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Original Article

Immunomodulatory and Antiviral Therapy Improved Functional Cure Rate in CHB Patients with High HBsAg Level Experienced NA

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Abstract

Background and Aims: A functional cure, or hepatitis B virus (HBV) surface antigen (HBsAg) loss, is difficult to achieve in patients with hepatitis B virus e antigen (HBeAg)-positive chronic hepatitis B. The HBV vaccine and granulocyte-macrophage colony-stimulating factor (GM-CSF) have been reported to help reduce HBsAg levels and promote HBsAg loss. In this prospective randomized trial, we evaluated HBsAg loss in patients receiving pegylated interferon-α2b (PEGIFN-α2b) and tenofovir disoproxil fumarate (TDF), with and without GM-CSF and HBV vaccination. ***Methods:*** A total of 287 patients with HBeAg positive chronic hepatitis B and seroconversion after nucleot(s)ide analog treatment were assigned randomly to three treatment groups for 48 weeks, TDF alone (control), PEGIFN-α2b + TDF, and PEGIFN-α2b + TDF + GM-CSF + HBV vaccine. The primary endpoints were the proportions of patients with HBsAg loss and seroconversion at 48 and 72 weeks. ***Results:*** The cumulative HBsAg loss rates in the control, PEGIFN-α2b + TDF, and PEGIFN-α2b + TDF + GM-CSF + HBV vaccine groups at week 48 were 0.0%, 28.3%, and 41.1%, respectively. The cumulative HBsAg seroconversion rates in these groups at week 48 were 0.0%, 21.7%, and 33.9%, respectively. Multivariate regression analysis showed that GM-CSF use plus HBV vaccination was significantly associated with HBsAg loss (p=0.017) and seroconversion (p=0.030). ***Conclusions:*** In patients with HBeAg-positive chronic hepatitis B and seroconversion after nucleot(s)ide analog treatment, immunomodulatory/antiviral treatment regimens effectively improved HBsAg loss, and the regimen including GM-CSF and HBV vaccination was most effective.

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Keywords: Chronic hepatitis B; HBsAg loss; Functional cure; Immunomodulatory/antiviral therapy; Pegylated interferon-α2b; Tenofovir disoproxil fumarate; Granulocyte-macrophage colony-stimulating factor; Hepatitis B virus vaccine.

Abbreviations: ALB, albumin; ALT, alanine aminotransferase; APRI, aspartate aminotransferase to platelet ratio index; AST, aspartate aminotransferase; BMI, body mass index; CHB, chronic hepatitis B; CI, confidence interval; CTL, cytotoxic T lymphocyte; FIB-4, fibrosis-4; GGT, γ-glutamyl transpeptadase; GM-CSF, granulocyte-macrophage colony-stimulating factor; HBeAg, hepatitis B virus e antigen; HBsAg, hepatitis B virus surface antigen; HBV, hepatitis B virus; IQR, interquartile range; NA, nucleot(s)ide analog; NEU, neutrophil; OR, odds ratio; PEGIFN-α, pegylated interferon alpha; PLT, platelet; RBC, red blood cell; TB, total bilirubin; TDF, tenofovir disoproxil fumarate; WBC, white blood cell.

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Introduction

Hepatitis B virus (HBV) is a hepatophilic virus that has infected approximately 2 billion people worldwide, of whom 3.5% have chronic and persistent infections, and are a major public health problem.[1,2] Without effective treatment, chronic HBV infection can progress to cirrhosis or hepatocellular carcinoma accompanied by a high risk of liver failure.[3,4] Currently, the main forms of antiviral therapy are nucleot(s)ide analogs (NAs) and pegylated interferon-α (PEGIFN-α). NAs are used widely for the treatment of chronic hepatitis B (CHB) because of their ease of use, good tolerability, and potent antiviral activity.[5] However, long-term or even lifelong treatment is required because of the difficulty of obtaining durable immune control and high virological and clinical relapse rates after drug discontinuation. NA treatment can achieve the complete suppression of HBV viral replication, but a functional cure, i.e., HBV surface antigen (HBsAg) loss, is difficult to achieve with NA or IFN treatment alone. The incidence of hepatocellular carcinoma is four times higher in patients with virological suppression alone than in those with HBsAg loss.[6]

Thus, the functional cure rate for CHB needs to be improved. The combined use of PEGIFN-α and NAs, which have different mechanisms of action, has been found to improve the rate of HBsAg loss, but only to 9.1%.[7] Thus, stronger immunomodulators need to be identified and applied to meet this goal.[7]

The HBV vaccine is often recommended as an alternative or complement to antiviral drugs for the treatment of CHB.[8] In some patients with CHB, HBV vaccination alone effectively maintains alanine aminotransferase (ALT) normalization and HBV e antigen (HBeAg) serological conversion.[9] In addition to preventing HBV infection by stimulating antibody production, HBV vaccines inhibit the replication of HBV DNA through a specific $CD4^+$ T-cell-mediated immune response; furthermore, this approach is inexpensive and side effects are rare.[10]

The immunomodulator granulocyte-macrophage colony-stimulating factor (GM-CSF) is also commonly used clinically to treat CHB. It increases granulocyte production and promotes innate immunity, and may enhance vaccine effects. Compared with HBV vaccination alone, an appropriate combined vaccination and GM-CSF-based drug treatment regimen can increase antibody levels by 8–10 times, increase the immune memory response by up to 5–10 times, and double cytotoxic T lymphocyte action, suggesting that GM-CSF contributes to the reduction of HBsAg levels and promotion of HBsAg loss.[11,12]

Our group has developed an immunomodulatory/antiviral regimen consisting of IFN-α, adefovir plus GM-CSF, and HBV vaccination, whose application resulted in a 9.2% HBsAg loss rate in HBeAg-positive patients with CHB. This prospective multicenter randomized controlled study was conducted to evaluate the efficacy and safety of different combination regimens using the more efficient PEGIFN-α2b and tenofovir disoproxil fumarate (TDF) in patients with HBeAg-positive CHB that seroconverted after NA treatment, to contribute to the overall goal of identifying an optimal antiviral treatment regimen for CHB.

Methods

Study design and treatment

In this prospective multicenter randomized controlled study, patients were assigned randomly in equal numbers to three treatment groups for 48 weeks: TDF alone (control), PEGIFN-α2b + TDF, and PEGIFN-α2b + TDF + GM-CSF + HBV vaccine. All patients subsequently received TDF alone for an additional 24 weeks. The drug sources and doses were TDF (Brilliant Pharmaceutical B, Fuzhou, China), 300 mg orally once daily; PEGIFN-α2b (Pegberon; Amoytop Biotech, Xiamen, China), 180 μg injected subcutaneously once a week; GM-CSF (Topleucon; Amoytop Biotech), 75 μg injected subcutaneously on Wednesday, Thursday, and Friday of weeks 1, 4, 12, 24, 36, and 48, a total of 18 injections; and recombinant Chinese hamster ovary cell HBV vaccine containing 20 μg HBsAg (North China Pharmaceutical Company Ltd. Shijiazhuang, China), injected intramuscularly on Saturday in weeks 1, 4, 12, 24, 36, and 48, a total of six injections. The study was approved by the Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine (No. 2018-515).

Patients

The study was conducted at nine centers in China. The inclusion criteria were (1) age 18–65 years, (2) CHB and HBeAg positivity with maintenance of the HBV DNA level below the detection limit and HBeAg seroconversion for >1 year after NA therapy, and (3) provision of written informed consent. The exclusion criteria were (1) other viral infection (e.g., hepatitis A, C, D, or E virus or human immunodeficiency virus), (2) cirrhosis or Child-Pugh score ≥ 7 at the time of enrollment, (3) liver disease of another cause (e.g., autoimmune liver disease, alcoholic liver disease, non-alcoholic fatty liver disease, or drug-related hepatitis), (4) serum creatinine level higher than normal, (5) liver malignancy or alpha-fetoprotein level >100 ng/mL at the time of enrollment, (6) other malignancy, (7) history of important organ transplantation, (8) interferon (IFN) contraindication (e.g., autoimmune, endocrine, or psychiatric disease combined with a history of serious heart, brain, kidney, retina, or other important organ/tissue disease), and (9) allergy to IFN, NAs, GM-CSF, or the HBV vaccine.

Assessment

The enrolled patients were tested routinely to detect and quantify hepatitis B markers, HBV DNA, other viral hepatitis markers, routine blood laboratory and biochemistry parameters, autoimmune liver disease-related indicators, and thyroid function. Abdominal color Doppler ultrasound examinations were also performed. In the first and second weeks of the treatment period, routine blood and liver function tests were performed; in the fourth week, routine blood and liver function tests, hepatitis B marker detection, and HBV DNA quantification were performed. Thereafter, assessments were performed every 4 weeks until 48 weeks, and then every 12 weeks until 72 weeks. Routine blood and liver function tests were performed every 4 weeks, and hepatitis B marker detection, HBV DNA quantification, blood biochemistry, thyroid function testing, antinuclear antibody detection, alpha-fetoprotein measurement, and the assessment of other indicators were performed every 12 weeks.

Outcomes

The primary endpoints were the proportions of patients with HBsAg loss (<0.05 IU/mL) and HBsAg seroconversion (HBsAg level <0.05 IU/mL and HBV surface antibody level >10 IU/mL) at week 48. Secondary endpoints were HBsAg decline from baseline at weeks 12, 24, 36, 48, 60, and 72.

Sample size estimation

For sample size estimation, we assumed HBsAg loss rates of 5% in the control group and 20% in one or both of the

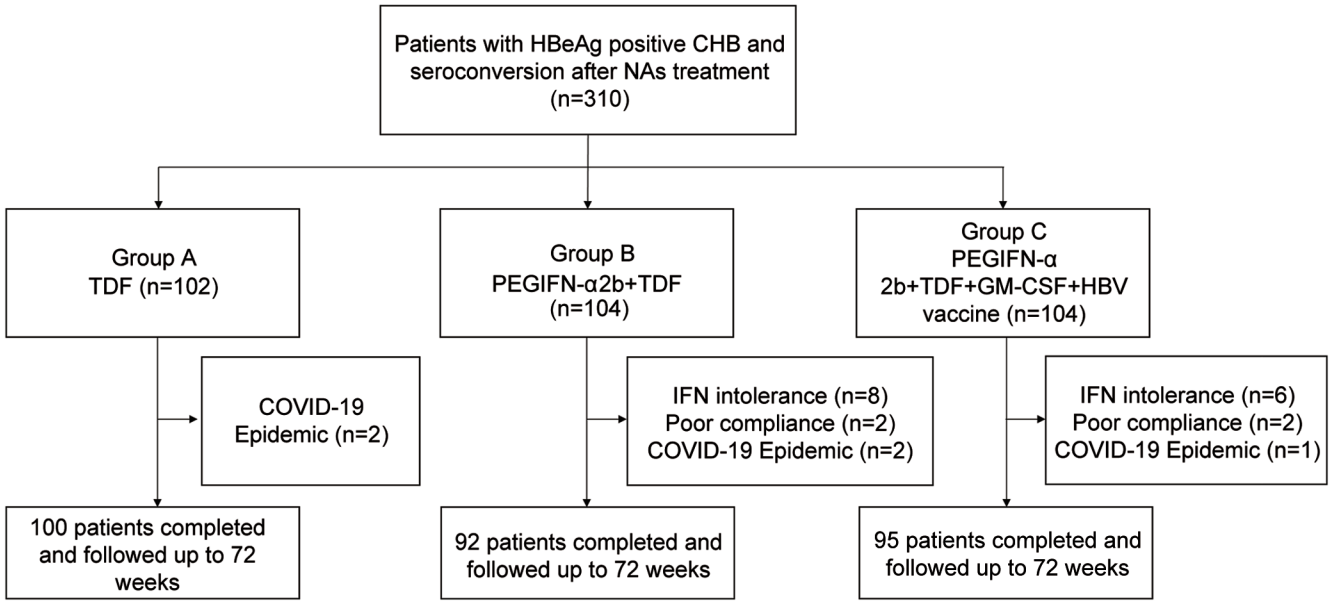

Fig. 1. Patient-selection. CHB, chronic hepatitis B; COVID-19, Corona Virus Disease 2019; GM-CSF, granulocyte-macrophage colony-stimulating factor; HBeAg, hepatitis B virus e antigen; HBV, hepatitis B virus; NA, nucleot(s)ide analog; PEGIFN-α, pegylated interferon alpha; TDF, tenofovir disoproxil fumarate.

other groups. With a type I error rate of 5%, 80% power, and 1:1:1 group allocation ratio, the expected absolute difference in risk between the PEGIFN-α groups and the control group was determined to be 15%. Considering a 15% dropout rate, we calculated that the final sample required was 291, with 97 per group.

Statistical analysis

Statistical analysis was performed using SPSS (version 26.0; IBM Corp., Armonk, NY, USA), and graphs were created using GraphPad Prism (version 9.0; GraphPad, San Diego, CA, USA). Categorical variables were reported as frequencies and percentages and were compared with chi-squared or Fisher's exact tests. Continuous variables were compared between groups with the *t*-test and the Mann-Whitney *U* test for variables expressed as means and standard deviations or medians and interquartile ranges (IQRs). Kaplan-Meier survival analysis was used to estimate cumulative HBsAg negativity and serological conversion rates. Logistic regression analysis was used to analyze factors associated with HBsAg loss and seroconversion. Statistical significance was set at $p<0.05$.

Results

Patient characteristics

Of the 310 patients enrolled in this study and randomized to treatment groups, 23 were lost because of IFN intolerance and the 2019 coronavirus disease epidemic (Fig. 1). Data from the remaining 287 patients (control, $n=100$; PEGIFN-α2b + TDF, $n=92$; PEGIFN-α2b + TDF + GM-CSF + HBV vaccine, $n=95$) were included in the final analysis. Most (77.4%) patients were male, and the mean age was 38.68±8.91 years. The baseline characteristics of the patients are shown in Table 1. No significant differences in age, sex, HBsAg level, liver function, or routine blood parameters were observed among the groups. The main NA used in the past was entecavir (ETV), and previous antiviral treatment durations ranged from 17 to 81 months.

HBsAg levels

In the control group, the mean HBsAg level decreased from 2.71±0.69 $\log_{10}$ IU/mL at baseline to 2.49±1.08 $\log_{10}$ IU/mL at 48 weeks and 2.44±1.06 $\log_{10}$ IU/mL at 72 weeks. In the PEGIFN-α2b + TDF group, the HBsAg levels at 0, 48, and 72 weeks were 2.74±0.47, 1.31±1.21, and 1.31±1.20 $\log_{10}$ IU/mL, respectively. In the four-drug group, these levels were 2.69±0.77, 0.93±1.12, and 1.09±1.19 $\log_{10}$ IU/mL, respectively (Fig. 2A). At weeks 12, 24, 36, 48, 60, and 72, the HBsAg level was higher in the control group than in the other two groups (all $p<0.05$; Fig. 2A). The decreases in the HBsAg level at 12, 24, 48, and 72 weeks in the control group (0.11±0.51, 0.19±0.59, 0.22±0.71, and 0.27±0.69 $\log_{10}$ IU/mL, respectively) were significantly lesser than those in the other two groups (all $p<0.05$), with no significant difference between the latter (Fig. 2B). During follow-up, the proportions of patients with HBsAg levels <3, <2, and <1 $\log_{10}$ IU/mL were significantly smaller in the control group than in the other two groups (all $p<0.001$), with no significant difference between the latter (Fig. 3).

HBsAg loss and serological conversion rates

At 48 weeks, the cumulative HBsAg negativity rates were 0.0% in the control group, 28.3% in the PEGIFN-α2b + TDF group, and 41.1% in the PEGIFN-α2b + TDF+ GM-CSF + HBV vaccine group ($p<0.001$; Fig. 4A). The cumulative HBsAg seroconversion rates at 48 weeks in these groups were 0.0%, 21.7%, and 33.9%, respectively ($p<0.001$; Fig. 4B). The rates at 72 weeks were similar (Fig. 4).

Safety

All three treatment regimens were generally tolerable, and no serious adverse events occurred (Table 2). Most events were related to PEGIFN-α2b, the most common being flu-like symptoms, including fever and malaise, followed by hair and weight loss. Neutropenia occurred in 80.43% (74/92) of patients in the PEGIFN-α2b + TDF group and 76.84% (73/95) of patients in the four-drug group. The proportions of patients

Table 1. Baseline characteristics of patients with HBeAg-positive CHB and seroconversion after NA treatment

Variable	TDF, *n*=100	PEGIFN-α2b + TDF, *n*=92	PEGIFN-α2b + TDF + GM-CSF + HBV vaccine, *n*=95	*p*-value
Age in years	39.68±8.03	39.23±9.28	37.08±9.30	0.097
Sex, male	76 (76.00%)	71 (77.17%)	75 (78.95%)	0.885
BMI in kg/m^2	23.00±2.76	23.38±3.36	22.76±3.14	0.390
HBsAg as log$_{10}$IU/mL	2.71±0.69	2.74±0.47	2.69±0.77	0.915
ALT in U/L	23.75±9.43	24.00±8.75	23.80±8.40	0.980
AST in U/L	24.17±7.01	24.01±8.24	23.43±7.66	0.780
GGT in U/L	21.0 (13.0–28.0)	20.0 (14.0–30.7)	18.0 (15.0–28.0)	0.657
TB in μmol/L	14.79±6.57	15.04±8.34	13.25±5.49	0.155
ALB in g/L	47.7 (46.0–50.0)	48.2 (45.6–50.0)	48.5 (46.8–50.0)	0.294
WBC as ×10^9/L	5.88±1.57	5.77±1.37	5.59±1.25	0.369
NEU as ×10^9/L	3.48±1.23	3.53±1.39	3.33±1.01	0.508
RBC as ×10^9/L	4.97±0.50	5.05±0.50	4.98±0.42	0.521
PLT as ×10^9/L	208.67±54.08	194.48±55.31	198.90±50.17	0.469
History of previous NAs, *n* (%)				
Entecavir	61 (61.00%)	51 (55.43%)	58 (61.05%)	0.667
Adefovir	25 (25.00%)	19 (20.65%)	13 (13.68%)	0.137
Tenofovir	31 (31.00%)	36 (39.13%)	39 (41.05%)	0.302
Lamivudine	14 (14.00%)	16 (17.39%)	17 (17.89%)	0.726
Duration of NAs in months	40 (20–65)	48 (18–81)	37 (17–64)	0.446

ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CHB, chronic hepatitis B; GGT, γ-glutamyl transpeptadase; GM-CSF, granulocyte-macrophage colony-stimulating factor;.HBeAg, hepatitis B virus e antigen; HBsAg, hepatitis B virus surface antigen; HBV, hepatitis B virus; NA, nucleot(s)ide analog; NEU, neutrophil; PEGIFN, pegylated interferon; PLT, platelet; RBC, red blood cell; TB, total bilirubin; TDF, tenofovir disoproxil fumarate; WBC, white blood cell.

with fever, malaise, hair loss, weight loss, neutropenia, thrombocytopenia, and abnormal thyroid function were significantly smaller in the control group than in the other two groups (all $p<0.001$), with no significant difference between the latter. All adverse reactions improved after PEGIFN-α2b discontinuation. No significant change in the fibrosis-4 (FIB-4) or aspartate aminotransferase to platelet ratio index (APRI) from baseline was observed in any group at 72 weeks (Table 3).

Factors associated with HBsAg loss and serological conversion in patients treated with PEGIFN-α2b

Univariate and multivariate regression analyses showed that

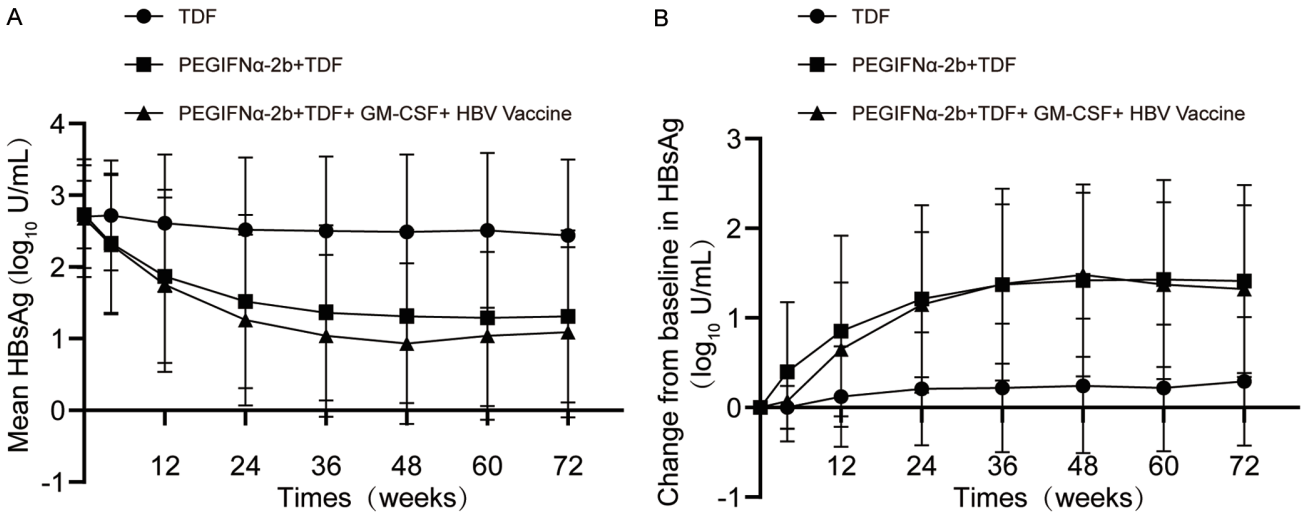

Fig. 2. Dynamic changes in HBsAg kinetics (A) during follow-up and magnitudes thereof (B). GM-CSF, granulocyte-macrophage colony-stimulating factor; HBsAg, hepatitis B virus surface antigen; HBV, hepatitis B virus; PEGIFN-α, pegylated interferon alpha; TDF, tenofovir disoproxil fumarate.

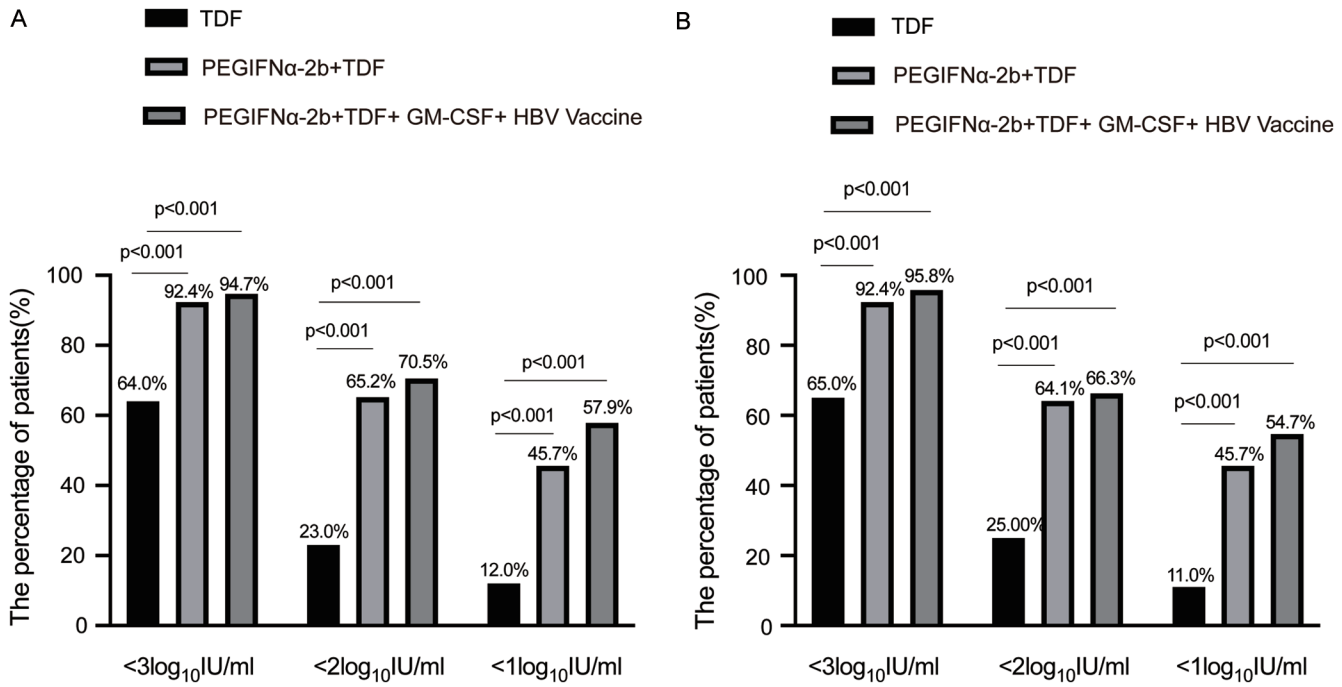

Fig. 3. HBsAg responses at 48 (A) and 72 (B) weeks. GM-CSF, granulocyte-macrophage colony-stimulating factor; HBsAg, hepatitis B virus surface antigen; HBV, hepatitis B virus; PEGIFN-α, pegylated interferon alpha; TDF, tenofovir disoproxil fumarate.

HBsAg loss was associated with baseline HBsAg levels <1,000 IU/mL [univariate $p<0.05$; odds ratio (OR) 0.47, 95% confidence interval (CI): 0.25–0.90, multivariate $p=0.02$)], peak ALT level in the first 12 weeks (OR 1.01, 95% CI: 1.00–1.01, both $p=0.02$), and GM-CSF and HBV vaccine use (univariate $p=0.04$; OR 2.17, 95% CI: 1.15–4.10, multivariate $p=0.02$). Age, sex, and BMI were not predictors of HBsAg negativity (Table 4). Similar results were obtained for HBsAg seroconversion, which was associated with peak ALT level in the first 12 weeks (OR 1.00, 95% CI: 1.00–1.01, $p=0.05$) and GM-CSF and HBV vaccine use (OR 2.07, 95% CI: 1.07–4.00, $p=0.03$) in multivariate analysis (Table 4).

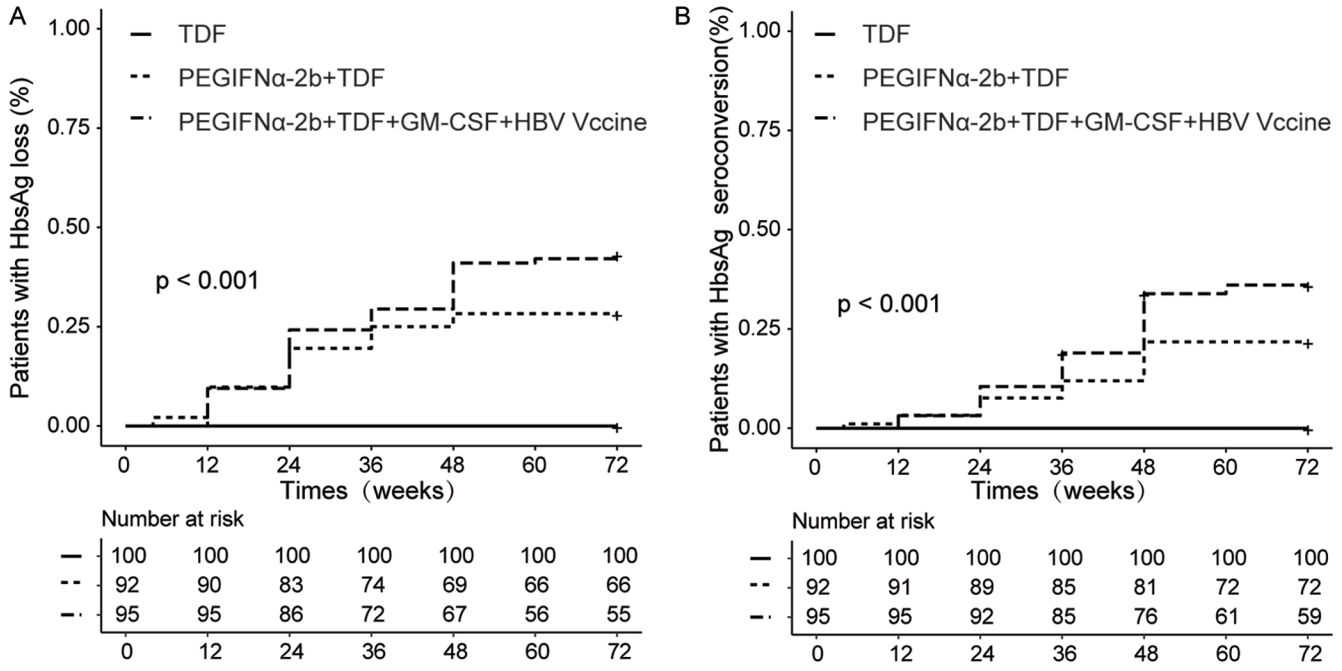

Fig. 4. Cumulative incidence of HBsAg loss (A) and seroconversion (B). GM-CSF, granulocyte-macrophage colony-stimulating factor; HBsAg, hepatitis B virus surface antigen; HBV, hepatitis B virus; PEGIFN-α, pegylated interferon alpha;TDF, tenofovir disoproxil fumarate.

Table 2. Adverse events occurring during follow-up

Variable	TDF, *n*=100	PEGIFN-α2b + TDF, *n*=92	PEGIFN-α2b + TDF + GM-CSF + HBV vaccine, *n*=95	*p*-value
Fever	0 (0.0%)	47 (51.1%)	51 (53.7%)	<0.001
Headache	0 (0.0%)	5 (5.4%)	5 (5.3%)	0.063
Fatigue	4 (4.0%)	46 (50.0%)	48 (50.5%)	<0.001
Nausea	0 (0.0%)	3 (3.3%)	2 (2.1%)	0.214
Hair loss	0 (0.0%)	22 (23.9%)	21 (22.1%)	<0.001
Weight loss	0 (0.0%)	12 (13.0%)	14 (14.7%)	<0.001
Neutropenia	1 (1.0%)	74 (80.4%)	73 (76.8%)	<0.001
Thrombocytopenia	0 (0.0%)	49 (53.3%)	49 (51.6%)	<0.001
Thyroid dysfunction	0 (0.0%)	9 (9.8%)	10 (10.5%)	<0.001

GM-CSF, granulocyte-macrophage colony-stimulating factor; HBV, hepatitis B virus; PEGIFN, pegylated interferon; TDF, tenofovir disoproxil fumarate.

Discussion

NAs and PEGIFNs are the main antiviral therapeutic agents in this context, but neither acts directly on covalently closed circular DNA. The formation of this DNA in the nucleus is a fundamental step in the HBV lifecycle and provides a template for future virus generations.[13] Due to the persistence of HBV covalently closed circular DNA, HBsAg loss is rarely achieved through spontaneous immune-mediated clearance or current therapies. NAs inhibit viral replication primarily by suppressing reverse transcription and have been used widely because they are easy to administer and well tolerated.[14] However, they require long-term or even lifelong treatment because they do not exert immunomodulatory effects and due to low HBeAg seroconversion and negativity rates during treatment and high relapse rates after NA discontinuation.[15,16] IFN has immunomodulatory and antiviral effects, and induces sustained HBeAg seroconversion and HBsAg negative conversion in some patients.[17,18] The HBV vaccine has recently been recommended as a complement to antiviral therapy for patients with CHB. Its combination with the immune adjuvant GM-CSF can significantly enhance the HBV-specific host immune response, which has potential value for the reduction of HBsAg levels and promotion of HBsAg loss.[8,12,19] A dose of GM-CSF as an adjuvant 24 h before HBV vaccine receipt significantly improved the serum conversion rate and serum protective antibody titer in individuals with poor serum conversion rates.[11,20] Wang *et al*.[12,21] reported that GM-CSF pretreatment once a day for 3 days before HBV vaccination eliminated HBsAg-positive hepatocytes compared with administration once or twice. National and international guidelines and several studies indicate that the addition of PEGIFN after NA use to suppress viral replication effectively reduces HBsAg levels and increases the rate of HBsAg clearance.[22–24] This study included patients with CHB who were HBV DNA and HBeAg negative after NA treatment, and the currently recommended first-line antiviral drugs TDF and PEGIFN-α2b were used, with and without the HBV vaccine and adjuvant GM-CSF, to identify strategies and methods for the effective improvement of the functional CHB cure rate. At 72 weeks of follow-up, the cumulative HBsAg clearance and serological conversion rates were 28.3% and 21.7% in the PEGIFN-α2b + TDF group and 41.3% and 33.9% in the PEGIFN-α2b + TDF + GM-CSF + HBV vaccine group, and HBsAg clearance was not achieved in control group. Thus, the immunomodu-

Table 3. Changes in noninvasive fibrosis markers (FIB-4 index and APRI) at 48 and 72 weeks

Groups	TDF, *n*=100	PEGIFN-α2b + TDF, *n*=92	PEGIFN-α2b + TDF + GM-CSF + HBV vaccine, *n*=95	*p*-value[a]
FIB4				
Baseline	1.11±0.53	1.11±0.52	0.95±0.42	0.064
48 weeks	1.06±0.47	2.71±2.25	1.82±1.06	<0.001
72 weeks	1.04±0.50	1.11±0.59	0.93±0.44	0.068
Change 72 weeks vs. baseline	−0.07±0.45	0.00±0.50	−0.02±0.33	0.432
p-value[b]	0.128	0.991	0.570	
APRI				
Baseline	0.34±0.15	0.35±0.18	0.32±0.15	0.431
48 weeks	0.33±0.15	1.26±1.14	0.98±0.97	<0.001
72 weeks	0.32±0.17	0.37±0.20	0.36±0.20	0.163
Change 72 weeks vs. baseline	−0.02±0.17	0.03±0.20	0.04±0.18	0.059
p-value[b]	0.222	0.163	0.153	

[a]Between groups and [b]within groups at 72 weeks vs. baseline. APRI, aspartate aminotransferase to platelet ratio index; FIB-4, fibrosis-4; GM-CSF, granulocyte-macrophage colony-stimulating factor; HBV, hepatitis B virus; PEGIFN, pegylated interferon; TDF, tenofovir disoproxil fumarate.

Table 4. Variables associated with HBsAg loss and seroconversion in patients receiving PEGIFN-α2b treatment

Variable	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
HBsAg loss at 72 weeks				
Age in years	0.99 (0.95, 1.02)	0.383		
Sex, male	0.79 (0.38, 1.66)	0.534		
BMI in kg/m^2	1.01 (0.92, 1.10)	0.888		
Baseline HBsAg (<1,000 IU/mL vs. >1,000 IU/mL)	0.54 (0.29, 1.00)	0.049	0.47 (0.25, 0.90)	0.022
Peak ALT in the first 12 weeks	1.004 (1.000, 1.008)	0.023	1.005 (1.001, 1.009)	0.019
GM-CSF+HBV vaccine	1.93 (1.05, 3.54)	0.035	2.17 (1.15, 4.10)	0.017
HBsAg seroconversion at 72 weeks				
Age in years	0.98 (0.94, 1.01)	0.198		
Sex, male	1.02 (0.48, 2.20)	0.950		
BMI in kg/m^2	0.97 (0.88, 1.07)	0.535		
Baseline HBsAg (<1,000 IU/mL vs. >1,000 IU/mL)	0.85 (0.45, 1.61)	0.617		
Peak ALT in the first 12 weeks	1.003 (1.000, 1.007)	0.058	1.004 (1.000, 1.007)	0.050
GM-CSF+ HBV vaccine	2.01 (1.05, 3.84)	0.035	2.07 (1.07, 4.00)	0.030

ALT, alanine aminotransferase; BMI, body mass index; CI, confidence interval; GM-CSF, granulocyte-macrophage colony-stimulating factor; HBsAg, hepatitis B virus surface antigen; HBV, hepatitis B virus; OR,odds ratio; PEGIFN, pegylated interferon.

latory/antiviral therapy improved the HBsAg loss rate significantly compared with NA monotherapy.

Reported HBsAg clearance and seroconversion rates in NA-experienced patients with CHB treated with PEGIFN-α2b and NAs for 48 weeks are as high as 50.93% and 48.15%, respectively,[25] significantly higher than in the present study. A possible reason for the difference is a difference in the baseline HBsAg level; 71.30% of patients in the previous study had baseline HBsAg levels <500 IU/mL,[26] and a low HBsAg level is a relevant factor for HBsAg negativity and serological conversion.[27] Of patients with CHB treated with PEGIFN and NAs whose HBsAg levels were <1,500 IU/mL after treatment, 26.4% had HBsAg clearance and 18.7% had HBsAg serological conversion at week 48.[22] We observed similar rates in the PEGIFN-α2b + TDF group in this study and higher rates in the four-drug group, which may be related to the enhancement of the HBV-specific immune response via the combined administration of the HBV vaccine and GM-CSF. Thus, the immunomodulatory/antiviral therapy was superior to NA monotherapy in terms of the reduction of the HBsAg level, with the addition of the HBV vaccine and GM-CSF further enhancing its efficacy.

Several studies have confirmed the association between low baseline HBsAg levels and HBsAg loss after treatment.[26–28] In a clinical study conducted at Xi'an Jiaotong University, the baseline HBsAg and ALT levels during the first 12 weeks of treatment were predictors of HBsAg loss.[22] In another study, the baseline HBsAg level and occurrence of 12-week ALT rebound were included in a simple scoring system that showed up to 0.78 and 0.81 efficacy for HBsAg clearance prediction in training and validation sets, respectively.[29] The findings are consistent with the results of this study. The monitoring of changes in these indicators during follow-up and the timely adjustment of the dosing regimen and treatment course according to such changes are crucial.

The study has several limitations. First, as it was conducted with NA-treated patients with CHB and HBV DNA levels below the detection limit at baseline, HBV genotypes were not known. Thus, the effects of different HBV genotypes on the treatment response could not be examined. Second, as the observed trends of HBsAg decrease or even loss during treatment are not necessarily maintained in the long-term, future studies should be conducted with longer follow-up periods to better understand the changes in HBsAg kinetics and the maintenance of HBsAg clearance.

Conclusions

Among patients with CHB and undetectable HBV DNA levels and HBeAg seroconversion after NA treatment, especially those with high HBsAg levels, immunomodulatory/antiviral treatment regimens effectively reduced the HBsAg level and improved HBsAg clearance. The regimen including GM-CSF and HBV vaccine administration was most effective.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Study concept and design (YY, HJ), conduct of the literature

search and writing of the manuscript (HJ, GY, JY), collection of patients' samples and medical information (XZ, LY), data analysis and generation of the tables and figures (BW, JZ), execution of research (LB, XZ, KW, PZ, DY, YrZ, YY, YmZ, JG, CY, HC, YL, DX, LY, JL, JH, SZ and CJ), and obtained funding and critically revised the manuscript (YY, HJ). All authors have made a significant contribution to this study and have approved the final manuscript.

Ethical statement

The study was approved by the Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine (No. 2018-515). All enrolled patients signed an informed consent form, and that the protocols conformed to the ethical guidelines of the latest version of the Declaration of Helsinki.

Data sharing statement

The datasets used to support the findings of this study are included within the article.

References

[1] Yuen MF, Chen DS, Dusheiko GM, Janssen HLA, Lau DTY, Locarnini SA, *et al*. Hepatitis B virus infection. Nat Rev Dis Primers 2018;4:18035. doi:10.1038/nrdp.2018.35, PMID:29877316.

[2] Wang G, Chen Z. HBV Genomic Integration and Hepatocellular Carcinoma. Advanced Gut & Microbiome Research 2022;2022:2140886. doi:10.1155/2022/2140886.

[3] Tang LSY, Covert E, Wilson E, Kottilil S. Chronic Hepatitis B Infection: A Review. JAMA 2018;319(17):1802–1813. doi:10.1001/jama.2018.3795, PMID:29715359.

[4] Li Y, Li S, Duan X, Yang C, Xu M, Chen L. Macrophage Phenotypes and Hepatitis B Virus Infection. J Clin Transl Hepatol 2020;8(4):424–431. doi:10.14218/JCTH.2020.00046, PMID:33447526.

[5] Hou J, Ning Q, Duan Z, Chen Y, Xie Q, Wang FS, *et al*. 3-year Treatment of Tenofovir Alafenamide vs. Tenofovir Disoproxil Fumarate for Chronic HBV Infection in China. J Clin Transl Hepatol 2021;9(3):324–334. doi:10.14218/JCTH.2020.00145, PMID:34221918.

[6] Yip TC, Wong GL, Chan HL, Tse YK, Lam KL, Lui GC, *et al*. HBsAg seroclearance further reduces hepatocellular carcinoma risk after complete viral suppression with nucleos(t)ide analogues. J Hepatol 2019;70(3):361–370. doi:10.1016/j.jhep.2018.10.014, PMID:30367899.

[7] Marcellin P, Ahn SH, Ma X, Caruntu FA, Tak WY, Elkashab M, *et al*. Combination of Tenofovir Disoproxil Fumarate and Peginterferon α-2a Increases Loss of Hepatitis B Surface Antigen in Patients With Chronic Hepatitis B. Gastroenterology 2016;150(1):134–144.e10. doi:10.1053/j.gastro.2015.09.043, PMID:26453773.

[8] Dahmen A, Herzog-Hauff S, Böcher WO, Galle PR, Löhr HF. Clinical and immunological efficacy of intradermal vaccine plus lamivudine with or without interleukin-2 in patients with chronic hepatitis B. J Med Virol 2002;66(4):452–460. doi:10.1002/jmv.2165, PMID:11857521.

[9] Mancini-Bourgine M, Fontaine H, Scott-Algara D, Pol S, Bréchot C, Michel ML. Induction or expansion of T-cell responses by a hepatitis B DNA vaccine administered to chronic HBV carriers. Hepatology 2004;40(4):874–882. doi:10.1002/hep.20408, PMID:15382173.

[10] Couillin I, Pol S, Mancini M, Driss F, Bréchot C, Tiollais P, *et al*. Specific vaccine therapy in chronic hepatitis B: induction of T cell proliferative responses specific for envelope antigens. J Infect Dis 1999;180(1):15–26. doi:10.1086/314828, PMID:10353856.

[11] Yağci M, Acar K, Sucak GT, Yamaç K, Haznedar R. Hepatitis B virus vaccine in lymphoproliferative disorders: a prospective randomized study evaluating the efficacy of granulocyte-macrophage colony stimulating factor as a vaccine adjuvant. Eur J Haematol 2007;79(4):292–296. doi:10.1111/j.1600-0609.2007.00912.x, PMID:17655695.

[12] Wang X, Dong A, Xiao J, Zhou X, Mi H, Xu H, *et al*. Overcoming HBV immune tolerance to eliminate HBsAg-positive hepatocytes via pre-administration of GM-CSF as a novel adjuvant for a hepatitis B vaccine in HBV transgenic mice. Cell Mol Immunol 2016;13(6):850–861. doi:10.1038/cmi.2015.64, PMID:26166767.

[13] Zoulim F. New insight on hepatitis B virus persistence from the study of intrahepatic viral cccDNA. J Hepatol 2005;42(3):302–308. doi:10.1016/j.jhep.2004.12.015, PMID:15710212.

[14] Dienstag JL. Benefits and risks of nucleoside analog therapy for hepatitis B. Hepatology 2009;49(5 Suppl):S112–S121. doi:10.1002/hep.22920, PMID:19399795.

[15] Chevaliez S, Hézode C, Bahrami S, Grare M, Pawlotsky JM. Long-term hepatitis B surface antigen (HBsAg) kinetics during nucleoside/nucleotide analogue therapy: finite treatment duration unlikely. J Hepatol 2013;58(4):676–683. doi:10.1016/j.jhep.2012.11.039, PMID:23219442.

[16] Zoutendijk R, Hansen BE, van Vuuren AJ, Boucher CA, Janssen HL. Serum HBsAg decline during long-term potent nucleos(t)ide analogue therapy for chronic hepatitis B and prediction of HBsAg loss. J Infect Dis 2011;204(3):415–418. doi:10.1093/infdis/jir282, PMID:21742840.

[17] Trépo C, Chan HL, Lok A. Hepatitis B virus infection. Lancet 2014;384(9959):2053–2063. doi:10.1016/S0140-6736(14)60220-8, PMID:24954675.

[18] Dienstag JL. Hepatitis B virus infection. N Engl J Med 2008;359(14):1486–1500. doi:10.1056/NEJMra0801644, PMID:18832247.

[19] Hoa PT, Huy NT, Thu le T, Nga CN, Nakao K, Eguchi K, *et al*. Randomized controlled study investigating viral suppression and serological response following pre-S1/pre-S2/S vaccine therapy combined with lamivudine treatment in HBeAg-positive patients with chronic hepatitis B. Antimicrob Agents Chemother 2009;53(12):5134–5140. doi:10.1128/AAC.00276-09, PMID:19770281.

[20] Lin C, Zhu J, Zheng Y, Chen Y, Wu Z, Chong Y, *et al*. Effect of GM-CSF in combination with hepatitis B vaccine on revacination of healthy adult non-responders. J Infect 2010;60(4):264–270. doi:10.1016/j.jinf.2010.01.011, PMID:20138189.

[21] Zhao W, Zhou X, Zhao G, Lin Q, Wang X, Yu X, *et al*. Enrichment of Ly6C(hi) monocytes by multiple GM-CSF injections with HBV vaccine contributes to viral clearance in a HBV mouse model. Hum Vaccin Immunother 2017;13(12):2872–2882. doi:10.1080/21645515.2017.1344797, PMID:28699816.

[22] Wu FP, Yang Y, Li M, Liu YX, Li YP, Wang WJ, *et al*. Add-on pegylated interferon augments hepatitis B surface antigen clearance vs continuous nucleos(t)ide analog monotherapy in Chinese patients with chronic hepatitis B and hepatitis B surface antigen ≤ 1500 IU/mL: An observational study. World J Gastroenterol 2020;26(13):1525–1539. doi:10.3748/wjg.v26.i13.1525, PMID:32308352.

[23] European Association for the Study of the Liver. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. J Hepatol 2017;67(2):370–398. doi:10.1016/j.jhep.2017.03.021, PMID:28427875.

[24] Lim SG, Yang WL, Ngu JH, Chang J, Tan J, Ahmed T, *et al*. Switching to or Add-on Peginterferon in Patients on Nucleos(t)ide Analogues for Chronic Hepatitis B: The SWAP RCT. Clin Gastroenterol Hepatol 2022;20(2):e228–e250. doi:10.1016/j.cgh.2021.04.031, PMID:33895361.

[25] Chen J, Qi M, Fan XG, Hu XW, Liao CJ, Long LY, *et al*. Efficacy of Peginterferon alfa-2b in Nucleoside Analogue Experienced Patients with Negative HBeAg and Low HBsAg: A Non-Randomized Clinical Trial. Infect Dis Ther 2021;10(4):2259–2270. doi:10.1007/s40121-021-00497-5, PMID:34309813.

[26] Zhang C, Yang Z, Wang Z, Dou X, Sheng Q, Li Y, *et al*. HBV DNA and HBsAg: Early Prediction of Response to Peginterferon α-2a in HBeAg-Negative Chronic Hepatitis B. Int J Med Sci 2020;17(3):383–389. doi:10.7150/ijms.39775, PMID:32132873.

[27] Liem KS, van Campenhout MJH, Xie Q, Brouwer WP, Chi H, Qi X, *et al*. Low hepatitis B surface antigen and HBV DNA levels predict response to the addition of pegylated interferon to entecavir in hepatitis B e antigen positive chronic hepatitis B. Aliment Pharmacol Ther 2019;49(4):448–456. doi:10.1111/apt.15098, PMID:30689258.

[28] Lee IC, Su CW, Lan KH, Wang YJ, Lee KC, Lin HC, *et al*. Virological and immunological predictors of long term outcomes of peginterferon alfa-2a therapy for HBeAg-negative chronic hepatitis B. J Formos Med Assoc 2021;120(9):1676–1685. doi:10.1016/j.jfma.2020.12.001, PMID:33339708.

[29] Ren P, Li H, Huang Y, Jiang J, Guo S, Cao Z, *et al*. A simple-to-use tool for predicting response to peginterferon in HBV DNA suppressed chronic hepatitis B patients in China. Antiviral Res 2021;194:105163. doi:10.1016/j.antiviral.2021.105163, PMID:34389410.