Predictors of therapeutic response to peginterferon α-2a and nucleos(t)ide analog combination therapy for HBeAg-negative chronic hepatitis B: 1-year follow-up after treatment

SHIMA MIMURA[1], KOJI FUJITA[1], KEI TAKUMA[1], MAI NAKAHARA[1], KYOKO OURA[1], TOMOKO TADOKORO[1], JOJI TANI[1], ASAHIRO MORISHITA[1], MASAFUMI ONO[1], TAKASHI HIMOTO[2] and TSUTOMU MASAKI[1]

[1]Department of Gastroenterology and Neurology, Kagawa University Hospital, Kita, Kagawa 761-0793; [2]Department of Medical Technology, Kagawa Prefectural University of Health Sciences, Takamatsu, Kagawa 761-0123, Japan

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Abstract. Chronic hepatitis B (CHB) is a major global health concern. Guidelines for the management of hepatitis B virus (HBV) indicate that the loss of hepatitis B surface antigen (HBsAg) is a key endpoint of interest. The present study aimed to examine long-term changes in HBsAg levels in HBV-DNA-negative, hepatitis B e-antigen (HBeAg)-negative patients treated with peginterferon (Peg-IFN) α-2a and nucleos(t)ide analog (NA), and to examine the conditions that make them susceptible to HBsAg decline. A total of 17 patients with CHB treated with NA and Peg-IFN were observed for 96 weeks (48 weeks of Peg-IFN therapy and 48 weeks of post-treatment follow-up). In this study, responders were defined as those with a 50% or greater decrease in HBsAg levels from baseline at week 96. Beginning at week 16 of Peg-IFN therapy, there was a significant difference in the decrease in HBsAg levels from baseline between the responders and non-responders. In responders, HBsAg levels tended to be >60% lower 16 weeks after Peg-IFN initiation than before initiation. Age at the start of NA use and the duration of NA use before Peg-IFN treatment initiation were significant pretreatment factors associated with HBsAg response. In conclusion, Peg-IFN was revealed to be more effective in HBeAg-negative patients with CHB who started NA at a young age and have been on long-term treatment, particularly if the HBsAg levels decreased to less than 60% of the starting level at week 16 after starting Peg-IFN treatment.

Introduction

Chronic hepatitis B (CHB) is a major global health concern. The main goal of CHB treatment is to reduce the risk of chronic liver disease and related complications and improve the quality of life and survival (1). Guidelines for the management of hepatitis B virus (HBV) infection indicate that hepatitis B surface antigen (HBsAg) loss is a key endpoint of interest and is strongly recommended (2). Currently, there are two main treatment options for CHB: treatment with a nucleos(t)ide analog (NA) or pegylated interferon α-2a (Peg-IFN).

Antiviral therapy aimed at reducing HBV DNA levels to the extent possible is important in the management of CHB, and NA renders HBV DNA undetectable in most patients with CHB. However, HBsAg clearance is unlikely to occur during a patient's lifetime, even if HBV replication is well-controlled (3,4). Peg-IFN is known to enhance innate immunity by preventing HBV protein formation and depleting the covalently closed circular DNA (cccDNA) pool in the liver (5-7). Therefore, PEG-IFN therapy may result in greater HBsAg reduction than NA therapy (8).

In previous studies, Peg-IFN add-on therapy to NA was reported to be superior to monotherapy in terms of promoting HBeAg loss and HBsAg reduction in patients with HBeAg-positive CHB (9,10). Regarding the impact of HBsAg levels, there are few studies with short-term follow-up after the end of treatment in HBeAg-negative CHB patients who received Peg-IFN in addition to NA; however, there are no reports of long-term follow-up. Moreover, the conditions under which HBsAg levels are further reduced in patients are not well understood.

The purpose of this study is to investigate the conditions under which Peg-IFN is effective by observing changes in HBsAg levels during and one year after Peg-IFN administration in HBeAg negative patients who received NA treatment and remained HBV DNA negative.

Patients and methods

Patients. This study included 17 patients with CHB who began Peg-IFN therapy at the Department of Gastroenterology and Neurology, Kagawa University Hospital, Japan, between September 2013 and April 2021. All patients were serum HBsAg-positive, HBsAb-negative, HBeAg-negative and HBeAb-positive. They had received NA therapy for more

Correspondence to: Dr Shima Mimura, Department of Gastroenterology and Neurology, Kagawa University Hospital, 1750-1 Ikenobe, Miki-cho, Kita, Kagawa 761-0793 Japan
E-mail: mimura.shima@ kagawa-u.ac.jp

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than 1 year, and their HBV DNA levels were below detection sensitivity. The patients had no history of interferon (IFN) treatment. All patients had hepatitis B and were free of other viruses, such as the hepatitis C virus or autoimmune hepatitis. None of the patients had a history of drug abuse or alcoholic hepatitis. The exclusion criteria were neutrophil count <1,500/mm^3, platelet count <90,000/mm^3, hemoglobin concentration <10 g/dl, cirrhosis, history of organ transplantation, and other known diseases for which Peg-IFN therapy was not suitable. This study was approved by the Ethics Committee of Kagawa University Hospital on June 20, 2019 (approval no. 2019032). An oral informed consent was obtained from all the participants. Written informed consent was not required by the ethics committee because the data involved routinely collected medical data. All data were obtained from patient medical records.

Peg-IFN therapy. Patients received subcutaneous injections of Peg-IFN α-2a (PEGASYS: Hoffmann-La Roche Inc., Basel, Switzerland) at 180 μg per week for 48 weeks. The dose of Peg-IFN was modified for adverse events according to the manufacturer's recommendations. The patients were followed up monthly for 12 months after treatment completion.

Laboratory tests. Virological and biochemical assessments were performed monthly and follow-up data were collected. HBsAg levels were measured using ARCHITECT i2000 (Abbott Laboratories, Chicago, IL, USA). HBV genotypes were determined using enzyme immunoassays. HBV DNA levels were measured using a Cobas®TaqMan®48 analyzer (Roche Molecular Systems, Branchburg, NJ, USA). Blood HBV DNA measurements were performed using the COBAS TaqMan HBV Test, v 2.0 (Roche Diagnostics K.K, Tokyo, Japan) (~2018/6) and the COBAS 6800/8800 HBV System (Roche Diagnostics K.K) (2018/7~) was used for this study, which is based on a polymerase reaction assay. HBV DNA values >1.3 log IU/ml were considered positive.

Assessment of effectiveness. In this study, responders were defined as those with a 50% or greater decrease in HBsAg levels from baseline at week 96. Conversely, non-responders were defined as those with a decrease in HBsAg levels of less than 50% from baseline at week 96.

Statistical analysis. Data on baseline characteristics are presented as median values. Patient characteristics were compared using unpaired Student's t-test. Fisher's exact tests were used to analyze categorical variables in both groups. Repeated measures ANOVA and the Tukey's test was performed to analyze the changes in serum HBsAg levels from baseline at weeks 0, 48 and 96. The mean value indicates a change in the amount of HBsAg. The results were considered significant at P<0.05. All statistical calculations were performed using the JMP software (version 15, SAS Institute, USA).

Ethical approval statement. This study was approved by the Ethics Committee of Kagawa University Hospital (approval no. 2019032). Oral informed consent was obtained from all participants. The ethics committee waived written informed consent because the data were collected routinely in medical practice. Information was removed from the data prior to analysis, and all methods were performed in accordance with relevant guidelines and regulations.

Table I. Clinical and demographic characteristics of patients at the start of Peg-IFN treatment.

Characteristic	Value
Sex, male/female	12/5
Age, years	55.5 (41.6-69.9)
Genotype, B/C/unknown	1/11/5
HBsAg titer, lU/ml	475 (4-19,049)
AST, IU/l	22 (18-43)
ALT, IU/l	23 (11-62)
Albumin, g/dl	4.4 (4.0-4.8)
Total bilirubin, mg/dl	0.8 (0.3-2.8)
Platelet, x10^3/μl	16.6 (8.4-45.7)
PT, %	85 (70-129)
Fib-4 index	0.8 (0.6-3.9)
NA use start age, years	51.2 (35.1-63.8)
NA use period until Peg-IFN treatment starts, years	4.8 (1.0-13.5)

Data are shown as median (range). HBsAg, hepatitis B surface antigen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; PT, prothrombin time; Fib-4, fibrosis-4; NA, nucleos(t)ide analog; Peg-IFN, peginterferon.

Table II. HBsAg levels from baseline.

Group	HBsAg ≥60%	HBsAg <60%	P-value
Responder (n)	5	5	<0.05
Non-responder (n)	7	0	

HBsAg, hepatitis B surface antigen.

Results

Baseline characteristics. The patients' baseline characteristics are summarized in Table I.

HBsAg response. The clinical course of HBsAg in all patients is shown in Fig. 1. In 10 of the 17 patients (58.8%), HBsAg levels decreased by more than 50% from baseline at week 96. No patient achieved HBsAg clearance. In two patients, HBsAg levels were higher at week 48 than at baseline. However, in these two cases, HBsAg levels decreased from baseline at week 96. In only one case, the HBsAg level increased at week 96 compared to that at baseline. In 9 of the 17 patients (52.9%), HBsAg levels were higher at week 96 than at week 48.

Fig. 2 shows the mean HBsAg levels at weeks 48 and 96 compared to baseline. In all groups, HBsAg levels were 43.5 and 47.7% at weeks 48 and 96, respectively (Fig. 2A). In the responder group, the percentages were 22.6 and 25.3%

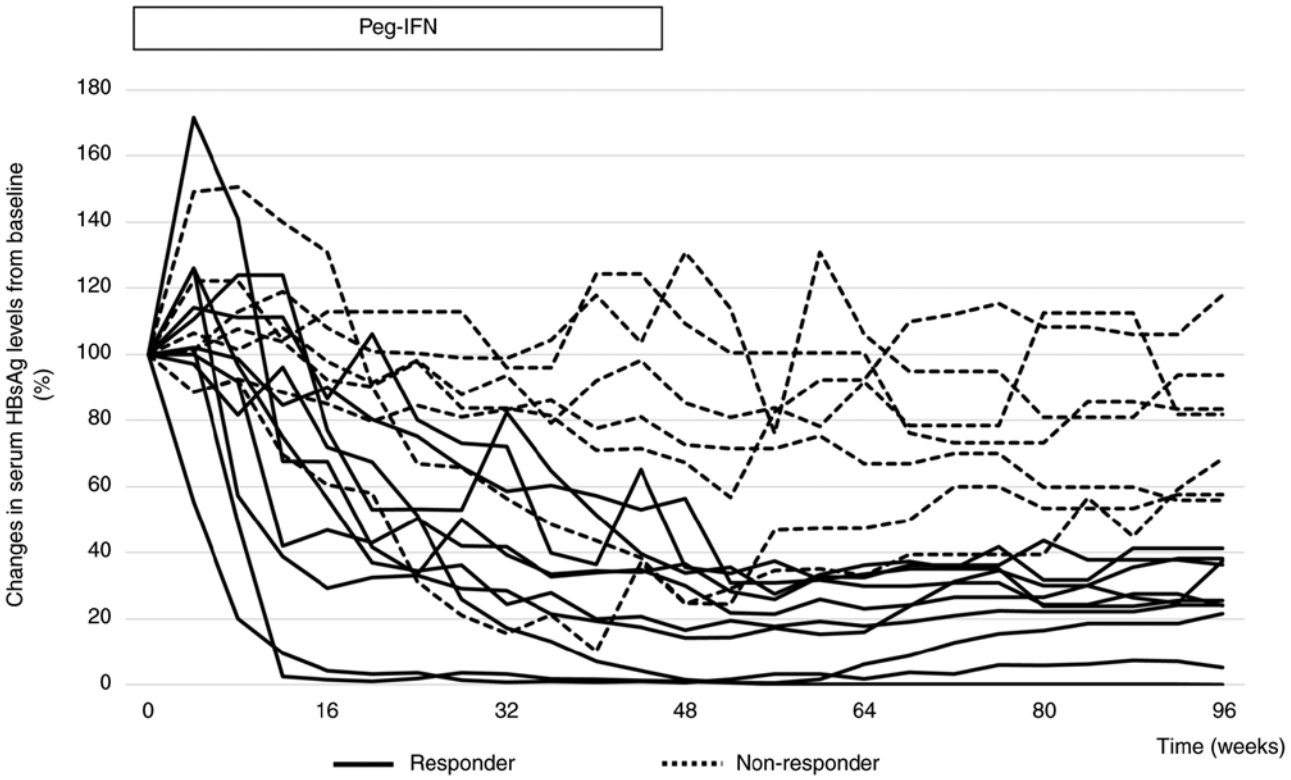

Figure 1. Clinical course of patients during the treatment and observation periods. HBsAg, hepatitis B surface antigen; Peg-IFN, peginterferon.

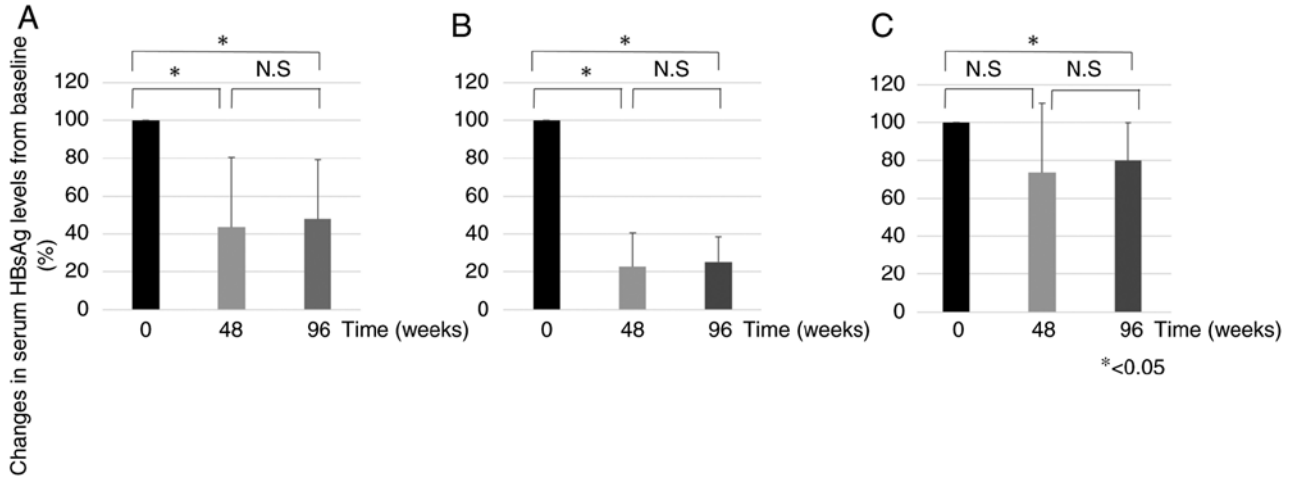

Figure 2. Changes in serum HBsAg levels at baseline, during treatment, at the end of treatment (week 48), and after 48 weeks of treatment (week 96). (A) All patients, (B) responder group and (C) non-responder group. *P<0.05. HBsAg, hepatitis B surface antigen; Peg-IFN, peginterferon; N.S, not significant.

at weeks 48 and 96 weeks, respectively (Fig. 2B). In the non-responder group, the percentages were 73.5 and 79.7% at 48 and 96 weeks, respectively (Fig. 2C). In the responder group, there was a significant decrease in HBsAg levels at week 48 compared with baseline, but not in the non-responder group. All groups showed a significant decrease in HBsAg levels at week 96 compared to baseline. All groups showed an increase in HBsAg levels at week 96 compared to week 48, but the difference was not significant.

Changes in serum HBsAg levels from baseline in the responder and non-responder groups are shown in Fig. 3. The mean declines in HBsAg levels from baseline were -0.97, and -0.92 log IU/ml at weeks 48 and 96, respectively, in the responder group, and -0.20, and -0.11 log IU/ml at weeks 48 and 96, respectively, in the non-responder group. From week 16 of Peg-IFN therapy, there was a significant difference in the decrease in HBsAg levels from baseline between responders and non-responders.

Fig. 4 shows the HBsAg levels at week 16 compared to baseline for each case in the responder and non-responder groups. In the responder group, 5 patients (50%) had a decrease in HBsAg level to 60% or less at week 16 compared to baseline. Conversely, there were no cases in the non-responder group in which the HBsAg levels decreased below 60%. The Fisher's exact test

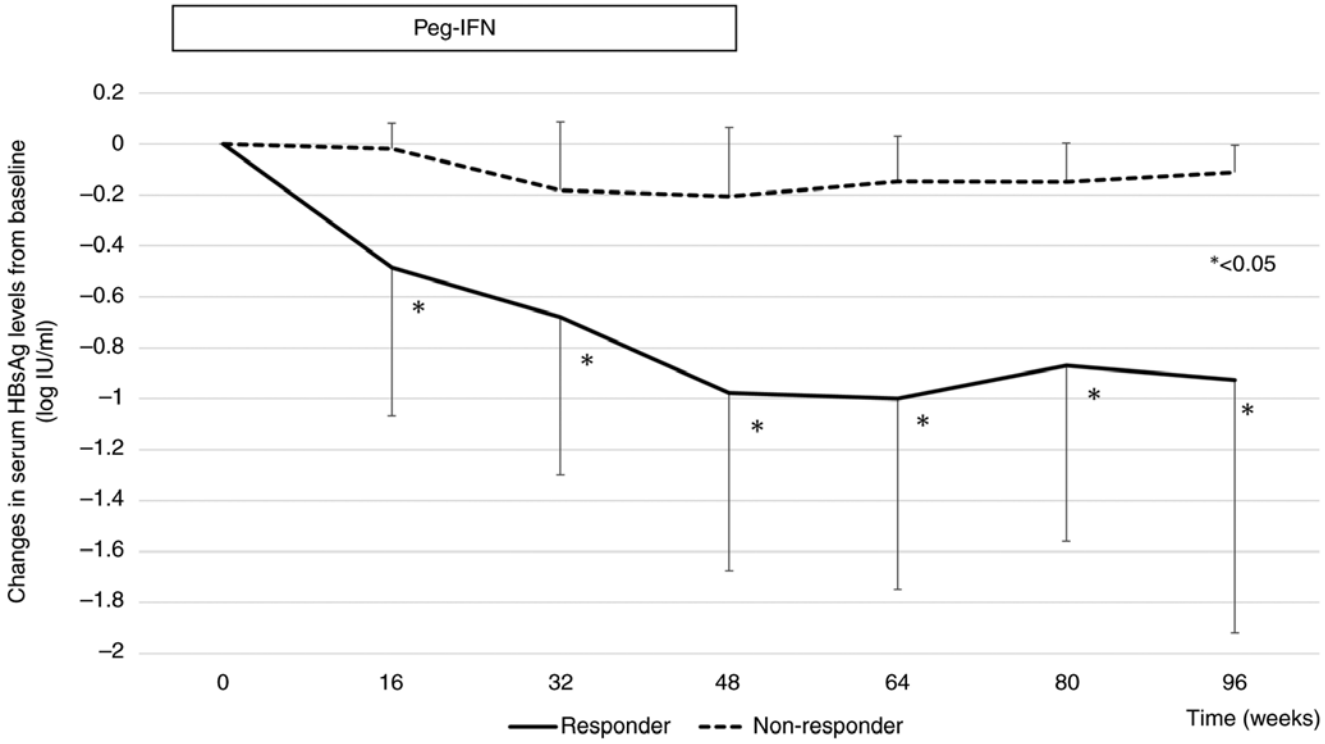

Figure 3. Changes in serum HBsAg levels from baseline in responder and non-responder groups. *P<0.05. HBsAg, hepatitis B surface antigen; Peg-IFN,

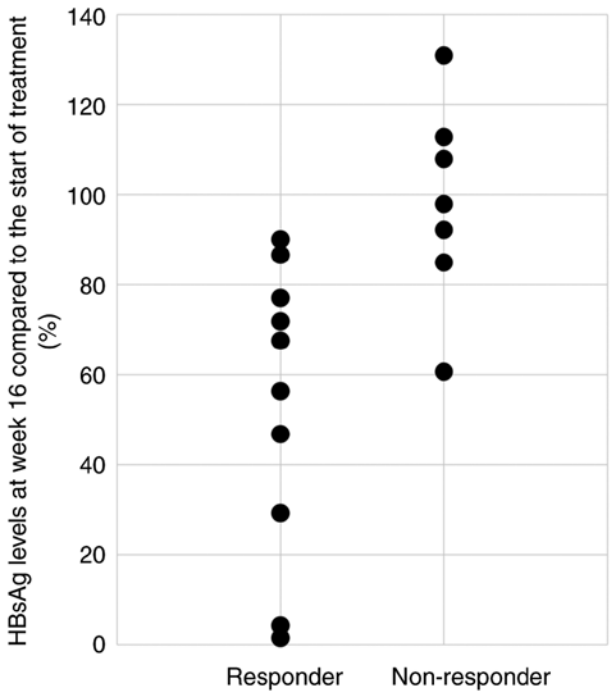

Figure 4. HBsAg levels at week 16 compared to the start of treatment in responder and non-responder groups. HBsAg, hepatitis B surface antigen.

showed a significant difference between the responding and non-responding groups in terms of whether HBsAg levels reached 60% or less at week 16 compared to baseline (Table II).

Contributing factors for prediction of responders. A comparison of pretreatment factors between responders and non-responders is presented in Table III. In the univariate analysis, age at the start of NA use and the duration of NA use until the start of treatment were significant pre-treatment factors associated with HBsAg response.

Discussion

In this study, we evaluated the effect of adding Peg-IFN to NA on HBsAg reduction in HBeAg-negative patients whose serum HBV DNA was undetectable after NA treatment. From a clinical perspective, predicting whether a patient can achieve HBsAg clearance before initiating Peg-IFN therapy is important. After 48 weeks of Peg-IFN therapy, the group whose HBsAg level was 50% of the level before initiation and after 48 weeks of observation (week 96) was defined as the responder group and was compared with the non-responder group.

Previous studies on a larger group of patients reported that Peg-IFN treatment was effective as long as the patient was in the immune reaction phase, regardless of whether the patient was HBeAg positive or negative (11,12). However, to our knowledge, the first study was on the administration of Peg-IFN in HBeAg-positive cases (11). The second study had a design in which patients who became HBeAg-negative with NAs were administered Peg-IFN in combination for several weeks, and then Peg-IFN was administered alone (12). Our study differs from theirs in that we limited our subjects to HBeAg-negative and HBeAb-positive cases and did not discontinue NA even after starting Peg-IFN therapy. Our study is the first report in this regard.

Although NAs strongly suppress HBV DNA, a large multicenter long-term NAs therapy cohort study showed a 10-year HBsAg loss rate of 2.1% and an annual incidence rate of only

Table III. Comparison of pre-treatment clinical and demographic characteristics between the HBsAg responder and non-responder groups.

Factors	Responder (n=10)	Non-responder (n=7)	P-value
Sex, male/female	8/2	4/3	0.314
Age, years	49.5 (41.6-69.9)	57.0 (52.0-67.0)	0.181
Genotype, B/C/unknown	0/6/3	1/5/2	0.190
HBsAg titer, IU/ml	433 (132-19,049)	726 (4-3,473)	0.575
AST, IU/l	21 (19-37)	23 (18-43)	0.428
ALT, IU/l	18 (13-62)	23 (11-43)	0.775
Albumin, g/dl	4.4 (4.0-4.8)	4.4 (4.1-4.7)	0.306
Total bilirubin, mg/dl	0.9 (0.3-1.6)	0.7 (0.4-2.8)	0.916
Platelet, $x10^3/\mu l$	18.0 (8.4-25.9)	16.4 (10.9-45.7)	0.414
PT, %	85 (70-98)	96 (78-129)	0.150
Fib-4 index	1.8 (0.8-2.8)	1.5 (0.6-3.9)	0.699
NA use start age, years	45.4 (35.1-61.1)	55.7 (47.2-63.8)	0.025
NA use period until Peg-IFN treatment starts, years	7.3 (3.0-13.5)	1.5 (1.0-6.1)	0.004

Data are shown as median (range). HBsAg, hepatitis B surface antigen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; PT, prothrombin time; Fib-4, fibrosis-4; NA, nucleos(t)ide analog; Peg-IFN, peginterferon.

0.22% with little hope of HBsAg seroclearance (13). According to a study by Papatheodoridis *et al*, HBsAg levels decreased by a median of 0.13 and 0.17 log IU/ml at 48 and 96 weeks, respectively, compared to baseline, in HBeAg-negative CHB patients administered NA monotherapy (14). The present study did not directly compare the HBsAg reduction rate between NA monotherapy and Peg-IFN and NA combination therapy. This is a potential limitation of this study. However, it seems that the addition of Peg-IFN to NA does not reduce HBsAg compared to NA monotherapy because the rate of decrease in HBsAg levels is 0.20 log IU/ml and 0.11 log IU/ml at 48 and 96 weeks, respectively, in non-responders. However, in the response group, the decrease in the amount of HBsAg from the baseline was 0.97 log IU/ml at 48 weeks and 0.92 log IU/ml at 96 weeks, which was remarkable. From this perspective, it seems worthwhile to consider targeting and administering Peg-IFN to patients who are expected to respond to it.

NAs have been demonstrated to suppress liver fibrosis and reduce the incidence of HCC (15-17). To obtain such effects, it is necessary to use NAs continuously for long periods, which may cause adherence and compliance problems. Additionally, patients from resource-limited countries or regions cannot afford the financial burden of long-term NAs therapy (18). Our study showed that the highest rate of HBsAg reduction was observed in patients who were younger when NA therapy was initiated, and had a longer history of NAs use. Based on this result, young patients taking NAs may also consider Peg-IFN combination therapy with the goal of achieving early seroclearance of HBsAg. Better use of different combinations of antiviral drugs could make the treatment more cost-effective and drug-free.

At week 16, a significant difference was observed between the responding and non-responding groups in terms of the rate of decrease in HBsAg levels. If the HBsAg level at week 16 was <60% of that at the start of Peg-IFN therapy, the response to Peg-IFN therapy was considered good. Based on these results, it may be necessary to observe the rate of decrease in HBsAg levels at week 16 and to consider continuing Peg-IFN as an add-on therapy.

While some studies have shown that pretreatment HBsAg titer is an important factor associated with the outcome of HBsAg loss in Peg-IFN therapy (19,20), long-term observational studies have reported that early serum HBsAg decline is highly predictive of HBsAg serum clearance (21-24). In a previous study, we reported that HBsAg titers <120 IU/ml may achieve HBsAg clearance with Peg-IFN monotherapy (25). In contrast, this study showed that when Peg-IFN was added to NA, there was no significant difference in the HBsAg levels between responders and non-responders at the start of treatment. In the current study, we focused on HBeAg-negative and HBeAb-positive patients; therefore, it is possible that the baseline HBsAg level was not high, and the small number of cases led to different results.

Although IFN-α therapy has a limited treatment period, it has the advantage of lasting effects even after treatment (26). In this study, we defined the responder and non-responder groups based on the HBsAg level at 1-year follow-up (week 96); however, we believe that extending the observation period will deepen our understanding of the therapeutic effects.

This study has certain limitations, the most important of which is its retrospective design. This increases the probability that the treatment assignment is subject to selection bias. The study required weekly hospital visits for Peg-IFN administration for 48 weeks and was limited to patients with time and financial resources. It is also possible that a selection bias may have been at work that excluded older individuals from the study, as they may not have benefited sufficiently from participation given their prognosis. In addition, the sample size was relatively small. This raises questions regarding whether the results can be generalized. Larger prospective studies are required to confirm and extend the results of this study.

In conclusion, in HBeAg-negative patients with CHB, the addition of Peg-IFN to NA therapy can further reduce the amount of HBsAg in a population that is younger when NA is initiated and undergoes NA therapy for a prolonged duration. In addition, a decrease in HBsAg below 60% of the pre-start level at week 16 after the start of Peg-IFN therapy could be a marker for predicting efficacy.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

SM, MO, TH and TM contributed to the study conception and design. Data collection and analysis were performed by SM, KF, KT, MN, KO, TT, JT and AM. The first draft of the manuscript was written by SM and TM. SM and KF confirm the authenticity of all the raw data. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Kagawa University Hospital (approval no. 2019032). Oral informed consent was obtained from all the participants. Written informed consent was not required by the ethics committee because the data involved routinely collected medical data.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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