Table 3 Differences between HBV reactivation in patients who are HBsAg carriers and patients with resolved infection

	Inactive carrier HBsAg(+) ($n = 20$)	Resolved infection HBsAg(−) ($n = 17$)	*P*-value
Age, years, median (range)	60 (29–84)	67 (48–86)	0.035
Male/female	11/9	11/6	NS
Disease types (F-A/F-SA/LOHF)	4/11/5	0/16/1	0.025
Prognosis (alive/died/LT)	2/16/2	0/17/0	NS
ALT, IU/L (mean ± SD)	1114 ± 1602	653 ± 1057	NS
Total bilirubin, mg/dL (mean ± SD)	13.6 ± 8.2	18.5 ± 6.3	NS
Prothrombin time (%), median (range)	28.8 (8.0–48.0)	30.3 (19.0–38.0)	NS
HBV DNA level, log copies/mL (mean ± SD)	7.6 ± 1.2	6.6 ± 1.5	NS
Treatment			
Lamivudine	13 (65)	9 (53)	NS
Entecavir	9 (45)	9 (53)	NS
Interferon	5 (25)	6 (35)	NS
Underlying disease			
NHL/MALT lymphoma	10 (50)	13 (76)	NS
Other onco-hematological	1 (5)	3 (18)	NS
Oncological	3 (15)	1 (6)	NS
Collagen disease	2 (10)	–	NS
Rheumatological	4 (20)	–	NS
HBV reactivation			
Under immunosuppressive therapy	10 (50)	2 (12)	0.015
After immunosuppressive therapy	3 (15)	14 (82)	<0.001
Type of immunosuppressive therapy			
CHOP	1 (5)	–	NS
R-CHOP	7 (35)	10 (59)	NS
Other rituximab-containing-therapy	1 (5)†	3 (18)‡	NS
Fludarabine plus prednisolone	–	1 (6)	NS
Anthracycline plus cyclophosphamide	2 (10)§	1 (6)	NS
Prednisolone	4 (20)	–	NS
Methotrexate	2 (10)	–	NS
Others	3 (15)¶	2 (12)††	NS

Unless otherwise indicated, data indicate the number of patients, and those in parenthesis indicate percentages of patients.
Laboratory data are at the onset of hepatic encephalopathy of coma grade greater than II. HBV DNA levels are at the onset of hepatitis.
Significant difference among group was assessed by Student's *t*-test, Mann–Whitney *U*-test and χ^2-test.
†One patient: only rituximab.
‡One patient: rituximab, etoposide. One patient: rituximab, pirarubicin, cyclophosphamide, vincristine, prednisolone. One patient: rituximab, etoposide, mitoxantrone, carboplatin, prednisolone.
§One patient: adriamycin, cyclophosphamide. One patient: epirubicin, cyclophosphamide, fluorouracil, dexamethasone.
¶One patient: imatinib mesylate. One patient: carboplatin, paclitaxel, prednisolone. One patient: tacrolimus, etanercept, infliximab, methotrexate, prednisolone.
††One patient: cyclophosphamide, prednisolone. One patient: vincristine, doxorubicin, dexamethasone (for peripheral blood stem cell transplantation).
ALT, alanine aminotransferase; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisolone; F-A, acute type fulminant hepatitis; F-SA, subacute type fulminant hepatitis; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; LOHF, late-onset hepatic failure; LT, liver transplantation; MALT, mucosa-associated lymphoid tissue lymphoma; NHL, non-Hodgkin's lymphoma; NS, not statistically significant; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone; SD, standard deviation.

HBsAg negative patients with HBcAb and/or HBsAb

The optimal screening and prophylactic strategy for prevention of HBV reactivation in patients with resolved infection remain unsettled. However, the most important first step in avoiding the serious morbidity associated with HBV reactivation is to identify patients at risk before the start of immunosuppressive therapy. In high HBV endemic areas, all patients should be screened for HBsAg. A highly sensitive assay is desirable to detect low levels of HBsAg and escape mutants of HBV.[55] In the next step, if HBsAg is negative, patients should be screened for HBcAb and HBsAb when they are receiving chemotherapy regimens that are associated with a high risk of reactivation. (e.g. intensive chemotherapy for hematological malignancies and hematopoietic stem cell transplantation [HSCT]). HBV reactivation during chemotherapy has been reported in HBsAb positive cases, HBcAb positive cases and cases positive only for HBcAb regardless of HBV DNA status.[33,35,56,57] In addition, while rare, there have been sporadic reports of cases in which HBV reactivation was observed after administration of rituximab in patients who were positive for HBsAb alone.[35,58] For the third step, if patients are HBcAb and/or HBsAb positive, they should be screened for HBV DNA. This screening can clarify the occult HBV infection.[58–60] Several reports have suggested an association between a decrease in HBsAb and HBcAb titers and risk of HBV reactivation, and monitoring these antibodies may provide an index for HBV reactivation.[61,62] However, this approach is not applicable to HBsAb negative and HBcAb positive patients. In addition, HBV reactivation has also been observed in patients with high HBsAb titers.[36] These may indicate that predicting reactivation only by monitoring HBsAb titers would be insufficient.

Using antiviral agents for patients with resolved infection in a prophylactic manner before the start of chemotherapy is probably as efficacious as in HBsAg positive carriers. However, there are issues such as obscure indications and cost-effectiveness. Alternatively, for patients with resolved infection, antiviral treatment can be deferred until seroconversion of HBsAg or detection of HBV DNA.[63–65] However, the appearance of HBV DNA in serum precedes HBsAg appearance, and there are cases of HBV reactivation without the appearance of HBsAg.[66] Therefore, periodic monitoring of HBV DNA may predict HBV reactivation, and it is therefore advantageous to combine these indices. Hui *et al.* reported that the median time from the elevation of serum HBV DNA to hepatitis onset was 18.5 weeks (range 12–28 weeks).[35] When monitoring for HBV reactivation, it is essential to identify HBV reactivation at an early stage. As previously shown in fulminant hepatitis cases, the start of nucleoside analogs after the onset of hepatitis cannot prevent fatal hepatitis. Therefore, commencement of nucleoside analogs at an early stage of HBV reactivation is important.[63,64]

The optimal duration of antiviral prophylaxis in HBsAg negative patients receiving immunosuppressive chemotherapy is not well understood. The intensity and duration of immunosuppression, as well as a number of host and viral factors, should be taken into consideration. In a previous study, in patients receiving rituximab plus steroid combination chemotherapy, discontinuation of lamivudine 4 weeks after completion of chemotherapy was followed by HBV reactivation, which occurred up to 6 months after treatment was withdrawn.[67] In the other setting of allogenic HSCT, HBV reactivation occurs later, in 40% at 2 years and 70% of patients at 5 years post-transplantation.[61]

GUIDELINES FOR PREVENTING HBV REACTIVATION

IN 2009, THE Intractable Hepatobiliary Disease Study Group in Japan and the Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis developed guidelines for preventing HBV reactivation.[68] These guidelines underwent minor revision in 2011, as shown in Figure 1.

The essential features of the guidelines are as follows. All patients should be screened for HBsAg before the start of chemotherapy. If HBsAg is positive, HBeAg, hepatitis B e antibody (HBeAb) and HBV DNA should be checked. Regardless of the patient's HBeAg, HBeAb or HBV DNA status, prophylactic therapy with entecavir before initiation is recommended. If HBsAg is negative, HBcAb and HBsAb testing should be performed. If HBcAb and/or HBsAb is positive, HBV DNA should be checked. When HBV DNA is detectable, antiviral prophylaxis before initiation is recommended. When HBV DNA is not detectable, HBV DNA and aspartate aminotransferase/ALT levels should be monitored monthly during and 12 months after completion of chemotherapy. Nucleoside analogs should be administrated immediately when HBV DNA becomes positive during this period. The timing of termination of nucleoside analog treatment will be determined in accordance with the treatment for type B chronic hepatitis, if HBsAg is positive. If HBcAb and/or HBsAb is positive,

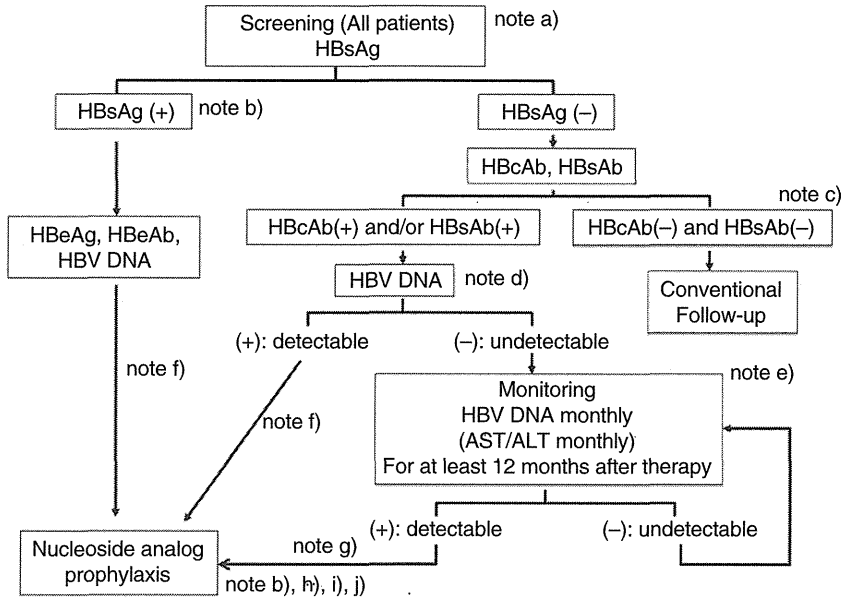

Figure 1 Guideline for preventing hepatitis B due to immunosuppressive therapy or chemotherapy (revised version). Reactivation of HBV can occur not only in HBsAg positive patients, but also in a proportion of HBsAg negative patients during and after intensive immunosuppressive therapy or chemotherapy of hematological malignancy. HBV reactivation deserves special attention because it can cause flare-up of hepatitis resulting in fulminant hepatitis. Appropriate measures are also necessary in patients receiving immunosuppressive therapy or chemotherapy for non-hematological malignancy in consideration of the risk of HBV reactivation. Because of a lack of evidence, there is no guarantee that prophylactic administration of nucleoside analog in these guidelines can prevent acute hepatic failure due to HBV reactivation. Notes: (a) HBV carriers and patients who have apparently recovered from HBV infection receiving immunosuppressive therapy or cytotoxic chemotherapy are at a risk of HBV reactivation. All patients should be screened for being HBV carriers by HBsAg. If results for HBsAg are negative, patients should be screened for evidence of previous infection by HBcAb and HBsAb. Highly sensitive detection methods for HBsAg, HBcAb and HBsAb are desirable. (b) HBsAg positive cases are subject to consultation with a hepatologist. Consultation with a hepatologist is desirable in all patients subject to administration of nucleoside analogs. (c) Detection of HBV DNA is desirable in those patients who have previously received immunosuppressive therapy or cytotoxic chemotherapy, and HBcAb and HBsAb are undermined before the start of the therapy. (d) Detection by PCR or real-time PCR is recommended. The sensitive real-time PCR method is desirable. (e) Patients receiving rituximab plus steroid combination therapy or hematopoietic stem cell transplantation are particularly at risk of HBV reactivation and deserve careful attention. Although there is a lack of evidence regarding the risk of HBV reactivation in patients receiving fludarabine, an intensive immunosuppressive agent, this still deserves careful attention in the future. (f) Prophylactic nucleoside analogs should be started as soon as possible before the start of immunosuppressive therapy or chemotherapy. (g) Nucleoside analogs should be administrated immediately when HBV DNA becomes positive during and after immunosuppressive therapy or chemotherapy. (h) Entecavir is recommended as the nucleoside analog. HBV DNA is monitored monthly during administration of nucleoside analogs. (i) Termination of nucleoside analog treatment is considered when the timing is as follows: If HBsAg is positive at screening, the timing of termination of nucleoside analog treatment will be determined in accordance to the treatment for type B chronic hepatitis. If HBcAb and/or HBsAb is positive at screening, nucleoside analog treatment will be discontinued when: (1) nucleoside analogs are administered for 12 months after the completion of immunosuppressive therapy or chemotherapy; (2) ALT levels are normal during the administration period, and (3) HBV DNA is negative during the administration period. (j) Patients should be closely observed for 12 months after treatment with nucleoside analogs. The follow up is according to the instruction method of each nucleoside analog. Nucleoside analogs should be re-administrated immediately when HBV DNA becomes positive during the observation period. These guidelines were jointly developed by the Intractable Hepatobiliary Disease Study Group in Japan and the Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis in 2009. The guidelines underwent minor revision in 2011. ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBcAb, hepatitis B core antibody; HBeAg, hepatitis B e antigen; HBeAb, hepatitis B e antibody; HBsAb, hepatitis B surface antibody; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; PCR, polymerase chain reaction.

nucleoside analog treatment will be discontinued if HBV DNA is negative and ALT levels are normal. Patients are closely observed for 12 months after treatment with nucleoside analogs.

Although reactivation of hepatitis B commonly occurs in the setting of cancer chemotherapy, it may also follow the use of immunomodulatory therapy for non-malignant conditions, for example, infliximab therapy for inflammatory bowel disease and therapy for rheumatological diseases with corticosteroids, methotrexate,[69,70] anti-tumor necrosis factor-α alone[71,72] or in combination with other therapies.[73–75] However, for both HBsAg positive patients and HBsAg negative resolved infection patients, the data are currently insufficient to provide information on the incidence of HBV reactivation of these agents. Consequently, careful attention is necessary when using new immunosuppressive agents.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Long-term efficacy of interferon therapy in patients with chronic hepatitis B virus infection in Japan

Fumitaka Suzuki · Yasuji Arase · Yoshiyuki Suzuki · Norio Akuta · Hitomi Sezaki · Yuya Seko · Yusuke Kawamura · Tetsuya Hosaka · Masahiro Kobayashi · Satoshi Saito · Kenji Ikeda · Mariko Kobayashi · Hiromitsu Kumada

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Abstract
Background Few studies have investigated the long-term effects of interferon (IFN) therapy for chronic hepatitis B (CHB). In this retrospective study, we investigated the efficacy of and predictors of response to IFN therapy in CHB patients.
Methods We analyzed data for 615 Japanese CHB patients (hepatitis B e antigen [HBeAg]-positive 414, HBeAg-negative 201) treated with IFN, and conducted follow up for a median duration of 8.1 years (range 0.5–23.2). Responders were defined as patients who showed continuously normalized alanine transaminase (ALT) levels, HBeAg clearance, and low hepatitis B virus (HBV) DNA levels at 6 months post-treatment or for a span of more than 6 months until each test point at 1, 3, 5, and 10 years.
Results The IFN response rates of all patients were 21, 18, 21, 23, and 25% at 6 months and 1, 3, 5, and 10 years, respectively. On multivariate analysis, significant determinants of the outcome of IFN therapy were as follows: at 6 months and 1 year, young age, low HBV DNA levels, and long duration of treatment; at 3 years, long duration of treatment, young age, and high level of albumin; at 5 years, high level of albumin, female, and pretreated with IFN; and at 10 years, HBeAg-negative. Sixty-nine of the 615 patients (11%) achieved seroclearance of hepatitis B surface antigen (HBsAg). On multivariate analysis, age ≥30 years, HBV genotype A, and male were all independent factors predicting the achievement of HBsAg seroclearance.
Conclusion HBeAg, HBV DNA level, age, sex, albumin, duration of treatment, pretreatment with IFN, and HBV genotype were important factors in determining long-term response to IFN therapy.

Keywords Interferon · Hepatitis B virus · Chronic hepatitis B · Genotype · Hepatitis B surface antigen

Abbreviations

CHB	Chronic hepatitis B
HBV	Hepatitis B virus
IFN	Interferon
HBeAg	Hepatitis B e antigen
ALT	Alanine transaminase
MU	Million units
HBsAg	Hepatitis B surface antigen
CLEIA	Chemiluminescent enzyme immunoassay
bDNA	Branched-chain DNA probe assay
TMA-HPA	Transcription-mediated amplification and hybridization protection assay
PCR	Polymerase chain reaction
ELISA	Enzyme-linked immunosorbent assay
AST	Aspartate transaminase
AFP	α Fetoprotein
OR	Odds ratio
CI	Confidence interval
HCC	Hepatocellular carcinoma

F. Suzuki (✉) · Y. Arase · Y. Suzuki · N. Akuta · H. Sezaki · Y. Seko · Y. Kawamura · T. Hosaka · M. Kobayashi · S. Saito · K. Ikeda · H. Kumada
Department of Hepatology, Toranomon Hospital, 2-2-2 Toranomon, Minato-ku, Tokyo 105-8470, Japan
e-mail: fumitakas@toranomon.gr.jp

M. Kobayashi
Research Institute for Hepatology, Toranomon Hospital, Tokyo, Japan

Introduction

Hepatitis B virus (HBV) infection is a common disease that can induce a chronic carrier state and is associated with the risk of developing progressive disease and hepatocellular carcinoma [1]. Interferon (IFN) and several nucleoside/nucleotide analogues such as lamivudine, adefovir dipivoxil, entecavir, and tenofovir disoproxil fumarate are currently approved as treatments for chronic hepatitis B (CHB) in most countries [2–5]. Successful treatment of CHB with clearance of hepatitis B e antigen (HBeAg), reduction in serum HBV DNA levels, and normalization of alanine transaminase (ALT) levels is associated with a favorable long-term outcome, independent of the antiviral drug used [6, 7].

A meta-analysis of IFN therapy published in 1993 reviewed 15 randomized controlled studies involving 837 adult patients who received IFN-α at doses of 5–10 million units (MU) administered at intervals ranging from daily to three times weekly for 4–6 months [8]. Clearance of HBeAg was noted in 33% of the treated patients compared with 12% of controls. Elimination of detectable HBV DNA and normalization of ALT levels were also more common in the treated patients than in the controls. The major pretreatment factors that correlated with a response were high ALT levels [9–11], low HBV DNA levels [9, 10], female sex, and elevated liver activity and fibrosis on liver biopsy [8]. Another recent meta-analysis of 24 randomized controlled trials concluded that the rates of persistent ALT normalization, clearance of HBeAg, and sustained elimination of HBV DNA (determined by hybridization) induced by IFN therapy were approximately 25% greater than the rates for controls. A more recent meta-analysis report showed that IFN increased the incidence of HBeAg and hepatitis B surface antigen (HBsAg) seroclearance after long-term follow up of 3–7 years [12].

However, specific data on the long-term effects of IFN therapy (median follow-up duration of 8.1 years), particularly among the Japanese, are limited. Moreover, few reports have investigated factors predicting the achievement of HBsAg seroclearance. To further evaluate factors influencing clinical outcome, we performed a retrospective cohort study on CHB patients treated with IFN in our hospital.

Patients and methods

Patients

We retrospectively examined 615 Japanese patients (151 females and 464 males) who commenced IFN treatment between June 1984 and April 2008 in the Department of Hepatology at Toranomon Hospital (Table 1). Several of the patients have been included in previous reports [13–15].

Table 1 Characteristics of patients at commencement of interferon therapy

Demographic data	
Total number	615
Sex, female/male	151/464
Age, years (range)	35 (15–68)
Previously treated with interferon	123 (20%)
Duration of treatment, weeks (range)	26 (4–981)
Follow-up period, years (range)	8.1 (0.5–23.2)
Laboratory data	
Aspartate transaminase, IU/L (range)	72 (18–990)
Alanine transaminase, IU/L (range)	138 (12–1578)
Bilirubin, mg/dL (range)	0.7 (0.2–8.8)
Albumin, g/dL (range)	3.9 (2.6–5.3)
Platelets, $\times 10^3/\mu L$ (range)	174 (48–500)
Staging of liver histology (F0/1/2/3/4/ND)	8/77/185/162/72/111
Serum HBV DNA, log copies/mL (range)	>7.6 (<2.6 to >7.6)
HBeAg (positive/negative)	414/201
HBV genotype (A/B/C/D/H/B + C/unknown)	24/37/504/1/1/1/47

Values are expressed as medians and ranges (in parentheses) or as numbers and percentages (in parentheses)

HBV hepatitis B virus, *HBeAg* hepatitis B e antigen, *ND* not done

All enrolled patients were followed up for a range of 0.5–23.2 years from completion of IFN treatment, with a median follow-up duration of 8.1 years. Before the commencement of IFN treatment, all patients had been positive for HBsAg in the serum for more than 6 months, and all were confirmed to have hepatitis caused by HBV and not by another vector, such as infection with hepatitis C virus or autoimmune hepatitis. None had a history of drug abuse or alcoholic hepatitis, and none had received nucleoside/nucleotide analogue therapy. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Toranomon Hospital Ethics Committee. Informed consent was obtained from each patient.

Interferon therapy and assessment of response to therapy

Patients received 3–12 MU of IFN-α or IFN-β (Sumiferon: Dainippon Sumitomo Pharma, Osaka, Japan; Canferon A: Takeda Chemical Industries, Osaka, Japan; Intron A: Schering-Plough MSD KK, Osaka, Japan; and Feron: Toray, Tokyo, Japan). The durations and regimens of treatment were as follows: 4 weeks (89 patients; daily for

4 weeks), 26 weeks (270 patients; daily for 4 weeks followed by 2 or 3 times a week), 52 weeks (103 patients; 2 or 3 times a week), 104 weeks (80 patients; 2 or 3 times a week), and more than 104 weeks (73 patients; 2 or 3 times a week). The median duration of treatment was 26 weeks (range 4–981).

The numbers of responders were evaluated at 6 months and 1, 3, 5, and 10 years after the completion of IFN therapy. In the baseline HBeAg-positive patients, responders were defined as patients who showed normalization of serum ALT level (normal level 6–30 IU/L), HBeAg clearance, and low HBV DNA level (<5 log copies/mL) at 6 months after completion of IFN therapy. In addition, baseline HBeAg-positive patients who showed continuous normalization of ALT levels, HBeAg clearance, and low HBV DNA level for more than 6 months until each test point at 1, 3, 5, and 10 years after completion of IFN therapy were also classified as "responders." In the baseline HBeAg-negative patients, responders were defined as those who showed sustained normalization of ALT level and low HBV DNA level (<4 log copies/mL) for more than 6 months until each test point after completion of IFN therapy.

All patients not considered to be responders were termed "non-responders." Patients receiving other therapies (IFN or nucleoside/nucleotide analogues) after the completion of IFN therapy were also termed non-responders.

Blood tests and serum viral markers

Routine biochemical tests were performed monthly via standard procedures during and for the first 12 months following the completion of IFN treatment and at least every 2 months thereafter. Levels of HBsAg, HBeAg, and anti-HBe were determined using radioimmunoassay kits (Abbott Diagnostics, Chicago, IL, USA) or a chemiluminescent enzyme immunoassay (CLEIA; Lumipulse System; Fujirebio, Tokyo, Japan). HBV DNA levels were measured using a branched-chain DNA probe assay (bDNA) (Chiron Laboratory Service, Van Nuys, CA, USA), a transcription-mediated amplification and hybridization protection assay (TMA-HPA) (Chugai Diagnostics Science, Tokyo, Japan), or a polymerase chain reaction (PCR)-based assay (COBAS Amplicor HBV Monitor Test or COBAS TaqMan HBV Test; Roche Diagnostics, Indianapolis, IN, USA).

HBV genotype

The major genotypes of HBV were determined using an enzyme-linked immunosorbent assay (ELISA; Institute of Immunology, Tokyo, Japan) or a PCR-invader assay (BML, Tokyo, Japan) according to the methods described by Usuda et al. [16] or Tadokoro et al. [17].

Statistical analysis

Differences between groups were examined for statistical significance using the χ^2 or Fisher's exact test and Mann–Whitney U-test where appropriate. Independent predictive factors associated with response to IFN treatment were determined using multivariate multiple logistic regression. The following 14 potential predictors of response to IFN treatment were assessed in this study: age, sex, pretreatment with IFN, duration of IFN treatment, severity of liver disease (CH or liver cirrhosis), HBV genotype, and levels of aspartate transaminase (AST), ALT, bilirubin, albumin, platelets, α fetoprotein (AFP), HBeAg, and HBV DNA. All factors found to be at least marginally associated with response to IFN therapy ($P < 0.10$) were entered into the multivariate multiple logistic regression analysis. The above calculations were performed using the Windows SPSS software package version 11.0.1 J (SPSS, Chicago, IL, USA).

The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to assess the relative risk. Independent risk factors predicting the achievement of HBsAg seroclearance were studied using stepwise Cox regression analysis. Potential factors predicting the achievement of HBsAg seroclearance assessed here were the above 14 variables, each transformed into categorical data consisting of two simple ordinal numbers for univariate and multivariate analyses. All factors found to be at least marginally associated with HBsAg seroclearance ($P < 0.10$) were tested in the multivariate Cox proportional hazard model. A Kaplan–Meier estimate was performed using the SPSS software, and P values were calculated using the Cox-Mantel log-rank test. A two-tailed P value of <0.05 was considered statistically significant.

Results

Study population

Twenty-four (4%), 37 (6%), 504 (82%), 1 (0.2%), 1 (0.2%), and 1 (0.2%) patients were infected with HBV genotypes A, B, C, D, H, and B + C, respectively. Genotype could not be measured in the remaining 47 patients. The baseline characteristics of the patients are shown in Table 1. Although few patients had genotypes A and B, the distribution of HBV genotype was similar to that in patients with CHB who had received care in our hospital, with a follow-up period of more than 2 years [18]. Twenty-two of 24 patients with genotype A, 14 of 37 with

genotype B, 342 of 504 with genotype C, 1 of 1 with genotype H, and 34 of 47 with unknown genotype were HBeAg-positive at the commencement of treatment. While we were able to measure HBV DNA levels in 254 patients at the commencement of IFN therapy, levels in the remaining 361 could not be measured owing to a lack of commercial kits before the bDNA assay was available. The numbers of patients receiving other additional therapies after the completion of IFN therapy were 111 (HBeAg-positive/-negative, 90/21), 92 (67/25), 34 (25/9), and 61 (39/22) at the 1-, 3-, 5-, and 10-year time points, respectively.

Response to interferon therapy in all patients

The IFN response rates in all patients were 21% (105/497), 18% (86/491), 21% (90/428), 23% (82/359), and 25% (59/235) at 6 months and 1, 3, 5, and 10 years, respectively, after completion of the IFN therapy (Fig. 1). In patients with genotype A, the response rate was highest at 6 months post-treatment and gradually decreased at subsequent time points from 1 to 10 years thence. In patients with genotype B, response rates were over 20% at all time points except for 6 months post-treatment, whereas rates in patients with genotype C were under 25% at all time points (Fig. 2a).

Evaluation of efficacy of IFN in relation to clinical factors in all patients

The data of all patients were subjected to univariate analyses to determine the clinical factors contributing to the efficacy of IFN at each time point. We then investigated the significance of response to IFN therapy using multivariate logistic regression analysis.

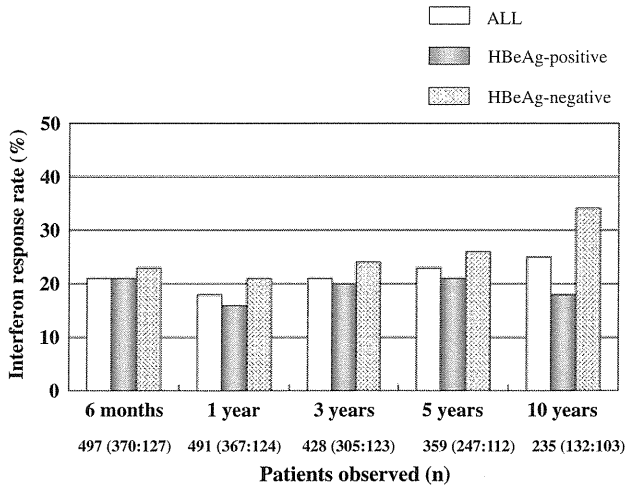

Fig. 1 Interferon response rates of all patients and hepatitis B e antigen (*HBeAg*)-positive and HBeAg-negative patients at 6 months and 1, 3, 5, and 10 years

Multivariate analyses including the variables noted above revealed several parameters that independently influenced the outcome of IFN therapy; namely, at 6 months: age ($P = 0.013$), HBV DNA level ($P = 0.019$), and duration of treatment ($P = 0.034$); at 1 year: HBV DNA level ($P < 0.001$) and age ($P = 0.001$); at 3 years: duration of treatment ($P < 0.001$), age ($P = 0.013$) and albumin level ($P = 0.013$); at 5 years: albumin level ($P = 0.004$), sex ($P = 0.005$), and pretreatment with IFN ($P = 0.039$); and at 10 years: HBeAg ($P < 0.001$) (Table 2).

Response to interferon therapy and evaluation of efficacy of IFN in relation to clinical factors in HBeAg-positive patients

Response rates in baseline HBeAg-positive patients were 21% (76/370), 16% (60/367), 20% (61/305), 21% (53/247), and 18% (24/132) at 6 months and 1, 3, 5, and 10 years, respectively (Fig. 1). In patients with genotype A, the response rate was highest at 6 months post-treatment and the rate was roughly equivalent to the 6 months post-treatment rate at subsequent time points from 1 to 10 years. Response rates in patients with genotype B in particular were above 40% at all time points except at 6 months, although few patients had genotype B. On the other hand, response rates in patients with genotype C were under 20% at all time points (Fig. 2a).

In addition, multivariate analyses in HBeAg-positive patients also revealed several parameters that independently influenced the outcome of IFN therapy–at 6 months: duration of treatment ($P = 0.001$) and age ($P = 0.014$); at 1 year: age ($P = 0.011$) and HBV DNA level ($P = 0.027$); at 3 years: sex ($P = 0.008$), duration of treatment ($P = 0.019$), age ($P = 0.020$), pretreatment with IFN ($P = 0.029$), and albumin level ($P = 0.043$); at 5 years: sex ($P = 0.002$) and pretreatment with IFN ($P = 0.005$); and at 10 years, genotype ($P = 0.019$) and AST ($P = 0.035$) (Table 3).

Response to interferon therapy and evaluation of efficacy of IFN in relation to clinical factors in HBeAg-negative patients

Response rates in baseline HBeAg-negative patients were 23% (29/127), 21% (26/124), 24% (29/123), 26% (29/112), and 34% (35/103) at 6 months and 1, 3, 5, and 10 years, respectively (Fig. 1). Rates in patients with genotype C were gradually increased at subsequent time points, whereas those in patients with genotype B remained under 30% at all time points (Fig. 2b).

In addition, univariate and multivariate analyses in HBeAg-negative patients revealed that duration of treatment (≥1 year) independently influenced the outcome of

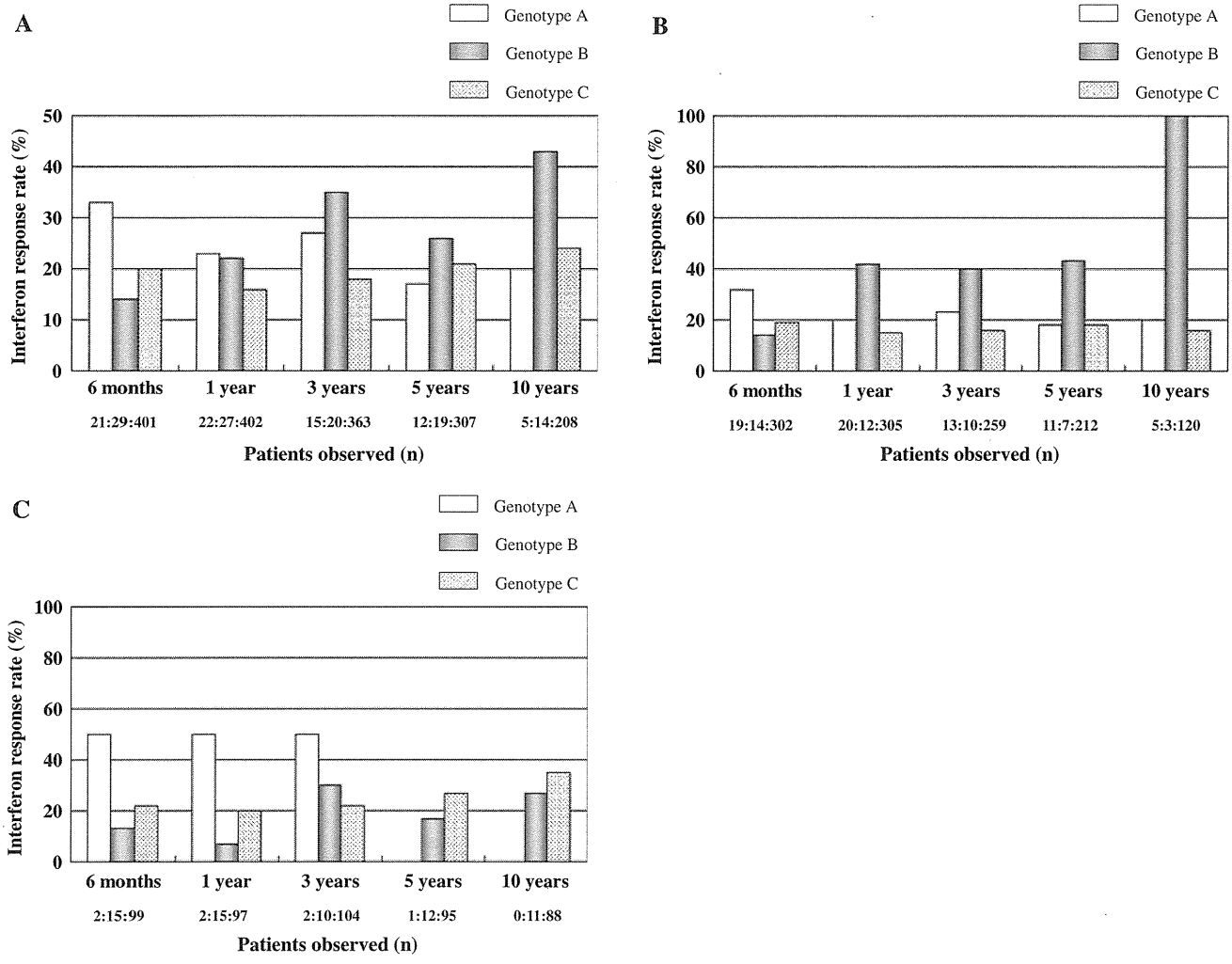

Fig. 2 Interferon response rates of patients with genotypes A, B, and C at 6 months and 1, 3, 5, and 10 years. **a** All patients, **b** HBeAg-positive patients, **c** HBeAg-negative patients

IFN therapy at 6 months, and at 1 and 3 years. No parameters independently influenced the outcome of IFN therapy at 5 or 10 years.

Evaluation of efficacy of IFN in relation to HBs antigen seroclearance

The HBsAg seroclearance rate in this study was obtained from patients who received IFN therapy alone; 69 of 615 patients (11%) achieved seroclearance of HBsAg. The cumulative HBsAg seroclearance rates in all patients from the commencement date of IFN therapy were 6.5% at 5 years, 15% at 10 years, 35% at 15 years, and 44% at 20 years (Kaplan–Meier method; Fig. 3a). No patients experienced the reappearance of HBsAg after seroclearance. Five factors found to be associated with achievement of HBsAg seroclearance on univariate analysis were: male sex ($P = 0.002$), age $\geq$30 years ($P = 0.011$), genotype A ($P = 0.038$), HBeAg-negativity ($P = 0.045$), and bilirubin $\leq$1.0 mg/dL ($P = 0.064$). On multivariate analysis, independent factors predicting the achievement of HBsAg seroclearance were: age $\geq$30 years, genotype A, and male sex (Table 4). The cumulative HBsAg seroclearance rate for genotype A patients was significantly higher than the rate for those with genotypes B or C ($P = 0.0116$) (Fig. 3b).

Relationship between the response to IFN and the development of hepatocellular carcinoma

Twenty-nine patients developed hepatocellular carcinoma (HCC) during the observation period, excluding 17 patients who received other additional therapies after the completion of IFN therapy and developed HCC thereafter. IFN response rates in the 29 patients who developed HCC were 5% (1/22), 5% (1/20), 10% (2/20), 13% (2/15), and 13% (2/16), respectively, at 6 months and 1, 3, 5, and 10 years after the completion of IFN. No patient developed HCC after HBsAg seroclearance.

Table 2 Factors associated with response to interferon therapy for all patients at 6 months and 1, 3, 5, and 10 years

Parameter	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*P*	OR (95% CI)	*P*
6 Months after completion of IFN therapy ($n = 229$)				
Duration of treatment (≥1 year)	2.680 (1.724–4.166)	<0.001	2.107 (1.058–4.198)	0.034
HBV DNA level (≤7.0 log copies/mL)	2.165 (1.107–4.219)	0.026	2.309 (1.148–4.630)	0.019
Age (<30 years)		0.057	2.451 (1.209–4.950)	0.013
1 year after completion of IFN therapy ($n = 231$)				
Duration of treatment (≥1 year)	2.553 (1.588–4.104)	<0.001		
HBV DNA level (≤7.0 log copies/mL)	3.268 (1.597–6.667)	0.001	4.464 (2.058–9.709)	<0.001
Age (<35 years)	1.799 (1.125–2.874)	0.014	3.831 (1.718–8.547)	0.001
3 years after completion of IFN therapy ($n = 397$)				
Duration of treatment (≥1 year)	2.410 (1.495–3.885)	<0.001	2.739 (1.618–4.634)	<0.001
Age (<30 years)	2.070 (1.215–3.521)	0.009	2.110 (1.171–3.802)	0.013
Albumin (≥3.9 g/dL)	1.697 (1.045–2.757)	0.030	2.009 (1.158–3.486)	0.013
Genotype (non-C)	2.155 (1.033–4.504)	0.041		
5 years after completion of IFN therapy ($n = 356$)				
Albumin (≥3.9 g/dL)	1.869 (1.108–3.153)	0.017	2.321 (1.316–4.093)	0.004
Pretreatment with IFN (positive)	1.770 (1.016–3.084)	0.048	1.821 (1.029–3.222)	0.039
Sex (female)		0.060	2.381 (1.297–4.367)	0.005
Duration of treatment (≥1 year)		0.080		
10 years after completion of IFN therapy ($n = 234$)				
HBeAg (negative)	2.315 (1.269–4.219)	0.006	2.252 (1.230–4.115)	0.009
ALT (≥100 IU/L)	1.972 (1.053–3.690)	0.036		
Pretreatment with IFN (positive)		0.058		

ALT alanine transaminase, *IFN* interferon, *HBV* hepatitis B virus, *HBeAg* hepatitis B e antigen, *CI* confidence interval, *OR* odds ratio, *n* number submitted to multivariate analysis, including all factors found to be associated with response to IFN therapy

Discussion

Although IFN has been reported to exert beneficial effects in CHB patients, the response rate is not high. A meta-analysis published in 1993 reviewed 15 randomized controlled studies involving 837 adult patients who received IFN-α for 4–6 months, and elimination of HBeAg occurred in 33% of the treated patients [8]. In previous studies, we found the response rates among HBeAg-positive patients at 6 months after the completion of therapy to be 20 and 31% for 6 months and 1 year of IFN therapy, respectively [13, 15]. Although a recent meta-analysis reported that IFN increased the incidence of HBeAg and HBsAg seroclearance after long-term follow up of 3–7 years [12], the factors that influenced the clinical outcome were unclear.

In Japan, from 1988, 4-week IFN treatment was reimbursed by the healthcare system, and since 2002, 24-week IFN treatment has been conducted. In the present study, these two regimens were the major ones, and other regimens were used in clinical studies at our hospital (including previously reported studies [14, 15]). Although the durations of treatment differed, we analyzed the factors associated with long-term response to IFN therapy, including the factor of duration of treatment.

In the present study, response rates were low among HBeAg-positive patients and relatively high among HBeAg-negative patients at all time points. Approximately 20% of the HBeAg-positive patients had sustained a response at 6 months to 10 years of follow up. Long-term follow-up studies after a four- to six-month course of IFN therapy in HBeAg-positive patients in European and Taiwanese studies showed higher (33–75%) response rates (HBeAg loss) than our study [7, 19, 20]. The difference in response rates between our present study and previous studies in other countries may be due to differences in ethnicity or HBV genotype (mainly genotype C in Japan). Moreover, the low IFN response rates at 1, 3, 5, and 10 years in the HBeAg-positive patients in our study were likely due to the change in treatments (IFN or nucleoside/nucleotide analogues). On the other hand, the response rates of HBeAg-negative patients in the present study were about 20% at 6 months and gradually increased thereafter. The sustained response rate in HBeAg-negative patients was usually <30% in European studies [21–23]. The response

Table 3 Factors associated with response to interferon therapy for HBeAg-positive patients at 6 months and 1, 3, 5, and 10 years

Parameter	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*P*	OR (95% CI)	*P*
6 months after completion of IFN therapy ($n = 279$)				
Duration of treatment (≥1 year)	2.449 (1.457–4.114)	0.001	2.801 (1.540–5.096)	0.001
Age (<35 years)	1.855 (1.112–3.096)	0.017	2.128 (1.164–3.891)	0.014
1 year after completion of IFN therapy ($n = 172$)				
Duration of treatment (≥1 year)	2.483 (1.407–4.380)	0.002		
HBV DNA level (≤7.0 log copies/mL)	3.509 (1.495–8.264)	0.005	3.003 (1.130–7.937)	0.027
Age (<35 years)	1.996 (1.133–3.521)	0.015	3.610 (1.351–9.615)	0.011
3 years after completion of IFN therapy ($n = 283$)				
Age (<35 years)	2.041 (1.155–3.597)	0.013	2.083 (1.122–3.861)	0.020
Duration of treatment (≥1 year)	2.055 (1.153–3.661)	0.016	2.130 (1.132–4.008)	0.019
Pretreatment with IFN (positive)	2.054 (1.050–4.019)	0.041	2.336 (1.091–4.998)	0.029
Albumin (≥3.9 g/dL)		0.055	1.974 (1.020–3.820)	0.043
Sex (female)		0.089	2.646 (1.284–5.464)	0.008
5 years after completion of IFN therapy ($n = 247$)				
Sex (female)	2.571 (1.328–4.975)	0.006	2.924 (1.477–5.814)	0.002
Pretreatment with IFN (positive)	2.460 (1.213–4.988)	0.015	2.870 (1.377–5.980)	0.005
10 years after completion of IFN therapy ($n = 122$)				
Genotype (non-C)	5.319 (1.222–23.26)	0.032	6.410 (1.364–30.30)	0.019
AST (≥100 IU/L)		0.081	2.932 (1.078–7.972)	0.035

AST aspartate transaminase, *IFN* interferon, *HBV* hepatitis B virus, *HBeAg* hepatitis B e antigen, *CI* confidence interval, *OR* odds ratio, *n* number submitted to multivariate analysis, including all factors found to be associated with response to IFN therapy

rates of HBeAg-negative patients in our present study and the studies in other countries [21–23] were similar.

Few reports have identified the factors associated with long-term virological response to IFN therapy. In our present study, HBeAg-negativity was the most important factor for predicting a long-term response (10 years). While the HBV DNA level was important for predicting the response at 6 months and 1 year for all patients and the response at 1 year for HBeAg-positive patients, other factors (age, sex, albumin level, AST, IFN pretreatment, and duration of treatment) were found to be important at some time points for all patients and for HBeAg-positive patients. The HBV DNA level may not have been associated with long-term response to IFN therapy because the follow-up period (median 5.7 years) in patients with an HBV DNA level measurable with commercial kits was significantly shorter than that in the other patients (median 11.2 years; $P < 0.001$).

Previous studies have reported that high ALT levels, low HBV DNA level, female sex, and elevated liver activity and level of fibrosis on liver biopsy were major pretreatment factors correlated with a response to IFN [8–11, 24]. However, in these studies the follow-up times for judging the response were short (typically 6 months to 1 year). Our present study has clarified that HBeAg, HBV DNA level, age, sex, IFN pretreatment, duration of treatment, and levels of albumin and AST are important factors in the long-term response to IFN. Further, non-C genotype was an important factor for long-term response in HBeAg-positive patients. Kao et al. [25] and Lin et al. [20] reported that HBV genotype B was associated with a higher response rate to IFN-α therapy than genotype C among CHB patients positive for HBeAg. Similarly, response rates among HBeAg-positive patients with genotype B in the present study were also higher than the response rates in those with genotype C in terms of long-term response (Fig. 2b). The long-term response rate among HBeAg-negative patients was relatively higher than that in HBeAg-positive patients. Previous reports have shown that response rates to a 6- to 12-month course of IFN-α in HBeAg-negative CHB patients range from 10 to 47% (average 24%) [26–29]. In addition, our previous report showed that 9 of 12 (75%) patients who received IFN-β twice per week for 24 weeks responded to the therapy [14]. However, the follow-up periods of these studies were short, and the long-term efficacy has not been clarified. While the efficacy of IFN in HBeAg-negative patients was high in the present study, the factors that might be useful in predicting a sustained response were less well-defined than those in HBeAg-positive patients, as previously reported [5].

A meta-analysis of IFN therapy published in 2010 reviewed 6 clinical controlled studies including 828 patients who received IFN [12]. The duration of follow-up

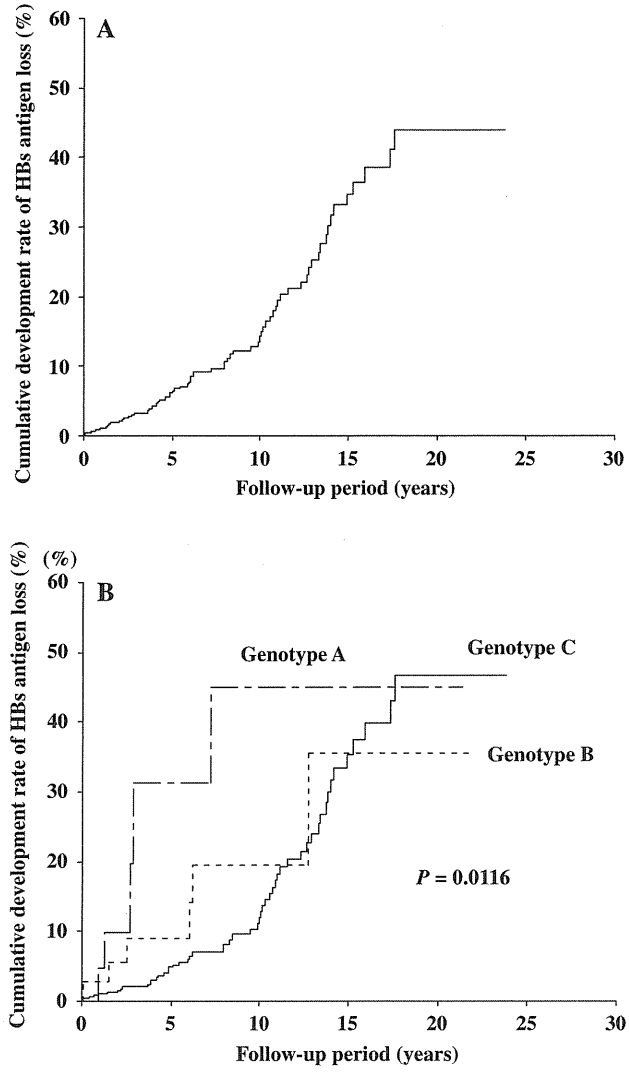

Fig. 3 Cumulative clearance of hepatitis B surface (*HBs*) antigen in patients treated with interferon (Kaplan–Meier method). **a** All patients, **b** patients stratified by genotypes A, B, and C

Table 4 Factors associated with HBsAg seroclearance by interferon therapy, determined by multivariate analysis

Parameter	Category	Hazard ratio	95% CI	*P*
Age	<30 years	1		0.002
	≥30 years	4.433	1.703–11.538	
Genotype	A	1		0.004
	B	0.296	0.087–1.005	
	C	0.199	0.075–0.528	
Sex	Female	1		0.005
	Male	2.962	1.387–6.327	

HBsAg hepatitis B surface antigen, *CI* confidence interval

ranged from 35.8 months to 7 years, and HBsAg seroclearance occurred in 9.5% (79/828). In the present study, we observed HBsAg seroclearance in 69 of 615 (11%) patients, with a median follow-up duration of 8.1 years. However, few reports have investigated factors predicting the achievement of HBsAg seroclearance. In our study, important factors for achieving HBsAg seroclearance were age ≥30 years, genotype A, and male sex. Patients with genotype A had primarily been infected during adulthood via sexual contact, and the average duration of infection was relatively short. In contrast, most Japanese carriers are infected perinatally and possess HBV genotype C, and therefore the efficacy of IFN therapy for patients with genotype C may be low. Male sex was also an important factor in determining potential to achieve HBsAg seroclearance, although female sex was an important factor in determining long-term response to IFN therapy. In our previous study of HBsAg seroclearance (mainly spontaneous seroclearance), we found that response rates were low among females (19%; 45/231) [30]. These present and previous findings indicate that male patients tended to achieve HBsAg seroclearance more frequently than females, although the reason is unclear. We previously reported that Kaplan–Meier analysis in 486 patients who received lamivudine therapy for 5 and 10 years showed an estimated loss of HBsAg in 3 and 13% of the patients, respectively, [31]. The cumulative clearance rates of HBsAg, also determined by Kaplan–Meier analysis, in patients treated with IFN were higher than those in the patients treated with lamivudine, albeit that there were differences in the baseline characteristics of the patients at the commencement of the respective therapies. The effects of IFN therapy in modulating the host immune response might induce HBsAg clearance.

In conclusion, we investigated the long-term efficacy of IFN therapy in Japanese CHB patients. Response rates were low among HBeAg-positive patients and relatively high among HBeAg-negative patients at all time points examined. HBeAg-negative status, HBV DNA level, age, sex, pretreatment with IFN, duration of treatment, and levels of albumin and AST were important factors in predicting long-term response for all patients and for HBeAg-positive patients. Age, genotype, and sex were important factors in predicting ability to achieve HBsAg seroclearance. Further studies exploring the efficacy of therapy over a longer duration may be necessary to confirm these findings and establish true response rates to IFN therapy, including treatment with pegylated IFN.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Determinants of the clinical outcome of patients with severe acute exacerbation of chronic hepatitis B virus infection

Nami Mori · Fumitaka Suzuki · Yusuke Kawamura · Hitomi Sezaki · Tetsuya Hosaka · Norio Akuta · Masahiro Kobayashi · Satoshi Saito · Yoshiyuki Suzuki · Yasuji Arase · Kenji Ikeda · Mariko Kobayashi · Hiromitsu Kumada

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Abstract
Background Severe acute exacerbation of chronic hepatitis B can sometimes occur and lead to hepatic failure and death. The objective of this study was to elucidate the predictors of progression to hepatic decompensation during severe acute exacerbation.
Methods We prospectively analyzed 37 consecutive patients with acute exacerbation of chronic hepatitis B (accompanied by jaundice and coagulopathy) for clinical outcome and factors that influenced the development of severe acute exacerbation, including viral kinetics.
Results Fourteen (37.8%) patients progressed to severe acute exacerbation (accompanied by encephalopathy). Multivariate analysis identified serum bilirubin (>5 mg/dl, $P = 0.002$) as a significant determinant of progression to hepatic failure and prothrombin activity (<45%, $P = 0.028$) and as a determinant of liver-related death. The hepatitis B virus (HBV) DNA level before therapy was measured in 25 patients. HBV DNA levels increased or did not change from before commencement of treatment in all 11 patients who progressed to severe acute exacerbation. On the other hand, HBV DNA levels did not change or increased in 8 of 14 patients (57%) with acute exacerbation ($P = 0.02$).
Conclusions Serum bilirubin and prothrombin activities were significant predictors of clinical outcome in patients with severe acute exacerbation of chronic hepatitis B. Viral kinetics until commencement of therapy can predict the severity of acute exacerbation of chronic hepatitis B.

Keywords Hepatitis B · Acute exacerbation · HBV DNA · Genotype · Encephalopathy

Abbreviations

AE	Acute exacerbation
ALT	Alanine aminotransferase
BCP	Basal core promoter
CS	Corticosteroid
HBV	Hepatitis B virus
IFN	Interferon
LMV	Lamivudine
NA	Nucleos(t)ide analogue
PC	Pre-core
PT	Prothrombin activity
SAE	Severe acute exacerbation

Introduction

More than 3 billion people worldwide and approximately 1.5 million people in Japan are chronically infected with hepatitis B virus (HBV), and chronic HBV infection is one of the most common causes of chronic hepatic failure and hepatocellular carcinoma (HCC) [1, 2]. Other complications of HBV infection include fulminant hepatitis and acute liver failure [3, 4]. Acute exacerbation (AE) in HBV carriers occurs either through a natural course [5, 6] or following intensive chemotherapy or immunosuppressive

N. Mori · F. Suzuki (✉) · Y. Kawamura · H. Sezaki · T. Hosaka · N. Akuta · M. Kobayashi · S. Saito · Y. Suzuki · Y. Arase · K. Ikeda · H. Kumada
Department of Hepatology, Toranomon Hospital, 2-2-2 Toranomon, Minato-ku, Tokyo 105-8470, Japan
e-mail: fumitakas@toranomon.gr.jp

M. Kobayashi
Research Institute for Hepatology, Toranomon Hospital, Tokyo, Japan

therapy [7, 8]. Some abrupt flares may be so severe that decompensation or even fulminant hepatic failure may occur [9–11]. Previous studies have identified pre-existing cirrhosis, high serum bilirubin levels, prolonged prothrombin time, pre-core/core promoter mutants, and high HBV DNA levels as factors associated with hepatic decompensation during AE in HBV carriers, though little is known about the predictive factors [9, 12, 13].

Liver transplantation is suitable therapy for acute hepatic failure, but the rate of liver transplantation has remained about 20% in Japan, where living donor liver transplantation is dominant [14, 15]. Thus, it is necessary to establish other effective therapies for patients with AE apart from liver transplantation. Steroids can rapidly inhibit excessive immune response and inflammatory reactions, and have been reported to be effective in cases of severe and potentially life-threatening exacerbation of chronic HBV (CHB) infection [16]. With the advent of oral nucleos(t)ide analogues (NAs), most guidelines recommend NAs for patients with AE of CHB infection [17–19], and several observational studies reported the use of NAs [9–11, 20, 21]. Timely use of potent anti-HBV agents, such as NAs, interferon (IFN), and steroids [22], during and/or after the development of hepatic decompensation could be potentially effective against various host- and virus-related factors.

The aim of the present study was to investigate the factor(s) that influence the rapid development of hepatic decompensation during AE of CHB.

Materials and Methods

Patients

The study subjects were patients with AE admitted to the Department of Hepatology, Toranomon Hospital, Tokyo, between 1984 and 2010. All patients were either followed up at our hospital with clinicopathologically proven CHB infection or were new patients with sudden-onset hepatic flares who visited our hospital outpatient clinic or were referred to our hospital from other clinics/hospitals. The diagnosis of CHB carrier state was established based on either positivity for hepatitis B surface antigen (HBsAg) for at least 6 months prior to the development of AE, or the presence of a high titer of anti-hepatitis B core antibodies (anti-HBcAb), together with negativity or a low titer of IgM anti-HBcAb. Chronic hepatitis and cirrhosis were confirmed by laparoscopy, needle biopsy, or ultrasonography, or treatment for these conditions for 1 year before the development of AE. AE of CHB infection was diagnosed by the following criteria: (1) an abrupt increase in serum alanine aminotransferase (ALT) levels to >300 IU/l in patients with original ALT levels of less than 5× the upper limit of normal or an abrupt two-fold increase in the serum ALT level to greater than 5× the upper limit of normal, (2) hyperbilirubinemia [serum bilirubin (Bil) >3.0 mg/dl], (3) evidence of coagulopathy with plasma prothrombin activity (PT) of <60% during the clinical course, and (4) lack of encephalopathy at admission. We also applied the following exclusion criteria: (1) the presence of viral markers other than HBV (hepatitis A, C, D, E, Epstein-Barr virus, cytomegalovirus, herpes simplex virus), (2) HBV reactivation induced by immunomodulators or chemo-/immunosuppressive therapy, (3) asymptomatic HBV carriers, (4) recent exposure to drugs and chemical agents as well as recent heavy alcohol intake, (5) breakthrough hepatitis caused by NAs, (6) evidence of decompensated liver disease before the onset of exacerbation as characterized previously, (7) HCC diagnosed by ultrasonography or computed tomography, and (8) coexistence of other serious medical conditions and other liver diseases, or metabolic diseases. Progression to severe acute exacerbation (SAE) was diagnosed by the development of hepatic encephalopathy of more than grade 2 within 8 weeks of onset associated with coagulopathy (PT <40%).

HBV DNA levels were measured serially to investigate the effects of HBV kinetics on the prognosis of patients with severe AE. HBV DNA levels were measured before treatment in 25 patients. "Before treatment" represented 1–8 weeks before commencement of treatment. HBV DNA levels were also measured after treatment in 27 patients. "After treatment" was defined as 2 weeks after commencement of therapy. Viral kinetics was assessed using the same assay in all individuals. The Local Ethics Committee of Toranomon Hospital approved the study, and informed consent was obtained from all patients.

Virological markers

Serial blood samples were obtained during the clinical course of AE and stored at −80°C until used for HBV molecular analysis. Serological tests for HBsAg, HBsAb, hepatitis e antigen (HBeAg), IgM anti-HBcAb, total anti-HBcAb, and anti-HBeAb were conducted using radioimmunoassay kits (Abbot Diagnostics, Chicago, IL, USA) according to the instructions provided by the manufacturer. Precore (PC) mutations were analyzed by PCR enzyme-linked mini-sequence assay (Roche Diagnostics, Tokyo, Japan), and basal core promoter (BCP) mutations were analyzed by PCR specific probe assay (Roche Diagnostics, Tokyo, Japan). HBV DNA was measured by Amplicor monitor assay (dynamic range 2.6–7.6 log copies/ml, Roche Diagnostics, Tokyo, Japan), COBAS TaqMan v.2.0 (dynamic range 2.1–9.0 log copies/ml, Roche Diagnostics), transcription-mediated amplification and hybridization

protect assay (TMA-HPA) (dynamic range 3.7–8.7 LGE/ml, Chugai Diagnostics Science Co., Tokyo) or sandwich hybridization assay with signal amplification using branched DNA (bDNA, dynamic range 0.7–3800 Meq/ml). The major genotype of HBV was determined using enzyme-linked immunosorbent assay (ELISA, Institute of Immunology, Tokyo, Japan) or PCR-invader assay (BML, Inc, Tokyo, Japan) based on the methods described previously [23, 24]. HBVDNA levels assessed by bDNA were re-measured by TaqMan PCR assay using stored serum samples.

Statistical analysis

Continuous variables were expressed as median (range), and compared by Mann–Whitney *U* test. Categorical variables were compared by χ^2 test or Fisher's exact test, as appropriate. Univariate analysis was applied to determine the relationship between SAE and each of the following factors: sex, age, presence of compensated cirrhosis, and various biological and virological markers as measured at baseline (bilirubin, PT, ALT, albumin, HBeAg, HBV DNA, and HBV genotype, PC and BCP mutations). Each continuous variable was transformed into two categories based on the value with the largest capacity to discriminate between patients for univariate and multivariate analyses. Factors that correlated significantly with SAE were entered into multiple logistic regression analysis, and the odds ratio (OR) with 95% confidence intervals (95% CI) were determined. All analyses were performed using The Statistical Package for Social Sciences (SPSS II v. 11.0, Chicago, IL, USA), and statistical significance was taken as a two-sided *P* value <0.05.

Results

Clinical features of severe acute exacerbation

A total of 37 patients (30 men and 7 women) fulfilled the criteria of AE and were included in this study. The baseline characteristics at the commencement of therapy of these 37 patients are shown in Table 1. Twenty-two patients were observed at our hospital, and 15 patients were referred from another hospital after the onset of hepatic flares. The majority of patients had genotype C, and 27 patients (72.9%) were HBeAg positive. The PC and BCP mutations were determined in 27 patients; 22 patients had mutations in the PC region, 16 patients had mutations in the BCP region, and 12 patients had mutations in both the PC and BCP regions. During the clinical course, the peak median values were: ALT 713 IU/l (range 307–2857), bilirubin 8.4 mg/dl (3.0–51.4), and PT 47.6% (12.0–60.0).

Table 1 Baseline characteristics of the 37 patients infected with HBV who developed severe acute exacerbation at the commencement of therapy

Number	37
Sex (male/female)	30/7
Age (years)	45 (23–63)
Family history (yes/no)	21/16
Cirrhosis (present/absent)	7/30
Albumin (g/dl)	3.4 (2.5–4.6)
Bilirubin (mg/dl)	4.7 (1.0–30.7)
AST (IU/l)	601 (64–2593)
ALT (IU/l)	657 (124–2142)
LDH (IU/l)	297 (106–594)
Platelets ($\times 10^4/mm^3$)	12.3 (6.2–32.0)
α-Fetoprotein (μg/ml)	62.0 (3.0–1600)
Prothrombin activity (%)	53 (26–80)
Genotype (A/B/C)	0/5/32
HBeAg (positive/negative)	27/10
HBV-DNA ($\log_{10}$ copies/ml)	8.5 (6.8–8.9)
PC (wild/mutant/ND)	5/22/10
BCP (wild/mutant/ND)	11/16/10

Data are median values (range) or number of patients

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *LDH* lactate dehydrogenase, *HBeAg* hepatitis B envelope antigen, *PC* pre core, *BCP* basal core promoter, *ND* not done

Treatment

NAs were used in 19 patients, IFN in 8, and corticosteroids (CS) in 20 patients. In addition, 7 patients were treated with a combination of NAs and CS; 2 patients were treated with three drugs (NAs, IFN, and CS). At the time of the study, lamivudine (LMV) was not yet available for the treatment of chronic hepatitis B, and thus IFN was used; 6 patients were treated with both IFN and CS. None of the patients underwent liver transplantation.

Prognosis of severe acute exacerbation and factors associated with progression to hepatic failure

Of the 37 patients admitted with CHB infection and AE, 23 (62.2%) did not develop SAE. The remaining 14 (37.8%) patients developed SAE; 9 (24.3%) patients died of liver-related death, but 5 (13.5%) survived. Further analysis showed that 8 (36.4%) of 22 patients who were observed in our hospital developed AE, and 6 (27.3%) of these patients died, whereas 6 (40.0%) of 15 patients who were referred from other hospitals after the onset of exacerbation developed AE, and 3 (20.0%) of these patients died. There was no significant difference in prognosis by treatment facility before AE. Ten of 37 patients experienced AE before 2000 when LMV was available in Japan, and 19

Table 2 Biochemical, virological and histological features of patients with severe acute exacerbation at the commencement of therapy

Case	Age (years)/ sex	Genotype	HBeAg	HBV-DNA (log copies/ ml)	Preexisting cirrhosis	Serum bilirubin (mg/dl)	ALT (IU/l)	PT (%)	Platelets ($\times 10^4$/ mm^3)	Therapy	Outcome (time from treatment to death, weeks)
1	63/M	B	−	8.4	No	5.8	1680	43	6.2	LMV + CS	Death (11)
2	32/M	B	−	>8.7	No	6.9	1340	41	13.4	CS	Death (1)
3	58/M	B	−	8.6	No	7.4	1446	36	7.7	CS	Death (2)
4	29/M	B	−	>8.7	No	15.6	307	26	10.0	LMV	Recovery (alive)
5	54/F	C	+	>8.7	No	2.4	2077	79	21.0	LMV + CS	Recovery (alive)
6	37/M	C	+	>8.7	No	4.1	552	53	8.9	CS	Recovery (alive)
7	62/M	C	+	7.0	No	12.0	220	53	7.1	LMV + CS + IFN	Recovery (alive)
8	33/F	C	+	>8.7	No	14.0	632	39	13.1	CS	Recovery (alive)
9	55/M	C	+	>8.7	Yes	4.0	1089	55	10.3	LMV + CS	Death (1)
10	37/F	C	+	7.1	Yes	5.8	1444	34	22.0	LMV + CS + IFN	Death (10)
11	49/M	C	+	8.0	Yes	8.8	834	58	9.9	CS	Death (10)
12	33/M	C	+	8.5	No	9.6	657	26	7.4	LMV + CS	Death (2)
13	54/M	C	+	7.8	Yes	12.1	364	36	15.8	LMV + CS	Death (2)
14	55/M	C	+	>8.7	No	24.2	520	44	8.3	CS	Death (5)

Abbreviations as in Table 1, *PT* prothrombin activity, *LMV* lamivudine, *CS* corticosteroids, *IFN* interferon-α

patients experienced AE after 2000. The other 8 patients experienced AE before 2000, but received LMV through participation in clinical trials or paid for the drug privately. The clinical features at the commencement of therapy of 14 patients who developed SAE are shown in Table 2 (median age 52 years, range 29–63). The mean time period between admission and death of 9 patients who developed SAE was 2 (range 1–11) weeks. Six patients who were admitted before the availability of LMV were treated with CS alone, 5 patients were treated with the combination of LMV and CS, 1 patient was treated with LMV alone, and 2 other patients were treated with LMV, CS, and IFN. Among 8 patients treated with LMV, of those who developed SAE, 5 died, and 2 patients developed complications caused by bacterial infection. Four patients had genotype B, while 10 patients had genotype C. HBeAg status was positive in 10 patients. The mean HBV DNA level was 8.7 (range 7.0–>8.7) log copies/ml, ALT 746 (220–2077) IU/l, serum bilirubin 8.1 (2.4–24.2) mg/dl, PT 42 (26–79)%, and platelet count was 10.0 (62–220) $\times$ $10^4/mm^3$.

Of the 5 patients who were treated successfully after progression to SAE, one later died of severe breakthrough hepatitis caused by emergence of LMV-resistant virus 3 years after SAE (case 7, Table 2). The other four survived (cases 4–6 and 8, Table 2).

Table 3 shows the results of univariate analysis. The following factors showed significant relationship with the development of SAE at the commencement of treatment: serum bilirubin (>5 mg/dl) and PT (<60%). Multivariate analysis identified serum bilirubin as a significant and independent determinant of the development of SAE (Table 3). On the other hand, two parameters showed significant relationships with liver-related death: serum bilirubin (>7 mg/dl, $P = 0.049$) and PT (<45%, $P = 0.003$). Multivariate analysis identified PT (OR 9.50, 95% CI 1.3–71.0, $P = 0.028$) as a significant determinant of death.

Viral kinetics associated with fulminant hepatic failure

To investigate the relationship between viral kinetics and SAE, HBV DNA levels were measured in 25 patients both before and commencement of treatment and also after treatment in 27 patients. Figure 1 shows the viral load of patients who developed and did not develop SAE at commencement of treatment compared with before treatment. Falls in the HBV DNA level occurred naturally. However, in 11 patients who developed SAE, HBV DNA levels increased in 6 patients and did not change in 5 patients. Among the latter 5, HBV DNA levels of 4 patients were >8.7 log copies/ml. In 14 patients who did not develop SAE, HBV DNA levels increased in 4 patients, were unchanged in 4 patients, and decreased in 6 patients. Hence, the HBV DNA level increased/was unchanged in 8 of 14 (57%) patients who did not develop SAE, compared with 11 of 11 (100%) patients who developed SAE. A significantly higher proportion of patients with SAE showed an increase/was unchanged in viral load compared to those who without SAE ($P = 0.02$). We also examined the viral kinetics in 27 patients by comparing HBV DNA levels at the commencement of treatment to after treatment.

Table 3 Univariate and multivariate analyses of host and viral factors associated with progression of severe acute exacerbation at commencement of treatment

Parameter	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Sex (female)	1.30 (0.15–4.11)	0.76		
Age (>55 years)	2.64 (0.57–12.3)	0.22		
Cirrhosis (present)	1.90 (0.39–9.26)	0.43		
Albumin (<3.5 g/dl)	1.75 (0.44–6.97)	0.85		
Bilirubin (>5 g/dl)	17.0 (2.92–99.1)	0.002	11.2 (1.71–73.8)	0.01
ALT (>800 IU/l)	1.88 (0.48–7.26)	0.36		
AST/ALT ratio (>1)	1.27 (0.31–5.19)	0.74		
Prothrombin activity (<60%)	11.9 (1.33–106.7)	0.03	8.22 (0.73–92.6)	0.09
Platelets (<15 × 10^4/mm^3)	0.81 (0.19–3.58)	0.89		
Genotype (B)	8.82 (0.87–89.1)	0.06		
HBeAg (positive)	0.89 (0.20–3.90)	0.89		
HBV-DNA (>8.7 log copies/ml)	2.34 (0.60–9.20)	0.70		
PC mutation	2.29 (0.22–24.1)	0.49		
BCP mutation	0.19 (0.034–1.08)	0.06		

Abbreviations as in Tables 1 and 2, *OR* odds ratio, *CI* confidence level

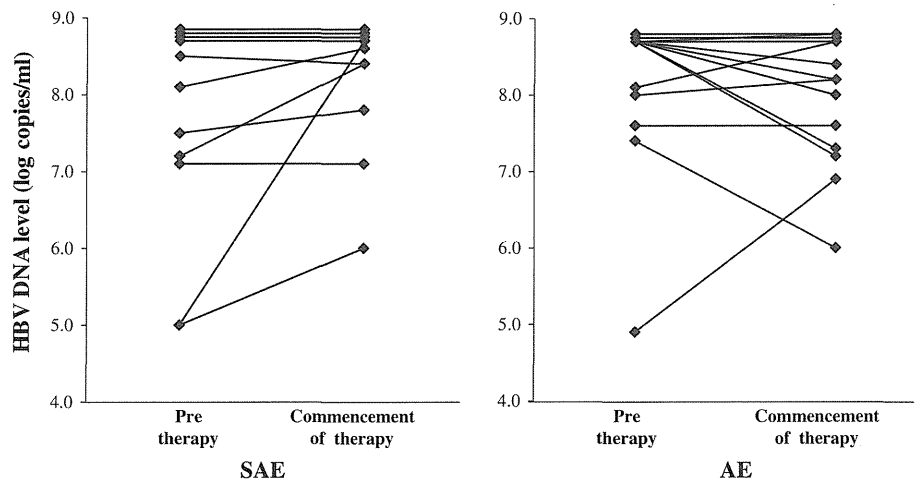

Fig. 1 Viral kinetics from pre-treatment to commencement of treatment in patients with acute exacerbation. Viral kinetics tended to increase or remained unchanged until treatment in 8 patients with acute exacerbation course ($n = 14$), while the viral load in all patients with severe acute exacerbation ($n = 11$) increased or remained unchanged ($P = 0.02$)

The HBV DNA level decreased more than 1 log copies/ml in 9 of 17 (52.9%) patients who did not develop SAE, compared with 3 of 10 (30.0%) patients who developed SAE, but the difference between the two groups was not significant.

Discussion

The results of the present study examined the predicting factors of progression to SAE accompanied by coagulopathy and encephalopathy in patients with AE of chronic hepatitis B, as well as the pattern of viral kinetics before and after commencement of therapy. Up to 30% of patients with CHB infection experience reactivation of hepatitis every year [5, 6], while some patients develop acute exacerbation with jaundice and coagulopathy, a severe life-threatening condition with high mortality [9, 12]. It is important to determine the predicting factors of progression to liver decompensation in patients with acute exacerbation. Multivariate analyses in previous studies indicated that pre-existing cirrhosis, a high Child–Pugh score, low albumin level, high serum bilirubin level, prolonged PT, and high HBV DNA levels were associated with the severity or mortality during acute exacerbation [9, 12, 13]. Our results are almost comparable to those of the above studies. Multivariate analysis in the present study identified the serum bilirubin level as a predictor of progression to liver decompensation. Moreover, there were no significant differences in viral load or therapeutic regimen. Genotype B was the predominant HBV strain in patients with SAE compared to patients with variable severity of liver diseases [25]. The frequencies of HBV genotype in patients with chronic hepatitis B admitted to our hospital were 3.0, 12.3, and 84.5%, for genotypes A, B, and C, respectively [26]. In the present study, although patients