

A Potent Hepatitis B Surface Antigen Response in Subjects With Inactive Hepatitis B Surface Antigen Carrier Treated With Pegylated-Interferon Alpha

Zhenhuan Cao,^{1*} Yali Liu,^{1*} Lina Ma,¹ Junfeng Lu,¹ Yi Jin,¹ Shan Ren,¹ Zhimin He,¹ Chengli Shen,² and Xinyue Chen¹

Hepatitis B surface antigen (HBsAg) clearance represents a clinical cure, although the clearance rate is extremely low. The aim of this study was to evaluate the feasibility and safety profiles of pegylated-interferon α -2a (PEG-IFN α -2a) as a therapeutic option for inactive HBsAg carriers. There were 144 inactive HBsAg carriers enrolled and divided into a therapeutic group (102 subjects) and a control group (42 subjects). PEG-IFN α -2a and PEG-IFN α -2a combined with adefovir dipivoxil were used for treatment group subjects with hepatitis B virus DNA <20 IU/mL and 20 IU/mL $\leq$ hepatitis B virus DNA $<2,000$ IU/mL, respectively. Total therapy duration was no more than 96 weeks. HBsAg clearance and seroconversion rates at therapeutic weeks 48 and 96 were used to evaluate the therapeutic efficacy. Per protocol analysis showed that the HBsAg clearance rate and seroconversion rate in the treatment group were 29.8% and 20.2% at week 48 and increased to 44.7% and 38.3% at week 96, respectively. However, the HBsAg clearance rate in the control group was 2.4% at weeks 48 and 96, and no subject achieved seroconversion. The quantitative HBsAg levels and changes during the early period of treatment (at week 12 and week 24) as well as the alanine aminotransferase elevation at week 12 were strong predictors of HBsAg clearance. The adverse events were similar to those with treatment for chronic hepatitis B patients. **Conclusion:** High rates of HBsAg clearance and seroconversion could be achieved by PEG-IFN α -2a-based treatments and the treatments were relatively safe for inactive HBsAg carriers. (HEPATOLOGY 2017;66:1058-1066).

Inactive hepatitis B surface antigen (HBsAg) carriers (IHCs), defined by normal alanine aminotransferase (ALT), hepatitis B virus (HBV) DNA ≤ 2000 IU/mL, and hepatitis B e antigen (HBeAg)-negative status, are generally stable and have a good prognosis. Therefore, treatment of IHCs is not recommended. More specifically, the disease progression is relatively slow, and achieving HBsAg clearance is difficult. The HBsAg clearance rate was $<1\%$ after 1-year

treatment with a nucleotide analogue, similar to the spontaneous clearance rate⁽¹⁾; and HBsAg clearance was only 3% after 1-year treatment with pegylated-interferon (PEG-IFN) and 1-year follow-up for chronic hepatitis B (CHB).⁽²⁾ However, during a mean follow-up of 12.3 years, IHCs had an annual relapse rate of 1.46%.⁽³⁾ Furthermore, a previous study showed that IHCs had a high risk of hepatocellular carcinoma and liver-related death compared with individuals not

Abbreviations: ADV, adefovir dipivoxil; ALT, alanine aminotransferase; AUROC, area under the receiver operator characteristic curve; CHB, chronic hepatitis B; CI, confidence interval; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; IHC, inactive HBsAg carrier; OR, odds ratio; PEG-IFN, pegylated interferon; PLT, platelet count.

Received June 24, 2016; accepted April 11, 2017.

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.29213/supinfo.

*These authors contributed equally to this work.

Supported by the Capital Health Research and Development Projects (2011-2018-08, 2016-1-2183), the Capital Characteristic Clinical Application Research and Extension of Achievements (Z151100004015181, Z161100000516018), the Beijing Municipal Administration of Hospitals' Youth Program (QML20161701), the Twelfth Five-Year Major Science and Technology Projects (2013ZX1000 2002-006), the Fengtai District Health System Science Research Project (2016-57), and the Beijing Youan Hospital Hepatic Disease & HIV Fund (YNK20150205, 20160021).

Copyright © 2017 by the American Association for the Study of Liver Diseases.

View this article online at wileyonlinelibrary.com.

DOI 10.1002/hep.29213

Potential conflict of interest: Nothing to report.

infected with HBV.⁽⁴⁾ HBV immunity could be easily reactivated by chemical drugs such as those used for the treatment of cancer or corticosteroids used for immune diseases,⁽⁵⁻⁷⁾ which would lead to liver damage from mild inflammation to liver failure.

HBsAg seroconversion after treatment is associated with good prognosis in CHB subjects.⁽⁸⁾ The European Association for the Study of the Liver suggested that HBsAg clearance was the aim of HBV treatment. Subsequently, the Asian Pacific Association for the Study of the Liver has replaced the term “inactive HBsAg carrier status” with “low-level viral replication of chronic HBV infection” and stated that HBsAg clearance was the goal of treating chronic HBV infection.⁽⁹⁾

Currently, it is not clear what the effects of HBsAg clearance are on the already benign outcome for IHCs. In our clinical practice, because of social discrimination, spiritual pressure, and career selections, etc., in China, some IHC subjects would like to get HBsAg cleared through treatment. The aim of this study was to assess the feasibility of HBsAg clearance and the safety profiles of antiviral treatment in IHCs. We enrolled 144 IHC subjects, 102 of whom received antiviral therapy. Based on our previous HBV antiviral treatment studies for CHB,⁽¹⁰⁻¹²⁾ we employed a 96-week PEG-IFN α -2a with or without adefovir dipivoxil (ADV) treatment strategy.

Patients and Methods

PATIENTS AND INCLUSION CRITERIA

A total of 144 IHCs were recruited in the study and received regular follow-up and clinical exams between September 2011 and October 2013 at Beijing You'an

Hospital. All IHCs met the criteria defined in the prevention and treatment guidelines for CHB (2010 edition)⁽¹³⁾: (1) HBsAg-positive for more than 6 months, HBeAg-negative, antibody to HBeAg-positive, antibody to hepatitis B core antigen-positive, HBV DNA <2000 IU/mL, normal level of ALT; (2) quantitative HBsAg <1,000 IU/mL; (3) total bilirubin <25 μ mol/L, albumin >40 g/L, international normalized ratio 0.8-1.2; (4) white blood cell count 4 to 10 $\times 10^9$ /L, platelet count (PLT) 100 to 300 $\times 10^9$ /L; (5) FibroScan value <6.4 kPa; (6) no antiviral therapy within 1 year; (7) age in the range 18-60 years; (8) exclusion of human immunodeficiency virus, hepatitis C virus, and hepatitis D virus coinfection, autoimmune diseases, decompensated liver function, severe metabolic disease complicated by hepatocellular carcinoma, liver cirrhosis, or tumors of other systems. All subjects provided written informed consent prior to the study. The protocol and the consent form for the study were approved by the research ethical committee of the Beijing You'an Hospital, Capital Medical University, China ([2011]04).

TREATMENT AND CLINICAL RESPONSE

After being informed of the benefits and risks of treatment, subjects were given the choice between joining either the treatment group or the control group. In the treatment group, subjects with undetectable HBV DNA (<20 IU/mL) were treated with a subcutaneous injection of PEG-IFN α -2a (180 μ g/week), and those with HBV DNA positive received a combination therapy of subcutaneous PEG-IFN α -2a and oral ADV (10 mg/day) (Supporting Fig. S1). Subjects in the control group did not receive antiviral therapy. The dosage of PEG-IFN α -2a was adjusted to 135 μ g/week if

ARTICLE INFORMATION:

From the ¹International Medical Department, Beijing Youan Hospital, Capital Medical University, Beijing, China; ²Section on Biomarkers and Prediction Modeling, Department of Medicine, University of Pittsburgh, Pittsburgh, PA.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

Xinyue Chen, M.S.
International Medical Department, Beijing Youan Hospital
Capital Medical University
Beijing, 100069 China
E-mail: chenxydoc@163.com
Tel: +86-10-83997159
or

Chengli Shen, M.D., Ph.D.
Section on Biomarkers and Prediction Modeling
Department of Medicine
University of Pittsburgh
Pittsburgh, PA 15261
E-mail: chs97@pitt.edu
Tel: +1-412-246-6958

neutrophil counts were $\leq 0.75 \times 10^9$ or PLT was $< 50 \times 10^9$. The treatment would be stopped if neutrophil counts were $\leq 0.50 \times 10^9$ or PLT was $< 25 \times 10^9$ or serious adverse events occurred. The total treatment duration was 96 weeks or less. In brief, if a subject cleared HBsAg before 72 weeks, this subject would receive 24 weeks of consolidation treatment and then stop treatment; if a subject cleared HBsAg during 72–96 weeks, this subject received consolidation treatment from the time of HBsAg clearance to week 96. So, the consolidation duration was less than 24 weeks, and the total treatment duration was 96 weeks; if a subject did not clear HBsAg during the treatment, the subject would be treated for 96 weeks and would not receive consolidation treatment. The consolidation treatment strategies were the same as the original treatment. The treatment effect was determined by HBsAg clearance. Subjects who achieved HBsAg clearance in 96 weeks were regarded as responders, and those in whom HBsAg was not cleared were considered nonresponders.

LABORATORY METHODS

ALT, total bilirubin, albumin, international normalized ratio, total white blood cell count, PLT, HBV DNA, HBsAg, and HBsAg antibody were measured at baseline and every 12 weeks during the treatment period. Thyroid hormone, thyroid antibodies, autoantibodies, and FibroScan were measured at baseline and every 24 weeks. In addition, white blood cell count, neutrophil counts, and PLT were measured weekly in the first 2 weeks of treatment. HBV DNA was quantified by fluorescence quantitative polymerase chain reaction with a detection limit of 20 IU/mL. HBsAg and HBsAg antibody were quantified by HBsAg quantitative Elecsys (Roche Diagnostics GmbH, Germany), with a detection limit of 0.05 IU/mL for HBsAg. Antibody to HBsAg > 10 IU/L was defined as positivity.

STATISTICAL ANALYSIS

Data were expressed as counts and percentages for categorical variables and means and standard deviations for continuous variables. The Student *t* test or rank-sum test was used for two-group comparisons of continuous variables, and the chi-squared or Fisher exact test was used for categorical variables, as appropriate. Receiver operator characteristic curves, which plot sensitivity by $1 - \text{specificity}$, and the area under

the receiver operator characteristic curve (AUROC) were used to evaluate the prognostic values of the quantitative HBsAg level and change at week 12 and week 24 in predicting HBsAg clearance. The cutoff point, which optimized the sensitivity and specificity, was selected for the subsequent prediction. Univariable and multivariable logistic regression analyses were conducted to evaluate the magnitude and significance of the association. A two-sided *P* value < 0.05 was considered statistically significant. Analyses were performed using the Statistical Package for the Social Sciences (SPSS, version 11.5) and STATA (version 14.1).

Results

THERAPY OUTCOMES

A total of 144 IHCs were enrolled in the study, 102 (70.8%) of whom received antiviral therapy as the treatment group and 42 (29.2%) of whom did not receive treatment as a control group. Eight subjects in the treatment group stopped treatment because of adverse events, which might be associated with PEG-IFN α -2a treatment; and 94 subjects (92.2%) completed the scheduled treatment. Forty subjects in the control group were followed up to week 96, and 2 subjects were lost follow-up at week 24 (Fig. 1).

BASELINE CHARACTERISTICS

The analysis was conducted on subjects who finished the scheduled treatment or follow-up. Among the subjects in the treatment group, 45 with HBV DNA level < 20 IU/mL received PEG-IFN α -2a (180 μ g/week) and 49 with HBV DNA 20–2,000 IU/mL received a combination therapy of PEG-IFN α -2a (180 μ g/week) with ADV. Baseline characteristics of gender, age, infection mode, baseline levels of ALT, HBV DNA, and HBsAg were not statistically different between the treatment and control groups (Table 1).

HBsAg CLEARANCE AND SEROCONVERSION RATES

Analyses were again restricted to subjects who finished the scheduled treatment or follow-up (per protocol analysis). The HBsAg clearance and seroconversion rates in the treatment group were 29.8% (28/94) and 20.2% (19/94) at week 48 and 44.7% (42/94) and 38.3% (36/94) at week 96, respectively. Only one subject in the control group achieved

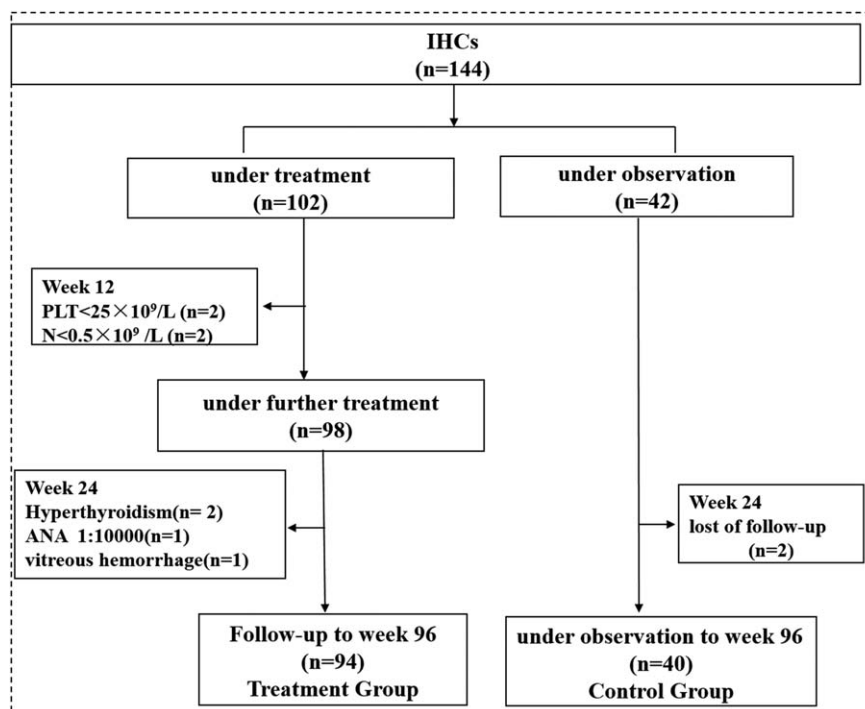


FIG. 1. Patient demographics. Abbreviation: ANA, antinuclear antibodies.

HBsAg clearance at week 48 and week 96. The HBsAg clearance rate in the control group was 2.4% (1/40), and no subject in the control group achieved seroconversion. The HBsAg clearance and seroconversion rates in the control group were significantly lower than those in the treatment group at weeks 48 and 96 (Fig. 2) ($P < 0.001$), respectively. The treatment group was divided into HBV DNA-negative (PEG-IFN α -2a monotherapy) and HBV DNA-positive (PEG-IFN α -2a with ADV). The HBsAg clearance and HBsAg seroconversion rates were compared with their respective control group (HBV DNA-negative in the control group and HBV DNA-positive in the control group) at week 96. The HBsAg clearance and seroconversion rates were 38.8% and 34.7% in the HBV DNA-positive (PEG-IFN α -2a with ADV) group and 51.1% and 42.2% in the HBV DNA-negative (PEG-IFN α -2a monotherapy) group, respectively, which were statistically significantly higher than their corresponding control groups ($P < 0.001$) (Supporting Tables S1 and S2). Furthermore, we also did an intention-to-treatment analysis (all subjects who were enrolled in the study were included in the analysis). Similar to the per protocol analysis, HBsAg clearance rates were significantly higher in the treatment group than in the control group.

HBV DNA UNDETECTABLE RATE

At week 48, 98.0% (48/49) of the subjects in the treatment group decreased from HBV DNA >20 IU/mL at baseline to <20 IU/mL after receiving the combination therapy of PEG-IFN α -2a with ADV. Only one subject had detectable HBV DNA at a concentration of 23.7 IU/mL. At week 96, HBV DNA levels were <20 IU/mL for all subjects. All 17 subjects in the control group with HBV DNA levels >20 IU/mL at

TABLE 1. Baseline Characteristics of Subjects

	Treatment Group (n = 94)	Control Group (n = 40)	P
Male, n (%)	62 (66.0%)	26 (65.0%)	1.000
Age (years)	38.8 $\pm$ 10.0	39.8 $\pm$ 10.6	0.665
Mode of transmission			
Vertical (%)	36 (38.3%)	13 (32.5%)	0.562
Others (%)	58 (61.7%)	27 (67.5%)	
ALT (IU/L)	28.3 $\pm$ 9.0	25.4 $\pm$ 8.4	0.549
HBV DNA, n (%)			
<20 IU/mL	45 (47.9%)	23 (57.5%)	0.516
20-1,000 IU/mL	25 (26.6%)	10 (25.0%)	
1,000-2,000 IU/mL	24 (25.5%)	7 (17.5%)	
HBsAg, n (%)			
<100 IU/mL	27 (28.7%)	14 (35.0%)	0.675
100-500 IU/mL	32 (30.4%)	14 (35.0%)	
500-1,000 IU/mL	35 (37.2%)	12 (30.0%)	
FibroScan value	4.6 $\pm$ 1.1	4.6 $\pm$ 1.0	0.903

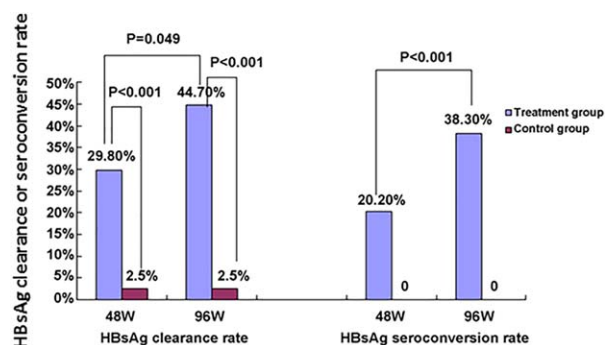


FIG. 2. HBsAg clearance and seroconversion rates in the treatment group and control group.

baseline did not change significantly during the follow-up period, indicating no spontaneous HBV DNA clearance.

WEEK 12 ALT ELEVATION AND HBsAg LEVEL ON HBsAg CLEARANCE

At week 12, 59.6% (56/94) of the subjects in the treatment group showed an elevated ALT level (>50 IU/L) with a maximum of 500.4 IU/L, and total bilirubin levels were all <40 μ mol/L. To evaluate the ALT change on HBsAg clearance, ALT was dichotomized into elevation or not. To assess the effects of the quantitative HBsAg levels and changes during the early period of treatment, we assessed the $\log_{10}$ HBsAg level at baseline, week 12, week 24, week 12 change from baseline, and week 24 change from baseline using univariable logistic regression analysis. The results showed that baseline HBsAg (odds ratio [OR], 2.34, $P = 0.005$), week 12 HBsAg (OR, 2.88, $P < 0.001$), week 24 HBsAg (OR, 3.03, $P < 0.01$), week 12 HBsAg change from baseline (OR, 5.98, $P < 0.001$), week 24 HBsAg change from baseline (OR, 4.34, $P < 0.001$), and week 12 ALT elevation (OR, 3.04, $P = 0.013$) were strong predictors for HBsAg clearance at week 96. Baseline gender, age, HBV DNA level, and treatment (PEG-IFN α -2a versus PEG-IFN α -2a + ADV) were not statistically significant (Table 2).

In order to further evaluate baseline and change of HBsAg in early treatment in predicting HBsAg clearance, multivariable logistic regressions were conducted for HBsAg levels at baseline, week 12, and week 24 as well as the week 12 and week 24 HBsAg change from baseline adjusted for age, gender, treatment, and an

increase of ALT. Similar to the univariable regression analysis results, all were significantly related to HBsAg clearance: the week 12 HBsAg decrease had the highest OR (5.34, 95% confidence interval [CI] 2.09–13.69), and the baseline HBsAg had the lowest OR (2.31, 95% CI 1.26–4.25). The OR of baseline HBsAg $<$ week 12 HBsAg $<$ week 24 HBsAg, indicating that HBsAg levels in early treatment were associated with HBsAg clearance and that early treatment HBsAg response (week 12 HBsAg change from baseline, week 24 HBsAg change from baseline) was related to HBsAg clearance, with an OR of 5.34 (95% CI 2.09–13.69) and 4.86 (95% CI 2.38–9.90). The OR of week 12 ALT elevation was 3.52 (95% CI 1.37–9.05) adjusted for age, gender, and treatment (Table 2).

RATES OF HBsAg CLEARANCE AMONG SUBJECTS WITH FAVORABLE BASELINE, WEEK 12, OR WEEK 24 ANTIVIRAL TREATMENT RESPONSE

Receiver operating characteristic curves were used to evaluate the performance of the above significant variables on HBsAg clearance. The AUROCs of HBsAg level were 0.64, 0.77, and 0.84 and the optimal cut points were <2.3 $\log_{10}$ IU/mL, <2.0 $\log_{10}$ IU/mL, and <1.7 $\log_{10}$ IU/mL at baseline, week 12, week 24, respectively. The AUROCs of week 12 HBsAg change from baseline and week 24 HBsAg change from baseline were 0.791 and 0.841, and the optimal cutoff points were >0.3 $\log_{10}$ IU/mL and >0.6 $\log_{10}$ IU/mL, respectively. For ALT, the AUROC of ALT was 0.629. The sensitivity and specificity of HBsAg levels for the predictions increased from 69.2% to 80.8% and from 54.8% to 73.8% from baseline to treatment week 24. The sensitivities were 71.4% and 76.2% and the specificities were both 80.8% for week 12 and week 24 HBsAg change from baseline, respectively (Fig. 3).

Based on the optimal cutoff values, in our data set the rates of HBsAg clearance were 57.5% (23/40), 70.7% (29/41), 73.8% (31/42), and 55.3% (31/56) for subjects with baseline HBsAg <2.3 $\log_{10}$ IU/mL, week 12 HBsAg change from baseline >0.3 $\log_{10}$ IU/mL, week 24 HBsAg change from baseline >0.6 $\log_{10}$ IU/mL, and week 12 ALT elevation, respectively (Fig. 4).

HBsAg quantitation, HBsAg response in early treatment, and week 12 ALT elevation were related to

TABLE 2. Baseline Variables and Change of HBsAg in Week 12 and Week 24 Associated With HBsAg Clearance

Variables	Univariable Analysis			Multivariable Analysis		
	OR	95% CI	P	OR	95% CI	P
Gender	0.944	(0.400-2.223)	0.896	—	—	—
Age	0.995	(0.955-1.037)	0.812	—	—	—
HBV DNA	0.605	(0.267-1.375)	0.231	—	—	—
Treatment*	0.605	(0.267-1.375)	0.231	—	—	—
Baseline HBsAg	2.341	(1.289-4.252)	0.005	2.313	(1.258-4.251)	0.007
Week 12 HBsAg	2.876	(1.750-4.726)	<0.001	3.159	(1.826-5.466)	<0.001
Week 24 HBsAg	3.025	(1.963-4.663)	<0.001	3.347	(2.050-5.465)	<0.001
Week 12 HBsAg change from baseline	5.976	(2.228-16.033)	<0.001	5.343	(2.085-13.689)	<0.001
Week 24 HBsAg change from baseline	4.338	(2.302-8.176)	<0.001	4.855	(2.380-9.902)	<0.001
Week 12 ALT elevation	3.043	(1.266-7.315)	0.013	3.520	(1.369-9.052)	0.009

*PEG-IFN α -2a versus PEG-IFN α -2a+ADV.

HBsAg clearance. Week 12 HBsAg change from baseline was highly correlated with week 12 HBsAg ($r = -0.81$), and week 24 HBsAg change from baseline was highly correlated with week 24 HBsAg ($r = -0.83$). The AUROCs of week 12 HBsAg change from baseline (0.791) and week 24 HBsAg change from baseline (0.841) were larger than the corresponding AUROCs of week 12 (0.768) and week 24 (0.836). To avoid redundancy in the variables, we used week 12 HBsAg change from baseline and week 24 HBsAg change from baseline instead of week 12 and week 24 HBsAg to calculate the HBsAg clearance rate. We

combined HBsAg decrease and week 12 ALT elevation together. Subjects with week 12 HBsAg change from baseline >0.3 log₁₀ IU/mL and ALT elevation at week 12 had an HBsAg clearance rate of 80.8% (21/26), and those with week 24 HBsAg change from baseline >0.6 log₁₀ IU/mL and ALT increase at week 12 had an HBsAg clearance rate of 84.0% (21/25). Only 17.2% (5/29) of subjects cleared HBsAg among those with week 12 HBsAg change from baseline ≤ 0.3 log₁₀ IU/mL and no ALT elevation and 14.3% (4/28) of subjects with week 24 HBsAg change from baseline ≤ 0.6 log₁₀ IU/mL and no ALT elevation

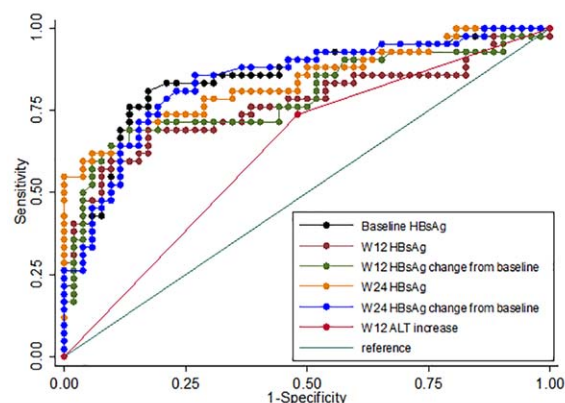


FIG. 3. Receiver operator characteristic curves of baseline HBsAg level, week 12 HBsAg, week 24 HBsAg, week 12 HBsAg change from baseline, week 24 HBsAg change from baseline, and week 12 ALT elevation on the prediction of HBsAg clearance. Abbreviation: SD, standard deviation.

HBsAg level or change	Area	SD	95% Confidence interval	Cut point	Sensitivity & specificity
Baseline HBsAg level	0.644	0.060	(0.526 0.762)	<2.3	69.2%, 54.8%
Week 12 HBsAg	0.768	0.053	(0.665 0.872)	<2.0	76.9%, 69.1%
Week 24 HBsAg	0.836	0.043	(0.751 0.922)	<1.7	80.8%, 73.8%
Week 12 HBsAg change from baseline	0.791	0.050	(0.693 0.889)	>0.3	71.4%, 80.8%
Week 24 HBsAg change from baseline	0.841	0.042	(0.759 0.923)	>0.6	76.2%, 80.8%
Week 12 ALT elevation	0.629	0.049	(0.533 0.724)	-	73.8%, 51.9%

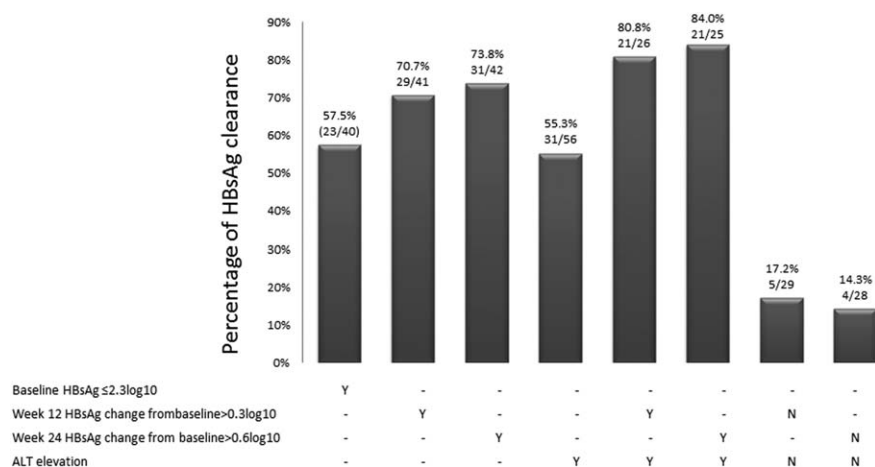


FIG. 4. Percentage of HBsAg clearance among patients stratified by baseline, week 12, and week 24 HBsAg levels; week 12 HBsAg change from baseline; week 24 HBsAg change from baseline; and week 12 ALT elevation.

(Fig. 4). The quantity of HBsAg in early treatment with the combination of ALT elevation at week 12 was a strong predictor for HBsAg clearance (Fig. 4). As mentioned earlier, because of the high correlation of week 12 and week 24 HBsAg with week 12 and week 24 HBsAg change from baseline, the combinations of week 12 and week 24 with ALT are not shown in the graph.

SAFETY

Of the 102 IHCs who received PEG-IFN α -2a with or without ADV, 8 withdrew from treatment because of side effects. At week 12, 2 subjects stopped treatment due to a PLT decrease ($9 \times 10^9/L$ and $18 \times 10^9/L$) and 2 due to a neutrophil count decrease ($0.48 \times 10^9/L$ and $0.40 \times 10^9/L$). At week 24, 4 subjects were withdrawn because of exclusionary diagnoses: 2 had hyperthyroidism, 1 had vitreous hemorrhage, and 1 had antinuclear antibodies 1:10,000 (Fig. 1). In addition, the most common side effects in the treatment group were fever in 57.8% (59/102) and slight decreases of neutrophil count and PLT in 38.2% (39/102) and 24.5% (25/102), respectively. In two subjects, neutrophil count decreased to $0.68 \times 10^9/L$ at week 48, and one decreased to $0.74 \times 10^9/L$ at week 72. The dosage of PEG-IFN α -2a was adjusted to 135 μg /week. The neutrophil count remained stable until the end of the treatment. The other most common adverse events were known to occur with PEG-IFN therapy and include fatigue, pyrexia, lipotrichia, and rash (Supporting Table S3). The lab tests and FibroScan values (median, minimum, and maximum) during treatment are shown in Supporting Table S4. The

treatment for 96 weeks was generally well tolerated, and the adverse events were similar with the 48-week treatment strategy for CHBs.

FOLLOW-UP AFTER DRUG WITHDRAWAL

Twelve subjects achieved HBsAg clearance before 96 weeks, 8 were followed for 24 weeks, 1 for 36 weeks, and 3 for 48 weeks. All maintained HBsAg clearance. The 52 subjects without HBsAg clearance after the treatment had no obvious increase of HBV DNA level after drug withdrawal, ALT levels in the normal range, and no aggravations observed.

Discussion

In this study, the HBsAg clearance and seroconversion rates were 44.7% and 38.3% at week 96, respectively. These rates were substantially higher than previously published reports that the HBsAg clearance rates for HBeAg-positive and HBeAg-negative CHB subjects were only 3%⁽²⁾ and 4%⁽¹⁴⁾ after 1-year PEG-IFN-based therapy and 0.5-year follow-up. Due to the common adverse reactions induced by PEG-IFN, such as decline of white blood cells and platelets, hyperthyroidism, and an abnormal level of autoantibodies, 8 IHCs in the therapy group stopped the treatment. Some subjects experienced fatigue, pyrexia, lipotrichia, and rash, which were tolerable. All of these adverse reactions reversed after drug withdrawal. In addition, 59.6% (56/94) of the subjects in the treatment group showed an elevated ALT level at week 12

after the treatment, and total bilirubin levels were all $<40 \mu\text{mol/L}$, which may be related to immune activation and anti-HBV response during IFN-related intervention. No liver function deterioration was observed in this study. Subjects who did not achieve HBsAg clearance did not show a rebounded HBV DNA level, and ALT remained normal. Therefore, PEG-IFN α -2a therapy for IHCs had a reasonable safety profile.

Even though the therapy period was extended to 96 weeks for IHCs with a low level of HBsAg, 60% of subjects could not achieve HBsAg clearance. Therefore, it is important to investigate the underlying reasons that can facilitate the development of an individualized therapeutic strategy and predict the therapeutic outcomes. The quantitative determination of HBsAg was important for antiviral treatment⁽¹⁵⁻¹⁸⁾ because it could be used as an indicator of immune control to predict HBeAg seroconversion, HBsAg clearance, and long-term prognosis. In addition, the early decline of HBsAg during treatment was an important predictor of sustained response to PEG-IFN treatment. The analysis of these factors with univariable logistic regression showed that the levels of HBsAg at baseline, week 12, and week 24; the decline of HBsAg during early therapy (at weeks 12 and 24) from baseline; and the elevation of ALT at week 12 were all statistically associated with HBsAg clearance. The results were similar with multivariable logistic regression adjusted for baseline variables. The quantity of HBsAg and ALT was assessed using the AUROCs; optimal cutoff points were selected based on sensitivity and specificity. Of subjects with week 12 HBsAg change from baseline $>0.3 \log_{10} \text{ IU/mL}$ and a week 12 ALT increase, 80.8% (21/26) cleared HBsAg. Of subjects with week 24 HBsAg change from baseline $>0.6 \log_{10} \text{ IU/mL}$ and a week 12 ALT increase, 84.0% achieved HBsAg clearance. Therefore, the elevation of ALT and decrease of HBsAg at weeks 12 and 24 were potential markers for the early prediction of HBsAg clearance in clinical practice. Although this study showed a high percentage of HBsAg clearance in IHCs, the long-term benefit of HBsAg clearance for IHCs is still not clear. We, therefore, seek to follow these subjects and investigate the long-term prognosis including the reappearance of HBsAg, chronic hepatitis, and other HBV-related diseases.

The limitations of this study included lack of randomization, an inability to evaluate the effect of HBV DNA on treatment (the different treatment strategies for the HBV DNA-positive and the HBV DNA-negative group), a relatively small sample size, and lack

of 1-year and long-term posttreatment HBsAg relapse data. However, despite these limitations, we provide a proof-of-concept prospective cohort study for the treatment of IHCs with high HBsAg clearance rates and relatively safe profiles.

Acknowledgment: We thank Dr. Douglas Landsittel for revision suggestions and critical reading.

REFERENCES

- 1) Chang TT, Gish RG, de Man R, Gadano A, Sollano J, Chao YC, et al. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. *N Engl J Med* 2006;354:1001-1010.
- 2) Lau GK, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, Cooksley G, et al. Peginterferon alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. *N Engl J Med* 2005;352:2682-2695.
- 3) Chu CM, Liaw YF. Spontaneous relapse of hepatitis in inactive HBsAg carriers. *Hepatology* 2007;45:311-315.
- 4) Chen JD, Yang HI, Iloeje UH, You SL, Lu SN, Wang LY, et al. Carriers of inactive hepatitis B virus are still at risk for hepatocellular carcinoma and liver-related death. *Gastroenterology* 2010;138:1747-1754.
- 5) Chung SJ, Kim JK, Park MC, Park YB, Lee SK. Reactivation of hepatitis B viral infection in inactive HBsAg carriers following anti-tumor necrosis factor- α therapy. *J Rheumatol* 2009;36:2416-2420.
- 6) Hwang JP, Lok AS. Management of patients with hepatitis B who require immunosuppressive therapy. *Nat Rev Gastroenterol Hepatol* 2014;11:209-219.
- 7) Hoofnagle JH. Reactivation of hepatitis B. *HEPATOLOGY* 2009;49(5 Suppl.):S156-S165.
- 8) Moucari R, Korevaar A, Lada O, Martinot-Peignoux M, Boyer N, Mackiewicz V, et al. High rates of HBsAg seroconversion in HBeAg-positive chronic hepatitis B patients responding to interferon: a long-term follow-up study. *J Hepatol* 2009;50:1084-1092.
- 9) Sarin SK, Kumar M, Lau GK, Abbas Z, Chan HL, Chen CJ, et al. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. *Hepatology* 2016;62:1-24.
- 10) Cao ZH, Ma LN, Zhang HW, Liu YL, Chen XY. Extended treatment with peginterferon alpha-2a in combination with lamivudine or adefovir for 96 weeks yields high rates of HBeAg and HBsAg seroconversion. *J Dig Dis* 2013;14:446-450.
- 11) Chen X, Cao Z, Liu Y, Zhang H, Zhang Y, Ma L, et al. Potent hepatitis B surface antigen response to treatment of hepatitis-B-e-antigen-positive chronic hepatitis B with alpha-interferon plus a nucleos(t)ide analog. *J Gastroenterol Hepatol* 2012;27:481-486.
- 12) Lu J, Zhang S, Liu Y, Du X, Ren S, Zhang H, et al. Effect of Peg-interferon alpha-2a combined with adefovir in HBV postpartum women with normal levels of ALT and high levels of HBV DNA. *Liver Int* 2015;35:1692-1699.
- 13) Chinese Society of Hepatology, Chinese Society of Infectious Diseases of CMA. The guideline of prevention and treatment for chronic hepatitis B (2010 version). [in Chinese] *Zhonghua Gan Zang Bing Za Zhi* 2011;19:13-24.
- 14) Marcellin P, Lau GK, Bonino F, Farci P, Hadziyannis S, Jin R, et al. Peginterferon alfa-2a alone, lamivudine alone, and the two

- in combination in patients with HBeAg-negative chronic hepatitis B. *N Engl J Med* 2004;351:1206-1217.
- 15) Ahn SH, Chan HL, Chen PJ, Cheng J, Goenka MK, Hou J, et al. Chronic hepatitis B: whom to treat and for how long? Propositions, challenges, and future directions. *Hepatol Int* 2010; 4:386-395.
- 16) Marcellin P, Bonino F, Lau GK, Farci P, Yurdaydin C, Piratvisuth T, et al. Sustained response of hepatitis B e antigen-negative patients 3 years after treatment with peginterferon alpha-2a. *Gastroenterology* 2009;136:2169-2179.
- 17) Sonneveld MJ, Rijckborst V, Boucher CA, Hansen BE, Janssen HL. Prediction of sustained response to peginterferon alfa-2b for hepatitis B e antigen-positive chronic hepatitis B using on-treatment hepatitis B surface antigen decline. *HEPATOLOGY* 2010; 52:1251-1257.
- 18) Brunetto MR, Moriconi F, Bonino F, Lau GK, Farci P, Yurdaydin C, et al. Hepatitis B virus surface antigen levels: a guide to sustained response to peginterferon alfa-2a in HBeAg-negative chronic hepatitis B. *HEPATOLOGY* 2009;49:1141-1150.

Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.29213/supinfo.