Research Article

Reduction of Hepatitis B Surface Antigen May Be More Significant in PEGylated Interferon-Alpha Therapy Combined with Nucleotide Analogues than Combined with Nucleoside Analogues in Chronic Hepatitis B Patients: A Propensity Score Matching Study

Yiran Xie [ID],[1] Haoxiang Zhu [ID],[1] Yifei Guo [ID],[1] Zhenxuan Ma [ID],[1] Xun Qi [ID],[2] Feifei Yang [ID],[1] Richeng Mao [ID],[1] and Jiming Zhang [ID][1,3]

[1]*Department of Infectious Diseases, Shanghai Key Laboratory of Infectious Diseases and Biosafety Emergency Response, Shanghai Institute of Infectious Diseases and Biosecurity, National Medical Center for Infectious Diseases, Huashan Hospital, Fudan University, Shanghai, China*
[2]*Shanghai Public Health Center of Fudan University, Shanghai, China*
[3]*Department of Infectious Diseases, Jing'An Branch of Huashan Hospital, Fudan University, Shanghai, China*

Correspondence should be addressed to Richeng Mao; njxiaomao@163.com and Jiming Zhang; jmzhang@fudan.edu.cn

Background. Nucleotide analogues (NTs) monotherapy may have a more significant effect on reducing hepatitis B surface antigen (HBsAg) than nucleoside analogues (NSs) due to their immunomodulatory function. However, this superiority remains unknown when combined with PEGylated interferon α (PegIFNα). Therefore, this study aimed to explore whether NTs have more significant antiviral effects than NSs in combination therapy with PegIFNα. *Methods*. Chronic hepatitis B (CHB) patients treated with PegIFNα plus nucleos(t)ide analogues (NAs) were retrospectively recruited. Efficacy and the predictors of hepatitis B surface antigen (HBsAg) reduction $>1 \log_{10}$ IU/mL after 48 weeks were analyzed. *Results*. A total of 95 patients were included and divided into the PegIFNα + NTs group and the PegIFNα + NSs group. Propensity score matching (PSM) was performed. The PegIFNα + NTs group had a greater reduction of HBsAg (-3.52 vs. $-2.33 \log_{10}$ IU/mL, $P = 0.032$) and a higher proportion of patients with HBsAg reduction $>1 \log_{10}$ IU/mL (100.0% vs. 72.2%, $P = 0.003$) even after PSM. However, HBsAg and hepatitis B e-antigen (HBeAg) loss rates, HBeAg seroconversion rates, degree of HBeAg and hepatitis B virus (HBV) DNA decline, HBV DNA undetectable rates, and alanine aminotransferase (ALT) normalization rates showed no significant differences. Subgroup analyses showed the difference in the reduction of HBsAg was particularly evident in HBeAg-positive and the "add-on" subgroups. PegIFNα plus NTs (OR = 36.667, 95% CI = 3.837–350.384) was an independent predictor for HBsAg reduction $>1 \log_{10}$ IU/mL after 48 weeks. *Conclusion*. This study suggests that PegIFNα plus NTs may lead to more HBsAg reduction, especially in HBeAg-positive and "add-on" patients.

1. Introduction

Chronic hepatitis B (CHB) is a global infectious disease. There are currently about 70 million people infected with hepatitis B virus (HBV) in China, with more than 20 million CHB patients. These patients are at high risks of liver cirrhosis and hepatocellular carcinoma (HCC), especially in developing countries [1], presenting an immense medical burden [2]. Covalently closed circular DNA (cccDNA) persistence within hepatocytes is relevant for chronic HBV infection [3]. Hepatitis B surface antigen (HBsAg) is a surrogate marker for cccDNA transcriptional activity [3–5]. The

disappearance of HBsAg, accompanied by a sustained virological response, loss of hepatitis B e-antigen (HBeAg), recovery of alanine aminotransferase (ALT), and improvement of liver tissue lesions, is defined as functional cure. Thus, important guidelines consider sustained HBsAg disappearance after drug withdrawal as an ideal treatment endpoint [6, 7].

However, HBsAg loss is not common with current standard antiviral strategies, including nucleos(t)ide analogues (NAs) and PEGylated interferon-alpha (PegIFNα). Reduced HBsAg level is often associated with better outcomes, including minimizing cirrhosis and HCC, and is conducive to HBsAg clearance. Therefore, it is often used as an efficacy indicator. NAs are economical and convenient but cannot directly act on cccDNA. Patients usually need to take long-term or even life-long medications, bringing unavoidable economic and psychological burdens and drug resistance problems. In contrast, PegIFNα can reduce HBsAg more thoroughly in a subset of patients [8]. The low virologic response rate in PegIFNα monotherapy and poor reduction of HBsAg in NAs monotherapy shed some light on combination strategies.

Previous studies have proven that PegIFNα combined with NAs had better clinical effects than those of PegIFNα or NAs monotherapy [9–11], particularly in reducing HBsAg [12] and enhancing HBsAg loss rate [13]. Additionally, NAs can vary in efficacy. Nucleotide analogues (NTs), including tenofovir disoproxil fumarate (TDF), adefovir dipivoxil (ADV), and tenofovir alafenamide (TAF), are not only structurally but also functionally different from nucleoside analogues (NSs) like entecavir (ETV) and lamivudine (LAM). According to a small randomized controlled trial, the reduction in HBsAg was significantly higher in the TDF arm than in the ETV arm in NAs-naive patients [14]. Furthermore, switching from ETV to TDF or TAF significantly declines HBsAg [15, 16]. Interestingly, NTs have also been found with an additional immunological effect in interferon lambda 3 (IFNλ3) induction compared to NSs [17]. Meanwhile, in some studies, TDF treatment was associated with a significantly lower risk of HCC than ETV [18, 19]. Still, the comparison remains controversial [20]. The HBsAg clearance rate could reach 9.1% after 48 weeks of therapy combining PegIFNα and TDF followed by TDF monotherapy until 72 weeks [9]. But the rate was only 0.8% when PegIFNα was combined with ETV for 48 weeks and followed up to even 96 weeks [11]. According to this indirect comparison, PegIFNα combined with TDF (which represents NTs) appears to reach a better HBsAg clearance rate than that of PegIFNα combined with ETV (which represents NSs) when the treatment durations are similar. However, the populations and the end-points were not totally consistent between the two studies, making comparison difficult. There is currently no study directly comparing the efficacy of these two combination therapies.

Therefore, comparing HBsAg reduction efficacy for PegIFNα therapy combined with NTs or NSs in CHB patients is valuable. Thus, we conducted a retrospective study using the data of CHB patients treated with a combination of PegIFNα plus different NAs.

2. Methods

2.1. Patients. Between October 2011 and December 2018, a total of 159 consecutive PegIFNα-naive CHB patients who received PegIFNα for at least 48 weeks and combined with NAs during the course were retrospectively enrolled from two clinical centers, Huashan Hospital of Fudan University (Shanghai, China) and Shanghai Public Health Center of Fudan University (Shanghai, China). Chronic HBV infection was defined as HBsAg-positive and/or HBV DNA-positive for at least six months before enrollment. The combination therapy strategies could be "add-on" (adding NAs on during the therapy of PegIFNα) and "NAs-experienced" (adding PegIFNα to NAs treatment which had been more than one year). The NAs used back then were kept consistent with the previous type. In total, 64 patients were excluded, with four having underlying chronic hepatitis C, autoimmune hepatitis, HIV, or tumor, seven having used PegIFNα for more than 48 weeks when NAs were added to the therapeutic regimen, one combining nucleoside analogues with nucleotide analogues, six using the combination therapy for less than 12 weeks, and 46 having a PegIFNα therapy duration less than 48 weeks or incomplete data at an important timepoint. In this study, 95 patients were ultimately included, with one group including those who received PegIFNα combined with nucleoside analogues (ETV) ($n = 18$), and the other group including patients treated with PegIFNα combined with nucleotide analogues (TDF or ADV) ($n = 77$). This retrospective study was conducted under the approval of the Ethics Committee for Huashan Hospital of Fudan University and following the Declaration of Helsinki. Written informed consent was obtained for all patients included.

2.2. Clinical Data. All patients' baseline clinical data and laboratory test results were recorded. Clinical data included demographic data, history of chronic hepatitis B, and treatment history (name, dose, time, and medication complications). Laboratory test results included blood routine, liver function, and hepatitis B-related indicators (HBsAg tilters, HBeAg titers, and HBV DNA levels). The baseline was defined as the start of PegIFNα therapy. The duration of PegIFNα therapy was at least 48 weeks, with combination therapy for a minimum of 12 weeks. Laboratory examination results at 0, 12, 24, 36, and 48 weeks and the medication changes during the treatment (complications, dose changes, and addition or withdrawal of NAs) were recorded in detail.

2.3. Definitions of Treatment Response. The primary endpoint was the reduction levels of HBsAg from the baseline at 48 weeks of treatment.

Serological responses after 48 weeks: (1) proportion of patients with HBsAg reduction >1 $\log_{10}$ IU/mL from baseline; (2) HBsAg loss rate; (3) reduction levels of HBeAg from baseline at 48 weeks; (4) HBeAg loss rate and HBeAg seroconversion (HBeAg loss with the appearance of anti-

HBe) rate. Virological responses after 48 weeks: (1) reduction of HBV DNA levels from baseline after 48 weeks; (2) HBV DNA undetectable rate (proportion of patients with DNA <500 IU/mL after 48 weeks according to the accuracy of the instrument at the time); (3) proportion of patients with HBsAg reduction >1 $\log_{10}$ IU/mL from baseline, and HBV DNA were undetectable after 48 weeks. Biochemical response at 48 weeks was defined as ALT normalization rate (proportion of patients with baseline ALT >1 upper limit of normal (ULN) and normal ALT after 48 weeks, ULN = 40 U/L).

2.4. Laboratory Measurements.
Serum HBsAg levels were determined by Elecsys HBsAg II assay (Roche Diagnostics GmbH, Mannheim, Germany; linear range, 0.05–52,000 IU/mL).

HBsAg loss was defined as HBsAg <0.05 IU/mL. HBV DNA was measured using TaqMan fluorescence quantification, and the lower detection limit was 500 IU/mL. Routine biochemical and hematological tests were performed locally. The normal upper limit of ALT was 40 IU/L. Data from laboratory assessments were collected at baseline and after 12, 24, 36, and 48 weeks of treatment.

2.5. Statistical Analysis.
HBsAg and HBV DNA levels and reduction levels were log (base 10) transformed.

The Kolmogorov–Smirnov test was conducted for normality testing. Continuous variables are represented by the mean ± standard deviation (SD) and median (interquartile range (IQR)). Independent t-tests were used to compare continuous variables with normally distributed data (Z-score between ±1.96, calculated by skewness and kurtosis), while Mann–Whitney U tests were used to compare continuous variables with a skewed distribution. The chi-squared test presented the categorical data as n (%). Differences among groups were evaluated using one-way analysis of variance (ANOVA) if the variances were homogeneous, and the LSD-T test was used for intergroup comparison. Otherwise, the Kruskal–Wallis test (K-W test) for nonparametric statistics was conducted. Multivariate logistic regression analysis was applied to determine the predictors that affected HBsAg reduction >1 $\log_{10}$ IU/mL from baseline at 48 weeks of treatment. To adjust for potential bias that could influence the results, including sample size with excessive deviation, we applied a balanced study based on the propensity score matching (PSM) technique at a 1 : 1 ratio with a caliper of 0.2 separately between the PegIFNα + ETV group and the PegIFNα + ADV group or the PegIFNα + ETV group and the PegIFNα + TDF group. Age, HBsAg, and prior treatment duration of NAs combined with PegIFNα were imputed for PSM. The balance of the variables between the groups was considered acceptable when the absolute value of the standard difference was less than 10%. Differences were considered significant at a two-tailed $P < 0.05$. All statistical analyses were carried out using SPSS software version 24.0 (IBM, Armonk, NY, USA).

3. Results

3.1. Baseline Characteristics.
A total of 95 cases were selected for effective analysis, including 18 patients who received a therapy combining PegIFNα with nucleoside analogues (PegIFNα + NSs) and 77 patients who received PegIFNα combined with nucleotide analogues (PegIFNα + NTs) (Figure 1). In detail, the PegIFNα + NTs group included the PegIFNα + ETV group, the PegIFNα + NTs group included the PegIFNα + ADV group and the PegIFNα + TDF group. There were no significant differences in baseline information between the two groups or among subgroups prior to PSM (Table 1). PSM was performed, yielding 18 patients matched in each subgroup. After PSM, relative multivariate imbalance L1 was lower than before matching, indicating a better balance. No covariate exhibited a significant imbalance, and all the covariates reached a balance within 10%, so the balance of the variables between groups was considered acceptable after PSM. No statistically significant differences were found among patients in each group after PSM (Table 1).

3.2. Primary Endpoint before and after PSM.
HBsAg level gradually decreased during treatment. After 48 weeks, patients in the PegIFNα + NTs group achieved more reduction in HBsAg levels (−3.45 vs. −2.33 $\log_{10}$ IU/mL, $P = 0.040$) than those in the PegIFNα + NSs group (Table 2). Both the PegIFNα + ADV group (−3.47 vs. −2.33 $\log_{10}$ IU/mL, $P = 0.029$) and the PegIFNα + TDF group (−3.44 vs. −2.33 $\log_{10}$ IU/mL, $P = 0.046$) reduced significantly more HBsAg levels than the PegIFNα + ETV group. After PSM, the change in HBsAg from baseline was −3.52 $\log_{10}$ IU/mL in the PegIFNα + NTs group and −2.33 $\log_{10}$ IU/mL ($P = 0.032$) in the PegIFNα + NSs group (Table 3). HBsAg declined significantly more in the PegIFNα + NTs group (Figures 2(a) and 2(d)). Both the PegIFNα + ADV group (−3.55 vs. −2.33 $\log_{10}$ IU/mL, $P = 0.035$) and the PegIFNα + TDF group (−3.49 vs. −2.33 $\log_{10}$ IU/mL, $P = 0.039$) reduced HBsAg more than the PegIFNα + ETV group (Table 3).

3.3. Serological Responses.
Before matching, the proportion of patients with an HBsAg reduction >1 $\log_{10}$ IU/mL after 48 weeks of treatment was significantly higher in the PegIFNα + NTs group than in the PegIFNα + NSs group (98.7% vs. 72.2%, $P = 0.001$). This difference was still present after matching (100% vs. 72.2%, $P = 0.003$) (Figure 3). Similarly, both the PegIFNα + ADV group and the PegIFNα + TDF group had a higher proportion of HBsAg reduction >1 $\log_{10}$ IU/mL after 48 weeks than the PegIFNα + ETV group before and after PSM (Tables 2 and 3) (Figure 3).

We further analyzed patients who had HBsAg loss after undergoing various treatments. Before PSM, four patients (22.2%) achieved HBsAg loss in the PegIFNα + NSs group, while only five patients (6.5%) in the PegIFNα + NTs group achieved HBsAg loss, but the difference was not statistically significant ($P = 0.109$) (Table 3). After PSM, patients achieving HBsAg loss in the PegIFNα + NTs and the
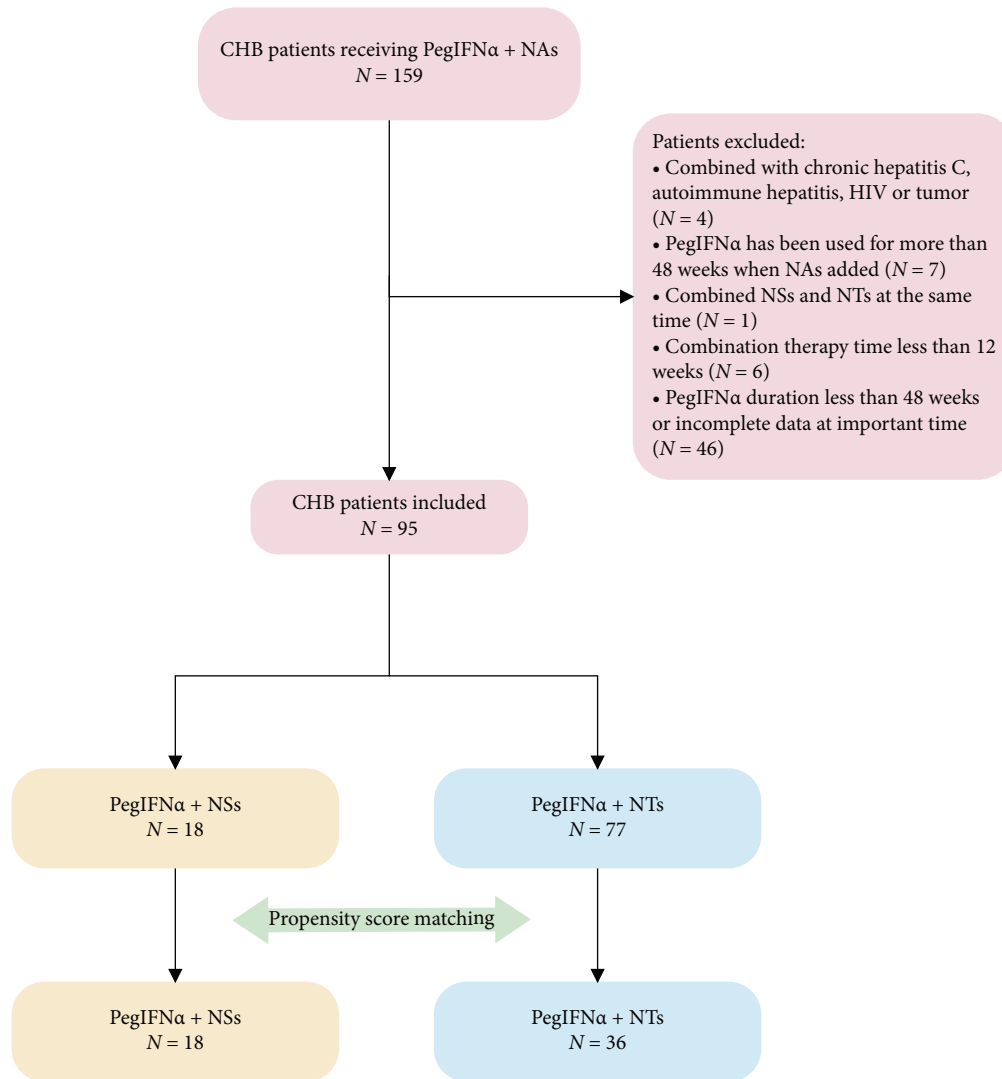
Figure 1: Flow diagram describing the selection of the study population.

PegIFNα + NSs group were three (8.3%) and four (22.2%), respectively, without significant statistical difference ($P = 0.205$) (Figure 3). Subgroup analysis did not show statistically significant differences (Tables 2 and 3).

After 48 weeks, the reduction in serum HBeAg from baseline was more pronounced in the PegIFNα + NTs group than in the PegIFNα + NSs group both before and after PSM. However, the differences were not statistically significant (before PSM: −532.27 vs. − 394.33 s/co, $P = 0.447$; after PSM: −478.72 vs. −394.33 s/co, $P = 0.667$) (Tables 2 and 3) (Figures 2(b) and 2(e)). HBeAg loss at 48 weeks occurred in 11 patients (16.9%) in the PegIFNα + NTs group and in three patients (23.1%) in the PegIFNα + NSs group before matching ($P = 0.895$) (Table 2). Meanwhile, eight (12.3%) and two (15.4%) patients from each group achieved HBeAg sero-conversion ($P = 1.000$) (Figure 3). After PSM, the HBeAg loss rate (23.1% vs. 13.8%, $P = 0.657$) and HBeAg seroconversion rate (15.4% vs. 10.3.%, $P = 0.637$) showed no significant differences between the two groups (Figure 3). No differences were observed among subgroups (Tables 2 and 3).

3.4. Virological Responses. Before matching, HBV DNA decreased by −4.57 $\log_{10}$ IU/mL from baseline in the PegIFNα + NTs group and −3.32 $\log_{10}$ IU/mL in the PegIFNα + NSs group ($P = 0.198$) (Table 2). After matching, the changes in HBV DNA from baseline were −4.72 $\log_{10}$ IU/mL and −3.32 $\log_{10}$ IU/mL in patients treated with PegIFNα + NTs and PegIFNα + NSs, respectively ($P = 0.194$) (Figures 2(c) and 2(f)). Meanwhile, the number of patients who reached HBV DNA below the lower detection limit (<500 IU/mL) after 48 weeks was 72 (94.7%) in the PegIFNα + NTs group and 17 (94.4%) in the PegIFNα + NSs group ($P = 1.000$) before matching, and 33 (94.3%) vs. 17 (94.4%), respectively, after matching ($P = 1.000$) (Figure 3). No differences were observed among subgroups (Tables 2 and 3).

Interestingly, the proportion of patients who simultaneously achieved both HBsAg reduction >1 $\log_{10}$ IU/mL and undetectable HBV DNA was 92.2% in the PegIFNα + NTs group and 72.2% in the PegIFNα + NSs group, with significant difference before matching ($P = 0.048$) (Figure 3).

Table 1: Baseline characteristics of the patients before and after PSM.

Variables	Before PSM				After PSM			
	PegIFNα + NSs (n = 18) / PegIFNα + ETV (n = 18)	P	PegIFNα + NTs (n = 77) / PegIFNα + ADV (n = 40)	PegIFNα + TDF (n = 37)	PegIFNα + NSs (n = 18) / PegIFNα + ETV (n = 18)	P	PegIFNα + NTs (n = 36) / PegIFNα + ADV (n = 18)	PegIFNα + TDF (n = 18)
NAs-experienced[c]	10 (55.6)	0.199	30 (39.0)		10 (55.6)	0.173	13 (36.1)	
	10 (55.6)	0.215	13 (32.5)	17 (45.9)	10 (55.6)	0.374	6 (33.3)	7 (38.9)
Duration of NAs before combined with PegIFNα (week)[b]	96 (42–168)	0.397	48 (14–192)		96 (42–168)	0.948	96 (10–384)	
	96 (42–168)	0.085	96 (24–384)	48 (11–60)	96 (42–168)	0.105	384 (170–456)	32 (9–96)
Weeks of adding-on PegIFNα (week)[a]	10.33 ± 13.90	0.857	10.94 ± 12.49		10.33 ± 13.90	0.715	11.67 ± 11.88	
	10.33 ± 13.90	0.728	12.03 ± 11.58	9.76 ± 13.45	10.33 ± 13.90	0.685	13.33 ± 11.56	10.00 ± 12.29
Total weeks of combination (week)[a]	36.50 ± 13.86	0.977	36.60 ± 12.74		36.50 ± 13.86	0.964	36.33 ± 13.86	
	36.50 ± 13.86	0.564	35.09 ± 12.02	38.24 ± 13.44	36.50 ± 13.86	0.731	34.7 ± 11.56	38.0 ± 12.29
Age (years)[a]	37.2 ± 6.3	0.222	34.8 ± 7.7		37.22 ± 6.32	0.154	34.56 ± 6.43	
	37.2 ± 6.3	0.332	35.5 ± 13.9	34.1 ± 8.5	37.22 ± 6.32	0.260	34.56 ± 6.0	34 ± 6.9
Male[c]	17 (94.4)	0.169	59 (76.6)		17 (94.4)	0.343	29 (80.6)	
	17 (94.4)	0.230	31 (77.5)	28 (75.7)	17 (94.4)	0.318	15 (83.3)	14 (77.8)
HBeAg-positive[c]	13 (72.2)	0.382	65 (84.4)		13 (72.2)	0.728	29 (80.6)	
	13 (72.2)	0.431	33 (82.5)	32 (86.5)	13 (72.2)	0.725	14 (77.8)	15 (83.3)
BMI (kg/cm²)[a]	23.64 ± 2.04	0.205	22.53 ± 2.60		23.67 ± 2.04	0.072	22.38 ± 1.78	
	23.64 ± 2.04	0.887	22.66 ± 2.58	22.40 ± 2.66	23.67 ± 2.04	0.195	22.3 ± 1.9	22.5 ± 1.7
WBC ($\times 10^9$/L)[a]	5.1 ± 0.7	0.259	5.2 ± 1.3		5.8 ± 1.5	0.113	5.2 ± 1.3	
	5.1 ± 0.7	0.245	5.1 ± 1.5	5.3 ± 0.9	5.8 ± 1.5	0.287	5.2 ± 1.7	5.2 ± 0.8
NEUT (%)[b]	62 (53–67)	0.076	55 (48–63)		58 (51–68)	0.246	56 (48–63)	
	62 (53–67)	0.174	57 (48–63)	57 (48–63)	58 (51–68)	0.510	55 (50–63)	56 (47–64)
RBC ($\times 109$/L)[a]	5.8 ± 1.2	0.351	4.8 ± 0.5		5.1 ± 0.4	0.144	4.9 ± 0.2	
	5.8 ± 1.2	0.308	4.9 ± 0.4	5.0 ± 0.6	5.1 ± 0.4	0.287	4.9 ± 0.1	4.9 ± 0.3
HGb (g/L)[a]	156 ± 10	0.260	152 ± 14		156 ± 10	0.244	152 ± 14	
	156 ± 10	0.504	152 ± 14	153 ± 15	156 ± 10	0.510	152 ± 15	152 ± 14
PLT ($\times 109$/L)[a]	198 ± 47	0.671	193 ± 42		198 ± 47	0.582	191 ± 43	
	198 ± 47	0.223	185 ± 44	202 ± 38	198 ± 47	0.392	182 ± 43	201 ± 41
ALB (U/L)[a]	48 ± 3	0.169	46 ± 4		48 ± 3	0.091	46 ± 4	
	48 ± 3	0.109	46 ± 3	47 ± 4	48 ± 3	0.203	46 ± 4	46 ± 4
ALT (U/L)[b]	48 (32–153)	0.269	97 (34–209)		48 (32–153)	0.210	99 (34–234)	
	48 (32–153)	0.340	111 (34–263)	90 (35–183)	48 (32–153)	0.407	97 (34–279)	101 (35–202)
ALT > ULN[c]	11 (61.1)	0.499	50 (69.4)		11 (61.1)	0.488	24 (70.6)	
	11 (61.1)	0.700	26 (72.2)	24 (66.7)	11 (61.1)	0.786	12 (70.6)	12 (70.6)
AST (U/L)[b]	27 (23–75)	0.214	48 (23–94)		27 (23–75)	0.136	53 (25–102)	
	27 (23–75)	0.415	48 (22–98)	47 (24–91)	27 (23–75)	0.314	42 (22–111)	57 (26–99)
GGT (U/L)[b]	23 (18–63)	0.980	18 (26–47)		23 (18–63)	0.610	33 (20–53)	
	23 (18–63)	0.958	29 (17–57)	24 (20–35)	23 (18–63)	0.860	36 (18–58)	32 (22–45)
TBIL (μmol/L)[a]	13.1 ± 7.5	0.980	13.1 ± 7.0		13.1 ± 7.5	0.677	14.1 ± 9.0	
	13.1 ± 7.5	0.676	12.4 ± 4.5	13.9 ± 8.9	13.1 ± 7.5	0.745	13.2 ± 4.7	15.1 ± 12.2
HBsAg ($\log_{10}$ IU/mL)[a]	3.25 ± 1.05	0.116	3.64 ± 0.91		3.25 ± 1.05	0.091	3.71 ± 0.86	
	3.25 ± 1.05	0.282	3.67 ± 0.90	3.61 ± 0.93	3.25 ± 1.05	0.228	3.77 ± 1.0	3.66 ± 0.7
HBsAg > 250 IU/mL[c]	15 (83.3)	0.477	71 (92.2)		15 (83.3)	0.388	33 (91.7)	
	15 (83.3)	0.417	36 (90.0)	35 (94.6)	15 (83.3)	0.557	16 (88.9)	17 (94.4)
HBeAg (s/co)[a]	411.38 ± 517.81	0.552	510.20 ± 617.34		411.38 ± 517.81	0.832	449.20 ± 616.64	
	411.38 ± 517.81	0.303	606.30 ± 95.87	406.40 ± 621.28	411.38 ± 517.81	0.469	568.45 ± 639.86	329.96 ± 585.93
HBV DNA ($\log_{10}$ IU/mL)[a]	4.68 ± 2.30	0.241	5.41 ± 2.21		4.68 ± 2.30	0.249	5.48 ± 2.20	
	4.68 ± 2.30	0.214	5.78 ± 2.13	4.68 ± 2.29	4.68 ± 2.30	0.479	5.66 ± 2.18	5.33 ± 2.28

[a]Variables are expressed as mean ± SD; [b]variables are expressed as median (IQR); [c]variables are expressed as n (%). ADV, adefovir dipivoxil; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate transaminase; BMI, body mass index; ETV, entecavir; GGT, gamma glutamyl transferase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HGb, hemoglobin; IQR, interquartile range; NAs, nucleos(t)ide analogues; NEUT, neutrophils; NTs, nucleotide analogues; PegIFNα, PEGylated interferon α; PLT, platelet; RBC, red blood cells; SD, standard deviation; TBIL, total bilirubin; TDF, tenofovir disoproxil fumarate; ULN, upper limit of normal; WBC, white blood cells.

The PegIFNα + TDF group had a significantly much higher rate than that of the PegIFNα + ETV group (97.3% vs. 72.2%, $P = 0.012$). However, the proportion in the PegIFNα + NTs group after PSM was not significantly higher (91.7% vs. 72.2%, $P = 0.205$) compared with the group treated with PegIFNα + NSs, still the PegIFNα + TDF group had a significantly higher proportion than the PegIFNα + ETV group (100.0% vs. 72.2%, $P = 0.045$) (Table 3) (Figure 3).

3.5. Biochemical Responses. The proportion of patients with elevated baseline ALT who returned to normal levels at 48 weeks differed between the two groups. However, the difference was not statistically significant. In all, 33 patients (43.4%) in the PegIFNα + NTs group and nine patients (52.9%) in the PegIFNα + NSs group achieved a biochemical response of serum ALT level <40 IU/L at the end of therapy before PSM ($P = 0.476$) (Table 2). After matching, 15 patients (42.9%) in the PegIFNα + NTs and nine patients (52.9%) in the PegIFNα + NSs group had biochemical responses, respectively ($P = 0.494$) (Figure 3). Biochemical responses did not vary substantially by subgroups (Tables 2 and 3).

3.6. Subgroup Analyses. Patients were divided into subgroups based on HBeAg status or combination strategies. No significant differences were found at baseline among patients treated with PegIFNα plus different oral drugs in HBeAg-positive, HBeAg-negative, or "add-on" patients (Tables S1–S3). Patients treated with PegIFNα + NSs had a lower baseline HBV DNA level in the "NAs-experienced" subgroup (Table S4). We found that there were more "add-on" patients in the HBeAg-positive subgroup (66.7% vs. 17.6%, $P = 0.001$), and the ALT level was also higher (104 vs. 34 U/L, $P = 0.001$) than in the HBeAg-negative subgroup (Table S5). Patients in the "NAs-experienced" subgroup had a longer duration of antiviral therapy before adding on PegIFNα. Therefore, the levels of HBsAg, HBeAg, and HBV DNA were lower than the "add-on" subgroup at baseline (Table S6).

In the HBeAg-positive subgroup, the reduction of HBsAg (−3.62 vs. −2.43 $\log_{10}$ IU/mL, $P = 0.002$) was significantly more and the proportion of patients with HBsAg reduction >1 $\log_{10}$ IU/mL after 48 weeks was significantly higher (100.0% vs. 69.2%, $P = 0.001$) in the PegIFNα + NTs group (Table S7). Antiviral effects in HBeAg-negative patients seemed to have no significant differences between the PegIFNα + NTs group and the PegIFNα + NSs group, although the sample size was too small for meaningful statistical analysis (Table S8). In the "add-on" subgroup, the reduction of HBsAg was significant more in the PegIFNα + NTs group than in the PegIFNα + NSs group (−3.89 vs. −2.27 $\log_{10}$ IU/mL, $P = 0.002$). HBsAg reduction was significantly more both in the PegIFNα + TDF group (−3.85 vs. −2.27 $\log_{10}$ IU/mL, $P = 0.008$) and in the PegIFNα + ADV group (−3.91 vs. −2.27 $\log_{10}$ IU/mL, $P = 0.003$) than in the PegIFNα + ETV group. The proportion of patients who achieved HBsAg reduction >1 $\log_{10}$ IU/mL was higher in the PegIFNα + NTs group than in the

PegIFNα + NSs group (100.0% vs. 75.0%, $P = 0.019$) (Table S9). In the "NAs-experienced" subgroup, no significant differences in the reduction of the HBsAg after 48 weeks were observed between the PegIFNα + NTs group and the PegIFNα + NSs group. However, the proportion of patients with an HBsAg reduction >1 $\log_{10}$ IU/mL at 48 weeks of treatment was significantly higher in the PegIFNα + NTs group than in the PegIFNα + NSs group (96.7% vs. 70.0%, $P = 0.042$), suggesting a significant difference in antiviral efficacy (Table S10). Because all the patients in the PegIFNα + NSs group had undetectable viral loads at baseline, so the HBV DNA level did not drop with treatment. Therefore, HBV DNA reduction levels were not comparable between two groups. Subgroup analyses were performed using data before PSM, given our sample size.

3.7. Predictors Associated with HBsAg Reduction >1 $\log_{10}$ IU/ mL at 48 Weeks. All patients were divided into two groups according to whether or not they achieved HBsAg reduction >1 $\log_{10}$ IU/mL after 48 weeks. Univariate analysis was performed to analyze the effect of clinical data and laboratory tests. Factors with a P value <0.1 or clinical significance were included in multivariate logistic regression analysis (forward: conditional method). As a result, we found that treatment with PegIFNα plus NTs (OR = 36.667, 95% CI = 33.837–350.384) (Table 4) was an independent predictor contributing to HBsAg reduction >1 $\log_{10}$ IU/mL at 48 weeks.

4. Discussion

To date, PegIFNα and NAs are important clinical first-line anti-HBV drugs with different mechanisms and effects on innate and adaptive immunity. NAs are oral direct antiviral drugs that reduce the viral load by inhibiting HBV DNA polymerase and reverse transcriptase. At the same time, they cannot directly inhibit the transcriptional activity of cccDNA. Therefore, obtaining durable immunological control is difficult, and the clearance and seroconversion of HBsAg and HBeAg are not easily achievable. As a result, long-term medication is often required. PegIFNα can enhance innate immunity, trigger T cell-mediated immune responses, prevent HBV protein formation, and deplete the cccDNA pool [21], resulting in superior effectiveness to NAs in reducing HBsAg [8]. Nearly one-third of PegIFNα responders achieve HBsAg clearance. In addition, strong inhibition of viral replication by NAs can assist PegIFNα's immunomodulatory effect [22]. Hence, a combination strategy with PegIFNα plus NAs is theoretically feasible and an inevitable trend for future development. However, before a new generation of effective drugs is introduced and popularized, exploration of the combination strategy has become a major focus of current research.

There have been several studies on the efficacy of combination therapy, among which many have shown combination therapy to be superior to monotherapy in reducing HBsAg levels [9, 23, 24] and found that combination therapy could even significantly increase HBsAg loss

Table 2: Efficacy results at week 48 before PSM.

Responses	PegIFNα + NSs (n = 18)	PegIFNα + NTs (n = 77)		P (total)	P (ETV vs. ADV)	P (ETV vs. TDF)	P (ADV vs. TDF)
	PegIFNα + ETV (n = 18)	PegIFNα + ADV (n = 40)	PegIFNα + TDF (n = 37)				
HBsAg reduction from baseline at week 48, $\log_{10}$ IU/mL	−2.33		−3.45	0.040	0.029	0.046	0.901
	−2.33	−3.47	−3.44	0.082			
HBeAg reduction from baseline at week 48, s/co	−394.33		−532.37	0.447	0.175	0.937	0.091
	−394.33	−654.90	−409.83	0.167			
HBV DNA reduction from baseline at week 48, $\log_{10}$ IU/ mL	−3.32		−4.57	0.198	0.112	0.481	0.287
	−3.32	−5.02	−4.10	0.251			
HBsAg loss, n (%)	4 (22.2)		5 (6.5)	0.109	0.068	0.200	0.667
	4 (22.2)	2 (5.0)	3 (8.1)	0.152			
HBeAg loss, n (%)	3 (23.1)		11 (16.9)	0.895	1.000	0.394	0.349
	3 (23.1)	7 (21.2)	4 (12.5)	0.562			
HBeAg seroconversion, n (%)	2 (15.4)		8 (12.3)	1.000	1.000	0.617	0.708
	2 (15.4)	5 (15.2)	3 (9.4)	0.742			
HBV DNA undetectable, n (%)	17 (94.4)		72 (94.7)	1.000	1.000	0.327	0.116
	17 (94.4)	35 (89.7)	37 (100)	0.062			
HBsAg reduction >1 $\log_{10}$ from baseline, n (%)	13 (72.2)		76 (98.7)	0.001	0.026	0.035	0.481
	13 (72.2)	40 (100)	36 (97.3)	0.004			
HBsAg reduction >1 $\log_{10}$ and DNA undetectable, n (%)	13 (72.2)		71 (92.2)	0.048	0.258	0.012	0.202
	13 (72.2)	35 (87.5)	36 (97.3)	0.024			
ALT normalization, n (%)	9 (52.9)		33 (43.4)	0.476	0.469	0.563	0.864
	9 (52.9)	17 (42.5)	16 (44.4)	0.764			

ADV, adefovir dipivoxil; ALT, alanine aminotransferase; ETV, entecavir; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; NSs, nucleoside analogues; NTs, nucleotide analogues; PegIFNα, PEGylated interferon α; TDF, tenofovir disoproxil fumarate.

Table 3: Efficacy results at week 48 after PSM.

Responses	PegIFNα + NSs (n = 18) PegIFNα + ETV (n = 18)	PegIFNα + NTs (n = 36) PegIFNα + ADV (n = 18)	PegIFNα + NTs (n = 36)	PegIFNα + NTs (n = 36) PegIFNα + TDF (n = 18)	P (total)	P (ETV vs. ADV)	P (ETV vs. TDF)	P (ADV vs. TDF)
HBsAg reduction from baseline at week 48, $\log_{10}$ IU/mL	−2.33	−3.55	−3.52	−3.49	0.032	0.035	0.039	0.853
HBeAg reduction from baseline at week 48, s/co	−394.33	−618.26	−478.72	−356.63	0.667	0.301	0.862	0.236
HBV DNA reduction from baseline at week 48, $\log_{10}$ IU/mL	−3.32	−4.85	−4.72	−4.60	0.194	0.240	0.311	0.840
HBsAg loss, n (%)	4 (22.2)	1 (5.6)	3 (8.3)	2 (11.1)	0.205	0.338	0.658	1.000
HBeAg loss, n (%)	3 (23.1)	2 (14.3)	4 (13.8)	2 (13.3)	0.657	0.648	0.639	1.000
HBeAg seroconversion, n (%)	2 (15.4)	1 (7.1)	3 (10.3)	2 (13.3)	0.637	0.596	1.000	1.000
HBV DNA undetectable, n (%)	17 (94.4)	15 (88.2)	33 (94.3)	18 (100.0)	1.000	0.603	1.000	0.229
HBsAg reduction >1 $\log_{10}$ from baseline, n (%)	13 (72.2)	18 (100.0)	36 (100.0)	18 (100.0)	0.003	0.045	0.045	—
HBsAg reduction >1 $\log_{10}$ and DNA undetectable, n (%)	13 (72.2)	15 (83.3)	33 (91.7)	18 (100.0)	0.205	0.691	0.045	0.229
ALT normalization, n (%)	9 (52.9)	7 (38.9)	15 (42.9)	8 (47.1)	0.494	0.404	0.732	0.625

ADV, adefovir dipivoxil; ALT, alanine aminotransferase; ETV, entecavir; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; NSs, nucleoside analogues; NTs, nucleotide analogues; PegIFNα, PEGylated interferon α; TDF, tenofovir disoproxil fumarate.
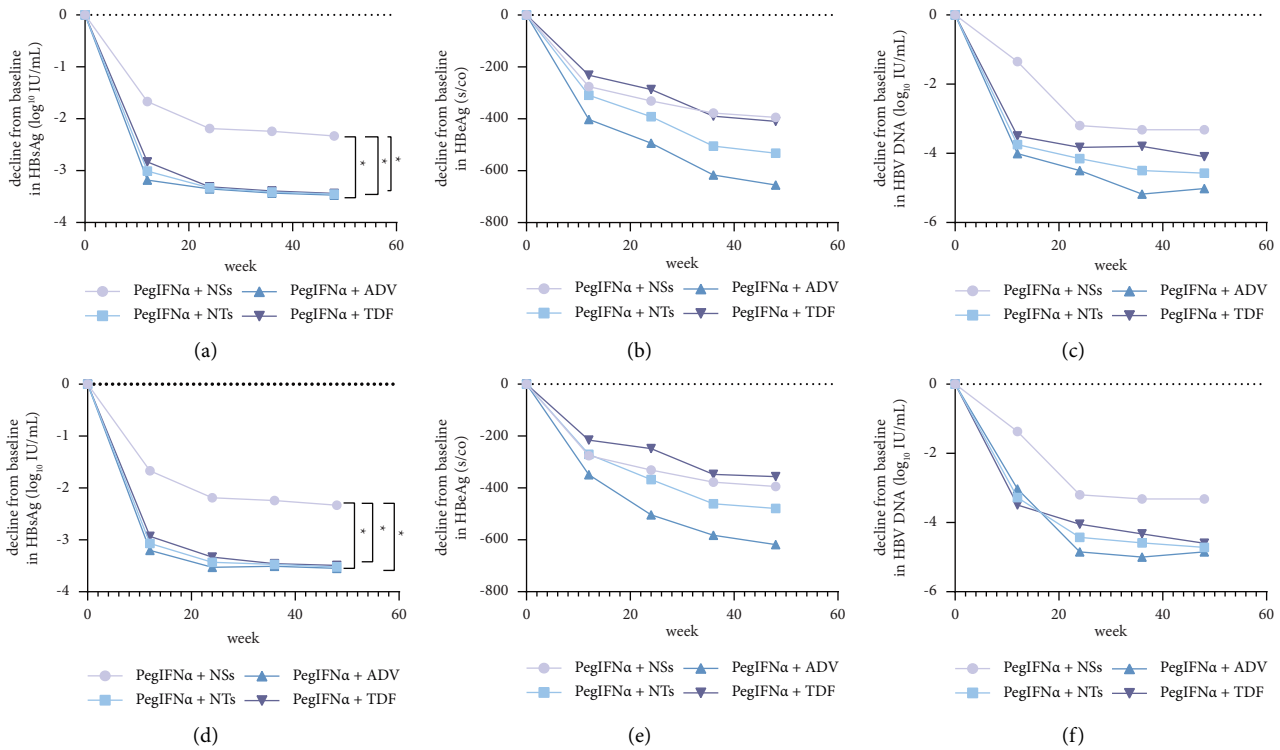
Figure 2: Mean reductions from baseline in different indicators. (a) HBsAg decline before matching; (b) HBeAg decline before matching; (c) HBV DNA decline before matching; (d) HBsAg decline after matching; (e) HBeAg decline after matching; (f) HBV DNA decline after matching. $^{*}P < 0.05$.

rate (9.1% vs. 2.8%) [24]. Furthermore, compared with NAs monotherapy, combination therapy resulted in a higher percentage of HBeAg loss (26% vs. 13%, at 96 weeks) [21] and a higher HBeAg seroconversion rate (15% vs. 5%, at 48 weeks) [25] as well. Therefore, it is evident that combination therapy has prominent advantages over monotherapy. However, combination therapy's baseline conditions, optimal treatment duration, and sustained response rate require further exploration.

At the same time, it is unclear whether efficacy differs between nucleotide analogues and nucleoside analogues when combined with PegIFNα. The two oral drugs are functionally different, especially in HBsAg reduction. Koike et al. found that TDF reduced significantly more HBsAg levels at week 24 (−0.147 vs. −0.027 $\log_{10}$ IU/mL, $P < 0.05$) and 48 (−0.208 vs. −0.051 $\log_{10}$ IU/mL, $P < 0.05$) in NAs-naive patients [14]. Furthermore, HBeAg-negative patients whose HBsAg had not been reduced during a 48-week ETV treatment had a significantly higher HBsAg reduction after switching to TDF or TAF than in the ETV continuation group [15]. HBV infection is a risk factor for hepatocarcinogenesis. Nevertheless controversial, previous research has shown that TDF treatment could be associated with a lower risk of HCC than ETV treatment. A large retrospective analysis in China found that over a median follow-up time of 3.6 years, 4.9% of ETV-treated patients developed HCC, while it occurred in only 0.6% of TDF-treated patients [19]. Similarly, a study in Korea consistently found that the annual incidence rate of HCC was

significantly lower in the TDF group than in the ETV group (0.64 vs. 1.06 per 100 person-year) [18]. Notably, researchers have indicated that patients treated with nucleotide analogues, especially ADV, have higher serum IFNλ3 levels than those treated with nucleoside analogues [26, 27]. The ability of IFNλ3 to induce interferon-stimulated genes (ISGs) in Huh7 cell lines is stronger than that of interferon lambda 1/2 (IFN-λ1/λ2), and this ability is weaker but longer-lasting than that of IFNα [26]. ISGs can encode antiviral proteins via complex intracellular signaling pathways, implying that IFNλ3 may be more effective against viruses than IFNα. Recombinant IFNλ3 had been shown to reduce HBsAg levels in vitro and had an additive antiviral effect with IFNα [17], further regulating the secretion of cytokines and enhancing antiviral immune function [28]. Hence, we supposed that a combination of PegIFNα with nucleotide analogues could have a better effect on reducing HBsAg levels than with nucleoside analogues. According to Ahn et al., the HBsAg clearance rate could reach 9.1% after 48 weeks of therapy combining PegIFNα and TDF, followed by TDF monotherapy until 72 weeks. No patient achieved HBsAg clearance in the TDF monotherapy group [9]. Liem et al. found that when PegIFNα was combined with ETV for 48 weeks and followed up for 96 weeks, only 0.8% of patients achieved HBsAg loss. No patients in the ETV monotherapy group achieved HBsAg clearance [11]. On the contrary, there are meta-analyses showing that the differences in HBsAg loss rates at the end of the combination therapy are not statistically significant among different NAs (ETV 11% vs. ADV
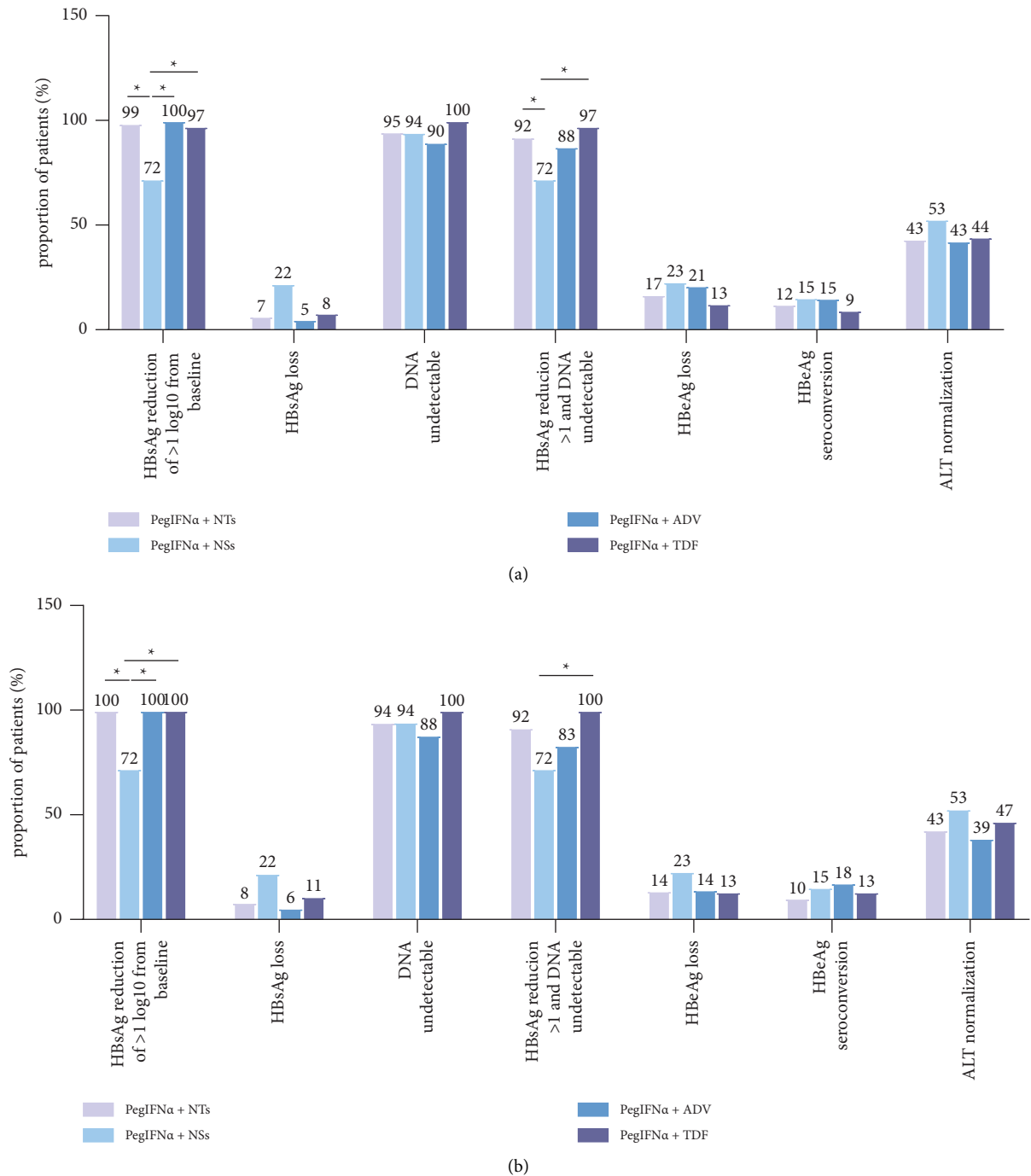
(a)

(b)

FIGURE 3: The rate of different indicators at the end of therapy. (a) Efficacy index before propensity score matching; (b) efficacy index after propensity score matching. $P < 0.05$.

12% vs. LAM 9% vs. TDF 6%, $P > 0.05$) and have found similar results for the HBsAg seroconversion rate (5% vs. 5% vs. 9% vs. 4%, $P > 0.05$) [29]. A prospective follow-up study found HBsAg loss occurred similarly in PegIFN + ADV (18.6%) and PegIFN + TDF (11.7%) patients up to five years after the end of a 48-week combination therapy. This study, however, did not provide the result when PegIFN combined with NSs [30]. Lin et al. recently found that the addition of

TDF to Peg-IFNα-2b in HBeAg-positive CHB patients with a poor response after 12 weeks of Peg-IFNα-2b monotherapy reduced HBsAg significantly more than the addition of ETV to Peg-IFNα-2b ($-1.799 \log_{10}$ IU/mL vs. $-1.078 \log_{10}$ IU/mL, $P = 0.0491$) [31]. It was an important result as it compared the addition of TDF or ETV to Peg-IFNα-2b directly. However, considering the small sample size and the restrictive conditions for the selected population, it lacks

TABLE 4: Multivariate logistic regression of HBsAg reduction $>1 \log_{10}$ IU/mL at 48 weeks.

Predictors	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P	OR (95% CI)	P
Age (years)	0.858 (0.780–0.944)	0.002		
Age > 40 years	22.812 (2.492–208.817)	0.006		
BMI (kg/cm^2)	0.753 (0.479–1.183)	0.218		
HBeAg-positive	0.405 (0.068–2.418)	0.322		
NAs-experienced	0.340 (0.059–1.953)	0.226		
PegIFNα plus NTs	29.231 (3.155–270.801)	0.003	36.667 (3.837–350.384)	0.002
PegIFNα plus NSs	1.181 (0.129–10.774)	0.883		
Week of PegIFNα adding NAs (week)	0.992 (0.931–1.058)	0.813		
Weeks of NAs before adding PegIFNα (week)	1.003 (0.992–1.015)	0.565		
Total weeks of combination (week)	1.004 (0.942–1.070)	0.909		
HBeAg at baseline (s/co)	1.001 (0.999–1.004)	0.263		
ALT at baseline (U/L)	1.007 (0.995–1.018)	0.259		
ALT > ULN	4.720 (0.812–27.452)	0.084		
ALT at week 12 (U/L)	1.025 (0.993–1.058)	0.124		
HBsAg at baseline (IU/mL)	3.338 (1.479–7.533)	0.004		
HBsAg > 250 IU/mL at baseline	5.857 (0.908–37.798)	0.063		
HBsAg at week 12 (IU/mL)	1.000 (1.000–1.001)	0.362		
HBsAg at week 24 (IU/mL)	1.000 (1.000–1.001)	0.310		
HBsAg decline at week 12 ($\log_{10}$ IU/mL)	0.813 (0.507–1.303)	0.390		
HBsAg decline at week 24 ($\log_{10}$ IU/mL)	0.538 (0.310–0.932)	0.027		
HBV DNA at baseline ($\log_{10}$ IU/mL)	1.317 (0.837–2.074)	0.234		
HBV DNA at week 12 ($\log_{10}$ IU/mL)	1.000 (1.000–1.000)	0.543		
HBV DNA decline at week 12 ($\log_{10}$ IU/mL)	0.905 (0.749–1.093)	0.300		
WBC ($\times 10^9$/L)	0.910 (0.451–1.836)	0.792		
NEUT (%)	0.962 (0.874–1.058)	0.420		
RBC ($\times 10^9$/L)	1.959 (0.394–9.734)	0.411		
HGb (g/L)	0.966 (0.898–1.039)	0.350		
PLT ($\times 10^9$/L)	1.024 (1.001–1.048)	0.037		0.040
ALB (U/L)	1.055 (0.793–1.404)	0.711		
AST (U/L)	1.018 (0.985–1.051)	0.293		
GGT (U/L)	1.023 (0.963–1.086)	0.461		
TBIL (μmol/L)	1.020 (0.888–1.172)	0.779		

ADV, adefovir dipivoxil; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate transaminase; BMI, body mass index; ETV, entecavir; GGT, gamma glutamyl transferase; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HGb, hemoglobin; NEUT, neutrophils; NAs, nucleos(t)ide analogues; NEUT, neutrophils; NSs, nucleoside analogues; NTs, nucleotide analogues; PegIFNα, PEGylated interferon α; PLT, platelet; RBC, red blood cells; TBIL, total bilirubin; TDF, tenofovir disoproxil fumarate; ULN, upper limit of normal; WBC, white blood cells.

universality, and a larger sample size study is required to verify the results. Therefore, whether PegIFNα combined with different NAs influences HBsAg reduction and clearance is still unclear. The loss rate of HBeAg after 48 weeks was similar between PegIFNα + TDF and PegIFNα + ETV (29.0% vs. 31.0%) [32]. Recent data from another study pointed out that PegIFNα combined with TDF could improve HBeAg responses in a short time. No advantages were found when PegIFNα was combined with LAM or ETV [33]. However, Lin et al. showed that the HBeAg loss rate was significantly higher in the TDF add-on group than in the ETV add-on group after 48 weeks (40% vs. 10%, $P = 0.028$) [31]. Interestingly, these studies suggested that PegIFNα combined with different NAs could have different efficacies, but direct evidence was required, and the mechanism underlying the differences must be discussed. We conducted this retrospective study to provide this evidence based on these findings. TAF has only been launched in recent years, and with insufficient studies discussing the efficacy of PegIFNα plus TAF, we did not include patients who received TAF in the current study. Meanwhile, patients in our cohort

who used LAM were excluded according to the exclusion criteria, so ETV was the only nucleoside analogues analyzed. To our knowledge, this study was the first to retrospectively compare HBsAg level reduction efficacy for CHB patients treated with different NAs in PegIFNα combination therapy, no matter which combination strategy was adopted. This could help prove that the difference in reduction was due to the types of NAs.

In order to minimize the impact of bias, PSM was performed to eliminate the inequality caused by excessive deviation of the general data and sample size. After PSM, the results showed that the HBsAg levels of the PegIFNα + NSs group decreased by an average of $-2.33 \log_{10}$ IU/mL from baseline at 48 weeks, while it decreased significantly more in the PegIFNα + NTs group, by an average of $-3.52 \log_{10}$ IU/mL ($P = 0.032$). The reductions of HBsAg in both groups were more than those in Lin et al.' study [31]. This could be because our study had a longer combination course and some patients had previously received NA treatment. The proportion of patients achieving HBsAg reduction $>1 \log_{10}$ IU/mL was significantly higher at 48 weeks in the

PegIFNα + NTs group compared to the PegIFNα + NSs group (100% vs. 72.2%, $P = 0.003$). However, even after PSM adjustment, no significant differences in the following indicators were found between the two groups: HBsAg loss rate, HBV DNA reduction levels, HBeAg reduction levels, HBeAg loss rate, HBeAg seroconversion rate, HBV DNA undetectable rate, and ALT normalization rate. The observation endpoint of this study was the 48th week of treatment, and subsequent follow-up had not yet been carried out, resulting in difficulty achieving HBsAg clearance, especially for antiviral treatment-naive patients. The ability to maintain steady HBsAg clearance after combination therapy cannot be confirmed. Another reason for the significant differences in decline levels, but not in HBsAg loss rates, maybe the small sample size. Based on the results of our study, we believe that NTs may significantly reduce more HBsAg than NSs when combined with PegIFNα. This reduction will contribute to achieving HBsAg clearance and even a functional cure. In our study, the proportion of patients who simultaneously reached HBV DNA below the lower detection limit and HBsAg reduction >1 $\log_{10}$ IU/mL from baseline at 48 weeks differed between the PegIFNα + ETV group and the PegIFNα + TDF group after PSM (100.0% vs. 72.2%, $P = 0.045$). This result exemplifies the dual effectiveness of PegIFNα combination therapy with TDF over combination therapy with ETV in inhibiting viral replication and reducing HBsAg levels simultaneously. Furthermore, the multivariate logistic regression also showed that treatment with PegIFNα plus NTs was an independent predictor for HBsAg decline >1 $\log_{10}$ IU/mL at 48 weeks, suggesting that the combination of PegIFNα and NTs can fasten HBsAg decline.

Combination strategies have been studied, including "De novo," "NAs-experienced," "add-on," and "switch-to." Several studies have shown that the "NAs-experienced" strategy seemed the best. The "switch-to" strategy was particularly effective and improved the HBsAg clearance rate [13, 29, 34]. This maybe because the direct antiviral activity of NAs can lead to virological suppression, which can further improve the immunomodulatory effect of PegIFNα, thereby maximizing the advantages of combination therapy. Since patients with different combination strategies and HBeAg status were enrolled, subgroup analyses were performed using data before PSM to determine whether the antiviral effects were different between "add-on" and "NAs-experienced" subgroups as well as between HBeAg-positive and HBeAg-negative subgroups. We found that in HBeAg-positive patients, the reduction of HBsAg was significantly more in the PegIFNα + NTs group. Possible mechanism may be the additional immunomodulatory effects of NTs combined with PegIFNα, which deregulate the immunosuppression caused by HBeAg [35, 36], resulting in a better clinical efficacy. However, further research is needed to investigate the different effects of NTs and NSs on the immune system. Regrettably, the number of HBeAg-negative patients was relatively small and was prone to bias. Therefore, no statistical analysis of this subpopulation was conducted, and further studies are warranted to confirm our findings. More significant reduction of HBsAg in the PegIFNα + NTs group was also observed in the "add-on" subgroup. Besides, patients who achieved a reduction in HBsAg >1 $\log_{10}$ IU/m were significantly more in the PegIFNα + NTs group in HBeAg-positive, "add-on," and "NAs-experienced" patients. We, therefore, infer that our findings were generally consistent across subgroups.

Limitations of our study include that it is a retrospective study with small sample size and short therapy duration without a long-term follow-up. Furthermore, the combination strategy was not precisely uniform, although the combination therapy duration was guaranteed at least 24 weeks. However, the prior treatment duration and drugs before combination for NAs-experienced patients, the weeks of adding-on NAs for "add-on" patients, and the total weeks of combination at baseline before and after PSM were not statistically different. In addition, the results were partially observable in subgroup analyses. So, the following analysis was considered reliable. However, further randomized controlled trials are required for verification.

5. Conclusion

In conclusion, reduction of HBsAg might be more pronounced in PegIFNα therapy combined with NTs than NSs, especially in HBeAg-positive patients and patients using "add-on" strategies. This finding will be beneficial for promoting further HBsAg clearance and functional cure. In addition, this finding can be used to make clinical decisions. Therefore, similar findings and mechanisms should be investigated further [37].

6. Consent

Informed consent was obtained from all patients.

Abbreviations

ADV:	Adefovir dipivoxil
ALB:	Albumin
ALT:	Alanine aminotransferase
AST:	Aspartate transaminase
BMI:	Body mass index
cccDNA:	Covalently closed circular DNA
CI:	Confidence interval
CHB:	Chronic hepatitis B
ETV:	Entecavir
GGT:	Gamma glutamyl transferase
HBeAg:	Hepatitis B e-antigen
HBsAg:	Hepatitis B surface antigen
HBV:	Hepatitis B virus
HCC:	Hepatocellular carcinoma
HGb:	Hemoglobin
IFN-λ1/λ2:	Interferon lambda 1/2
IFNλ3:	Interferon lambda 3
IQR:	Interquartile range
ISGs:	Interferon-stimulated genes
LAM:	Lamivudine
NAs:	Nucleos(t)ide analogues
NEUT:	Neutrophils

NSs: Nucleoside analogues
NTs: Nucleotide analogues
PegIFNα: PEGylated interferon-alpha
PLT: Platelet
PSM: Propensity score matching
RBC: Red blood cells
SD: Standard deviation
TAF: Tenofovir alafenamide
TBIL: Total bilirubin
TDF: Tenofovir disoproxil fumarate
ULN: Upper limit of normal
WBC: White blood cells.

Data Availability

All data generated or analyzed during this study are included within the article.

Ethical Approval

This study was approved by the Institutional Ethics Committee of Huashan Hospital, Fudan University, China (KY2018–251).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

JZ and RM designed and supervised the study. RM revised the manuscript. YX and HZ drafted the manuscript. YX and HZ contributed equally. FY provided clinical data. YX, ZM, and XQ collected clinical data and interpreted the data. YX performed statistical analysis. All authors approved the final version of the manuscript.

Acknowledgments

Supplementary Materials

Table S1: baseline characteristics in the HBeAg-positive subgroup. Table S2: baseline characteristics in the HBeAg-negative subgroup. Table S3: baseline characteristics in the "add-on" subgroup. Table S4: baseline characteristics in the "NAs-experienced" subgroup. Table S5: baseline characteristics of patients with different HBeAg status. Table S6: baseline characteristics of patients with different combination strategies. Table S7: efficacy results at week 48 in the HBeAg-positive subgroup. Table S8: efficacy results at week 48 in the HBeAg-negative subgroup. Table S9: efficacy results at week 48 in the "add-on" subgroup. Table S10: efficacy results at week 48 in the "NAs-experienced" subgroup. (*Supplementary Materials*)

References

[1] D. Li, S. Sedano, R. Allen, J. Gong, M. Cho, and S. Sharma, "Current treatment landscape for advanced hepatocellular carcinoma: patient outcomes and the impact on quality of life," *Cancers*, vol. 11, no. 6, p. 841, 2019.

[2] J. Liu, W. Liang, W. Jing, and M. Liu, "Countdown to 2030: eliminating hepatitis B disease, China," *Bulletin of the World Health Organization*, vol. 97, no. 3, pp. 230–238, 2019.

[3] G. Fattovich, F. Bortolotti, and F. Donato, "Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors," *Journal of Hepatology*, vol. 48, no. 2, pp. 335–352, 2008.

[4] H. L. Chan, V. W. Wong, A. M. Tse et al., "Serum hepatitis B surface antigen quantitation can reflect hepatitis B virus in the liver and predict treatment response," *Clinical Gastroenterology and Hepatology*, vol. 5, no. 12, pp. 1462–1468, 2007.

[5] R. Moucari, V. Mackiewicz, O. Lada et al., "Early serum HBsAg drop: a strong predictor of sustained virological response to pegylated interferon alfa-2a in HBeAg-negative patients," *Hepatology*, vol. 49, no. 4, pp. 1151–1157, 2009.

[6] P. Lampertico, K. Agarwal, T. Berg et al., "EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection," *Journal of Hepatology*, vol. 67, no. 2, pp. 370–398, 2017.

[7] W. Guiqiang, W. Fusheng, Z. Hui, L. Taisheng, Z. Sujun, and Z. Hong, "Guidelines for the prevention and treatment of chronic hepatitis B (version 2019) (In Chinese)," *Journal of Clinical Hepatology*, vol. 35, no. 12, pp. 2648–2669, 2019.

[8] G. L. H. Wong, V. W. S. Wong, and H. L. Y. Chan, "Combination therapy of interferon and nucleotide/nucleoside analogues for chronic hepatitis B," *Journal of Viral Hepatitis*, vol. 21, no. 12, pp. 825–834, 2014.

[9] S. H. Ahn, P. Marcellin, X. Ma et al., "Hepatitis B surface antigen loss with tenofovir disoproxil fumarate plus peginterferon alfa-2a: week 120 analysis," *Digestive Diseases and Sciences*, vol. 63, no. 12, pp. 3487–3497, 2018.

[10] M. Enomoto, S. Nishiguchi, A. Tamori et al., "Sequential therapy involving an early switch from entecavir to pegylated interferon-alpha in Japanese patients with chronic hepatitis B," *Hepatology Research*, vol. 48, no. 6, pp. 459–468, 2018.

[11] K. S. Liem, M. J. H. van Campenhout, Q. Xie 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," *Alimentary Pharmacology & Therapeutics*, vol. 49, no. 4, pp. 448–456, 2019.

[12] W. P. Brouwer, Q. Xie, M. J. Sonneveld et al., "Adding pegylated interferon to entecavir for hepatitis B e antigen-positive chronic hepatitis B: a multicenter randomized trial (ARES study)," *Hepatology*, vol. 61, no. 5, pp. 1512–1522, 2015.

[13] Q. Ning, M. Han, Y. Sun et al., "Switching from entecavir to PegIFN alfa-2a in patients with HBeAg-positive chronic hepatitis B: a randomised open-label trial (OSST trial)," *Journal of Hepatology*, vol. 61, no. 4, pp. 777–784, 2014.

[14] K. Koike, K. Suyama, H. Ito, H. Itoh, and W. Sugiura, "Randomized prospective study showing the non-inferiority of tenofovir to entecavir in treatment-naive chronic hepatitis B patients," *Hepatology Research*, vol. 48, no. 1, pp. 59–68, 2018.

[15] N. Tamaki, M. Kurosaki, S. Kirino et al., "Hepatitis B surface antigen reduction as a result of switching from long-term entecavir administration to tenofovir," *JGH Open*, vol. 4, no. 3, pp. 429–432, 2020.

[16] T. Kumada, H. Toyoda, T. Tada, S. Yasuda, N. Miyake, and J. Tanaka, "Comparison of the impact of tenofovir alafenamide and entecavir on declines of hepatitis B surface antigen levels," *European Journal of Gastroenterology and Hepatology*, vol. 32, no. 2, pp. 255–260, 2021.

[17] K. Murata, M. Asano, A. Matsumoto et al., "Induction of IFN-λ3 as an additional effect of nucleotide, not nucleoside, analogues: a new potential target for HBV infection," *Gut*, vol. 67, no. 2, pp. 362–371, 2018.

[18] J. Choi, H. J. Kim, J. Lee, S. Cho, M. J. Ko, and Y. S. Lim, "Risk of hepatocellular carcinoma in patients treated with entecavir vs tenofovir for chronic hepatitis B: a Korean nationwide cohort study," *Journal of the American Medical Association Oncology*, vol. 5, no. 1, pp. 30–36, 2019.

[19] T. C. F. Yip, V. W. S. Wong, H. L. Y. Chan, Y. K. Tse, G. C. Y. Lui, and G. L. H. Wong, "Tenofovir is associated with lower risk of hepatocellular carcinoma than entecavir in patients with chronic HBV infection in China," *Gastroenterology*, vol. 158, no. 1, pp. 215–225, 2020.

[20] F. Su, K. Berry, and G. N. Ioannou, "No difference in hepatocellular carcinoma risk between chronic hepatitis B patients treated with entecavir versus tenofovir," *Gut*, vol. 70, no. 2, pp. 370–378, 2021.

[21] M. Bourliere, P. Rabiega, N. Ganne-Carrie et al., "Effect on HBs antigen clearance of addition of pegylated interferon alfa-2a to nucleos(t)ide analogue therapy versus nucleos(t)ide analogue therapy alone in patients with HBe antigen-negative chronic hepatitis B and sustained undetectable plasma hepatitis B virus DNA: a randomised, controlled, open-label trial," *The Lancet Gastroenterology & Hepatology*, vol. 2, no. 3, pp. 177–188, 2017.

[22] R. Thimme and M. Dandri, "Dissecting the divergent effects of interferon-alpha on immune cells: time to rethink combination therapy in chronic hepatitis B?" *Journal of Hepatology*, vol. 58, no. 2, pp. 205–209, 2013.

[23] J. M. Kittner, M. F. Sprinzl, A. Grambihler et al., "Adding pegylated interferon to a current nucleos(t)ide therapy leads to HBsAg seroconversion in a subgroup of patients with chronic hepatitis B," *Journal of Clinical Virology*, vol. 54, no. 1, pp. 93–95, 2012.

[24] P. Marcellin, S. H. Ahn, X. Ma et al., "Combination of tenofovir disoproxil fumarate and peginterferon alpha-2a increases loss of hepatitis B surface antigen in patients with chronic hepatitis B," *Gastroenterology*, vol. 150, no. 1, pp. 134–144, 2016.

[25] H. Chi, B. E. Hansen, S. Guo et al., "Pegylated interferon alfa-2b add-on treatment in hepatitis B virus envelope antigen-positive chronic hepatitis B patients treated with nucleos(t)ide analogue: a randomized, controlled trial (pegon)," *The Journal of Infectious Diseases*, vol. 215, no. 7, pp. 1085–1093, 2017.

[26] C. R. Bolen, S. Ding, M. D. Robek, and S. H. Kleinstein, "Dynamic expression profiling of type I and type III interferon-stimulated hepatocytes reveals a stable hierarchy of gene expression," *Hepatology*, vol. 59, no. 4, pp. 1262–1272, 2014.

[27] Y. Duan, S. Guan, K. Yang et al., "Serum level of interferon -λ3 in patients with chronic hepatitis B and its clinical significance (In Chinese)," *Journal of Clinical Hepatology*, vol. 35, no. 2, pp. 315–318, 2019.

[28] K. Murata, S. Tsukuda, F. Suizu et al., "Immunomodulatory mechanism of acyclic nucleoside phosphates in treatment of hepatitis B virus infection," *Hepatology*, vol. 71, no. 5, pp. 1533–1545, 2020.

[29] K. Qiu, B. Liu, S. Y. Li et al., "Systematic review with meta-analysis: combination treatment of regimens based on pegylated interferon for chronic hepatitis B focusing on hepatitis B surface antigen clearance," *Alimentary Pharmacology & Therapeutics*, vol. 47, no. 10, pp. 1340–1348, 2018.

[30] R. Erken, V. V. Loukachov, A. de Niet et al., "A prospective five-year follow-up after peg-interferon plus nucleotide analogue treatment or no treatment in HBeAg negative chronic hepatitis B patients," *Journal of Clinical and Experimental Hepatology*, vol. 12, no. 3, pp. 735–744, 2022.

[31] S. Lin, Y. Fu, W. Wu et al., "The efficacy of addition of Tenofovir Disoproxil Fumarate to Peg-IFNα-2b is superior to the addition of Entecavir in HBeAg positive CHB patients with a poor response after 12 weeks of Peg-IFNα-2b treatment alone," *International Journal of Medical Sciences*, vol. 17, no. 10, pp. 1458–1463, 2020.

[32] F. Xiong, X. Bao, N. Gu et al., "The combination therapy of Peginterferonα and entecavir for HBeAg-positive chronic hepatitis B with high HCC risk," *Infection, Genetics and Evolution*, vol. 78, Article ID 104101, 2020.

[33] F. Zhu, Q. Zhang, Q. Zhang, and D. Zhang, "Effects of IFN monotherapy versus combined therapy on HBeAg seroconversion or seroclearance in HBeAg-positive chronic hepatitis B patients: a meta-analysis," *Microbial Pathogenesis*, vol. 139, Article ID 103912, 2020.

[34] P. Hu, J. Shang, W. Zhang et al., "HBsAg loss with peg-interferon alfa-2a in hepatitis B patients with partial response to nucleos(t)ide analog: new switch study," *Journal of Clinical and Translational Hepatology*, vol. 6, no. 1, pp. 1–10, 2018.

[35] Y. F. Liaw, G. K. K. Lau, J. H. Kao, and E. Gane, "Hepatitis B e antigen seroconversion: a critical event in chronic hepatitis B virus infection," *Digestive Diseases and Sciences*, vol. 55, no. 10, pp. 2727–2734, 2010.

[36] F. Yang, X. Yu, C. Zhou et al., "Hepatitis B e antigen induces the expansion of monocytic myeloid-derived suppressor cells to dampen T-cell function in chronic hepatitis B virus infection," *PLoS Pathogens*, vol. 15, no. 4, Article ID e1007690, 2019.

[37] Y. Xie, H. Zhu, Y. Guo et al., "Reduction of hepatitis B surface antigen more pronounced in pegylated interferon alpha therapy combined with nucleotide analogues than nucleoside analogues in chronic hepatitis B patients. Research square," 2021, https://assets.researchsquare.com/files/rs-510467/v1/bbd3bd9c-0ca5-4d82-9a68-e56d3b6834f4.pdf?c=1631884218.