

Figure 3 Survival based on difference of age after HBsAg seroclearance.

Histologic Changes of Liver after HBsAg Seroclearance

Thirteen patients with normal alanine aminotransferase levels after HBsAg seroclearance were examined for histologic changes of the liver before and after HBsAg seroclearance (Table 3). The median age of HBsAg seroclearance for the 13 patients with assessable liver biopsy was 45 years (range 24-61 years). The median interval between liver biopsies before and after HBsAg seroclearance was 101.6 months (range 16.0-207.7 months). Moreover, the median interval between HBsAg seroclearance and liver biopsies after HBsAg seroclearance was 18.9 months (range 2.3-69.8 months). The histologic changes of liver biopsies before and after HBsAg seroclearance are listed in Table 3. All patients showed marked improvement of necroinflammation of the liver, but only 2 of the 13 patients showed no liver fibrosis.

Cirrhosis-related Complications and Hepatocellular Carcinoma Appearance Rates

Liver cirrhosis and hepatocellular carcinoma did not develop in any of the 164 patients without evidence of liver cirrhosis at the time of HBsAg seroclearance. Cumulative hepatocellular carcinoma appearance rates are shown in Figure 2 by the Kaplan-Meier method. Two patients with liver cirrhosis at the time of HBsAg seroclearance had an occurrence of hepatocellular carcinoma at 5 and 5.8 years after seroclearance of HBsAg. Cumulative hepatocellular carcinoma appearance rates were 2.5% in the 5th year and 5.5% in the 10th year in the liver cirrhosis group. Pathologic confirmation using fine-needle biopsy or surgically resected specimens showed hepatocellular carcinoma. On the other hand, none of the patients without liver cirrhosis had hepatocellular carcinoma. Table 4 shows recent studies on the outcomes after HBsAg sero-

Table 5 Predictive factors for death after HBsAg seroclearance

Factor	Category	Odds ratio	95% CI	P value
Age (years)	<60/≥60	1/3.42	1.24-9.45	.018
US	Non-LC/LC	1/2.12	0.80-6.12	.127
Sex	Female/Male	1/1.34	0.42-4.21	.620
Platelet ($\times 10^4/\text{mm}^3$)	>20/≤20	1/1.33	0.357-4.97	.670
AST (IU/L)	≥38/<38	1/0.437	0.19-12.40	.680
Total protein (g/dl)	<8/≥8	1/0.657	0.082-5.26	.693
ALT (IU/L)	≥50/<50	1/0.420	0.19-12.18	.693
HBV-genotype	B/C	1/1.03	0.20-5.27	.971

ALT = alanine aminotransferase; AST = aspartate aminotransferase; CI = confidence interval; Hb = hemoglobin; HBV = hepatitis B virus; US = ultrasonography

clearance. These studies indicated that patients with hepatitis C virus have a high tendency for hepatocellular carcinoma.

Survival

Survivals are shown in Figure 3. During the observation period, 15 patients died of various causes; 8 died of malignant tumors (3 with gastric carcinomas, 2 with malignant lymphomas, 1 with tongue carcinoma, 1 with prostatic carcinoma, and 1 with ovarian carcinoma); 2 died of pneumonia; 3 died of heart failure; and 2 died of cerebral infarction. No patients died of liver failure or hepatocellular carcinoma. Hepatic decompensation did not develop in any patients after seroclearance of HBsAg. A Cox proportional hazards model was used to analyze the factors contributing to their survival: factors examined included age, gender, histologic findings, HBV genotype, and interferon administration. By Cox regression analysis, the relative risk of death incidence in patients aged less than 60 years was 3.58 compared with that of patients aged 60 years or more. Survival time was longer in patients aged less than 60 years ($P = .017$) (Table 5).

DISCUSSION

The results of this study indicate that patients with HBsAg clearance have a good prognosis. None of the patients with liver cirrhosis who had lost serum HBsAg progressed to decompensated liver cirrhosis. Moreover, no patients without liver cirrhosis with HBsAg clearance progressed to liver cirrhosis and/or hepatocellular carcinoma. Our findings agree with the published data by Chen et al¹⁶ (Table 4). However, 2 of 67 patients with liver cirrhosis at the time of HBsAg clearance had hepatocellular carcinoma during follow-up, as Huo et al¹⁵ showed. Fortunately, these 2 patients could be treated with radical resection.

Chen et al¹⁶ reported that the prognosis after spontaneous HBsAg seroclearance is excellent, except in patients with liver cirrhosis or concurrent hepatitis virus infection. On the other hand, Huo et al¹⁵ reported that adverse events were not rare in patients with chronic HBV infection even after HBsAg clearance. The discrepancy among these studies may be attributable to differences in the backgrounds of the patients who were followed up. These discrepancies might depend on concurrent hepatitis, severity of liver disease, ages, and other factors. For example, chronic hepatitis C virus infection is considered to be one of the major causes of hepatocellular carcinoma in many countries, and we suggest a role for hepatitis C virus in the origin of hepatocellular carcinoma in the patients with HBsAg clearance. Patients with hepatitis C virus-RNA after HBsAg seroclearance tend to have occurrences of hepatocellular carcinoma frequently when compared with patients without hepatitis C virus-RNA. Moreover, most asymptomatic carriers with seroclearance of HBsAg have a tendency not to consult a doctor. On the other hand, generally,

symptomatic carriers after seroclearance of HBsAg tend to consult a doctor and are followed up. Thus, hepatocellular carcinoma development rates might be high in clinical institutions with a high rate of symptomatic carriers.

In the present study, we assessed the prolonged prognosis in a large number of Japanese patients with HBsAg seroclearance. This article excludes the patients with concurrent hepatitis virus infection. Moreover, most asymptomatic carriers with HBsAg seroclearance and normalization of alanine aminotransferase could be followed up and were included for analysis. This was because most patients in our hospital were civil servants and were frequently examined with liver function tests.

Most patients had a good prognosis after HBsAg clearance, and thus good survival. Moreover, there was no significant difference in survival between the liver cirrhosis and non-liver cirrhosis groups. There was no significant difference in survival between those with HBV genotype B and those with HBV genotype C. Thus, most patients with HBsAg seroclearance also showed clinical improvement and prolonged survival. In fact, all 15 patients died of causes unrelated to liver cirrhosis and/or hepatocellular carcinoma. The Cox proportional hazard model indicated that only age was associated with survival.

Next, it is important to decide how long the patients with seroclearance of HBsAg should be followed up. Our present findings showed the following: (1) The patients with liver cirrhosis at the time of HBsAg seroclearance have a possibility of hepatocellular carcinoma appearance. (2) No patient without liver cirrhosis at seroclearance had hepatocellular carcinoma. Thus, considering cost-effectiveness, it seems reasonable to increase the interval of follow-up after HBsAg seroclearance for patients without liver cirrhosis with chronic HBV infection alone. However, patients with liver cirrhosis should be carefully followed up.

In regard to histologic changes after marked reduction of HBV, most patients showed improvement in liver histology.^{10,22-24} However, liver fibrosis resolves significantly less frequently and less quickly than necroinflammation. This may be because slight liver fibrosis can be reduced for a long period, but advanced liver fibrosis cannot be reduced sufficiently.

In regard to the appearance of anti-hepatitis Bs during follow-up, patients treated with interferon and/or CS showed the high cumulative appearance of anti-hepatitis Bs by log-rank test. This result is consistent with other studies that suggest immunomodulation of interferon^{25,26} and CS.^{27,28} The authors concluded that interferon and CS might stimulate the production of anti-HBs in vivo.

CONCLUSION

The prognosis after HBsAg clearance was excellent except in patients with liver cirrhosis. However, patients

with liver cirrhosis should be closely monitored for predictable complications.

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Anticarcinogenic Impact of Interferon on Patients with Chronic Hepatitis C: A Large-Scale Long-Term Study in a Single Center

Kenji Ikeda Yasuji Arase Satoshi Saitoh Masahiro Kobayashi
Takashi Someya Tetsuya Hosaka Hitomi Sezaki Norio Akuta
Fumitaka Suzuki Yoshiyuki Suzuki Hiromitsu Kumada

Department of Gastroenterology, Toranomon Hospital, and Okinaka Memorial Institute for Medical Research, Tokyo, Japan

Key Words

Cirrhosis · Fibrosis · Hepatitis C virus · Hepatocellular carcinoma · Interferon

Abstract

Background: The anticarcinogenic capacity of interferon (IFN) was assessed in a cohort of Japanese patients with chronic hepatitis C en masse. **Patients and Methods:** The rate of hepatocarcinogenesis was analyzed in 2,166 patients with chronic hepatitis C, of whom 1,654 had received IFN therapy while 512 had not. **Results:** Crude rates of hepatocarcinogenesis in treated and untreated patients were 2.6 and 4.6% at the end of the 5th year, 5.8 and 12.7% at the 10th year and 13.9 and 23.9% at the 15th year (after completion of IFN therapy for those treated) ($p < 0.001$). IFN decreased the hazard ratio of carcinogenesis to 0.42 ($p < 0.001$) in multivariate analysis with adjustments for significant covariates including fibrotic stage, γ -glutamyl transpeptidase level, gender, platelet count and age. Among the 1,654 patients treated with IFN, 606 (36.6%) achieved persistent loss of hepatitis C virus (HCV) RNA and an additional 266 (16.1%) gained normal levels of alanine aminotransferase without loss of HCV RNA for 6 months or longer after the completion of IFN therapy. Cumulative rates of hepatocarcinogenesis in sustained virological responders and biochemical responders were 1.4 and 2.0% at the end of the 5th year,

1.9 and 3.6% at the 10th year and 1.9 and 7.5% at the 15th year, respectively. The hazard ratio of sustained virological response was 0.10 ($p < 0.001$), and that of biochemical response was 0.12 ($p < 0.001$). Normalization of aminotransferase levels after IFN therapy without loss of serum HCV RNA decreased hepatocarcinogenesis. **Conclusion:** IFN significantly decreased the rate of hepatocarcinogenesis in patients with chronic hepatitis C as a whole in Japan, even in those who fail to clear HCV RNA from serum.

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Introduction

In most developed countries, hepatitis B virus (HBV) and hepatitis C virus (HCV) infections account for the great majority of hepatocellular carcinoma (HCC), with incidence rates dependent on the regional prevalence of these hepatitis viruses. HCV-associated HCC typically develops through a sequence of events that progress from chronic inflammation through fibrosis and cirrhosis accompanying dysplasia and ultimately to HCC. In our previous cohort study on Japanese patients with HCV-related cirrhosis [1], cumulative rates of developing HCC at 5, 10 and 15 years were 21.5, 53.2 and 75.2%, respectively. According to our observations of untreated patients with chronic hepatitis C [2], rates of hepatocarcino-

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Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

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Kenji Ikeda, MD
Department of Gastroenterology, Toranomon Hospital
Toranomon 2-2-2, Minato-ku
Tokyo 105-8470 (Japan)
Tel. +81 44 877 5111, Fax +81 44 860 1623, E-Mail ikedakenji@tora.email.ne.jp

genesis at 5, 10 and 15 years were estimated to be 4.8, 13.6 and 26.0%, respectively. The life expectancy of patients with HCV-related cirrhosis is largely influenced by the development of HCC in the clinical course. As the efficacy of radically curative therapies for HCC remains limited at best, and since a severe organ shortage does not provide with sufficient chances for liver transplantation, the prevention of HCC in patients with chronic liver disease is of great importance at the present.

Interferon (IFN) is effective in eliminating HCV and reducing serum levels of alanine aminotransferase (ALT) in some patients with chronic hepatitis C [3–6]. Reduced incidence of HCC in HCV-associated cirrhosis by IFN has been reported by many investigators including ourselves [7–14]; only a few studies have failed to find its benefit [15, 16]. However, many published studies had shortcomings in the study design, in terms of pooling patients who received IFN in diverse regimens, relatively short periods of follow-up despite a long incubation period of HCC, large numbers of dropouts and retrospective studies with historical controls. Moreover, almost all studies evaluated the activity of IFN to prevent HCC by comparing responders and nonresponders to the treatment. Due to difficulties in studying patients with chronic hepatitis C, a number of nonrandomized studies examined the effect of IFN on the incidence of hepatocarcinogenesis [17–20]. With invariable limitations in study design and interpretation of the results, these studies have disclosed useful information as regards the treatment of patients with chronic HCV infection.

In order to evaluate whether IFN can reduce the rate of carcinogenesis in patients with chronic hepatitis C, we compared 1,654 patients with IFN therapy with 512 patients without treatment in a single clinical center, who were adjusted for background features by the multivariate analysis. Therefore, the principal aims of our study were to show the role of IFN in preventing HCC in chronic hepatitis type C en masse and to establish the extent to which IFN decreases the rate of carcinogenesis as a sequel to chronic hepatitis C in a society.

Patients and Methods

Study Population

A total of 2,166 patients with chronic hepatitis were examined, whose initial sera tested negative for hepatitis B surface antigen by radioimmunoassay (Ausria, Dainabot, Tokyo, Japan) and positive for anti-HCV by the second-generation enzyme-linked immunosorbent assay (Dainabot); anti-HCV was tested in sera that had been stored frozen at -80°C . They included 1,421 men and 745

women aged 14–78 with a median of 50 years. They were all diagnosed with chronic hepatitis by liver biopsy with or without peritoneoscopy between 1970 and 2000 at the Department of Gastroenterology in Toranomon Hospital, Tokyo, Japan. Patients who had possibly developed HCC already at the time of diagnosis of hepatitis were strictly excluded from the study. In order to exclusively investigate hepatocarcinogenesis in HCV-related cirrhosis, patients coinfecting with HBV were excluded.

Among the 2,166 patients with HCV-related hepatitis, 1,654 (76.4%) received IFN therapy, mostly since 1987 when IFN was available in Japan; new antivirals or anticarcinogenic treatments of viral cirrhosis, except for IFN, were not introduced in 1987 or thereafter in Japan. The remaining 512 patients did not receive IFN or any other antiviral therapies. This is a retrospective cohort study with historical controls composed of patients before 1987 and those who refused or could not receive IFN for various reasons since 1987.

Background and Laboratory Findings

Table 1 shows demographic profiles and laboratory data for the 1,654 patients treated with IFN and the 512 without receiving IFN since they were diagnosed with chronic hepatitis. There were more males, with a median age 3 years lower in treated than in nontreated patients. There were 299 treated patients (18.1%) with a history of alcohol intake ≥ 500 kg until the diagnosis of chronic hepatitis (corresponding to daily consumption of 3,000 ml of beer or 300 ml of whiskey for 20 years) and 113 (22.1%) untreated patients ($p < 0.001$). Because IFN was introduced to our hospital in 1987, the observation period was significantly shorter in the treated than in untreated patients (median 10.4 vs. 12.3 years; $p < 0.0001$).

Although all patients tested positive for HCV RNA during their clinical courses, tests for the concentration of HCV RNA in the initial serum was possible in 1,863 (86.5%) patients. HCV genotypes were analyzed by the serological typing method with a commercial kit (Kokusai Diagnostic Corporation, Kobe, Japan) in which the serological group 1 represented genotypes 1a and 1b, and group 2 stood for 2a and 2b genotypes. HCV in the serological group 2 was significantly more frequent in patients with IFN treatment than in those without. Concentration of HCV RNA was determined in the initial sera from 1,873 (86.5%) patients by the competitive polymerase chain reaction (PCR) method with the HCV probe assay (Chiron Corp., Emeryville, Calif., USA) or by PCR with Amplicor HCV Monitor kits (Roche Diagnostics Japan Co., Tokyo, Japan). High concentration of HCV ($\geq 10^6$ copies/ml by the competitive PCR or $\geq 10^6$ equivalents/ml by the HCV probe assay) was significantly more frequent in untreated than in treated patients ($p < 0.0001$). The stage of hepatic fibrosis was not different between the two groups.

Interferon Treatment and Judgment of the Effect

A total of 1,654 patients underwent IFN therapy in one or more treatment courses: 1,358 patients (82.1%) received IFN once, 240 patients (14.5%) twice, and the remaining 56 patients (3.4%) three times or more. Initial treatment was performed with natural or recombinant IFN- α ($n = 1,238$), natural IFN- β ($n = 386$) or both ($n = 30$). Regimens of IFN were variable: 926 (56.0%) patients received IFN 6–9 million units (MU) daily for 8 weeks, followed by 2 or 3 times per week for 16 weeks; 329 (20.0%) received IFN 6–9 MU daily for 2–4 weeks, followed by 3 times per week for 20–22 weeks; 185 (11.2%) underwent a short-course therapy with IFN

Table 1. Patient profiles and laboratory data at the diagnosis of chronic hepatitis

Factors	Interferon therapy		p value
	yes (n = 1,654)	no (n = 512)	
Male	1,110 (67.1%)	311 (60.7%)	0.024
Age, years	50 (16–72)	53 (21–78)	<0.001
History of transfusion	607 (36.7%)	229 (44.7%)	0.001
Family member with liver disease	426 (25.8%)	140 (27.3%)	0.47
Alcohol intake \geq 500 kg	299 (18.1%)	113 (22.1%)	0.044
Observation period, year	10.4 (0.1–33.6)	12.3 (0.1–33.6)	<0.001
Laboratory data			
ALT, IU/l	63 (4–1,266)	67 (4–704)	0.098
AST, IU/l	106 (9–1,660)	96 (12–832)	0.0001
γ -GTP, IU/ml	62 (6–1,118)	70 (3–850)	0.39
Platelet counts, $\times 1,000/\text{mm}^3$	169 (27–433)	165 (35–560)	0.091
ICG R ₁₅ , %	14 (1–90)	16 (1–95)	0.003
AFP, ng/ml	4 (1–90)	5 (1–1,180)	0.42
HCV serological group			
Group 1, genotypes 1a/1b	1,021 (66.1%)	259 (81.4%)	<0.0001
Group 2, genotypes 2a/2b	488 (31.6%)	48 (15.1%)	
Undetermined	36 (2.3%)	11 (3.5%)	
HCV RNA concentration			
High ^a	937 (58.4%)	191 (71.3%)	<0.0001
Low ^b	668 (41.6%)	77 (28.7%)	
Histological stage of hepatitis			
F1, slight fibrosis	1,029 (62.2%)	298 (58.2%)	0.10
F2/F3, moderate/severe fibrosis	625 (37.8%)	214 (41.6%)	

AST = Aspartate aminotransferase; AFP = α -fetoprotein; ICG R₁₅ = retention of indocyanine green at 15 min.

^a HCV RNA concentration $\geq 10^6$ copies/ml by the competitive PCR or $\geq 10^6$ equivalents/ml by the HCV probe assay.

^b HCV RNA concentrations less than high concentrations.

daily for 4–8 weeks; 128 (7.7%) were administered with intermittent IFN 3 times per week for 24 weeks; 72 (4.4%) had a prolonged course of IFN for 8–36 months; 8 (0.5%) received IFN- β 6 MU daily for 6–18 months, and the remaining 6 (0.4%) were given IFN- α combined with IFN- β for 4 months. The median dose of 624 MU was administered during the median period of 24 weeks. IFN for 24 weeks or longer was given to 83.2% of the patients. IFN therapy was usually initiated within a few months after the diagnosis of chronic hepatitis, and all patients were started on it within 12 months. The median interval between liver biopsy and initiation of IFN was 9 days.

Almost all the patients given IFN showed varied degrees of fever, chills, myalgia, headache and general malaise after the first injection. Most patients developed leukocytopenia and thrombocytopenia in various degrees. A significant thrombocytopenia $\leq 40,000/\text{mm}^3$ required a reduction of the IFN dose in 39 patients. IFN therapy was discontinued due to psychosis in 35 patients and ophthalmological symptoms in 12 patients. None of the patients developed decompensated liver disease with ascites, encephalopathy, jaundice or variceal bleeding. Although only 88 (5.3%) patients could not continue injection with IFN, studies for carcinogenesis were analyzed on the intention-to-treat basis.

The efficacy of IFN was judged by the clearance of HCV RNA from serum and ALT levels 12 months after the completion of treatment. Sustained virological response (SVR) was defined as persistent disappearance of HCV RNA after therapy, biochemical response (BR) as normal ALT levels without elimination of HCV RNA for at least 6 months after therapy, and no response (NR) as persistently elevated or transiently normalized ALT levels without loss of HCV RNA lasting for less than 6 months.

Follow-Up of Patients and Diagnosis of HCC

Patients were followed up monthly after diagnosis of chronic hepatitis in our outpatient clinic and monitored for hematological, biochemical and virological parameters. With their admission, during and after the treatment with IFN, weekly or biweekly follow-up was performed in almost all patients who received IFN. Imaging diagnosis was made once or twice per year in the majority of patients with ultrasonography or computed tomography. Angiography was performed only when HCC was highly suspected on imaging by ultrasonography or computed tomography.

When angiography pictured a characteristic hypervascular nodule specific for HCC in patients, histological confirmation was not required in the majority of them. Microscopic examinations of liv-

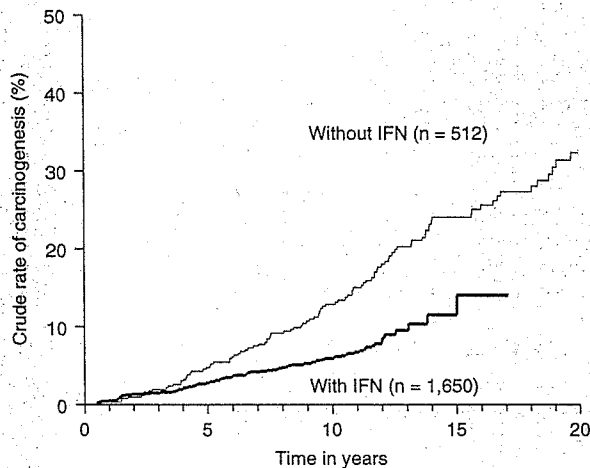


Fig. 1. Crude rates of hepatocarcinogenesis in patients treated with IFN and those untreated. The carcinogenesis rate was significantly lower in treated than in untreated patients (log-rank test, $p < 0.0001$).

er tissues obtained by a fine-needle biopsy were performed in 14 patients whose angiogram could not portray a typical image of HCC. There were 89 patients in whom HCC was confirmed histologically on liver specimens obtained at surgery or autopsy. Detection of serological tumor markers and increase with time were also taken into account in the diagnosis of HCC.

There were 223 (10.3%) patients lost to follow-up, including 164 (9.9%) treated and 59 (11.5%) untreated. Rates of annual dropouts in treated and untreated patients were 0.95 and 0.93%, respectively. In 9 patients, the response to IFN was judged by information on aminotransferase levels determined in other clinics and by persistent HCV RNA, as well as aminotransferase levels at 6 months after the completion of therapy in an additional 3 patients. Therefore, the response to IFN could be judged in all patients including the 12 who were lost to our follow-up early. Since the eventual outcome with respect to the development of HCC was not confirmed in these patients, their data were censored in statistical analyses [21]. Deaths unrelated to liver disease were censored and withdrawn from the analysis. The date of the last follow-up in this study was May 1, 2004, and the median observation period of studied patients was 10.7 years, with a range of 0.1–33.6 years.

Statistical Analysis

Nonparametric Mann-Whitney U test and χ^2 test were used for analysis of background characteristics of patients. The rate of HCC development was calculated by the Kaplan-Meier method [22]; it was based on the duration between diagnosis of chronic hepatitis by liver biopsy and detection of HCC. Differences in slopes of carcinogenesis curves were evaluated by the log-rank test. To gain a robust statistical power for the anticarcinogenic activity of IFN, observation of treated patients was initiated at the commencement of IFN therapy, in lieu of the diagnosis of chronic hepatitis. Independent factors associated with the development of HCC were studied using the stepwise Cox regression analysis [23]. The follow-

ing 18 variables were analyzed for potential covariates in hepatocarcinogenesis at the time when hepatitis was diagnosed: age, sex, total alcohol intake, family history of liver disease, history of blood transfusion, stage of hepatic fibrosis, aspartic aminotransferase, ALT, albumin, bilirubin, globulin, γ -glutamyl transpeptidase (γ -GTP), platelet count, retention of indocyanine green at 15 min, serological grouping of HCV, HCV RNA level and IFN treatment.

Although continuous variables without conversion of data were evaluated in multivariate analyses, several variables were transformed into categorical data consisting of two or three ordinal numbers in calculating hazard ratios. All factors found to be marginally associated with hepatocarcinogenesis with p values < 0.15 were tested by the multivariate Cox proportional hazard model. All analyses of data were performed with the computer program SPSS version 11 [24], and a p value < 0.05 was considered significant.

Results

Response to IFN

Response to IFN was judged 12 months after the completion of therapy by both HCV RNA and serial ALT readings. Among the 1,654 patients with IFN treatment, SVR (elimination of HCV RNA) was achieved by 606 (36.6%), BR (ALT normalized for at least 6 months without clearance of HCV RNA from serum) in 266 (16.1%) and NR (elevated or transiently decreased ALT levels without loss of serum HCV RNA) in 782 (47.3%).

Crude Rates of Hepatocarcinogenesis

During the median observation period of 10.7 years, HCC developed in 199 of the 2,166 (9.2%) patients, including 96 of the 1,654 (5.8%) patients treated with IFN and 103 of the 512 (20.1%) patients without IFN (fig. 1). Among the 199 patients with HCC, 140 (70.4%) imaged a typical hypervascular stain on angiography and dynamic computed tomography, while 59 failed to exhibit tumor stains on angiography. HCC in these 59 patients was confirmed histologically on liver specimens obtained at surgery or by fine-needle biopsy.

Crude rates of hepatocarcinogenesis in patients treated with IFN and those untreated were 1.3 and 1.8% at the end of the 3rd year (after the completion of therapy), 2.6 and 4.6% at the end of the 5th year, 5.8 and 12.7% at the 10th year and 13.9 and 23.9% at the 15th year, respectively (fig. 1). The carcinogenesis rate was significantly lower in patients treated with IFN than in untreated patients (log-rank test, $p < 0.0001$).

Impact of IFN on Hepatocarcinogenesis

During the observation period, HCC developed in 96 of the 1,654 (5.8%) patients treated with IFN, including

11 patients (1.8%) with SVR, 10 (3.8%) with BR and 75 (9.6%) with NR to IFN. Rates of hepatocarcinogenesis in patients with SVR, BR and NR were 0.7, 0.8 and 2.0% at the end of the 3rd year, 1.4, 2.0 and 3.8% at the 5th year, 1.6, 2.9 and 6.5% at the 7th year, 1.9, 3.6 and 9.6% at the 10th year and 1.9, 7.5 and 27.6% at the end of 15th year (fig. 2). Hepatocarcinogenesis was significantly less frequent in patients with SVR or BR than in patients with NR and those untreated (log-rank test, $p < 0.0001$).

Factors Influencing Hepatocarcinogenesis

Univariate analysis identified 9 factors significantly associated with carcinogenesis. They were fibrotic stage ($p < 0.001$), age ($p < 0.001$), α -fetoprotein ($p < 0.001$), aspartic aminotransferase ($p = 0.001$), retention of indocyanine green at 15 min ($p = 0.002$), total alcohol intake ($p = 0.002$), γ -GTP ($p = 0.005$) and HCV serotype ($p = 0.045$). IFN therapy ($p = 0.064$), histological activity of hepatitis ($p = 0.069$) and ALT ($p = 0.70$) were marginally associated with carcinogenesis.

In order to prove the role of IFN on carcinogenesis in patients with chronic hepatitis type C en masse, multivariate analysis was performed by non-time-dependent proportional hazard analysis. Fibrotic stage, γ -GTP, gender, IFN therapy, platelet count and age independently influenced the development of HCC in the cohort (table 2). Advanced liver fibrosis in F2/F3 stages imposed a higher risk for carcinogenesis with a hazard ratio of 8.68, 95% confidence interval (CI) 5.08–14.81, compared with the F1 stage. Similarly, higher γ -GTP levels (hazard ratio 2.64), male sex (2.38), low platelet count (2.22) and older age (1.90) posed higher carcinogenesis risks. After adjusting background clinical biases between treated and untreated patients for the 5 significant covariates identified in the multivariate analysis, IFN therapy significantly decreased the hepatocarcinogenesis rate in the entire patients with chronic hepatitis C with a hazard ratio of 0.42 (95% CI 0.29–0.61) in comparison with untreated patients.

Based on the multivariate analysis, curves of carcinogenesis rates were theoretically illustrated in treated and untreated patients with the average histological stage, average γ -GTP value, average ratio of male to female, average platelet count and average age (fig. 3).

Hazard of Hepatocarcinogenesis Stratified by the Response to IFN

Since the carcinogenesis rate in patients with SVR or BR was significantly lower than that of patients with NR or untreated patients by the product limit method, a mul-

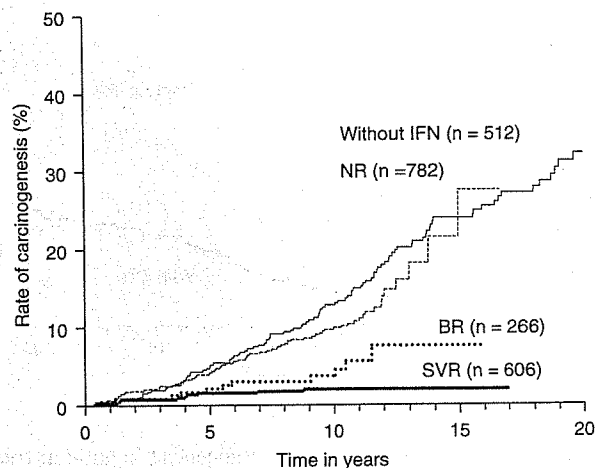


Fig. 2. Rates of hepatocarcinogenesis in patients with SVR, BR and NR to IFN. The rate in patients with NR (persistently elevated ALT or transiently normalized ALT for less than 6 months) was significantly higher than that in patients with SVR or BR.

Table 2. Factors associated with hepatocarcinogenesis in patients with chronic hepatitis C^a

Factors	HR	95% CI	p value
Fibrosis stage			
F1	1		
F2–F3	8.68	(5.08–14.81)	<0.001
γ -GTP, IU/ml			
<50	1		
≥ 50	2.64	(1.58–4.42)	<0.001
Gender			
Women	1		
Men	2.38	(1.56–3.70)	<0.001
IFN therapy			
No	1		
Yes	0.42	(0.29–0.61)	<0.001
Platelet count, $\times 10^3/\text{mm}^3$			
≥ 100	1		
<100	2.22	(1.47–3.44)	<0.001
Age, years			
<50	1		
≥ 50	1.90	(1.27–2.85)	0.002

HR = Hazard ratio.

^a Evaluated by the Cox proportional hazard analysis.

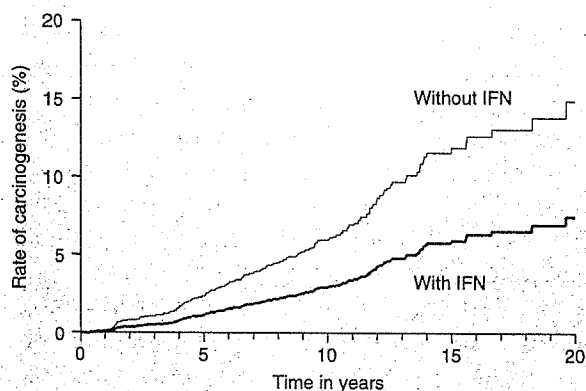


Fig. 3. Theoretical curves of hepatocarcinogenesis in patients treated with IFN and those untreated who have the average histological stage, average γ -GTP value, average ratio of male to female, average platelet count and average age. They are based on the analysis of 1,654 patients treated with IFN and 512 untreated patients.

Table 3. Factors associated with hepatocarcinogenesis in patients with chronic hepatitis C who had distinct responses to IFN therapy^a

Factors	HR	95% CI	p value
Fibrosis stage			
F1	1		
F2–F3	9.90	(4.19–23.40)	<0.001
Gender			
Women	1		
Men	3.44	(1.89–6.25)	<0.001
γ -GTP, IU/ml			
<50	1		
≥ 50	2.68	(1.30–5.54)	0.008
Age, years			
<50	1		
≥ 50	2.56	(1.50–4.38)	0.001
AFP, ng/ml			
<20	1		
≥ 20	2.32	(1.34–4.02)	0.003
Platelet count, $\times 10^3/\text{mm}^3$			
≥ 100	1		
<100	2.09	(1.14–3.75)	0.013
Response to IFN			
Without IFN	1		
NR	0.57	(0.13–2.56)	0.46
BR	0.12	(0.04–0.35)	<0.001
SVR	0.10	(0.03–0.30)	<0.001

HR = Hazard ratio; AFP = α -fetoprotein.

^a Evaluated by the Cox proportional hazard analysis.

tivariate analysis was performed taking into account the response to IFN. Hazard ratios of patients with SVR and BR to IFN therapy were 0.10 (95% CI 0.03–0.30, $p < 0.001$) and 0.12 (95% CI 0.04–0.35, $p < 0.001$), respectively, in comparison with that of untreated patients, when the other 5 factors served as significant covariates (table 3). The hazard ratio of NR at 0.57 (95% CI 0.13–2.56) was less than 1, but fell short of making a significant difference against untreated patients.

Mortality and Causes of Death

During the observation period, 116 of the 2,166 (5.4%) patients died, including 52 of the 1,654 (3.1%) subjects treated with IFN and 64 of the 512 (12.5%) subjects without IFN. Estimated survival rates in the treated and untreated patients were 99.3 and 98.3% at 5 years, 97.8 and 96.0% at 10 years and 93.8 and 86.9% at 15 years, respectively. The survival rate of treated patients was significantly higher than that of untreated patients (log-rank test, $p < 0.0001$).

Discussion

Based on our epidemiological data obtained by long-term observations of patients with chronic hepatitis [2] and patients with cirrhosis [1], the life expectancy of patients with HCV-related chronic liver disease heavily depends on the development of HCC. The possibility of eventually developing HCC in patients with HCV infection and cirrhosis is staggeringly high at 75% [1]. Theoretically, the treatment of chronic HCV infection with IFN can prevent the development of HCC. From the ethical point of view, a prospective randomized trial with control untreated patients is not to be allowed at present when IFN has become the standard radical therapy for chronic hepatitis C; everyone can receive IFN, as expenses are being covered for by the medical insurance in Japan. Another difficulty involves the informed consent in prospective randomized studies. It requires at least 5 years in order that IFN can decrease the incidence of carcinogenesis in chronic hepatitis C, with a statistical difference in the carcinogenesis rate between treated and 'untreated' patients. Since any randomized studies are considered extremely difficult in the future, we attempted to carry out this retrospective study by the multivariate analysis with statistical adjustments for possible covariates.

In the product limit analysis, IFN significantly decreased the crude rate of hepatocarcinogenesis in the

entire cohort of 2,166 patients with chronic hepatitis C. Since there were some background differences between treated and untreated patients, we tried to correct for biases including stage of fibrosis, γ -GTP value, sex, platelet count and age, which significantly affect the carcinogenesis rate. Demographic, histological and biochemical factors having been adjusted, IFN is proven to bring about a significant decrease in the hazard of carcinogenesis in patients with chronic hepatitis C en masse (hazard ratio 0.42, $p < 0.001$ by the non-time-dependent model). Taking into consideration that a significant number of patients without IFN had received anti-inflammatory medicines, which might have contributed to suppression of hepatocarcinogenesis, the actual anticarcinogenic activity of IFN may be higher than the observed. Having published results of a similar study on a cohort of 1,643 patients with a median observation period of 5.4 years in 1999 [18], we could not establish the anticarcinogenic activity of IFN because of a low risk of carcinogenesis in untreated patients (1.2% per year). Nevertheless, we expected a significant statistical difference if we could extend the median observation period to longer than 7 or 10 years in our studied patients. This has been realized in the present study, in which 2,166 patients with and without IFN therapy were observed for a median of more than 10 years. As far as we are aware, it represents the first study that has demonstrated preventive effects of IFN on the carcinogenesis rate in a large cohort of patients in a single center, in correlation with distinct responses to it, such as SVR, BR and NR.

Treatment of patients with chronic HCV infection using IFN- α and ribavirin has led to sustained loss of serum HCV RNA in 40–50% of recipients with HCV genotype 1 and 75–80% with HCV genotype 2 or 3. However, to date, the combination therapy with IFN- α and ribavirin has not been evaluated for its impact on the risk of developing HCC. Monotherapy with IFN- α achieves sustained clearance of serum HCV RNA in only 20–30% of patients; the impact of IFN- α on the development of HCC has been evaluated only in patients who had received IFN- α without ribavirin [17–20, 25–27].

Multivariate analysis definitively demonstrated that IFN lessens the carcinogenesis risk in the patients whose ALT levels decreased after therapy. Furthermore, the anticarcinogenic capacity of IFN was demonstrated not only in the patients with persistent aminotransferase normalization, but also in those with transient normalization of ALT for at least 6 or 12 months. Many authors have already described that the activity of IFN to suppress the

development of HCC in patients with HCV RNA clearance (SVR) is similar to that in patients with ALT normalization in the absence of eliminating HCV RNA (BR) [18, 25–27]. Based on these compelling lines of evidence, the anticarcinogenic activity of IFN is ascribed to the suppression of inflammatory and regenerative processes in hepatocytes. Moreno and Muriel [28] reported that IFN reverts liver fibrosis, and therefore, control of the necro-inflammatory process can suppress the growth of HCC. Tarao et al. [29] reported that high aminotransferase levels increase the rate of HCC recurrence in patients with cirrhosis. Our results stand in favor of the view that the carcinogenic process in patients with chronic hepatitis C would be enhanced by fluctuating as well as persistently elevated levels of aminotransferases. It does seem that IFN exerts suppressive effects on HCC through reduction or complete remission of inflammatory activity. Recently, a few authors reported that even transient disappearance of HCV RNA during IFN therapy contributed to a low carcinogenesis rate in the clinical course of hepatitis [17, 27]. The significance of transient HCV in decreasing hepatocarcinogenesis should be further explored and confirmed by multicenter clinical studies with rigorous virological assessments.

HCC developed in a few patients with SVR 5 years after the HCV infection had been terminated by IFN, along with normalized ALT levels. These patients would have developed minute HCC in their livers already while receiving IFN which escaped the detection by imaging modalities or screening for serological tumor markers. This would indicate the limitation of IFN in preventing HCC. IFN will not be able to suppress HCC once it has developed, even when it succeeds in eliminating HCV and suppressing necroinflammatory processes in the liver.

With many difficulties in vaccine development, the recent progress in treatment of chronic HCV infection, from IFN monotherapy to combination therapy with ribavirin, is very auspicious. SVR and BR can be achieved in up to 56% of patients with combined IFN and ribavirin [30]. There is evidence that a sustained virological response can lead to decrease in fibrosis and even reversal of cirrhosis [31]. Because HCV-associated HCC occurs almost exclusively in patients with cirrhosis, successful treatment for SVR in patients without cirrhosis is likely to prevent future development of HCC [32]. However, once cirrhosis has been established, a preventive benefit of IFN monotherapy is restricted to the patients who can achieve SVR or BR. In their meta-analysis of 3 randomized and 11 nonrandomized controlled trials, Camma et

al. [33] have reported a low but statistically significant preventive effect.

In conclusion, IFN significantly decreases the rate of hepatocarcinogenesis in patients with chronic hepatitis C, irrespective of the response to it.

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Virological Outcomes in Patients Infected Chronically With Hepatitis B Virus Genotype A in Comparison With Genotypes B and C

Mariko Kobayashi,¹ Norio Akuta,² Fumitaka Suzuki,² Yoshiyuki Suzuki,² Yasuji Arase,² Kenji Ikeda,² Tetsuya Hosaka,² Satoshi Saitoh,¹ Masahiro Kobayashi,^{2*} Takashi Someya,² Junko Sato,¹ Sachiyo Watabiki,¹ Yuzo Miyakawa,³ and Hiromitsu Kumada²

¹Research Institute for Hepatology, Toranomon Hospital, Tokyo, Japan

²Department of Gastroenterology, Toranomon Hospital, Tokyo, Japan

³Miyakawa Memorial Research Foundation, Tokyo, Japan

In a single hospital in Tokyo, the 87 patients infected persistently with hepatitis B virus (HBV) genotype A, the 413 with B, and the 3,389 with C were compared for virological outcome. Hepatitis B surface antigen (HBsAg) was cleared from the serum in 12% (3/26), 2% (2/112), and 3% (23/826) of patients with genotypes A, B, and C, respectively, at 5 years of follow-up ($P=0.0395$). Hepatitis B e antigen (HBeAg) was cleared from serum more frequently in patients with genotype B than those with A or C (78% [32/41] vs. 58% [11/19] or 45% [251/562], $P=0.00001$) at 5 years. Of the 45 individuals infected with genotype A and followed for 3 years or longer, HBeAg was more frequent (16% [3/19] vs. 73% [19/26], $P=0.0002$) and levels of HBV DNA higher (median <2.6 [range: <2.6 – 5.6] vs. >7.6 [<2.6 – >7.6] log copies/ml, $P=0.001$) in the 26 patients with biopsy-proven chronic hepatitis than the 19 asymptomatic carriers. Among the 26 hepatitis patients infected with HBV genotype A, decreases in HBV DNA were less frequent (20% [1/5] vs. 93% [13/14] or 86% [6/7], $P=0.0095$) and increases in serum levels of hyaluronic acid ≥ 10 ng/ml commoner (80% [4/5] vs. 14% [2/14] or 14% [1/7], $P=0.017$) in the patients who kept HBeAg than in those who seroconverted or who remained HBeAg-negative. In conclusion, patients persistently infected with HBV genotype A fare better than those with genotype B or C. However, high levels of HBV DNA continue in those in whom HBeAg persists along with fibrosis in the liver. **J. Med. Virol.** 78:60–67, 2006. © 2005 Wiley-Liss, Inc.

KEY WORDS: chronic hepatitis; cirrhosis; hepatitis B e antigen; hepatitis B surface antigen; hepatocellular carcinoma; sexual transmission

INTRODUCTION

There are an estimated 350 million people in the world who are persistently infected with hepatitis B virus (HBV), some of whom develop a spectrum of chronic liver disease ranging from chronic hepatitis through cirrhosis to hepatocellular carcinoma [Lee, 1997]. New HBV infections have been prevented by mass vaccination of neonates [Tsen et al., 1991; Chen et al., 1996] and immunoprophylaxis of babies born to mothers carrying HBV [Noto et al., 2003]. However, there are individuals who have been infected, and they need to be identified for receiving treatment as required. Clinical outcomes and the response to antiviral treatment are influenced by many host factors, such as ethnicity, gender, and the age at infection, as well as viral factors represented by HBV genotypes.

HBV has a partially double-stranded DNA genome of approximately 3,200 nucleotides (nt) [Tiollais et al., 1981]. Eight HBV genotypes have been classified by a sequence divergence in the entire genome exceeding 8% [Okamoto et al., 1988], and they are named by capital Alphabet letters from A to H [Okamoto et al., 1988; Norder et al., 1992; Stuyver et al., 2000; Arauz-Ruiz et al., 2002]. Recently, HBV genotypes have attracted an increasing attention because they influence the clinical outcome and treatment response in patients with chronic liver disease [Tsubota et al., 2001; Kao, 2002; Miyakawa and Mizokami, 2003; Schaefer, 2005; Yu et al., 2005]. Due to their uneven geographical

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*Correspondence to: Masahiro Kobayashi, BS, Research Institute for Hepatology, Toranomon Hospital, 1-3-1, Kajigaya, Takatsu-ku, Kawasaki City 213-8587, Japan.
E-mail: vj7m-kbys@asahi-net.or.jp

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distribution, however, only two HBV genotypes prevail in most countries; the United States is the only exception with seven (A–G) genotypes [Chu et al., 2003; Westland et al., 2003]. Thus, genotypes A and D are common in Europe and India, while genotypes B and C are frequent in Asia [Magnius and Norder, 1995; Miyakawa and Mizokami, 2003]. Therefore, comparison has been restricted between patients infected with genotypes A and D, as well as those with B and C [Zhang et al., 1996; Mayerat et al., 1999; Kao et al., 2000; Orito et al., 2001; Chu et al., 2002; Thakur et al., 2002].

During 31 years from 1973 to 2003, 4,121 patients visited Toranomon Hospital in the Metropolitan Tokyo, and HBV genotypes were determined in them. There were 128 patients with genotype A, of whom 87 were chronically infected with HBV at the presentation. They were followed along with the 413 patients chronically infected with genotype B and the 3,389 with genotype C for seroclearance of hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg). Furthermore, patients with genotype A were grouped by the presence or absence of HBeAg at the presentation, as well as seroconversion during the follow-up, and they were compared for virological and clinical outcomes.

MATERIALS AND METHODS

Patients Chronically Infected With HBV

During 31 years from April 1973 to December 2003, genotypes of HBV DNA were determined in 4,121 patients with HBsAg in the Department of Gastroenterology at Toranomon Hospital in the Metropolitan Tokyo. Genotypes were A in 128 (3.11%) patients, B in 431 (10.46%), C in 3,434 (83.32%), D in 4 (0.97%), E in 1 (0.02%), and F in 3 (0.07%); they were not classifiable in the remaining 120 (2.91%) patients.

Of the 128 patients infected with HBV genotype A, 41 (32%) presented with acute hepatitis B as diagnosed by high-titered IgM antibody to hepatitis B core antigen. The remaining 87 (68%) patients were chronically infected with HBV genotype A when they visited our hospital. Their diagnoses were asymptomatic carriers with persistently normal ALT levels in 38 (44%) and chronic hepatitis in 39 (45%). In addition, nine (10%) patients presented with cirrhosis and one (1%) with hepatocellular carcinoma. Chronic hepatitis was diagnosed by liver biopsies performed under laparoscopy, and liver cirrhosis by liver biopsy and/or ultrasonographic images plus laparoscopic findings. Hepatocellular carcinoma was diagnosed by imaging modalities, such as ultrasonography, computed tomography, and magnetic resonance imaging, and by liver biopsy if necessary.

The 87 patients infected chronically with HBV genotype A had the median age of 34 years (range: 11–67 years), included 72 (83%) men and were followed for the median of 5.0 years (0.1–22 years). Only two (2%) had a history of blood transfusion, and three (3%) were co-infected with hepatitis C virus. They had the median

serum HBV DNA level at 4.2 log copies/ml, and HBeAg was detected in sera from 32 (37%). Subgenotypes of A [Bowyer et al., 1997; Sugauchi et al., 2004] were Aa (Asian or African type) in 5 (6%) and Ae (European type) in 65 (75%); they were not classifiable in the remaining 17 (19%).

Serological Markers of HBV Infection

HBsAg was determined by hemagglutination (MyCell; Institute of Immunology Co., Ltd., Tokyo, Japan) or enzyme-linked immunosorbent assay (ELISA) (ELISA, F-HBsAg; Sysmex, Kobe, Japan), and HBeAg by ELISA (ELISA, F-HBe; Sysmex). HBV DNA was determined by quantitative polymerase chain reaction (PCR) (Amplicor HBV Monitor Test; Roche Molecular Systems, Inc., New Jersey) and the results were expressed in log copies/ml within a detection range from 2.6 to 7.6.

Genotypes of HBV

The six major genotypes (A–F) were determined serologically by ELISA (HBV GENOTYPE EIA; Institute of Immunology). The method utilizes the combination of epitopes on preS2-region products that is specific for each genotype [Usuda et al., 1999, 2000]. Genotype G was determined by preS2 serotype for genotype D and HBsAg subtype adw, and H was recognized by serotype for genotype C and subtype adw, respectively; these combinations were specific for genotypes G and H, respectively [Kato et al., 2001, 2004].

Subgenotypes of A designated Ae prevalent in Europe and Aa frequent in Africa as well as Asia [Sugauchi et al., 2004] (corresponding to A' originally reported by Bowyer et al. [1997]), were determined by the nucleotide sequence in the S gene [Sugauchi et al., 2004]. Briefly, nucleic acids were extracted from serum and a sequence of the large S gene was amplified by PCR with nested primers. The first-round PCR was performed with BGF1 (sense, 5'-CTG TGG AAG GCT GGC ATT CT-3' [nt 2757–2776]) and BGR2 (antisense, 5'-GGC AGG ATA GCC GCA TTG TG-3' [nt 1050–1079]) primers, and the second-round PCR with PLF5Bm (sense, 5'-TGT GGA TCC TGC ACC GAA CAT GGA GAA-3' [nt 136–162]) and BR112 (antisense, 5'-TTC CGT CGA CAT ATC CCA TGA AGT TAA GGG A-3' [nt 865–895]) as well as BGF5 (sense, 5'-TGC GGG TCA CCA TAT TCT TG-3' [nt 2811–2830]) and BGR6 (antisense, 5'-AGA AGT CCA CCA CGA GTC TA-3' [nt 249–268]) for 35 cycles each (94°C, 1 min [5 min in the first cycle]; 53°C, 2 min; and 72°C, 3 min [7 min in the last cycle]). Amplification products were run on gel electrophoresis and stained with BIG Dye (Applied Biosystems, California), purified by Qquick PC purification kit (Qiagen, Hilden, Germany) and then sequenced in AGI Prism 310 Genetic Analyzer (Applied Biosystems). The large S-gene sequences were analyzed phylogenetically along with reference Aa and Ae sequences by six-parameter and neighbor-joining methods [Gojobori et al., 1982; Saitou and Nei, 1987].

Determination of Hyaluronic Acid in Serum

Hyaluronic acid was determined by the agglutination of microparticles coated with proteins that specifically bind with it (Elpia-Ace HA, Fujirepio, Tokyo, Japan).

Statistical Analysis

Frequencies were compared between groups by the Mann-Whitney *U*-test and Fisher's exact test, and means by the Wilcoxon signed rank test. Loss of HBeAg or HBsAg was compared in the Kaplan-Meier life table, and differences were evaluated by log-rank test after the production limit method. A *P*-value less than 0.05 was considered significant.

RESULTS

Patients Infected Chronically With HBV Genotype A

There were 45 patients who were infected chronically with HBV genotype A and had been followed for 3 years or longer. Of them, 19 had persistently normal ALT levels (asymptomatic carriers), while the remaining 26 with elevated ALT levels possessed biopsy-proven chronic hepatitis. Table I compares demographic and virological characteristics at the baseline between the 19 asymptomatic carriers and 26 patients with chronic hepatitis. HBeAg was more frequent and the median HBV DNA level higher in patients with chronic hepatitis than asymptomatic carriers. The majority of asymptomatic carriers (79% [15/19]) and patients with chronic hepatitis (73% [19/26]) were infected with subgenotype Ae. There were three (12%) patients infected with subgenotype Aa and two of them had chronic hepatitis. Subgenotypes were not classifiable in the remaining four (21%) asymptomatic carriers and four (15%) patients with chronic hepatitis. Liver disease worsened in a single patient with chronic hepatitis. He was 47 years old at the presentation and infected with subgenotype Ae. Cirrhosis developed followed by hepatocellular carcinoma in him.

HBsAg and HBeAg in Patients With Chronic Hepatitis Infected With HBV Genotype A

Of the 26 patients infected with HBV genotype A, 4 (15%) lost HBsAg during follow-up, in comparison with

16 of the 116 (14%) patients with genotype B and 68 of the 862 (8%) with genotype C. Figure 1 compares seroclearance of HBsAg among patients with genotype A, B, or C. The loss of HBsAg at 5 years was significantly more frequent in patients with genotype A than B or C (12% vs. 2% or 3%, *P* = 0.0395).

Of the 26 hepatitis patients with genotype A, 19 (75%) possessed HBeAg at the presentation. HBeAg was cleared from serum in 14 (74%) of them during follow-up, in comparison with the seroclearance in 36 of the 41 (88%) patients with genotype B and in 347 of the 562 (62%) with genotype C. Figure 2 compares seroclearance of HBeAg among patients with genotype A, B, or C. At 5 years of follow-up, HBeAg was cleared more frequently in patients with genotype B than in those with genotype A or C (78% vs. 58% or 45%, *P* = 0.00001).

Development of Cirrhosis and Hepatocellular Carcinoma in Patients Infected With HBV of Various Genotypes

Figure 3 compares the development of cirrhosis in patients infected with genotype A, B, or C. Of the patients with genotype A, cirrhosis developed in only one at 5 years, but not any more during follow-up for 20 years. In contrast, cirrhosis increased steadily in patients with genotype B or C; it developed twice more often in patients with genotype C than B (30% vs. 14%).

Hepatocellular carcinoma developed in the single cirrhotic patient with genotype A, but did not in any others with genotype A during follow up for 20 years (Fig. 4). It increased with time, however, in patients with genotype B or C. Hepatocellular carcinoma tended to develop more frequently in patients with genotype C than B at 20 years (15% vs. 11%).

Changes in HBV DNA Levels and Hyaluronic Acid in the Patients Infected With HBV Genotype A

Of the 26 patients with genotype A, 14 (54%) seroconverted for the loss of HBeAg, while 5 (19%) kept it throughout follow-up longer than 3 years; the remaining 7 (27%) patients were without HBeAg at the presentation and thereafter. Table II compares demographic and virological characteristics of the three

TABLE I. Baseline Characteristics of the 45 Patients Infected With HBV Genotype A Who Were Followed for Longer Than 3 Years

Feature	Asymptomatic carriers (n = 19)	Chronic Hepatitis (n = 26)	Differences
Age (years) ^a	29 (11-48)	32 (13-59)	NS ^c
Male	15 (79%)	24 (92%)	NS
Follow-up (years) ^a	6.5 (3.4-17.7)	6.8 (3.5-18.6)	NS
History of transfusion	0 (0%)	1 (4%)	NS
Anti-HCV	0 (0%)	1 (4%)	NS
HBeAg positive	3 (16%)	19 (75%)	<i>P</i> = 0.0002
HBV DNA (log copies/ml)	<2.6 (<2.6-5.9)	>7.6 (<2.6->7.6)	<i>P</i> = 0.001
Subgroups (Aa/Ae/ND) ^b	0%/79%/21%	12%/73%/15%	NS

^aMedian values are shown with the range in parentheses.

^bNot determined.

^cNot significant.

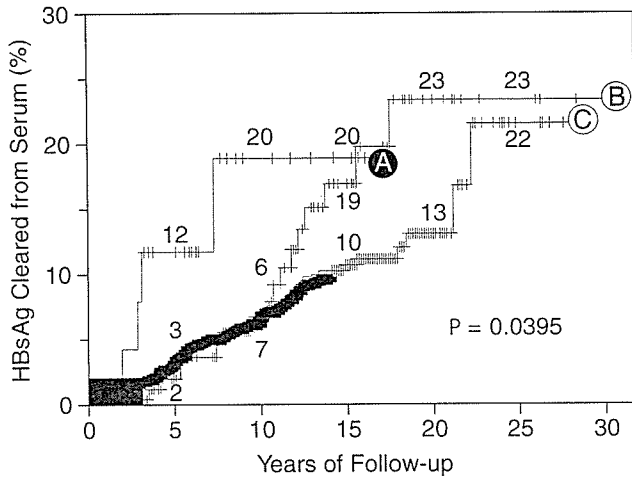


Fig. 1. Seroclearance of HBsAg during follow-up. Clearance rates of HBsAg are compared among patients with chronic hepatitis B who were infected with genotypes A, B, or C by the Kaplan–Meier life table. Differences are significant between genotype A and genotypes B and C at 5 and 10 years, as well as between genotypes B and C at 20 years by the log-rank test. Seroclearance of HBsAg did not spontaneously occur in all of them.

groups of patients at the baseline. Levels of HBV DNA were significantly lower in the patients without HBeAg than in those whom HBeAg persisted or who seroconverted within 3 years ($P = 0.03$).

Figure 5 compares changes in HBV DNA levels among patients infected with genotype A in whom HBeAg persisted, who seroconverted and who had remained negative for HBeAg. HBV DNA levels >7.6 log copies/ml continued for longer than 3 years in four of the five (80%) patients with persistent HBeAg. HBV DNA levels decreased in 13 of the 14 (93%) patients with seroconversion; they slightly changed from 6.7 to 7 log copies/ml in the remaining one patient. HBV DNA decreased to

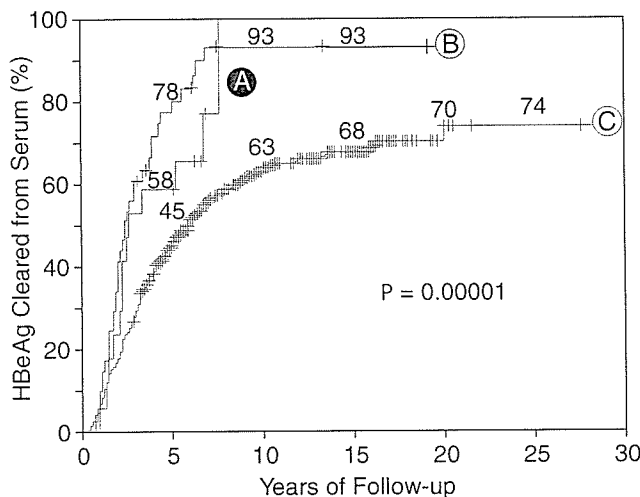


Fig. 2. Seroclearance of HBeAg during follow-up. Clearance rates of HBeAg are compared among patients with chronic hepatitis B who were infected with genotypes A, B, or C by the Kaplan–Meier life table. Differences are significant among genotypes A–C at 5 years as well as between genotypes B and C since 10 years or later by the log-rank test. Seroclearance of HBeAg did not spontaneously occur in all of them.

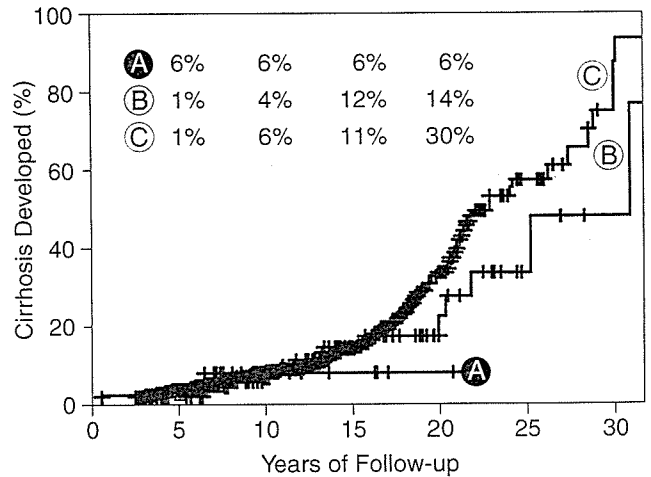


Fig. 3. Development of cirrhosis in patients infected with HBV genotype A, B, or C.

levels below the detection limit in 4 of the 14 (29%) patients with seroconversion and 1 of the 7 (14%) without HBeAg at the baseline. Of the 7 patients without HBeAg, 4 (57%) kept HBV DNA in detectable levels, comparable to 9 of the 14 (64%) patients with seroconversion. Decreases in HBV DNA during follow-up for 3 years or longer were significantly more frequent in the patients with seroconversion and those without HBeAg than in those with persistent HBeAg (93% [13/14] and 86% [6/7] vs. 20% [1/5], $P = 0.0095$ by the Fisher's exact test).

Figure 6 compares serum levels of hyaluronic acid among patients infected with genotype A in whom HBeAg persisted, who seroconverted and who had remained HBeAg-negative. Hyaluronic acid increased in four of the five (80%) patients in whom HBeAg persisted in contrast to only one of the seven (14%) patients without HBeAg. Increases in serum levels of hyaluronic acid ≥ 10 ng/ml was more frequent in the

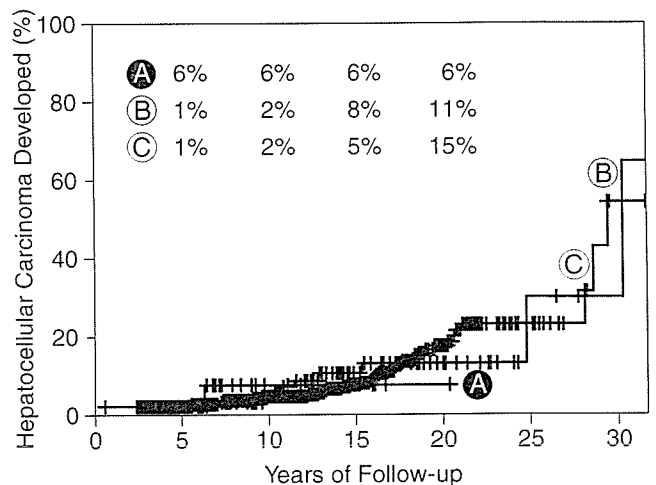


Fig. 4. Development of hepatocellular carcinoma in patients infected with HBV genotype A, B, or C.

TABLE II. Baseline Characteristics of the 26 Patients Infected With HBV Genotype A in Whom HBeAg Persisted, Who Seroconverted and Who Were Without HBeAg at the Presentation

Feature	HBeAg persisted (n = 5)	Seroconverted (n = 14)	Without HBeAg (n = 7)	Differences
Age (years) ^a	49 (24–59)	30 (13–60)	33 (14–41)	NS ^c
Male	5 (100%)	14 (100%)	5 (71%)	NS
Follow-up (years) ^a	6.2 (3.7–7.4)	9.2 (3.0–21)	8.1 (3.9–17)	NS
History of transfusion	0	1 (7%)	0	NS
Anti-HCV	0	0	1 (14%)	NS
HBV DNA (log copies/ml)	>7.6 (all patients)	>7.6 (6.7–>7.6)	4.1 (<2.6–7.1)	<i>P</i> = 0.03
Subgroups (Aa/Ae/ND ^b)	(0%/80%/20%)	(7%/79%/14%)	(29%/57%/14%)	NS

^aMedian values are shown with the range in parentheses.

^bNot determined.

^cNot significant.

patients with persistent HBeAg than in those with seroconversion and those without HBeAg (80% [4/5] vs. 14% [2/14] and 14% [1/7], *P* = 0.017 by the Fisher's exact test).

Of the 19 hepatitis patients presenting with serum HBeAg, 16 received antiviral and/or steroid withdrawal therapies, and 11 (69%) responded by the loss of HBeAg, while the remaining 4 failed to do so (Table III). There were three patients in whom HBeAg disappeared without receiving treatments. In total, therefore, seroconversion was accomplished in 14 of the 19 (74%) patients with genotype A.

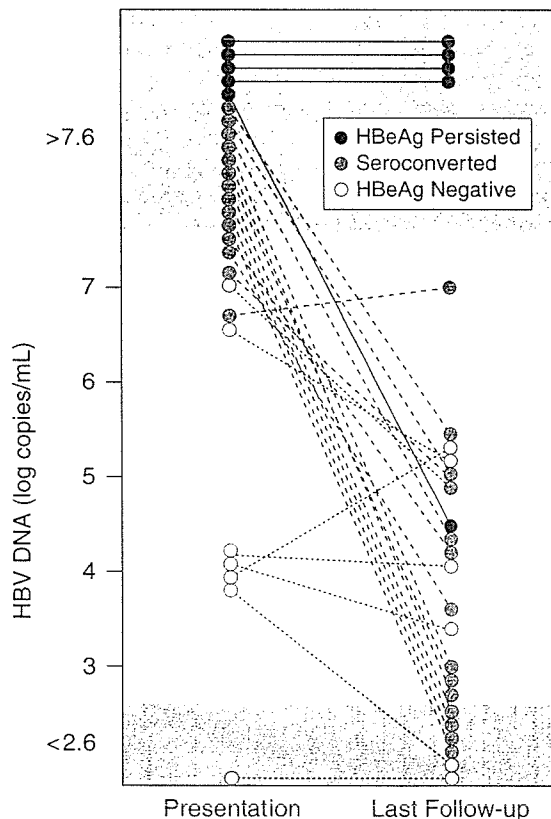


Fig. 5. Changes in serum levels of HBV DNA from the baseline to the last follow-up. Patients in whom HBeAg persisted, who seroconverted and who were without HBeAg at the baseline are compared.

DISCUSSION

Of the eight genotypes of HBV, E, and F are local, and confined to Central Africa and Central/South America, respectively [Magnius and Norder, 1995; Miyakawa and Mizokami, 2003]. Genotype H is genetically close to F and distributes in Central America [Arauz-Ruiz et al., 2002]. Genotype G occurs very rarely [Stuyver et al., 2000; Chu et al., 2003; Kato et al., 2004], and is always

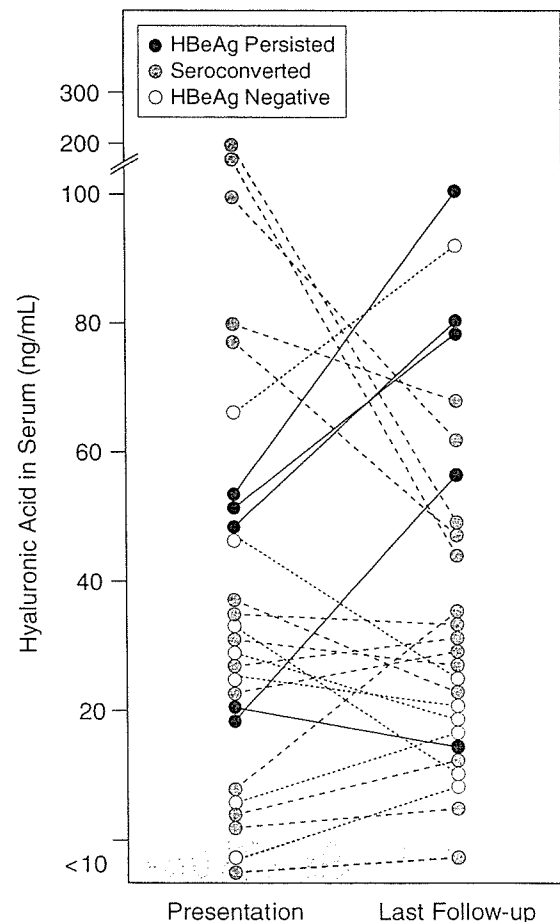


Fig. 6. Changes in serum levels of hyaluronic acid from the baseline to the last follow-up. Patients in whom HBeAg persisted, who seroconverted and who were without HBeAg at the baseline are compared.

TABLE III. Loss of HBeAg in the 19 Hepatitis Patients Infected With HBV Genotype A Who Had Been Followed for Longer Than 3 Years

Case No.	Sex/age	Pathology	Sub-group	Treatment	HBeAg Lost
1	M23	F1/A1	Ae	Interferon	Yes
2	M33	F2/A1	Ae	Interferon	Yes
3	M44	F3/A1	Ae	Interferon	Yes
4	M57	F2/A1	Ae	Interferon	Yes
5	M13	F1/A1	Ae	Steroid withdrawal	Yes
6	M16	F1/A1	Ae	Steroid withdrawal	Yes
7	M28	F1/A1	ND	Steroid withdrawal	Yes
8	M47	F2/A1	Aa	Steroid withdrawal	Yes
9	M17	F1/A1	Ae	Steroid/Interferon	Yes
10	M29	F1/A1	Ae	Lamivudine	Yes
11	M38	F1/A1	Ae	Lamivudine	Yes
12	M30	F1/A0	Ae	None	Yes
13	M39	F1A1	Ae	None	Yes
14	M47	F3/A2	Ae	None	Yes
15	M24	F2/A2	Ae	Interferon and others ^b	No
16	M43	F2/A1	Ae	Steroid/Interferon	No
17	M48	F1/A2	Ae	Interferon/Lamivudine	No
18	M49	F1/A1	ND ^a	Steroid withdrawal	No
19	M59	F1A1	Ae	Interferon	No

^aNot determined.

^bThe patient received interferon, lamivudine interferon/lamivudine, and then lamivudine plus entecavir.

co-infected with HBV of the other genotypes [Kato et al., 2002, 2003]. Thus, only four genotypes (A–D) are left for comparison in epidemiological and clinical studies in most countries of the world. Since even these four genotypes have distinct geographical distributions, comparison with respect to severity of liver disease or response to antiviral treatment is hardly feasible among them, except in multi-national studies on patients of diverse ethnicities [Westland et al., 2003; Janssen et al., 2005].

In the Toranomon Hospital in Tokyo, by far the most patients presenting with HBsAg were infected with HBV of genotype B (10.5%) or C (83.3%), and genotype A infected only a minority (3.10%) of them. During 31 years, 128 patients with genotype A visited there. Unlike most infections with genotype B and C transmitted perinatally from carrier mothers with HBeAg [Okada et al., 1976], genotype A infection in Japan is often acquired in the adulthood by men having extra-marital sexual contacts either with men or women; there has been no evidence for maternal transmission of HBV genotype A in Japan [Kobayashi et al., 2002, 2003; Ogawa et al., 2002; Suzuki et al., 2005]. HBV infection prevails among homosexuals in Western countries where genotype A is frequent, who poorly respond to vaccines [Goilav and Piot, 1989]. Genotype A infection in Japan has a propensity to become chronic and tends to respond to antiviral therapies better than genotype B or C infection [Kobayashi et al., 2002, 2003; Suzuki et al., 2005].

In the present study, we have compared the virological outcome among infections with HBV genotypes A, B, and C, and found substantial differences. Patients with genotype A fared better than those with genotype B or C in that they cleared HBsAg and HBeAg faster during follow-up (Figs. 1 and 2). It is not certain, however, whether or not the observed differences are influenced

by the duration of HBV infection. HBV genotype A is contracted predominantly by men in the adulthood and genotypes B or C had been transmitted perinatally until 1986 when the national immunoprophylaxis started. It needs to be pointed out that this study is retrospective in nature, and most patients with HBeAg had received interferon, lamivudine or steroid withdrawal, or combination thereof. Of the 16 patients with genotype A who received treatment, 11 (69%) responded and cleared HBeAg from serum. In addition, three patients lost HBeAg spontaneously. Hence seroconversion was achieved in 14 of the 19 (74%) patients with genotype A. In view of lamivudine, adefovir dipivoxil, and pegylated interferon that are reported efficacious in treatment of chronic hepatitis B [Perrillo et al., 2000; Hadziyannis et al., 2003; Kumada, 2003; Janssen et al., 2005], it would be unethical to evaluate genotype-dependent differences in the natural course of persistent HBV infection.

Of the 45 individuals chronically infected with HBV genotype A and had been followed for 3 years or longer, HBeAg was more frequent and HBV DNA levels higher in the 26 patients with biopsy-proven chronic hepatitis than in the 19 asymptomatic carriers. Among the 26 patients with genotype A, HBeAg persisted throughout the observation in 5 (19%) and disappeared in 14 (54%); HBeAg remained negative in the other 7 (27%) patients. HBV DNA stayed in high levels more frequently ($P=0.0095$) in the patients with persistent HBeAg (80% [4/5]) than in those who seroconverted (7% [1/14]) or remained HBeAg-negative (29% [2/7]). Furthermore, increases in serum hyaluronic acid ≥ 10 ng/ml were more frequent ($P=0.017$) in the patients with persistent HBeAg (80% [4/5]) than in those with seroconversion (14% [2/17]) or HBeAg-negative (14% [1/7]). Although the patients with genotype A fare better than those with genotype B or C, persistent HBeAg refractory to

treatment would predict ongoing liver disease with fibrosis in progress.

Recently, subgenotypes have been recognized and they may influence the biology of HBV and liver disease. For instance, a subgenotype of B having the recombination with genotype C (Ba) induces more severe liver disease with poorer response to lamivudine than that without the recombination (Bj) [Sugauchi et al., 2002, 2003; Akuta et al., 2003]. As for genotype A, there are two subgenotypes with different geographical distributions. Subgenotype Ae is common in Europe and the United States, while Aa is prevalent in Asia and Africa [Bowyer et al., 1997; Sugauchi et al., 2004]. In a case-control study, HBeAg was more frequent and HBV DNA levels higher in carriers of Ae than Aa [Tanaka et al., 2004]. The majority of genotype A strains from our patients (86%) were found to be Ae; they were probably introduced to Japan by immigrants and visitors from foreign countries [Kobayashi et al., 2004]. Cirrhosis and hepatocellular carcinoma developed in only one of the 19 (5%) patients infected with subgenotype Ae, in remarkable contrast to frequent hepatocellular carcinoma in Africa where infection with subgenotype Aa is common during the infancy [Kew et al., 2005].

Although there have been accumulating lines of evidence for virological and clinical influence of HBV genotypes, there are conflicting views on them. Differences between genotypes B and C in Asia [Kao et al., 2000; Orito et al., 2001; Tsubota et al., 2001; Chan et al., 2004; Yu et al., 2005] have not been reproduced, probably due to selection bias for the patients with severe disease [Sumi et al., 2003] or subgenotypes of B different between Japan (Bj) and Hong Kong (Ba) [Yuen et al., 2004]. Liver disease, once advanced beyond a certain severity, will progress spontaneously irrespective of HBV genotypes. Subgenotype Ba having the recombination with genotype C may be endowed with a higher disease-inducing capacity than subgenotype Bj without the recombination [Sugauchi et al., 2002].

Of patients infected with three different genotypes in Japan, the virological outcome of persistent HBV infection was more favorable for those with genotype A than B and C in that order. It is not known where genotype D stands, although it fares worse than genotype A in chronic HBV infection [Thakur et al., 2002; Janssen et al., 2005]. In ranking the four major genotypes (A–D) in disease-inducing capacity and response to antiviral therapies, perinatals, or adulthood transmission, as well as subgenotypes inherent to countries, would have to be taken into considerations [Sugauchi et al., 2002, 2004; Norder et al., 2004].

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