in the risk for HCC in patients with chronic hepatitis C treated with interferon.[42–46] The reduction of occurrence of HCC was more convincingly shown in patients with sustained virologic response as compared with nonresponders and nontreated patients. The weight of evidence from these studies suggests that antiviral treatment reduces the risk of HCC in patients with hepatitis C.[16]

Future Research Needs

The origin of the majority of cases of HCC in Japan is HBV and HCV infection. The molecular mechanisms responsible for carcinogenesis with HCV and HBV infection have not been elucidated. Such clarification is urgently needed in that it may provide insights into new approaches to therapy and prevention of HCC.

With the introduction of passive and active immunization against HBV infection, especially in the case of perinatal transmission by carrier mothers to their infants, the prevalence of chronic HBV infections has decreased markedly. With the introduction of routine screening of blood donors for anti-HCV, transfusion-related transmission of HCV has been reduced, which has led to a decrease in frequency of hepatitis C in the population. Unfortunately, needle stick-related transmission of HCV in hospital employees and transmission because of injection drug use are still common causes of new cases of

hepatitis C. In this regard, development of an HCV vaccine is urgently needed.

Antiviral treatment appears to reduce the risk of HCC among patients with hepatitis C. However, patients with chronic hepatitis C whose HCV genotype is 1b and whose HCV viral load is high are often resistant to optimal regimens of interferon and ribavirin combination therapy. In addition, mutation of the HBV genome during lamivudine therapy with development of viral resistance is a growing problem in the treatment of hepatitis B. More effective antiviral treatments with fewer side effects for both viral infections are needed.

The age of patients with precancerous stage liver disease and with HCC is rising. This has made use of aggressive regimens for therapy of HCC more and more difficult. A relatively noninvasive but effective therapeutic strategy is needed for elderly patients with HCC.

During the next 1 to 2 decades, the metabolic problems of obesity, hepatic steatosis, and diabetes are expected to increase in frequency in the general Japanese population. These may be risk factors for HCC and account for a proportion of cases that are not related to HBV or HCV. Studies on the relationship between HCC and these metabolic diseases need to be actively pursued in Japan.

Finally, the striking differences in frequency, risk patterns, and epidemiology of HCC in Japan in comparison with other countries, including the United States, have yet to be fully explained, and collaborative efforts in epidemiologic research between Japan and other nations should be encouraged to help elucidate the reasons for these differences, which might well provide clues to the etiology of HCC both in Japan and the rest of the world.

References

1. Yoshizawa H. Hepatocellular carcinoma associated with hepatitis C virus infection in Japan: projection to other countries in the foreseeable future. Oncology 2002;62(Suppl. 1):8–17.
2. Tanaka Y, Hanada K, Mizokami M, Yeo AE, Shih JWK, Gojobori T, Alter HJ. A comparison of the molecular clock of hepatitis C virus in the United States and Japan predicts that hepatocellular carcinoma incidence in the United States will increase over the next two decades. Proc Nat Acad Sci U S A 2002;99:15584–15589.
3. The Liver Cancer Study Group of Japan. Primary liver cancer in Japan, sixth report. Cancer 1987;60:1400–1411.
4. Arii S, Yamaoka Y, Futagawa S, Inoue K, Kobayashi K, Kojiro M, Makuuchi M, Nakanuma Y, Okita K, Yamada R, The Liver Cancer Study Group. Results of surgical and non-surgical treatment for small-sized hepatocellular carcinoma: a retrospective and nationwide survey in Japan. Hepatology 2000;32:1224–1229.
5. Ikai I, Okita K, Omata M, Kojiro M, Kobayashi K, Nakanuma Y, Fujiwara S, Makuuchi M, Yamaoka Y. Report of the 15th follow-up survey of primary liver cancer. Hepatol Res 2004;28:21–29.
6. Kiyosawa K, Akahane Y, Nagata A, Koike Y, Furuta S. The significance of blood transfusion in non-A, non-B chronic liver disease in Japan. Vox Sang 1982;43:45–52.
7. Kiyosawa K, Sodeyama T, Tanaka E, Gibo Y, Yoshizawa K, Nakano Y, Furuta S, Akahane Y, Nishioka K, Purcell RH, Alter HJ. Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. Hepatology 1990;12:671–675.
8. Vital Statistics of Japan 2001. Statistics and Information Department, Minister's Secretariat, Ministry of Health, Labor and Welfare. Health and Welfare Statistics Association. Tokyo: Ohwa Sogo Insatsu. Co., 2003.
9. Tong MJ, El-Farra N, Reikes AR, Co RL. Clinical outcomes after transfusion-associated hepatitis C. N Engl J Med 1995;332: 1463–1466.
10. Tanaka E, Kiyosawa K, Matsushima T, Ishikawa K, Hino K, Tanaka S, Nose H, Kimada H, Iino S, Kamimura T, Unohara M, Mizokami M, Okanoue T, Kuroki T, Yamada G, Miura T, Yano M, Tsubouchi H, Kohara K, Sato S, Hattori N, and Genotyping Elisa Study Group. Epidemiology of genotypes of hepatitis C virus in Japanese patients with type C chronic liver diseases: a multi-institute analysis. J Gastroenterol Hepatol 1995;10:535–545.
11. Hamada H, Yatsuhashi H, Yano K, Daikoku M, Arisawa K, Inoue O, Koga M, Nakata K, Eguchi K, Yano M. Impact of aging on the development of hepatocellular carcinoma in patients with post-transfusion chronic hepatitis C. Cancer 2002;95:331–339.
12. Poynard T, Ratziu V, Chalotte F, Goodman Z, McHutchison J, Albrecht J. Rates and risk factors of liver fibrosis progression in patients with chronic hepatitis C. J Hepatol 2001;34:730–739.
13. Bosch X, Ribes J, Borras J. Epidemiology of primary liver cancer. Semin Liver Dis 1999;19:271–285.
14. Colombo M, de Francis R, Del Ninno E, Sangiovanni A, De Fazio C, Tommasini M, Donato MF, Piva A, Di Carlo V, Dioguardi N. Hepatocellular carcinoma in Italian patients with cirrhosis. N Engl J Med 1991;325:675–680.
15. Tsukuma H, Hiyama T, Tanaka S. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. N Engl J Med 1993;328:1797–1801.
16. Yoshida H, Tateishi R, Sata M, Fujiyama S, Nishiguchi S, Ishibashi H, Yamada G, Yokosuka O, Shiratori Y, Omata M. Benefit of interferon therapy in hepatocellular carcinoma prevention for individual patients with chronic hepatitis C. Gut 2004; 53:425–430.
17. Kubo S, Kinoshita H, Hirohashi K, Tanaka H, Tsukamoto T, Shunto T, Kuroki T. High malignancy of hepatocellular carcinoma in alcoholic patients with hepatitis C virus. Surgery 1997;121: 425–429.
18. Aizawa Y, Shibamoto Y, Takagi I, Zeniya M, Toga G. Analysis of factors affecting the appearance of hepatocellular carcinoma in patients with chronic hepatitis C: a long-term follow-up study after histologic diagnosis. Cancer 2000;89:53–59.
19. Chiba T, Matsuzaki Y, Abei M, Shoda J, Aikawa T, Tanaka N, Osuga T. Multivariative analysis of risk factors for hepatocellular carcinoma in patients with hepatitis C virus-related liver cirrhosis. J Gastroenterol 1996;31:552–558.
20. El-Serag HB, Tran T, Everhart JE. Diabetes increases the risk of chronic disease and hepatocellular carcinoma. Gastroenterology 2004;126:460–468.
21. Moriya K, Yotsuyanagi H, Shintani Y, Fujie H, Ishibashi K, Matsuura Y, Miyamura T, Koike K. Hepatitis C virus core protein induces hepatic steatosis in transgenic mice. J Gen Virol 1997; 78:1527–1531.
22. Shintani Y, Fujie H, Miyoshi H, Tsutsumi T, Tsukamoto K, Kimura S, Moriya K, Koike K. Hepatitis C virus infection and diabetes: direct involvement of the virus in the development of insulin resistance. Gastroenterology 2004;126:840–848.
23. Shirota Y, Kaneko S, Honda M, Kawai HF, Kobayashi K. Identification of differentially expressed genes in hepatocellular carcinoma with cDNA microarrays. Hepatology 2001;33:832–840.

24. Kim JW, Ye Q, Forgues M, Chen Y, Budhu A, Sime J, Hofseth LJ, Kaul R, Wang XW. Cancer-associated molecular signature in the tissue samples of patients with cirrhosis. Hepatology 2004;39: 518–527.
25. Orito E, Ichida T, Sakugawa H, Sata M, Horiike N, Hino K, Okita K, Okanoue T, Iino S, Tanaka E, Suzuki K, Watanabe H, Hige S, Mizokami M. G[e]ographic distribution of hepatitis B virus (HBV) genotypes in patients with chronic HBV infection in Japan. Hepatology 2001;34:590–594.
26. Kao JH, Chen PJ, Lai MY, Chen DS. Hepatitis B genotypes correlate with clinical outcomes in patients with chronic hepatitis B. Gastroenterology 2000;118:554–559.
27. Orito E, Mizokami M. Hepatitis B virus genotypes and hepatocellular carcinoma in Japan. Intervirology 2003;46:408–412.
28. Kew MC. Hepatitis B virus in the etiology of hepatocellular carcinoma. In: Tabor E, ed. Viruses and liver cancer. Amsterdam: Elsevier Science, 2000:17–30.
29. Torbenthon M, Thomas DL. Occult hepatitis B. Lancet Infect Dis 2002;2:479–486.
30. Uchida T, Kaneita Y, Gotoh K, Kanagawa H, Kouyama H, Kawanishi T, Mima S. Hepatitis C virus is frequently coinfected with serum markers-negative hepatitis B virus: provable replication promotion of the former by the latter as demonstrated by in vitro cotransfection. J Med Virol 1997;52:399–405.
31. Koike K, Kobayashi M, Gondo M, Hayashi I, Osuga T, Takada S. Hepatitis B virus DNA is frequently found in liver biopsy samples from hepatitis C virus-infected chronic hepatitis patients. J Med Virol 1998;54:249–255.
32. Shintani Y, Yotsuyanagi H, Moriya K, Fujie H, Tsutsumi T, Takayama T, Makuuchi M, Kimura S, Koike K. The significance of hepatitis B virus DNA detected in hepatocellular carcinoma of patients with hepatitis C. Cancer 2000;88:2478–2486.
33. Pollicino T, Squadrito G, Cerenzia G, Cacciola I, Raffa G, Craxi A, Farinati F, Missale G, Smedile A, Tiribelli C, Villa E, Raymond G. Hepatitis B virus maintains its pro-oncogenic properties in the case of occult HBV infection. Gastroenterology 2004;126:102–110.
34. Kobayashi M, Tanaka E, Sodeyama T, Urushihara A, Matsumoto A, Kiyosawa K. The natural course of chronic hepatitis C: a comparison between patients with genotypes 1 and 2 hepatitis C viruses. Hepatology 1996;23:695–699.
35. Sun Z, Lu P, Gail MH. Increased risk of hepatocellular carcinoma in male hepatitis B surface antigen carriers with chronic hepatitis who have detectable aflatoxin metabolite M1. Hepatology 1999; 30:379–383.
36. Kiyosawa K. Proposals for decreasing liver cancer. Hepatol Res 2002;24:S68–S73.
37. Handbook of hepatitis virus screening. Kanenuirusu R-J, Kenkyuukai K, eds. Tokyo, October 2003.
38. Seeff LB, Miller RN, Rabkin CS, Buskell-Bales Z, Straley-Eason KD, Smoak BL. Forty-five-year follow-up of hepatitis C virus infection in healthy young adults. Ann Intern Med 2000;132:105–111.
39. U.S. Preventive Service Task Force. Screening for hepatitis C virus infection in adults: recommendation statement. Ann Intern Med 2004;140:462–464.
40. Chou R, Clark EC, Helfand M. Screening for hepatitis C virus infection: a review of the evidence for the U.S. preventive service task force. Ann Intern Med 2004;140:465–479.
41. Nishigutchi S, Kuroki T, Nakatani S, Morimoto H, Takeda T, Nakajima S, Shiomi S, Seki S, Kobayashi K, Ohtani S. Randomized trial of effects of interferon-α on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. Lancet 1995;346:1051–1055.
42. Kasahara A, Hayashi N, Mochizuki K, Takayanagi M, Yoshioka K, Kakumu S, Iijima A, Urushihara A, Kiyosawa K, Okuda M, Hino K, Okita K. Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. Hepatology 1998;27:1394–1402.
43. Imai Y, Kawata S, Tamura S, Yabuuchi I, Noda S, Inada M, Maeda Y, Shirai Y, Fukuzaki T, Kaji I, Ishikawa H, Matsuda Y, Nishikawa M, Seki K, Matsuzawa Y. Relation of interferon therapy and hepatocellular carcinoma in patients with chronic hepatitis C. Ann Intern Med 1998;129:94–99.
44. Ikeda K, Saitoh S, Arase K, Chayama K, Suzuki Y, Kobayashi M, Tsubota A, Nakamura I, Murashima N, Kumada H, Kawanishi M. Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis C: a long-term observation study of 1,643 patients using statistical bias correction with proportional hazard analysis. Hepatology 1999;29:1124–1130.
45. Yoshida H, Shiratori Y, Moriyama M, Arakawa Y, Ide T, Sata M, Inoue O, Yano M, Tanaka M, Fujiyama S, Nishiguchi S, Kuroki T, Imazeki F, Yokosuka O, Kinoyama S, Yamada G, Omata M. Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. Ann Intern Med 1999; 131:174–181.
46. Okanoue T, Itoh Y, Minami M, Sakamoto S, Yasui K, Sakamoto M, Murakami Y, Kashima K. Interferon therapy lowers the rate of progression to hepatocellular carcinoma in chronic hepatitis C but not significantly in advanced stage: a retrospective study in 1148 patients. J Hepatol 1999;30:653–659.

Address requests for reprints to: Kendo Kiyosawa, M.D., Department of Internal Medicine, Gastroenterology, Shinshu University School of Medicine, 3-1-1, Asahi, Matsumoto, Nagano-Prefecture 390-8621, Japan. e-mail: kkiyosa@hsp.md.shinshu-u.ac.jp; fax: (81) 263-32-9412.

Supported in part by a grant from the Ministry of Health, Labor and Welfare of Japan and a grant from the Ministry of Education, Culture, Sport, Science and Technology of Japan (No. 15590633).

Data sources used in this paper: Vital statistics of Japan, which are reported every year by the Ministry of Health, Labor and Welfare of Japan (1958-2001). Nationwide follow-up surveys by the Liver Cancer Study Group of Japan (1980–1999). The Liver Cancer White Paper published by the Japan Society of Hepatology (JSH) in 1999. Handbook of Aged People Health Screening for Hepatitis Virus, October 2003, 1 Published paper by Japanese researchers up to March, 2004 (References 5, 10, 16, 25, 27, 36,40, 41, 42, 43, 44 and 45). The cross-sectional studies in Shinshu University Hospitals. (References 6 and 7).

Available online at www.sciencedirect.com
SCIENCE DIRECT
Hepatology Research 28 (2004) 73–78
Hepatology Research
www.elsevier.com/locate/ihepcom

Changes in virus loads and precore mutations in chronic hepatitis B patients treated with 4 weeks of daily interferon alfa-2a therapy

Michio Kato [a,*], Nobukazu Yuki [a], Akira Kaneko [a], Keiji Yamamoto [a], Manabu Masuzawa [b], Norio Hayashi [c]

[a] *Department of Gastroenterology, Osaka National Hospital, Hoenzaka 2-1-14, Chuo-ku, Osaka 540-0006, Japan*
[b] *Department of Internal Medicine, Osaka Police Hospital, Japan*
[c] *Department of Molecular Therapeutics, Osaka University Graduate School of Medicine, Japan*

Received 27 November 2002; received in revised form 14 August 2003; accepted 23 October 2003

Abstract

Interferon (IFN) alfa-2a was administered to 23 patients with chronic hepatitis B daily for 4 weeks and the relation between the efficacy of the treatment and changes in total hepatitis B virus (HBV) DNA and precore mutant levels was investigated. At 6 and 12 months after the completion of IFN therapy, 39.1% (9/23) and 36.8% (7/19) of patients, respectively, showed alanine transaminase (ALT) normalization; 31.3% (5/16) and 50.0% (7/14), respectively, became negative for HBe-antigen (HBeAg); and 42.1% (8/19) and 41.2% (7/17), respectively, became undetectable for HBV DNA. All 18 of the patients who were positive for HBeAg at baseline nevertheless had the precore mutation. The level of precore mutant as a proportion of the total HBV DNA level was constant at baseline, and 3 and 6 months after the completion of therapy. Thus, the investigation showed that in chronic hepatitis B, the precore mutation occurs at a constant proportion beginning in the HBeAg-positive phase, and IFN therapy inhibits the growth of the wild-type and precore mutant viruses equally.

Keywords: Chronic hepatitis B; rIFNα-2a; HBV DNA; Precore mutant

1. Introduction

Drugs that have been used to treat chronic hepatitis B include interferon (IFN), propagermanium, and steroids. Recently, lamivudine has been introduced and its use in combination with IFN has attracted interest. IFN therapy was first reported by Greenberg et al. [1] in 1976, whose work shows that IFN inhibits viral growth. In the US and Europe, IFN monotherapy generally consists of long-term administration of 5–10MU per day three times per week for 4–6 months [2,3]. In Japan, in 1986, the National Health Insurance coverage established 4 weeks as the standard treatment period. Consequently, in the present study, rIFN alfa-2a was administered daily for 4 weeks at a dose of 9MU per day for the first 3 days and 18MU per day thereafter.

The efficacy of IFN therapy is estimated by seroconversion from HBe-antigen (HBeAg) to HBe-antibody (HBeAb), undetectable response for hepatitis B virus (HBV) DNA, and normalization of alanine transaminase (ALT). Factors reported to be associated with response to IFN therapy are the baseline levels of HBV DNA and ALT [2,3]. Moreover, the clinical significance of infection with the precore mutant virus, which does not produce HBeAg, has recently drawn attention. We therefore, quantitatively analyzed precore mutant levels and examined the changes in these levels with IFN monotherapy.

2. Materials and methods

The subjects were 23 patients with chronic hepatitis B, 16 males and 7 females, with a mean age of 36.3 ± 9.8 years. Eighteen of the patients were positive for HBeAg and five were negative. Although all 23 patients were positive for HBV DNA in the polymerase chain reaction (PCR) assay, two patients were below detection limits by the bDNA probe assay. The precore mutant level was not less than 10^7 copies/ml in 12 patients and less than 10^7 copies/ml in 11

* Corresponding author. Tel.: +81-6-6942-1331; fax: +81-6-6943-6467.
E-mail address: katom@onh.go.jp (M. Kato).

1386-6346/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.hepres.2003.10.002

Table 1
Baseline characteristics of patients

Patients number	Sex	Age	Grading[a]	Staging[a]	Interval from prior IFN (Month)	ALT (IU/l)	HBeAg (index)	HBeAb (%)	HBVDNA bDNA-p (Meq/ml)	HBVDNA PCR (copy/ml)	Precore mutant (copy/ml)
1	Female	35	–	–	38	287	0.8	96.4	96	8×10^7	8×10^7
2	Male	46	A1	F1	–	67	0.8	95.1	<0.7	5×10^3	<100
3	Female	52	A2	F1	10	310	179.8	0	1900	3×10^8	3×10^8
4	Female	29	–	–	24	304	3.6	57.8	3.4	3×10^6	3×10^6
5	Male	34	A1	F0	–	74	53.5	0	44	3×10^7	8×10^6
6	Female	22	A1	F1	–	259	407.7	0	78	3×10^7	3×10^7
7	Female	34	A1	F1	7	116	385.4	0	190	7×10^7	4×10^7
8	Male	43	A2	F2	14	206	113.3	0	1800	4×10^8	7×10^8
9	Male	56	A3	F2	–	148	7.2	58.0	1.6	9×10^5	9×10^5
10	Male	33	A1	F0	–	142	271.9	0	350	1×10^8	1×10^5
11	Male	33	–	–	41	87	292.2	0	150	3×10^7	3×10^7
12	Male	40	A2	F0	–	995	451.8	0	2100	4×10^8	4×10^8
13	Male	28	A1	F1	–	42	0.8	82.1	710	8×10^8	8×10^8
14	Male	44	A2	F2	49	57	1.8	17.2	2.8	5×10^6	8×10^5
15	Female	30	A2	F1	–	211	183.4	0	3800<	8×10^8	8×10^8
16	Female	27	A2	F2	–	87	9.2	23.3	<0.7	9×10^4	5×10^3
17	Male	36	–	–	10	448	231.8	0	25	1×10^7	1×10^6
18	Male	33	A3	F3	7	44	5.2	54.7	1	3×10^6	1×10^6
19	Male	27	A1	F2	5	184	53.9	0	400	3×10^7	3×10^7
20	Male	58	A3	F3	13	367	0.8	87.6	74	3×10^7	3×10^7
21	Male	26	A1	F3	8	115	172.8	0	1000	2×10^8	1×10^8
22	Male	28	A2	F0	10	338	174.7	0	490	1×10^7	7×10^6
23	Male	42	–	–	96	191	2.2	78.6	2.1	1×10^5	5×10^3

[a] Five cases were not measured.

patients, 1 of whom had a precore mutant level of less than 10^2 copies/ml. Fourteen patients had previously received IFN therapy for intervals from 5 to 96 months, during which they had been administered 477MU daily for 4 weeks. Baseline ALT was 34 to 66 IU/l in three patients and not less than 67 IU/l in 20 patients. Eighteen patients underwent liver biopsy, of whom four had a fibrosis score of F0, six a score of F1, five a score of F2, and three a score of F3 (Table 1).

rIFN alfa-2a was initially administered at a dose of 9MU per day for three consecutive days and 18MU per day for the subsequent 25 days (total dose, 477MU). Excluded were patients who had received an antiviral agent or immunomodulator within 3 months before the study; those who had received an injectable agent containing glycyrrhizin/cysteine/glycine or shosaiko-to (Chinese herbal medicine) within 1 month before the study; and those with a white blood cell (WBC) count of less than 3000/mm^3 or a platelet count of less than 100,000/mm^3.

The virological tests performed were the total amount of HBV DNA, using a bDNA probe assay (Quantiplex, Chiron) and competitive polymerase chain reaction assay (nested-PCR, Otsuka Assay), and the HBV precore mutant levels, using a quantitative mutation-site specific polymerase chain reaction assay (PCR-MSSA, Otsuka Assay). Using PCR-MSSA assay, precore point mutation (G–A, 83rd base of precore region) was examined using a mutation-trapped oligonucleotide primer, which yields a polymerase chain reaction amplification product only with precore mutantsand within the detection limits of 10^2 to 10^9 copies/ml [4]. Each measurement was performed immediately before treatment initiation, at treatment completion, and 6 months after treatment completion. HBeAg and HBeAb levels were measured immediately before treatment initiation, at treatment completion, and 3, 6, and 12 months after treatment completion. They were measured by radioimmunoassay (RIA), and a cutoff index higher than 2.1 for HBeAg was judged to be positive, and an inhibition percent higher than 50 for HBeAb was judged to be positive. Liver histology findings were assessed according to the Knodell histologic activity index [5] and the Desment scoring system [6].

The efficacy of the treatment was evaluated at its completion and at 6 and 12 months after completion according to ALT normalization and loss of HBeAg and HBV DNA.

The statistical analysis was performed using Fisher's exact test and the Wilcoxon 2-sample test.

3. Results

3.1. Efficacy

The rate of patients with normalized ALT levels was 4.3% (1/23) at treatment completion, 39.1% (9/23) at 6 months after completion, and 36.8% (7/19) at 12 months after completion. Although all measurement rates changed during the follow-up, there were many cases with normalized ALT levels after the treatment completion. Of the patients who were positive for HBeAg at baseline, the rate of patients who

Table 2
The rate of biochemical and virological response

	At treatment completion	3 months after treatment completion	6 months after treatment completion	12 months after treatment completion
ALT normalized	4.3%	34.8%	39.1%	36.8%
	1/23	8/23	9/23	7/19
HBeAg lost[a]	38.9%	29.4%	31.3%	50.0%
	7/18	5/17	5/16	7/14
HBV DNA cleared[b]	47.6%	40.0%	42.1%	41.2%
	10/21	8/20	8/19	7/17

Reduction of the number of patients during the follow-up is caused by without patient's consent.
[a] Five patients were excluded because negative at study initiation.
[b] Two patients were excluded because undetectable at study initiation.

became negative for HBeAg was 38.9% (7/18) at treatment completion, 31.3% (5/16) at 6 months after completion, and 50.0% (7/14) at 12 months after completion. Thus, the highest negative rate was at 12 months after the treatment completion. The rate of patients who became undetectable for HBV DNA (bDNA probe assay) was 47.6% (10/21) at treatment completion, 42.1% (8/19) at 6 months after completion, and 41.2% (7/17) at 12 months after completion. A rate of more than 40% undetectable was maintained after the treatment completion. The inability to obtain consent resulted in a reduction in the number of patients followed (Table 2).

3.2. *Efficacy according to patient baseline characteristics*

Examination of baseline patient characteristics, ALT normalization, and loss of HBeAg and HBV DNA at 6 months after treatment completion revealed a trend toward greater efficacy with respect to the rate of ALT normalization and

Table 3
Efficacy according to baseline characteristics of patients

Features			6 Months after treatment completion		
		n	ALT normalized ($n = 23$)	HBeAg lost[a] ($n = 16$)	HBV DNA cleared[b] ($n = 19$)
Sex	Male	16	25.0% (4/16)	20.0% (2/10)	38.5% (5/13)
	Female	7	71.4% (5/7)	50.0% (3/6)	50.0% (3/6)
Age	<40	15	40.0% (6/15)	25.0% (3/12)	38.5% (5/13)
	40≦	8	37.5% (3/8)	50.0% (2/4)	50.0% (3/6)
Prior IFN therapy	Yes	14	35.7% (5/14)	40.0% (4/10)	53.8% (7/13)
	No	9	44.4% (4/9)	16.7% (1/6)	16.7% (1/6)
Staging	F0,F1	10	50.0% (5/10)	28.6% (2/7)	25.0% (2/8)
	F2,F3	8	37.5% (3/8)	20.0% (1/5)	66.7% (4/6)
	Non-perform	5	20.0% (1/5)	50.0% (2/4)	40.0% (2/5)
ALT (IU/ml)	67≦	20	35.0% (7/20)	33.3% (5/15)	37.5% (6/16)
	34–66	3	66.7% (2/3)	0% (0/1)	66.7% (2/3)
HbeAg (index)	100–1000	11	27.3% (3/11)	22.2% (2/9)	33.3% (3/9)
	2.1–100	7	42.9% (3/7)	42.9% (3/7)	50.0% (3/6)
	<2.1	5	60.0% (3/5)	–	50.0% (2/4)
HBeAb (%)	50–100	8	62.5% (5/8)	50.0% (2/4)	42.9% (3/7)
	0–50	15	26.7% (4/15)	25.0% (3/12)	41.7% (5/12)
HBV DNA (Meq./ml)	100≦	11	27.3% (3/11)	25.0% (2/8)	33.3% (3/9)
	0.7–100	10	40.0% (4/10)	42.9% (3/7)	50.0% (5/10)
	<0.7	2	100% (2/2)	0% (0/1)	–
HBV DNA (copies/ml)	10^7–10^9	16	31.3% (5/16)	27.3% (3/11)	35.7% (5/14)
	10^2–10^7	7	57.1% (4/7)	40.0% (2/5)	60.0% (3/5)
	<10^2	0	–	–	–
Precore mutant (copies/ml)	10^7–10^9	12	41.7% (5/12)	37.5% (3/8)	45.5% (5/11)
	10^2–10^7	10	30.0% (3/10)	25.0% (2/8)	37.5% (3/8)
	<10^2	1	100% (1/1)	–	–

[a] Five patients were excluded because negative at study initiation.
[b] Two patients were excluded because undetectable at study initiation.

further report that high precore mutant concentrations correlate with a high total HBV DNA level in such patients, indicating that the inflammation associated with the hepatitis is severe, and acts to indirectly promote carcinogenesis. In addition, it is reported that a primary infection by HBV with a gene mutation contributes to the infection becoming fulminant and severe [22]. However, it is also reported that mutation of the precore region during the natural course of chronic hepatitis B is related to quiescence of the hepatitis and a decrease in the virus level [23].

The time required to reach the true endpoint of the present study, carcinogenesis or survival, makes it difficult to provide conclusions regarding whether the hepatic lesions in patients will progress or whether the hepatitis will become quiescent and the patients will become asymptomatic carriers. However, it is already evident that IFN treatment inhibits the growth of both the wild-type and precore mutant viruses seen in chronic hepatitis B and that it is also effective in patients who are positive for HBeAg and have a predominance of the wild-type virus.

References

[1] Greenberg HG, Pollard RB, Lutwick LI, Gregory PB, Robinson WS, Merigan TC. Effect of human leukocyte interferon on hepatitis B virus infection in patients with chronic active hepatitis. N Engl Med 1976;295:517–22.

[2] Lee WM. Hepatitis B virus infection. N Engl J Med 1997;337:1733–45.

[3] Hoofnagle JH. Therapy of viral hepatitis. Digestion 1998;59:563–78.

[4] Kinoshita M, Seno T, Fukui T, Shin S, Tsubota A, Kumada H. A detection method for point mutation in the precore region of human hepatitis B virus (HBV)-DNA using mutation-site-specific assay. Clin Chim Acta 1994;228:83–90.

[5] Knodell RG, Ishak KG, Black WC, Chen TS, Craig R, Kaplowitz N, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology 1981;1:431–5.

[6] Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology 1994;19:1513–20.

[7] Hadziyannis SJ, Papatheodoridis GV, Dimou E, Laras A, Papaioannou C. Efficacy of long-term lamivudine monotherapy in patients with hepatitis B e antigen-negative chronic hepatitis B. Hepatology 2000;32:847–51.

[8] Someya T, Suzuki Y, Arase Y, et al. Interferon therapy for flare-up of hepatitis B virus infection after emergence of lamivudine-induced YMDD motif mutant. J Gastroenterol 2001;36:139–41.

[9] Mutimer D, Dowling D, Cane P, Ratcliffe D, Tang H, O'Donnell K, et al. Additive antiviral effects of lamivudine and alpha-int0erferon in chronic hepatitis B infection. Antivir Ther 2000;5:273–7.

[10] Matsumura N, Yoshikawa O, Kondo M, Kawakami H, Kishida T. Therapeutic effect of a low dosage of human leukocyte interferon on chronic hepatitis B virus interferon. Digestion 1983;26:205–12.

[11] Niederau C, Heintges T, Lange S, Goldmann G, Niederau CM, Mohr L, et al. Long-term follow-up of HBeAg-positive patients treated with interferon alfa for chronic hepatitis B. N Engl J Med 1996;334:1422–7.

[12] Lin SM, Sheen IS, Chien RN, Chu CM, Liaw YF. Long-term beneficial effect of interferon therapy in patients with chronic hepatitis B virus infection. Hepatology 1999;29:971–5.

[13] Perrillo R, Tamburro C, Regenstein F, Balart L, Bodenheimer H, Silva M, et al. Low-dose, titratable interferon alfa in decompensated liver disease caused by chronic infection with hepatitis B virus. Gastroenterology 1995;109:908–16.

[14] Brunetto MR, Giarin M, Saracco G, Oliveri F, Calvo P, Capra G, et al. Hepatitis B virus unable to secrete e antigen and response to interferon in chronic hepatitis B. Gastroenterology 1993;105:845–50.

[15] Fattovich G, McIntyre G, Thursz M, Colman K, Giuliano G, Alberti A, et al. Hepatitis B virus precore/core variation and interferon therapy. Hepatology 1995;22:1355–62.

[16] Muraoka H, Sanefuji T, Keida R, Tsuji R, Abe H, Uchimura Y, et al. A case of acute exacerbation of chronic hepatitis B accompanied by antibody to HBeAg with remission of liver damage after long-term treatment with interferon. Kurume Med J 1995;42:307–11.

[17] Aikawa T, Kanai K, Kako M, Kawasaki T, Hino K, Iwabuchi S, et al. Interferon-alpha 2a for chronic hepatitis B with e antigen or antibody: comparable antiviral effects on wild-type virus and precore mutant. J Viral Hepat 1995;2:243–50.

[18] Kako M, Kanai K, Aikawa T, Iwabuchi S, Takehira Y, Kawasaki T, et al. Response to interferon-alpha 2a in patients with e antigen-negative chronic hepatitis B. J Clin Gastroenterol 1997;25:440–5.

[19] Shindo M, Okuno T. Genomic variations in precore and cytotoxic T lymphocyte regions in chronic hepatitis B in relationship to interferon responsiveness. Liver 2000;20:136–42.

[20] Okamoto H, Yotsumoto S, Akahane Y, Yamanaka T, Miyazaki Y, Sugai Y, et al. Hepatitis B viruses with precore region defects prevail in persistently infected hosts along with seroconversion to the antibody against e antigen. J Virol 1990;64:1298–303.

[21] Murashima N, Arase Y, Chayama K, Ikeda K, Kumada H, Saitoh S, et al. Relationship of hepatocellular carcinogenesis with precore mutant virus and serum hepatitis B virus DNA concentration. A longitudinal analysis of patients with cirrhosis. Hepatol Res 1998;10:142–55.

[22] Aritomi T, Yatsuhashi H, Fujino T, Yamasaki K, Inoue O, Koga M, et al. Association of mutations in the core promoter and precore region of hepatitis virus with fulminant and severe acute hepatitis in Japan. J Gastroenterol Hepatol 1998;13:1125–32.

[23] Karino Y, Toyota J, Sato T, Ohmura T, Yamazaki K, Suga T, et al. Early mutation of precore (A1896) region prior to core promoter region mutation leads to decrease of HBV replication and remission of hepatic inflammation. Dig Dis Sci 2000;45:2207–13.