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Ⅲ. 研究成果の刊行物・別刷

Original Article

Factors associated with the effect of interferon- α sequential therapy in order to discontinue nucleoside/nucleotide analog treatment in patients with chronic hepatitis B

Akihiro Matsumoto,¹ Hiroshi Yatsunami,² Shinya Nagaoka,² Yoshiyuki Suzuki,³ Tetsuya Hosaka,³ Masataka Tsuge,⁴ Kazuaki Chayama,⁴ Tatsuo Kanada,⁵ Osamu Yokosuka,⁵ Shuhei Nishiguchi,⁶ Masaki Saito,⁶ Shiho Miyase,⁷ Jong-Hon Kang,⁸ Noboru Shinkai,⁹ Yasuhito Tanaka,⁹ Takeji Umemura¹ and Eiji Tanaka¹

¹Department of Medicine, Shinshu University School of Medicine, Matsumoto, ²The Clinical Research Center, National Hospital Organization Nagasaki Medical Center, Omura, ³Department of Hepatology, Toranomon Hospital, Tokyo, ⁴Department of Gastroenterology and Metabolism, Applied Life Sciences, Institute of Biomedical and Health Sciences, Hiroshima University, Hiroshima, ⁵Department of Gastroenterology and Nephrology, Graduate School of Medicine, Chiba University, Chiba, ⁶Division of Hepatobiliary and Pancreatic Diseases, Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya, ⁷Department of Gastroenterology and Hepatology, Kumamoto Shinto General Hospital, Kumamoto, ⁸Center for Gastroenterology, Teine Keijinkai Hospital, Sapporo, and ⁹Department of Virology and Liver Unit, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan

Aim: The factors associated with the outcome of sequential therapy with interferon- α (IFN- α) in order to halt nucleoside/nucleotide analog (NUC) maintenance treatment for chronic hepatitis B were analyzed.

Methods: A total of 50 patients with chronic hepatitis B who underwent IFN- α sequential therapy for cessation of NUC were enrolled retrospectively. The subjects received NUC plus IFN- α for 4 weeks followed by IFN- α alone for 24 weeks. Natural IFN- α of 6-MU doses was administered three times a week. A successful response to NUC/IFN- α sequential therapy was defined as serum hepatitis B virus (HBV) DNA below 4.0 log copies/mL, serum alanine aminotransferase (ALT) below 30 IU/L, and hepatitis B e-antigen negativity at 24 months after completing the treatment.

Results: Multivariate analysis revealed that hepatitis B surface antigen (HBsAg) of 3.0 log U/mL or more ($P < 0.002$) and hepatitis B core-related antigen (hepatitis B core-related antigen [HBcrAg])

of 4.5 log U/mL or more ($P < 0.003$) at the start of IFN- α administration were significant factors associated with a 24-month non-response. Maximal levels of ALT and HBV DNA during the follow-up period after completing IFN- α therapy were significantly related ($P < 0.001$), and receiver-operator curve analysis showed that both maximal ALT ($P < 0.001$) and HBV DNA ($P < 0.001$) were significantly related to the final 24-month response.

Conclusion: The combinational use of HBsAg and HBcrAg levels may be useful to predict the 24-month outcome of NUC/IFN- α sequential therapy. Maximal levels of ALT and HBV DNA during post-treatment follow-up may also help monitor responses to IFN- α sequential therapy.

Key words: hepatitis B core-related antigen, hepatitis B surface antigen, interferon- α , nucleoside/nucleotide analogs, sequential therapy

INTRODUCTION

HEPATITIS B VIRUS (HBV) infection is a widespread health problem with an estimated 350–400 million carriers worldwide. Prolonged infection with HBV can

cause chronic hepatitis, which may eventually develop into liver cirrhosis and hepatocellular carcinoma (HCC).^{1–3} Currently available antiviral treatments for hepatitis B include nucleoside/nucleotide analogs (NUC) and interferon- α (IFN- α).⁴ NUC are p.o. administered and are associated with low rates of adverse effects. Although treatment with NUC, such as lamivudine (LVD), adefovir dipivoxil and entecavir (ETV), induces virological and biochemical responses in most patients, NUC therapy also carries the risk of drug resistance. Furthermore, patients with hepatitis B are required to undergo extended

Correspondence: Eiji Tanaka, Department of Medicine, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan.
Email: etanaka@shinshu-u.ac.jp
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treatment with NUC because early discontinuance often leads to relapse.^{5,6} In contrast, the remission of chronic hepatitis B by IFN- α is prolonged, but is achieved only in a small percentage of patients.

Serfaty *et al.*⁷ conducted a pilot study on sequential therapy using LVD and IFN- α and concluded that this treatment could induce a sustained virological response in patients with chronic hepatitis B who did not respond to IFN- α alone. However, ensuing reports⁸⁻¹² were unable to confirm such a cooperative effect. Because the clinical backgrounds of the enrolled patients also differed among the above reports, it has become necessary to clarify the factors associated with the outcome of IFN- α sequential therapy in order to estimate its clinical significance.

We previously analyzed patients with chronic hepatitis B who ceased NUC therapy and showed that lower hepatitis B surface antigen (HBsAg) and hepatitis B core-related antigen (HBcrAg) levels were associated with a favorable clinical outcome in subjects negative for hepatitis B e-antigen (HBeAg) and HBV DNA at NUC discontinuation.^{13,14} Although we identified patients in whom NUC could be safely halted with high reliance, such patients accounted for a relatively minor percentage. Therefore, we conducted the present study to analyze the effect of IFN- α sequential therapy on successfully stopping NUC.

This report retrospectively analyzes the factors associated with outcome of IFN- α sequential therapy following NUC treatment. As the subjects were followed long term, treatment responses at 24 months after stopping IFN- α were evaluated and compared with those at 6 and 12 months.

METHODS

Patients

A TOTAL OF 50 patients with chronic hepatitis B who underwent IFN- α sequential therapy in order to halt NUC therapy between May 2002 and September 2010 were enrolled. Subjects received NUC plus IFN- α for 4 weeks followed by IFN- α alone for 20 weeks (Fig. 1). Natural IFN- α (Sumiferon; Sumitomo Dainippon Pharma, Tokyo, USA) at a dose of 6 MU was administered three

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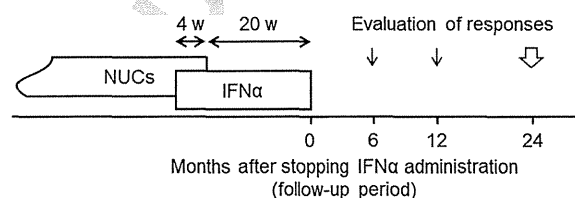


Figure 1 Experimental design of the present study. IFN, interferon; NUC, nucleoside/nucleotide analog; w, weeks.

times a week. Doses were reduced to 3 MU during exceptional circumstances, such as side-effects. All patients completed 24 weeks of IFN- α administration and received over 80% of the scheduled dose. Patients were recruited retrospectively from eight hospitals across Japan (Shinshu University Hospital, National Hospital Organization Nagasaki Medical Center, Toranomon Hospital, Hiroshima University Hospital, Chiba University Hospital, The Hospital of Hyogo College of Medicine, Kumamoto Shinto General Hospital, and Teine Keijinkai Hospital). The demographic data of the subjects are presented in Table 1. The median age at NUC cessation was 35 years. Approximately three-fourths of the patients were men. Genotype C HBV was predominant as has earlier been reported for Japan.¹⁵ Eighty-six percent of patients began NUC therapy with LVD and 14% did so with ETV. The duration of NUC administration ranged from 4 to 121 months. The follow-up period was defined as the point of stopping IFN- α administration up until the last visit or to when NUC were re-administered due to reactivation of hepatitis B. NUC were recommenced in 25 (50%) of the 50 patients enrolled. Among them, 17 were treated before judgment of the 24-month response to sequential therapy. All patients requiring re-administration

Table 1 Demographic data of 50 enrolled patients

Characteristic	Value
Age at start of NUC administration (years)†	34 (21–57)
Age at end of NUC administration (years)†	35 (22–62)
Sex (male: female)	38:12
Genotype (B:C: undetermined)	3:36:11
NUC at start (LVD: ETV)	43:7
NUC at end (LVD: ETV: LAM + ADV: ETV + ADV)	40:8:1:1
Duration of NUC administration (months)†	6 (4–121)
HBeAg positivity at start of NUC‡	70% (35/50)
HBeAg positivity at end of NUC‡	42% (21/50)
Follow-up period after stopping IFN- α administration (months)†	28 (2–102)
Patients requiring re-administration of NUC‡	50% (25/50)
Patients developing HCC‡	0% (0/50)

†Data are expressed as the median (range).

‡Data are expressed as a positive percentage (positive number/total number).

ADV, adefovir dipivoxil; ETV, entecavir; HBeAg, hepatitis B e-antigen; HCC, hepatocellular carcinoma; IFN, interferon; LAM, lamivudine; LVD, lamivudine; NUC, nucleoside/nucleotide analog.

of NUC possessed alanine aminotransferase (ALT) levels of over 80 IU/L and HBV DNA levels of over 5.8 log copies/mL at or just before the point of NUC re-continuation, which fulfilled the established requirements for restarting NUC.^{13,14,16}

Hepatitis B surface antigen was confirmed to be positive on at least two occasions at least 6 months apart in all patients before NUC treatment. Tests for hepatitis C virus and HIV antibodies were all negative. Patients complicated with HCC or signs of hepatic failure at the cessation of NUC administration were excluded from the study. No such complications were observed during follow up.

With few exceptions, patients were seen at least once a month during the first year of follow up, at least once every 3 months during the second year and at least once every 6 months afterwards. No patient developed HCC or hepatic failure during the follow-up period. Stored serum samples were kept frozen at -20°C or below until assayed. This study was approved by the ethics committees of all participating institutions (approval reference 1117 for Shinshu University Hospital, 24085 for National Hospital Organization Nagasaki Medical Center, 758 for Toranomon Hospital, 321 for Hiroshima University Hospital, 934 and 977 for Chiba University Hospital, 779 for The Hospital of Hyogo College of Medicine, 411 for Kumamoto Shinto General Hospital, and "Analysis of efficacy of IFN- to stop NUC in patients with chronic hepatitis B" for Teine Keijinkai Hospital).

Hepatitis B viral markers

Serological markers for HBV, including HBsAg, HBeAg and antibody to HBeAg, were tested using commercially available enzyme immunoassay kits (Abbott Japan, Tokyo, Japan; Fujirebio, Tokyo, Japan; and/or Sysmex, Kobe, Japan) at each hospital. Quantitative measurement of HBsAg¹⁷ was performed using a chemiluminescence enzyme immunoassay (CLEIA)-based HISCL HBsAg assay manufactured by Sysmex (Kobe, Japan). The assay had a quantitative range of $=1.5$ to 3.3 log IU/mL. End titer was determined by diluting samples with normal human serum when initial results exceeded the upper limit of the assay range.

Serum HBV DNA was determined using a COBAS TaqMan HBV kit (Roche, Tokyo, Japan)¹⁸ with a quantitative range of 2.1–9.0 log copies/mL. According to the manufacturer's instructions, detection of a positive signal below the quantitative range was described as a positive signal, and no signal detection was regarded as a negative signal. Six HBV genotypes (A–F) were evaluated according

to the restriction patterns of DNA fragments from the method reported by Mizokami *et al.*¹⁹

Serum HBcrAg levels were measured using a CLEIA HBcrAg assay kit with a fully automated Lumipulse System analyzer (Fujirebio) as described previously.^{20,21} The HBcrAg assay measures all antigens transcribed and translated from the precore and core genes of the HBV genome, which include hepatitis B e, core and p22cr antigens.^{14,20} HBcrAg concentration was calculated based on a standard curve generated using recombinant pro-HBeAg. The immunoreactivity of pro-HBeAg at 10 fg/mL was defined as 1 U/mL. We expressed HBcrAg in terms of log U/mL, with a quantitative range set at 3.0–6.8 log U/mL.

Evaluation of response to NUC/IFN- α sequential therapy

The clinical conditions of a successful response to NUC/IFN- α sequential therapy were set at serum HBV DNA below 4.0 log copies/mL, serum ALT below 30 IU/L and negative HBeAg, according to established Japanese guidelines in which patients who meet these conditions are not recommended to start antiviral therapy.²² We assessed the final response at approximately 24 months after completing IFN- α sequential therapy and compared results to those at 6 and 12 months after the treatment.

Statistical analyses

Fisher's exact and Pearson's χ^2 -tests were adopted to test for differences between subgroups of patients. The Mann–Whitney *U*-test was employed to compare continuous data. Each cut-off value was decided using receiver–operator curve (ROC) analysis, and results were evaluated by measuring the area under the ROC (AUC). Multivariate analysis was performed using a logistic model for the 24-month response to NUC/IFN- α sequential therapy. Correlations between maximal values of ALT and HBV DNA were calculated using Spearman's rank correlation coefficient test. The non-relapse rate was analyzed by the Kaplan–Meier method.

All tests were performed using the IBM SPSS Statistics Desktop for Japan version 19.0 (IBM Japan, Tokyo, Japan). $P < 0.05$ was considered to be statistically significant.

RESULTS

Factors associated with the 24-month response to NUC/IFN- α sequential therapy

OF THE 50 patients enrolled, 18 were judged as responders at 24 months after completing IFN- α sequential therapy (i.e. 24-month responders), while the

remaining 32 were classified as 24-month non-responders. The clinical backgrounds of both groups are compared in Table 2. The median age at NUC commencement and sex distribution did not differ remarkably between the groups. Genotype C was similarly predominant. The types of NUC administered at the start and end of treatment were comparable between the groups, but the duration of NUC administration was significantly longer in responders. Re-administration of NUC due to aggravation of hepatitis B before judgment of the 24-month response was observed in approximately half of the 32 non-responders. After the final evaluation at 24 months, re-continuation of NUC was seen in only one of the 18 responders versus roughly half of the 15 non-responders who had previously not required it. The follow-up period was significantly longer in responders because observation was discontinued when NUC were re-administered.

Biochemical and virological markers were compared between 24-month responders and non-responders at the start of NUC, at the start of IFN- α and at the end of IFN- α (Table 3). Positivity for the HBeAg was significantly lower in responders at all time points. HBsAg and HBcrAg levels did not differ between the groups at the start of NUC, but became significantly lower in responders at the start and end-points of IFN- α administration. A significant difference in HBV DNA level was seen between the groups at the end of IFN- α administration only. ALT levels did not differ between the groups at any point.

Multivariate analysis revealed that HBsAg and HBcrAg levels of 3.0 or more and 4.5 log U/mL or more, respectively, at the start of IFN- α administration were significant factors associated with a 24-month non-response to NUC/IFN- α sequential therapy (Table 4). The factors adopted for this logistic model were as follows: age at

end of NUC of 37 years or more, duration of NUC administration of 18 months or more, sex, type of NUC at start, HBV genotype, HBeAg positivity at the start of IFN- α , HBsAg level at the start of IFN- α of 3.0 log IU/mL or more, and HBcrAg level at the start of IFN- α of 4.5 log U/mL or more. The corresponding cut-off values for each factor were determined by ROC analysis.

Of the 50 patients enrolled, 23 (46%) had HBsAg of 3.0 log IU/mL or more and HBcrAg of 4.5 log U/mL or more, 27 (54%) had HBsAg of less than 3.0 log IU/mL or HBcrAg of less than 4.5 log U/mL, and none had HBsAg of less than 3.0 log IU/mL and HBcrAg of less than 4.5 log U/mL at the start of IFN- α administration. Whereas none of the 23 patients with the highest HBsAg and HBcrAg levels were responders, 18 (67%) of the remaining 27 patients responded to NUC/IFN- α sequential therapy ($P=0.005$).

Comparison of responses to NUC/IFN- α sequential therapy at different time points

We assessed the responses to NUC/IFN- α sequential therapy at 6 and 12 months after completing IFN- α administration using the same criteria as those for determining the 24-month outcome. Responses were in 78% agreement ($P<0.001$) between 6 and 24 months and 80% agreement ($P<0.001$) between 12 and 24 months.

Prediction of response to NUC/IFN- α sequential therapy using maximal levels of ALT and HBV DNA

The maximal levels of ALT and HBV DNA during follow up were found to be significantly related ($r=0.777$, $P<0.001$). ROC analysis showed that both maximal ALT

Table 2 Comparison of clinical backgrounds between 24-month responders and non-responders

Clinical background	24-month responders (n = 18)	24-month non-responders (n = 32)	P
Age at start of NUC (years)†	36 (21–56)	34 (21–57)	0.486
Sex (male : female)	15:3	23:9	0.497
Genotype (B:C:undetermined)	1:16:1	2:20:10	0.101
NUC at start (LVD : ETV)	16:2	27:5	1.000
NUC at end (LVD : ETV : LAM + ADV : ETV + ADV)	16:2:0:0	24:6:1:1	0.610
Duration of NUC administration (months)†	51 (5–121)	5 (4–72)	0.001
Follow-up period after stopping IFN- α administration (months)†	30 (23–102)	22 (2–81)	0.014
Re-administration of NUC before judging 24-month response‡	0% (0/18)	53% (17/32)	<0.001
Re-administration of NUC after judging 24-month response‡	6% (1/18)	47% (7/15)	0.012

†Data are expressed as the median (range).

‡Data are expressed as a positive percentage (positive number/total number).

ADV, adefovir dipivoxil; ETV, entecavir; HBeAg, hepatitis B e-antigen; HCC, hepatocellular carcinoma; IFN, interferon; LAM, lamivudine; LVD, lamivudine; NUC, nucleoside/nucleotide analog.

Table 3 Comparison of ALT level and viral markers between 24-month responders and non-responders at the time points of starting NUC administration, starting IFN- α administration and stopping IFN- α administration

ALT/viral marker	24-month responders (n = 18)	24-month non-responders (n = 32)	P
At start of NUC administration			
ALT (IU/L)†	242 (32–2274)	281 (22–1044)	0.872
HBeAg‡	44% (8/18)	84% (27/32)	0.008
HBV DNA (log copies/mL)†	8.0 (<2.1–>9.0)	7.8 (<2.1–>9.0)	0.866
HBsAg (log IU/mL)†	3.5 (1.8–4.9)	3.5 (2.5–4.4)	1.000
HBcrAg (log U/mL)†	>6.8 (3.7–>6.8)	>6.8 (<3.0–>6.8)	0.121
At start of IFN- α administration			
ALT (IU/L)†	29 (12–103)	29 (12–111)	0.779
HBeAg‡	11% (2/18)	59% (19/32)	0.001
HBV DNA (log copies/mL)†	<2.1 (neg.–3.9)	<2.1 (neg.–4.8)	0.142
HBsAg (log IU/mL)†	2.9 (1.5–4.1)	3.7 (2.5–4.3)	0.028
HBcrAg (log U/mL)†	3.6 (<3.0–5.9)	5.6 (<3.0–>6.8)	0.002
At end of IFN- α administration			
ALT (IU/L)†	25 (10–48)	28 (12–134)	0.384
HBeAg‡	6% (1/18)	59% (19/32)	<0.001
HBV DNA (log copies/mL)†	<2.1 (neg.–4.1)	4.6 (<2.1–>9.0)	<0.001
HBsAg (log IU/mL)†	2.8 (1.9–4.0)	3.6 (2.6–4.7)	0.007
HBcrAg (log U/mL)†	3.4 (<3.0–5.5)	5.5 (<3.0–>6.8)	0.017

†Data are expressed as the median (range).

‡Data are expressed as a positive percentage (positive number/total number).

ALT, alanine aminotransferase; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; IFN, interferon; neg., negative; NUC, nucleoside/nucleotide analog.

Table 4 Multivariate analysis of factors associated with 24-month non-responders to NUC/IFN- α sequential therapy

Selected factor	Odds ratio	95% CI	P
HBsAg \geq 3.0 log IU/mL at start of IFN- α	17.7	2.9–108.2	0.002
HBcrAg \geq 4.5 log U/mL at start of IFN- α	15.0	2.5–88.6	0.003

CI, confidence interval; HBcrAg, hepatitis B core-related antigen; HBsAg, hepatitis B surface antigen; IFN, interferon; neg., negative; NUC, nucleoside/nucleotide analog.

and HBV DNA levels were significantly associated with the treatment response (Fig. 2), with an AUC for each parameter of over 0.8. The cut-off values providing the highest significance in ROC analysis were 128 IU/L for ALT and 4.5 log copies/mL for HBV DNA. The existence of a second cut-off value was also identified for HBV DNA (6.0 log copies/mL) to discriminate between 24-month responders and non-responders. These results indicated that patients reaching a maximal ALT level of over 128 IU/L or maximal HBV DNA level of over 6.0 log copies/mL during post-treatment follow up were likely to be non-responders.

Lastly, we analyzed the changes in cumulative non-relapse rate of hepatitis B during and after IFN- α

administration by tentatively defining relapse as ALT level exceeding 128 IU/L during follow up. We selected maximal ALT instead of maximal HBV DNA because: (i) the inflection point to distinguish a response was clear for maximal ALT but ambiguous for maximal HBV DNA; (ii) the value for “sensitivity + specificity – 1” as calculated by ROC analysis was larger for maximal ALT (7.5 vs 6.5); and (iii) the maximal levels of ALT and HBV DNA were closely associated, and thus ALT values were considered to represent those of HBV DNA. The cumulative non-relapse rate decreased rapidly after completely halting NUC until just prior to 6 months after stopping IFN- α and then was seen to plateau until the study end-point (Fig. 3). This suggests that the recurrence of hepatitis associated with a 24-month non-response can be expected to occur primarily during the first 6 months after stopping IFN- α administration.

DISCUSSION

THE COOPERATIVE EFFECT of NUC/IFN- α sequential therapy has been controversial.^{7–12} Enomoto *et al.*¹⁰ first analyzed the results of ETV/IFN- α sequential therapy in patients with HBeAg positive chronic hepatitis B and detected several differences. Although their results were negative, they witnessed that patients who had achieved HBeAg

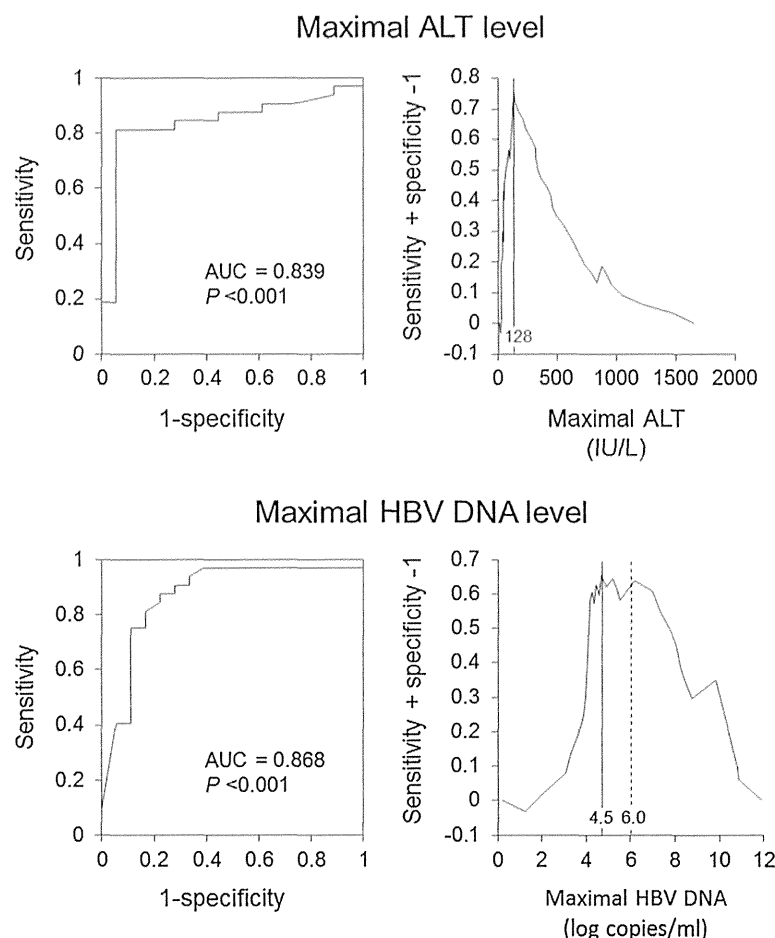


Figure 2 Receiver-operator curve analysis of maximal alanine aminotransferase (ALT) and hepatitis B virus (HBV) DNA levels to discriminate between 24-month responders and non-responders. Vertical solid lines indicate the actual values of markers corresponding to main inflection points and the vertical broken line indicates the actual value of the marker corresponding to a second inflection point; AUC, area under the receiver-operator curve.

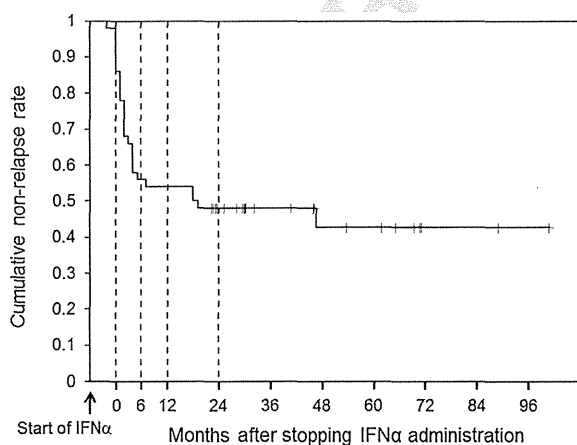


Figure 3 Kaplan-Meier analysis of the non-relapse rate after starting interferon (IFN)- α administration by defining relapse of hepatitis B as alanine aminotransferase (ALT) level exceeding 128 IU/L.

seroconversion by the time of IFN- α commencement experienced a significantly higher sustained virological response rate than those in whom the HBeAg persisted. Thus, it appeared beneficial to further clarify the factors associated with the response to NUC/IFN- α sequential therapy.

The present study analyzed the factors associated with a long-term response to IFN- α sequential therapy in order to safely discontinue NUC therapy. All patients were treated with natural IFN- α for 6 months and followed for at least 24 months after completing the sequential therapy, with the exception of those who required re-administration of NUC due to aggravation of hepatitis B. The type and duration of NUC administration were not fixed in this study because IFN- α sequential therapy was implemented to discontinue NUC in patients who were undergoing maintenance treatment. Although a prospective study would have been ideal to elucidate the factors associated with

IFN- α sequential therapy outcome, we undertook this retrospective trial because no variables have been sufficiently analyzed to date. Furthermore, we were able to address the long-term response to IFN- α sequential therapy in relation to the results of earlier retrospective studies. It has been reported that pegylated IFN- α (PEG IFN- α) provides a higher HBV response rate than does conventional IFN- α .²³ Therefore, additional prospective studies of sequential therapy using PEG IFN- α are needed as well.

Both HBsAg and HBcrAg levels at the time of NUC cessation were factors significantly associated with the response to NUC/IFN- α sequential therapy. HBsAg has been closely linked with PEG IFN- α therapy outcome.²⁴⁻²⁷ Moucari *et al.*²⁶ analyzed HBeAg negative hepatitis B patients who had been treated with PEG IFN- α for 48 weeks and concluded that an early serum HBsAg drop was strongly predictive of a sustained virological response. Sonneveld *et al.*²⁴ assessed HBeAg positive hepatitis B patients who had received PEG IFN- α with or without LVD for 52 weeks and observed that patients who experienced no decline in HBsAg level from baseline at week 12 had little chance of achieving a sustained response and no possibility of HBsAg loss. HBcrAg includes antigens that are transcribed and translated from precore and core genes of the HBV genome, and HBeAg is a primary component of these antigens. Thus, our results were consistent with those described by Enomoto *et al.*¹⁰ that the proportion of patients losing HBeAg positivity during ETV treatment was significantly higher in responders to ETV/IFN- α sequential therapy than in non-responders.

Hepatitis B surface antigen and HBcrAg levels have both been associated with intrahepatic HBV cccDNA, which is a key molecule in HBV replication whose value is closely related to HBV replication activity.^{21,27,28} Several reports^{27,29,30} have shown that HBV cccDNA level is associated with the response to antiviral therapy, such as with PEG IFN- α and NUC. Sung *et al.*²⁹ analyzed HBeAg positive hepatitis B patients who had been treated with either LVD monotherapy or a combination of PEG IFN- α and LVD and concluded that intrahepatic HBV cccDNA level at the end of therapy was superior to serum HBV DNA in predicting a sustained virological response. Serum HBV DNA is associated with intrahepatic HBV cccDNA and is widely used as a marker for HBV replication activity. However, such associations may be incompatible with antiviral therapies, and especially NUC treatment, because NUC directly hamper production of the HBV virion by inhibiting reverse transcription of pre-genomic RNA without affecting HBV cccDNA directly. As serum levels of HBsAg and HBcrAg are easier to measure than intrahepatic HBV cccDNA, these two antigen assays may be more suitable

as surrogate markers for HBV replication activity in patients undergoing antiviral therapy. We previously reported that the combinational use of HBsAg and HBcrAg was beneficial to forecast the risk of hepatitis relapse after discontinuation of NUC.^{13,14} The present study confirms this notion; it is possible that HBsAg and HBcrAg have complimentary roles in monitoring antiviral effects because the production of these two antigens is regulated by alternative enhancer-promoter systems in the HBV genome.

It is noteworthy that ROC analysis revealed maximal levels of ALT and HBV DNA to be closely associated with the 24-month response to NUC/IFN- α sequential therapy. We observed that patients with ALT higher than 128 IU/mL or HBV DNA higher than 6.0 log copies/mL during follow up were likely to be non-responders. When a relapse of hepatitis B was tentatively defined as ALT exceeding 128 IU/L during observation, relapses occurred frequently during the first 6 months after ceasing IFN- α and then became more sporadic afterwards. The timing of judgment of a virological response to NUC/IFN- α sequential therapy is critical when evaluating treatment efficacy. As this period is usually set at 6 months after completing therapy, our results confirm that 6 months is indeed appropriate. Our findings also suggest that maximal levels of ALT and HBV DNA are useful for monitoring the results of NUC/IFN- α sequential therapy. Accordingly, patients who are likely to be non-responders can now be identified as early as 24 weeks in advance and alternative strategies for treatment may be considered in a more timely fashion.

In conclusion, the combinational use of HBsAg and HBcrAg levels may be useful to predict the response to NUC/IFN- α sequential therapy. Maximal levels of ALT and HBV DNA during follow up may also be employed for monitoring the results of IFN- α sequential therapy.

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Retrospective Study

Mutations of pre-core and basal core promoter before and after hepatitis B e antigen seroconversion

Nozomi Kamijo, Akihiro Matsumoto, Takeji Umemura, Soichiro Shibata, Yuki Ichikawa, Takefumi Kimura, Michiharu Komatsu, Eiji Tanaka

Nozomi Kamijo, Akihiro Matsumoto, Takeji Umemura, Soichiro Shibata, Yuki Ichikawa, Takefumi Kimura, Michiharu Komatsu, Eiji Tanaka, Department of Medicine, Shinshu University School of Medicine, Matsumoto 390-8621, Japan

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Correspondence to: Takeji Umemura, MD, PhD, Associate Professor, Department of Medicine, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390-8621, Japan. tumemura@shinshu-u.ac.jp

Telephone: +81-263-372634
Fax: +81-263-329412

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e antigen (HBeAg) seroconversion.

METHODS: The proportion of pre-core (G1896A) and basal core promoter (A1762T and G1764A) mutant viruses and serum levels of hepatitis B virus (HBV) DNA, hepatitis B surface antigen (HBsAg), and HB core-related antigen were analyzed in chronic hepatitis B patients before and after HBeAg seroconversion ($n = 25$), in those who were persistently HBeAg positive ($n = 18$), and in those who were persistently anti-HBe positive ($n = 43$). All patients were infected with HBV genotype C and were followed for a median of 9 years.

RESULTS: Although the pre-core mutant became predominant (24% to 65%, $P = 0.022$) in the HBeAg seroconversion group during follow-up, the proportion of the basal core promoter mutation did not change. Median HBV viral markers were significantly higher in patients without the mutations in an HBeAg positive status (HBV DNA: $P = 0.003$; HBsAg: $P < 0.001$; HB core-related antigen: $P = 0.001$). In contrast, HBV DNA ($P = 0.012$) and HBsAg ($P = 0.041$) levels were significantly higher in patients with the pre-core mutation in an anti-HBe positive status.

CONCLUSION: There is an opposite association of the pre-core mutation with viral load before and after HBeAg seroconversion in patients with HBV infection.

Key words: Seroconversion; Hepatitis B core-related antigen; Pre-core; Basal core promoter; Mutation; Hepatitis B surface antigen; Hepatitis B virus DNA

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Abstract

AIM: To investigate the role of pre-core and basal core promoter (BCP) mutations before and after hepatitis B

Core tip: The exact roles of pre-core (pre-C) and basal core promoter (BCP) mutations remain unclear before and after hepatitis B e antigen (HBeAg) seroconversion.