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# Predictors for Efficacy of Combination Therapy with a Nucleos(t)ide Analogue and Interferon for Chronic Hepatitis B*

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**Summary:** This study aims to explore the efficacy of interferon-α (IFN-α) combined with either entecavir (ETV) or adefovir (ADV) therapy *versus* IFN-α mono-therapy for chronic hepatitis B (CHB) patients, and to identify the factors associated with treatment outcomes. Totally, 159 CHB patients receiving interferon-based treatment for 48 weeks were enrolled in this retrospective study, including IFN-α mono-therapy group (group A, *n*=44), IFN-α plus ADV group (group B, *n*=53) and IFN-α plus ETV group (group C, *n*=62). The primary measures of efficacy assessments were the changes in HBsAg. Cox regression analysis was used to identify the predictors of treatment outcomes. The predictive values of the factors were assessed by ROC analysis. For patients with baseline hepatitis B surface antigen (HBsAg) level <1000 IU/mL, the reductions in mean HBsAg levels at week 48 were greater in group C than that in group A ($P$<0.05). Higher rate of HBeAg seroconversion was achieved in the combined therapy group than in IFN-α mono-therapy group at week 48 ($P$<0.05). Two factors were independently associated with HBeAg seroconversion: baseline HBeAg level <2.215 $\log_{10}$ index/mL and △HBeAg (decline in HBeAg from baseline) >0.175 $\log_{10}$ at week 12. In conclusion, interferon-α plus ETV therapy can accelerate HBsAg decline as compared with interferon-α mono-therapy in CHB patients with lower baseline HBsAg levels, and the combination therapy was superior to IFN-α mono-therapy in increasing the rate of HBeAg seroconversion. Baseline HBeAg and △HBeAg at week 12 can independently predict HBeAg seroconversion in patients subject to interferon-based therapy for 48 weeks.
**Key words**: chronic hepatitis B; adefovir; entecavir; combination therapy; interferon-α; ROC analysis

Chronic hepatitis B (CHB) is a major public health problem. About 400 million people are chronically infected with hepatitis B virus (HBV) worldwide[1]. In China, the proportion of patients with cirrhosis and hepatocellular carcinoma (HCC) caused by HBV infection is as high as 60% and 80%, respectively[2]. Currently, two types of antiviral agents [conventional interferon-α/pegylated-interferon-α (PEG-IFN-α) and nucleos(t)ide analogues (NAs)] are approved for the antiviral treatment of CHB patients[3–5]. These treatment regimens maintain several limitations. NAs are effective inhibitors of HBV replication, but they seldom result in cure. Therefore, long-time (potentially lifelong) use of NAs is required in the majority of CHB patients, which would arouse drug resistance and compliance issues[6, 7]. Although interferon arouses higher rates of HBeAg and HBsAg loss with a finite duration, only about a third of patients will respond, and its use was precluded by less tolerated and common side effects[8, 9]. International guidelines on the management of CHB recommend that either PEG-IFN-α or NAs such as entecavir (ETV) or tenofovir (TDF) can be used as first-line therapy for CHB patients[3–5]. Nevertheless, these first line antiviral drugs are not widely available or used in low- and middle-income countries[1].

Earlier researches have revealed that a higher opportunity of HBV relapse had been observed in CHB patients once NAs treatments were terminated, even though they had achieved HBeAg seroconversion or maintained long-term HBeAg negative status[10, 11]. There is still an urgent need to look for optimized treatment regimen. Since IFN-α and NAs exert roles with different mechanisms of action, a new treatment strategy aiming to improve the overall efficacy is being explored. Several randomized controlled trials[12–15] have evaluated the effects of interferon combined with NAs for CHB patients, but the results were inconclusive. And NAs with lower barrier to resistance were used [such as lamivudine (LAM) and adevior (ADV)] in the combination group, more potent NAs (such as ETV and TDF) are limited[16, 17]. Besides, other studies reported that ETV did not offer a benefit in declining HBsAg levels compared to the drugs with lower barrier to resistance[18, 19], and it also showed no superiority to ADV in increasing the rate of HBeAg seroconversion in Asian patients according to a pilot meta-analysis[20]. Nevertheless, TDF has been introduced into China recent years and the price has declined since last year, the data about patients receiving TDF plus interferon therapy were very limited. Since

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conventional interferon-α and ADV are still widely used in Asian countries, the efficacy of IFN-α plus ETV widely used *versus* IFN-α plus ADV for CHB patients is also worthy to be clarified.

This study compared the efficacy of IFN-α combined with either ETV or ADV therapy *versus* IFN-α mono-therapy for CHB patients in clinical practice, and the factors associated with treatment outcomes of interferon-based therapy were also retrospectively analyzed.

## 1 MATERIALS AND METHODS

### 1.1 Study Design

In this single-center and retrospective study, 210 Chinese CHB patients were investigated who commenced interferon-based therapy between January 2015 and September 2016 in the liver clinics of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology. As a result of the baseline factors mismatching, only 159 CHB patients were finally enrolled for the analysis after Propensity Score Matching (PSM). Totally, 159 patients were given interferon-based treatment for 48 weeks and 99 patients were followed up to 72 weeks (fig. 1).

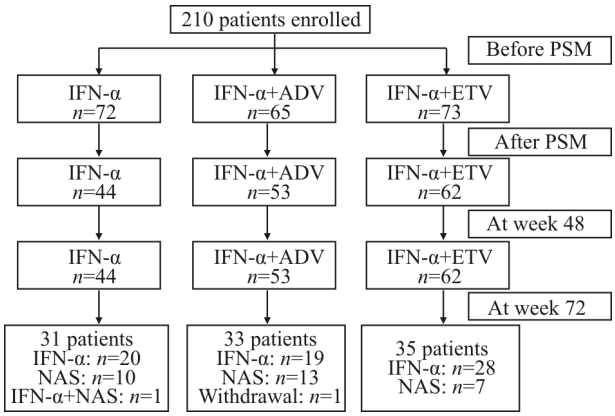


**Fig. 1** Study design

IFN-α: interferon-α; ADV: adefovir; ETV: entecavir; NAs: nucleos(t)ide analogues; PSM: Propensity Score Matching

This study involved three therapeutic regimens groups: IFN-α mono-therapy group (group A, *n*=44), IFN-α plus ADV group (group B, *n*=53) and IFN-α plus ETV group (group C, *n*=62). Interferon-α2b (Beijing Kawin Technology share-holding Co. Ltd., China) was administered (5 MIU once every two days), ETV (Suzhou Dawnrays Pharmaceutical Co. Ltd., China) 0.5 mg daily, and ADV (Chia Tai Fine Pharmaceutical Group Co. Ltd., China) 10 mg daily.

All patients signed an informed consent. This study was approved by the Ethics Committee of the Union Hospital, Tongji Medical College, Huazhong University of Science and Technology. It was conducted according to the principles of the Declaration of Helsinski.

### 1.2 Study Inclusion and Exclusion Criteria

Inclusion criteria were as follows: HBsAg positive for more than 6 months before screening; age between 18 and 65 years; HBV DNA levels higher than $2\times10^4$ IU/mL for HBeAg positive and $2\times10^3$ IU/mL for HBeAg negative patients; and alanine aminotransferase (ALT) levels between 2 and 10 times the upper limit of normal (ULN)[12].

Exclusion criteria included: interferon administration, immunomodulatory treatment, or NAs treatment within 6 months prior to screening; signs of cirrhosis or liver decompensation; co-infection with hepatitis A, C, D, E, or HIV; history of alcohol use; estimated glomerular filtration rate (eGFR) <90 mL/(min·1.73 $m^2$); alfa-fetoprotein (AFP) >100 ng/mL; pregnancy; evidence of HCC, autoimmune diseases, endocrine system disease, severe coronary artery disease, renal transplant disease, seizures and psychiatric illness, retinopathy; and any other serious medical conditions[12].

### 1.3 Follow-up

Study visits occurred every 4 weeks until 48 weeks and every 12 weeks until 72 weeks.

At every visit, patients were checked for drug compliance and adverse events. Blood was taken for examinations containing liver function tests and complete blood counts every 4 weeks in the first 48 weeks. Other tests were taken every 12 weeks including HBV DNA and serological measurements, renal and thyroid function tests, serum levels of AFP and electrolyte. All these data were recorded in the computer databases and outpatient medical history records.

### 1.4 Laboratory Assay

Blood samples were collected at every visit time. Assays were performed at the laboratory of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology. Serum HBV DNA levels were determined by a commercial Real Time PCR assay (Hepatitis B viral DNA Quantitative Fluorescence Diagnostic Kit, China) with a sensitivity threshold of 500 IU/mL (quantitative range: $5\times10^2$ to $5\times10^9$ IU/mL). Serological markers for HBV, including HBsAg, HBeAg, antibody to HBsAg, antibody to HBeAg and antibody to HBcAg were tested using commercially available enzyme immunoassay kits. Quantitative measurements of HBsAg levels were performed using a chemiluminescence enzyme immunoassay (HBsAg *in vitro* Diagnostic

Kit, China), which had a quantitative range of 0.1 to $1\times10^3$ IU/mL. Quantitative measurements of HBeAg levels were performed using a chemiluminescence enzyme immunoassay (HBeAg *in vitro* Diagnostic Kit, China), which had a quantitative range of 2.5 to $1\times10^3$ index/mL.

The eGFR was calculated by the modification of diet in renal disease (MDRD) formulas [eGFR=175× serum Cr–1.154×age–0.203×1.212 (if black)×0.742 (if female)][21].

**1.5 Efficacy Measures**

The primary efficacy measures were the changes in HBsAg. Secondary efficacy measure was the rate of HBeAg seroconversion (HBeAg loss and anti-HBeAg positive) or HBeAg loss for HBeAg positive patients. Other efficacy measures included the incidence of HBsAg loss, HBsAg seroconversion, normalization of ALT levels and the suppression of HBV DNA levels to below 500 IU/mL.

Efficacy assessments were conducted every three months in the first 48 weeks of consecutive treatment.

**1.6 Statistical Analysis**

To avoid the interference of confounding factors, PSM was adopted. The values of HBV DNA, HBeAg and HBsAg were logarithmically transformed for analysis. Continuous variables were compared between two groups using the Student's *t* test and Mann-Whitney test respectively. Chi-square test or Fisher's exact test was used to evaluate categorical variables. Analysis of variance (ANOVA) and Kruskal-Wallis tests were used to compare differences between different groups accordingly. The cumulative incidence of HBsAg loss was estimated by Kaplan-Meier method. Cox regression analysis was used to identify the predictors of treatment outcomes. ROC curves and the area under the curve (AUC) measurements were used to evaluate the diagnostic value of the predictors. The variables with a *P* value of <0.05 in the multivariate Cox regression analysis were incorporated into the multivariate ROC curve analysis. A two-tailed *P* value of <0.05 was considered statistically significant. Software package SPSS version 22.0 (IBM Corp., USA) was used for analysis.

## 2 RESULTS

**2.1 Baseline Characteristics of the Patients**

The baseline characteristics of the patients in each group after PSM are summarized in table 1. After matching, the covariates were balanced (*P*>0.05). Totally, 76.1% patients were male and 49.69% patients were HBeAg positive.

**Table 1 The baseline characteristics of patients in the three groups**

| Covariates | IFN-α (*n*=44) | IFN-α+ADV (*n*=53) | IFN-α+ETV (*n*=62) | *P* value |
|---|---|---|---|---|
| Age (years), $x\pm s$ | 27±6 | 28±6 | 28±8 | 0.64 |
| Male, *n* (%) | 32 (72.72%) | 42 (79.25%) | 47 (75.81%) | 0.75 |
| ALT (IU/mL), $x\pm s$ | 155.57±85.15 | 148.57±77.49 | 158.58±76.24 | 0.59 |
| HBeAg positive, *n* (%) | 24 (54.55%) | 24 (45.28%) | 31 (50%) | 0.66 |
| HBV DNA ($\log_{10}$ IU/mL), $\bar{x}\pm s$ | 6.33±1.58 | 5.91±1.55 | 5.82±1.61 | 0.23 |
| Median, 25%–75% percentile | 6.96 (4.94–7.67) | 5.34 (4.48–7.47) | 5.43 (4.45–7.51) | |
| HBsAg <3 $\log_{10}$ IU/mL, Yes, *n* (%) | 26 (59.09%) | 34 (64.15%) | 37 (59.68%) | 0.89 |
| HBsAg* ($\log_{10}$ IU/mL), $x\pm s$ | 2.04±0.83 | 1.84±0.91 | 2.10±0.57 | 0.33 |
| Median, 25%–75% percentile | 2.46 (1.66–2.96) | 2.03 (1.56–2.89) | 2.23 (1.69–2.97) | |
| BUN (mmol/L), $\bar{x}\pm s$ | 5.21±1.28 | 5.05±1.43 | 4.72±1.23 | 0.14 |
| CR (μmol/L), $x\pm s$ | 78.13±13.77 | 73.79±12.59 | 77.99±15.23 | 0.23 |
| eGFR (mL/min), $\bar{x}\pm s$ | 151.8±24.87 | 141.7±22.94 | 142.6±26.77 | 0.11 |

*Baseline HBsAg < 3 $\log_{10}$ IU/mL

**2.2 HBV DNA Suppression and ALT Normalization**

At week 48, the proportions of patients with undetectable HBV DNA (<500 IU/mL) were 77% (34 out of 44), 87% (46 out of 53) and 90% (56 out of 62) in groups A, B and C, respectively (*P*=0.0282) (fig. 2A). The details in HBV DNA suppression according to the HBeAg positive and negative status are shown in fig. 2B and fig. 2C.

The proportions of patients with ALT normalization was 93.18% (41 out of 44), 96.23% (51 out of 53), and 96.77% (60 out of 62) at week 48 in groups A, B and C, respectively (*P*=0.3776). Differences in the proportions of ALT normalization were not statistically significant (Data not shown).

**2.3 HBeAg Loss and Seroconversion**

For HBeAg positive patients, the percentages of patients with HBeAg loss were 12.5% (3 out of 24), 25% (6 out of 24) and 25.81% (8 out of 31) at week 48 in groups A, B and C, respectively (*P*=0.0442). The percentage of HBeAg loss was higher in the combined therapy group than in the mono-therapy group at week 48 (group A *vs*. group B: *P*=0.0464; group A *vs*. group C: *P*=0.0313) (fig. 3A).

The percentage of HBeAg seroconversion was 8.33% (2 out of 24), 20.83% (5 out of 24) and 22.58% (7 out of 31) for HBeAg positive patients at week 48 in groups A, B and C, respectively (*P*=0.0098). The percentage of HBeAg seroconversion was higher in the combined therapy group than in the mono-therapy group at week 48 (group A *vs*. group B: *P*=0.0149; group A *vs*. group C: *P*=0.0056) (fig. 3B).

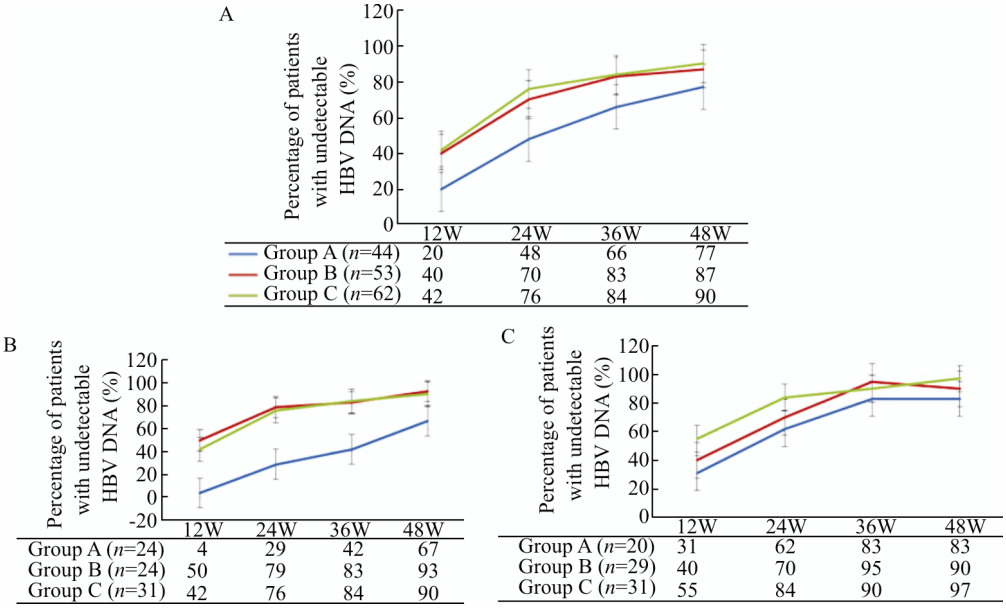


**Fig. 2** The proportions of patients with undetectable HBV DNA from baseline to week 48

The proportion of HBV DNA suppression was higher in the combined therapy group than that in the mono-therapy group at each visit ($P$<0.05). The proportion of HBV DNA suppression in group B did not differ from that in group C at each visit ($P$>0.05). A: all patients positive or negative for HBeAg; B: patients positive for HBeAg; C: patients negative for HBeAg

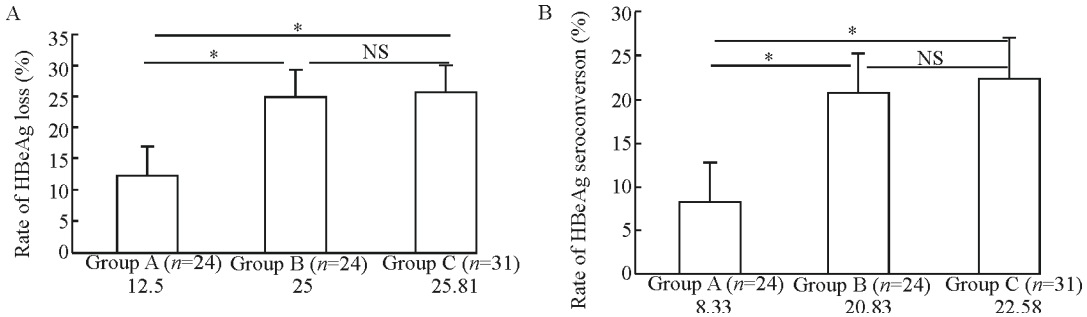


**Fig. 3** The rates of patients with HBeAg loss or HBeAg seroconversion for HBeAg-positive patients at week 48

A: At week 48, the rate of HBeAg loss in group B did not differ from that in group C ($P$=1.0); B: At week 48, the rate of HBeAg seroconversion in group B did not differ from that in group C ($P$=0.8646). *$P$<0.05; NS: not significant

The multivariate Cox regression analysis identified that two factors were independently correlated with HBeAg seroconversion: baseline HBeAg level and △HBeAg level at week 12 (table 2).

**2.4 Changes in HBsAg**

As the ULN in HBsAg is $10^3$ IU/mL, to overcome this shortcoming, the changes in HBsAg were compared among different groups by stratified method.

For patients with baseline HBsAg <1000 IU/mL, the levels of HBsAg decreased gradually over time in each group in the first 48 weeks ($P$<0.05). And the reductions in mean HBsAg levels (△HBsAg)(±SD) were 0.46(±0.91), 0.79(±0.7) and 0.84(±0.85) $\log_{10}$ IU/mL at week 48 in groups A ($n$=26), B ($n$=34) and C ($n$=37), respectively ($P$=0.1651). At week 48, the levels of △HBsAg in group C were higher than those in group A ($P$=0.0422) (fig. 4).

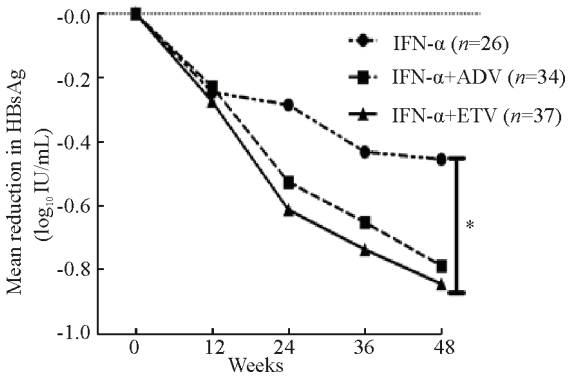


**Fig. 4** The reductions in HBsAg levels for patients with baseline HBsAg <1000 IU/mL

For the comparisons of the reductions in HBsAg levels for patients with baseline HBsAg <1000 IU/mL in each group at week 12, 24, 36 and 48, the $P$ values were 0.9003, 0.1533, 0.2913 and 0.1547, respectively. At week 48, the reductions of HBsAg levels in group B did not differ from those in group C ($P$>0.05).

**Table 2 Factors associated with HBeAg seroconversion for HBeAg positive patients**

| Covariates | Univariate analysis | | | | Multivariate analysis | | | |
|---|---|---|---|---|---|---|---|---|
| | *P* value | HR | 95% CI | | *P* value | HR | 95% CI | |
| | | | Lower limit | Upper limit | | | Lower limit | Upper limit |
| Baseline HBV DNA | 0.104 | 0.72 | 0.485 | 1.07 | 0.1 | 0.015 | 0.01 | 2.251 |
| Regimen* | 0.001 | 0.091 | 0.021 | 0.387 | 0.728 | 0.51 | 0.011 | 2.655 |
| Sex** | 0.69 | 1.339 | 0.32 | 5.605 | | | | |
| Age | <0.0001 | 0.946 | 0.926 | 0.966 | 0.324 | 0.862 | 0.641 | 1.158 |
| ALT | 0.764 | 1.001 | 0.995 | 1.007 | | | | |
| Baseline HBsAg# | 0.065 | 0.327 | 0.099 | 1.073 | 0.278 | 0.103 | 0.002 | 6.25 |
| △12W HBV DNA | 0.794 | 0.949 | 0.639 | 1.409 | | | | |
| △24W HBV DNA | <0.0001 | 1.503 | 1.256 | 1.797 | 0.138 | 0.024 | 0.02 | 3.339 |
| Baseline HBeAg | 0.0001 | 0.177 | 0.072 | 0.433 | 0.005 | 0.003 | 0.01 | 0.181 |
| △12W HBeAg | 0.038 | 1.931 | 1.036 | 3.602 | 0.009 | 0.015 | 0.001 | 0.352 |
| △24W HBeAg | 0.343 | 0.729 | 0.38 | 1.401 | | | | |

HR, Hazard ratio; CI, confidence interval; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen. *combined therapy group *vs*. mono-therapy group; **male *vs.* female; #baseline HBsAg <1000 IU/mL patients *vs.* baseline HBsAg ≥ 1000 IU/mL patients

For patients with baseline HBsAg ≥1000 IU/mL, the proportion of HBsAg <1000 IU/mL increased gradually over time in each group in the first 48 weeks ($P$<0.05). And the proportions of patients with HBsAg <1000 IU/mL at week 48 were 56% (5 out of 18), 58% (6 out of 19) and 60% (8 out of 25) in groups A, B and C, respectively ($P$=0.8486).

The multivariate Cox regression analysis showed that △HBsAg level at week 24 was independently associated with a decline of HBsAg level >1.0 $\log_{10}$ IU/mL at week 48 for patients with baseline HBsAg level <1000 IU/mL (table 3). No factors were found to be significantly correlated with HBsAg <1000 IU/mL at week 48 for patients with baseline HBsAg ≥1000 IU/mL by multivariate Cox regression analysis (data not shown).

**2.5 HBsAg Loss and Seroconversion**

At week 48, the cumulative rates of HBsAg loss were 3.94%, 9.62% and 8.95% in groups A, B and C, respectively ($P$=0.5911) (fig. 5). The baseline characteristics of the patients with HBsAg loss ($n$=7) are summarized in table 4. HBsAg seroconversion was only observed in two patients (one in group B, and the other in group C) at week 72.

**2.6 The Predictive Value of Predictors for Treatment Outcomes**

ROC analysis showed that△HBsAg level at week 24 was significantly correlated with a decline of HBsAg level >1.0 $\log_{10}$ IU/mL at week 48 for patients with baseline HBsAg level <1000 IU/mL, with an AUC of 0.867 (95% CI: 0.785–0.949, $P$<0.0001) (fig. 6A). The best cut-off point was 0.41 ($\log_{10}$ IU/mL), which would lead to a positive predictive value (PPV) of 84.31% for a >1.0 $\log_{10}$ decrease in HBsAg at week 48 (sensitivity: 73.9%; specificity: 89.3%) for patients with baseline HBsAg <1000 IU/mL.

**Table 3 Factors associated with △HBsAg >1.0 $\log_{10}$ for patients with baseline HBsAg <1000 IU/mL**

| Covariates | Univariate analysis | | | | Multivariate analysis | | | |
|---|---|---|---|---|---|---|---|---|
| | *P* value | HR | 95% CI | | *P* value | HR | 95% CI | |
| | | | Lower limit | Upper limit | | | Lower limit | Upper limit |
| Baseline HBVDNA | 0.947 | 1.01 | 0.761 | 1.339 | | | | |
| Regimen* | 0.21 | 0.497 | 0.166 | 1.485 | 0.763 | 0.81 | 0.205 | 3.193 |
| Sex** | 0.63 | 0.773 | 0.27 | 2.209 | | | | |
| Age | 0.199 | 0.959 | 0.899 | 1.022 | 0.661 | 0.981 | 0.902 | 1.067 |
| ALT | 0.499 | 1.002 | 0.997 | 1.007 | | | | |
| △12W HBV DNA | 0.712 | 1.064 | 0.765 | 1.48 | | | | |
| △24W HBV DNA | 0.53 | 1.107 | 0.806 | 1.521 | | | | |
| Baseline HBsAg# | 0.017 | 0.483 | 0.266 | 0.876 | 0.706 | 0.854 | 0.375 | 1.943 |
| △12W HBsAg | 0.001 | 0.108 | 0.03 | 0.393 | 0.248 | 3.818 | 0.394 | 3.034 |
| △24W HBsAg | <0.0001 | 0.054 | 0.015 | 0.193 | 0.001 | 0.025 | 0.003 | 0.211 |

HR, Hazard ratio; CI, confidence interval; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen.
*combined therapy group *vs*. mono-therapy group; **male *vs*. female; #baseline HBsAg <1000 IU/mL patients *vs*. baseline HBsAg ≥ 1000 IU/mL patients

Based on the multivariate analysis, baseline HBeAg (PRE-1) and △12W HBeAg (PRE-2) were selected to establish the new model (PRE-3) by the liner score [PRE-3 =38.932–3.306× (baseline HBeAg value)–2.21 ×(△12W HBeAg value)], to identify the predictive value of HBeAg seroconversion. ROC analysis showed that PRE-1 and PRE-3 were significantly correlated with HBeAg seroconversion, with an AUC of 0.866 (95% CI: 0.784–0.948, $P$<0.0001) and 0.914 (95% CI: 0.846–0.983, $P$<0.0001), respectively (fig. 6B). The best cut-off point was 2.215 ($\log_{10}$ index/mL) for PRE-1, which would result in a PPV of 98.38% in HBeAg seroconversion for HBeAg positive patients (sensitivity: 92.9%; specificity: 73.8%). The best cut-off point was 0.253 for the new model (PRE-3), which would result in a PPV of 96.53% in HBeAg seroconversion (sensitivity: 85.7%; specificity: 86.2%).

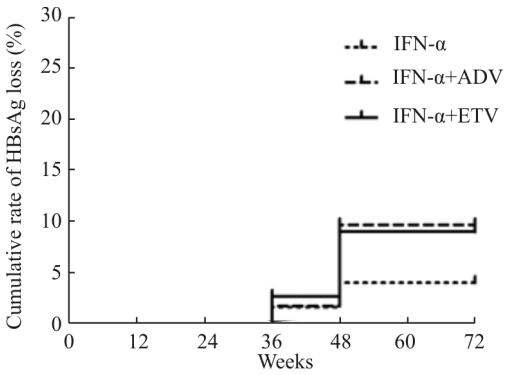


**Fig. 5** The cumulative rate of HBsAg loss by Kaplan-Meier method

**Table 4 The baseline characteristics of the patients with HBsAg loss**

| Covariates | HBsAg <0.1 IU/mL patients |
|---|---|
| Age (years), mean (range) | 26 (18, 33) |
| Male, *n* (%) | 6 (85.71%) |
| ALT (IU/mL), mean (range) | 145 (95–230) |
| HBV DNA ($\log_{10}$ IU/mL), mean (range) | 5.486 (3.32–7.7) |
| HBsAg ($\log_{10}$ IU/mL), mean (range) | 1.356 (1.081–1.603) |

ALT, alanine aminotransferase; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen

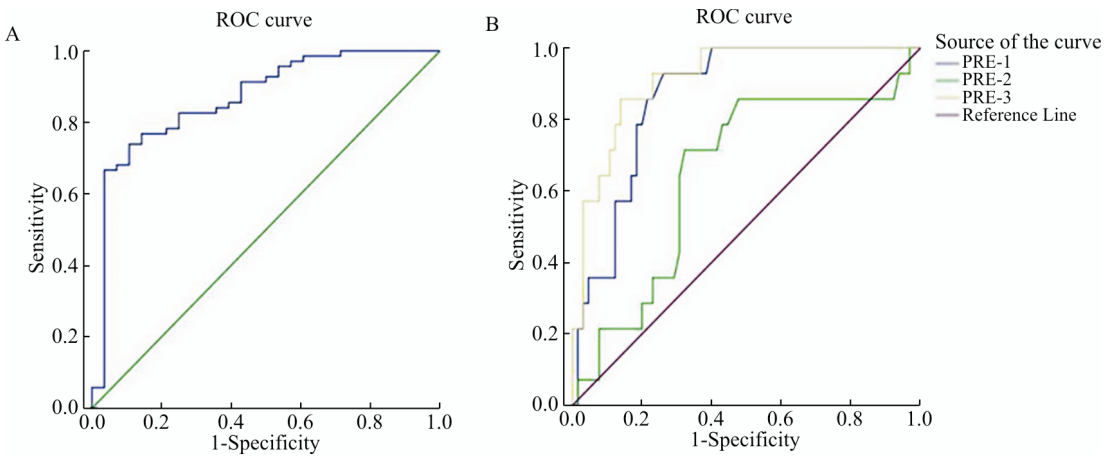


**Fig. 6** Receiver operating characteristic (ROC) curves of predictors with treatment outcomes
A:ROC curves of serum △24W HBsAg level with △HBsAg level >1.0 $\log_{10}$ IU/mL at week 48 for patients with baseline HBsAg <1000 IU/mL; B: ROC curves of the factors with HBeAg seroconversion for HBeAg positive patients. ROC analysis showed that PRE-2 was not significantly correlated with HBeAg seroconversion, with an AUC of 0.648 (95% CI: 0.487–0.81, $P$=0.082).

### 2.7 Adverse Events and Safety

Apart from influenza-like syndrome (such as fever, headache, muscle pain, fatigue and so on), the most frequently recorded treatment-related adverse effects (AEs) were abnormal laboratory results. Eighty (50.31%) patients experienced decreases in white blood cells and neutrophils (29, 26 and 25 in groups A, B and C, respectively, $P$>0.05). Five (9.4%) patients developed hypophosphatemia in group B. The most serious AEs were thyroid dysfunction, which was detected in the three groups with a similar probability (2, 3 and 4 in groups A, B and C, respectively, $P$>0.05). These patients discontinued interferon therapy after 48 weeks of treatment, and most patients (6 out of 9) were cured by medication since then. The level of eGFR was not significantly different in the subgroup of combination therapy ($P$>0.05). No patient developed eGFR <90 mL/(min·1.73 $m^2$) or drug resistance mutations.

## 3 DISCUSSION

In this study, we found that IFN-α plus ETV showed similar efficacy to IFN-α plus ADV after 48 weeks of therapy for CHB patients. But the HBsAg levels de-

creased more in IFN-α plus ETV group than in IFN-α mono-therapy group for patients with baseline HBsAg <1000 IU/mL. And the combination therapy was superior to IFN-α mono-therapy in increasing the rate of HBeAg seroconversion. The dynamics of HBsAg and HBeAg levels was significantly correlated with treatment outcomes of interferon-based treatment for CHB patients.

There were very few reports comparing the efficacy in the subgroups of interferon combined with different NAs. A retrospective study by Hagiwara *et al*[22] showed that PEG-IFN-α plus ETV group had a better antiviral effect than PEG-IFN-α plus LAM group. Another randomized controlled trail[23] in HBeAg positive patients gave either a 4-week ETV or ADV treatment followed by a 48-week combination therapy of PEG-IFN-α, and displayed that there was no difference in the rate of HBeAg seroconversion after 24 weeks of therapy. Furthermore, the effects were compared among interferon plus LAM group, interferon plus ADV group and interferon plus ETV group in a observational study from China[24], which indicated that the rate of HBeAg and HBsAg seroconversion was highest in interferon plus ADV group. The patients enrolled in the above studies showed different baseline characteristics and modes of drug administration. Besides, the sample size was too small, so the results required further certification.

In addition, the reports about the effects of interferon-α plus NAs *versus* interferon-α in the aspects of changes in HBsAg and rate of HBeAg seroconversion were also inconclusive. Wursthorn *et al*[15] reported that PEG-IFN-α plus ADV therapy induced strong HBsAg reductions in patients with CHB, but this study did not set the control arms. Recently, Ning *et al*[16] and Lampertico *et al*[25] illustrated that the levels of HBsAg were significantly reduced in PEG-IFN-α plus ETV group as compared with those in PEG-IFN-α mono-therapy group, which was consistent with our findings. However, another recent research[26] reported that PEG-IFN-α with ETV combination therapy did not improve the reductions in HBsAg levels over PEG-IFN-a mono-therapy, which was contrary to our findings. Moreover, two meta-analyses showed that the rate of HBeAg seroconversion was higher in PEG-IFN-α plus NAs group than in PEG-IFN-α mono-therapy group[27, 28]. However, these studies were limited by small sample size and lower level of quality. Therefore, more evidence from the studies with higher quality (such as larger number of samples, standard inclusion criteria and longer follow-up time) may be required for reliable evaluation.

The mechanisms of interferon combined with different NAs are not well clear. NAs may strengthen the antiviral effects of IFN-α via targeting HBV DNA polymerase, which would accelerate the decline of viral load[7, 28]. As some previous researches suggested, the reductions in viral roads by NAs may promote the immune response to IFN-α[29, 30]. The de novo formation of cccDNA can be inhibited by IFN-α and cccDNA amplification can be inhibited by NAs, resulting in a decreased production of virions, a reduced recycling of viral nucleocapsids to the nucleus of uninfected liver cells, and theoretically a decline of viral cccDNA and proteins secretion (including HBsAg and HBeAg)[17]. Although ETV was superior to ADV in HBV DNA suppression[31], it did not offer a benefit in declining the levels of HBsAg or increasing the rate of HBeAg seroconversion when combined with interferon. It is probably due to that NAs only interfere with HBV polymerases and have minimal effects on the formation of cccDNA or proteins secretion[7, 28].

The factors associated with a decline of HBsAg level >1.0 $\log_{10}$ for patients with baseline HBsAg <1000 IU/mL were first analyzed in this study. Some studies reported that higher rates of HBsAg loss or response to antiviral therapy were observed in patients with baseline HBsAg <1000 IU/mL than in the patients who had baseline HBsAg ≥1000 IU/mL[32, 33]. Since HBsAg loss is uncommon with current standard treatment regimens, several studies suggested that the early on-treatment predictors of having >1 $\log_{10}$ decline in HBsAg could indicate subsequent HBsAg clearance[32, 34]. Our study was supported by these researches[34–37] which showed that monitoring the dynamics of HBsAg or HBeAg levels during antiviral treatment would provide insights into the extend of immune control or HBsAg loss for CHB patients. Because serum HBsAg levels correlate with intrahepatic cccDNA levels, and the HBsAg decline at early time may therefore reflect the effects of interferon in decreasing intrahepatic cccDNA and consequently predict a favorable treatment outcome subsequently[34, 36].

Our study provides definitive evidence in real-world that interferon combined with either ETV or ADV therapy was well tolerated and had a relatively better clinical profile than interferon mono-therapy. Besides ETV, ADV can also be chosen to combine with interferon in increasing the rate of HBeAg seroconversion without increasing the chances of AEs for CHB patients in China. The combined use of interferon with ETV therapy may accelerate HBsAg decline for patients with relatively lower HBsAg levels (<1000 IU/mL), which can be used to guide clinical treatment decisions and support the notion of finite duration for patients with CHB.

Some limitations need to be considered in our research. First, this was a single-center and retrospective study, more samples are needed to confirm our results. Second, the patients' HBV genotype data were lacking, so we could not stratify the patients in this angel. Third, since the efficacy of combination therapy was only evaluated for 48 weeks, longer follow-up period would provide additional insights. Finally, although TDF (a more potent NA with a higher barrier to resistance) or PEG-IFN-α (more convenient to be administrated) has many advantages than our domestic antiviral drugs, it still takes some time to translate combination therapy with TDF and PEG-IFN-α into current clinical practice because of economic burden or other reasons in developing countries. Recently, Marcellin *et al*[38] reported that patients receiving TDF plus PEG-IFN-α therapy for 48 weeks achieved higher proportion of HBsAg loss than either mono-therapy, and this also would be our future focus. Of course, there is still a need for further research on how to combine these two types of drugs more suitably and identify more optimized treatment strategy.

In conclusion, interferon-α plus ETV therapy can accelerate HBsAg decline compared with interferon-α mono-therapy in CHB patients with lower baseline HBsAg levels. IFN-α plus ETV showed similar efficacy with IFN-α plus ADV after 48 weeks of therapy. And the combination therapy was superior to IFN-α

mono-therapy in increasing the proportion of HBeAg seroconversion. Monitoring the dynamics of HBsAg and HBeAg levels had a good application value in the prediction of treatment outcomes for CHB patients with interferon-based therapy.

**Conflict of Interest Statement**

All authors declare that there are no conflicts of interest.

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