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

Yiran Xie
Huashan Hospital Fudan University Department of Infectious Diseases
Haoxiang Zhu
Huashan Hospital Fudan University Department of Infectious Diseases
Yifei Guo
Huashan Hospital Fudan University Department of Infectious Diseases
Zhenxuan Ma
Huashan Hospital Fudan University Department of Infectious Diseases
Xun Qi
Shanghai Public Health Clinical Center
Feifei Yang
Huashan Hospital Fudan University Department of Infectious Diseases
Richeng Mao
Huashan Hospital Fudan University Department of Infectious Diseases
Jiming Zhang (jmzhang@fudan.edu.cn)
Huashan Hospital, Fudan University https://orcid.org/0000-0001-5115-2037

Research

Keywords: chronic hepatitis B, hepatitis B surface antigen, nucleoside analogues, nucleotide analogues, pegylated interferon alfa

Posted Date: June 7th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-510467/v1

Abstract

Background: Nucleotide analogues (NTs) monotherapy may have a greater 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). The study aimed to explore whether NTs have greater 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 at 48 weeks were analyzed.

Results: A total of 95 patients were investigated, including in PegIFNα plus NSs group and in PegIFNα plus NTs group. Propensity score matching (PSM) was performed. The PegIFNα + NTs group had a greater reduction of HBsAg (−3.48 vs −2.33 $\log_{10}$ IU/mL, P = 0.038) 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. Higher platelet counts (OR = 1.043, 95%CI = 1.002–1.085) and PegIFNα plus NTs (OR = 77.861, 95%CI = 3.923–1545.273) were independent predictors for HBsAg reduction > 1 $\log_{10}$ IU/mL at 48 weeks.

Conclusion: This study suggests that PegIFNα plus NTs led to more HBsAg reduction.

Introduction

Chronic hepatitis B (CHB) is a global infectious disease. There are currently about 70 million people infected with chronic hepatitis B virus (HBV) in China, of whom more than 20 million are CHB patients. Those patients are at high risk of liver cirrhosis and hepatocellular carcinoma (HCC) especially in developing countries[1] presenting an immense medical burden[2]. The persistence of covalently closed circular DNA (cccDNA) 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, major guidelines consider sustained HBsAg disappearance after drug withdrawal an ideal treatment end point[6, 7].

However, HBsAg loss is not common with current standard antiviral strategies including nucleos(t)ide analogues (NAs) and pegylated interferon-alpha (PegIFNα). Reduction of HBsAg level is often associated with better outcomes including minimizing cirrhosis and HCC and is conductive to HBsAg clearance, therefore, it is often used as an efficacy indicator. NAs are economic 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, as well as drug resistance problems. In contrast, PegIFNα can reduce HBsAg more thoroughly in a subset of patients[8]. Low virologic response rate in PegIFNα monotherapy and poor reduction of HBsAg in NAs monotherapy shed light on combination strategies.

Previous studies have proven that PegIFNα combined with NAs had better clinical effects than PegIFNα or NAs monotherapy[9–11], particularly in reducing HBsAg level[12] and enhancing HBsAg loss rate[13]. Additionally, different NAs can vary in efficacy. Nucleotide analogues, including tenofovir disoproxil fumarate (TDF), adefovir dipivoxil (ADV), and tenofovir alafenamide (TAF), are not only structurally but also functionally different from nucleoside analogues like entecavir (ETV) and lamivudine (LAM). The reduction in HBsAg was significantly greater in the TDF arm than the ETV arm in NAs naïve patients according to a small randomized controlled trail[14]. Switching from ETV to TDF or TAF lead to significantly more decline of HBsAg[15, 16]. Interestingly, nucleotide analogues have also been found with an additional immunological effect in interferon lambda 3 (IFN-λ3) induction compared to nucleoside analogues[17]. Meanwhile, TDF treatment could be associated with a significantly lower risk of HCC than ETV based on recent studies[18, 19]. Still, the comparison remains controversial[20]. In combination strategies, PegIFNα combined with TDF can reach an HBsAg clearance rate as high as 10.4%[9], but the rate is only 0.8% when combined with ETV[11]. According to this indirect comparison, PegIFNα combined with TDF (which represents nucleotide analogues) appears to reach a better HBsAg clearance rate than PegIFNα combined with ETV (which represents nucleoside analogues) when the treatment durations are similar. However, there is currently no study directly comparing the efficacy of these two types of combination therapy.

Therefore, it is useful to compare HBsAg reduction efficacy for PegIFNα therapy combined with NTs or NSs in CHB patients so that we conducted a retrospective study using the data of CHB patients treated with a combination of PegIFNα plus different NAs at Huashan Hospital of Fudan University from October 2011 to December 2018.

Methods

Patients

Between October 2011 and December 2018, a total of 159 consecutive PegIFNα-naïve 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). Chronic HBV infection was defined as being HBsAg positive and/or HBV DNA positive for at least six months before enrollment. The combination therapy could be add-on (adding on NAs during the therapy of PegIFNα) and NAs experienced. NAs used were maintained consistent with the prior type. Sixty-four patients in total were excluded: four had underlying chronic hepatitis C, autoimmune hepatitis, HIV or tumor; seven had used PegIFNα for more than 48 weeks when NAs were added to the therapeutic regimen; one combined nucleoside analogues and nucleotide analogues at the same time; six used the combination therapy for less than 12 weeks; and forty-six had a PegIFNα therapy duration less than 48 weeks or incomplete data at an important time. In this study, 95 patients were ultimately included, of which one group included those who received PegIFNα combined with nucleoside analogues (ETV) (n = 18), and the other group included 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 in accordance with the Declaration of Helsinki. Written informed consent was obtained for all patients included.

Clinical data

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

Definition of treatment response

The primary endpoint was a reduction of HBsAg levels from the baseline at 48 weeks of treatment. Serological responses: (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 rate (HBeAg loss with appearance of anti-HBe). Virological responses: (1) Reduction of HBV DNA levels from baseline at 48 weeks; (2) HBV DNA undetectable rate (proportion of patients with DNA < 500 IU/mL at 48 weeks); (3) Proportion of patients with HBsAg reduction > 1 $\log_{10}$IU/mL from baseline and HBV DNA undetectable at 48 weeks. Biochemical response was defined as ALT normalization rate (proportion of patients with baseline ALT > 1 upper limit of normal [ULN] and normal ALT at 48 weeks, ULN = 40 U/L)

Laboratory measurements

Serum HBsAg levels were determined by Elecsys HBsAg II assay (Roche Diagnostics GmbH, Mannheim, Germany; linear range, 0.05 to 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 limit of detection was 500 IU/mL. Routine biochemical and hematological tests were performed locally. The upper normal limit of ALT was 40 IU/L. Data from laboratory assessments were collected at baseline, and at 12, 24, 36, and 48 weeks of treatment.

Statistical analysis

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, which was calculated by skewness and kurtosis), while Mann-Whitney U tests were used to compare continuous variables with a skewed distribution. Categorical data were presented as n (%) and analyzed by the chi-squared test. Differences among groups were evaluated using one-way analysis of variance (*ANOVA*), if the variances were homogeneous and *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 on the basis of the propensity score-matching (PSM) technique at a 1:1 ratio with a caliper of 0.2 separately between PegIFNα + ETV group and PegIFNα + ADV group or PegIFNα + ETV group and PegIFNα + TDF group. Age, HBsAg, and prior treatment duration of NAs before combined with PegIFNα were imputed for PSM. When the absolute value of the standard difference was less than 10%, the balance of the variables between the groups was considered acceptable. Differences were

considered significant at a two-tailed $P < 0.05$. All statistical analyses were carried out using SPSS statistical software version 24.0 (IBM, Armonk, NY, USA).

Ethical approval

This study was approved by the Institutional Ethics Committee of Huashan Hospital, Fudan University, China (KY2018–251). Informed consent was obtained from all patients.

Results

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) (Fig. 1). Subgroups of different drugs combined were PegIFNα + ETV, PegIFNα + ADV and PegIFNα + TDF. Before PSM, there was no significant difference in baseline information between the two groups or among different drugs (Table 1). PSM was performed, yielding 18 patients matched in each group. After PSM, relative multivariate imbalance L1 waslower than the imbalance before PSM, indicating a better balance. No covariate exhibited a large imbalance, and all of the covariates reached a balance within 10%. There were no statistically significant differences among patients in each group after PSM (Table 1).

TABLE 1 Comparison of general data before and after matching between two groups

	Before PSM				After PSM			
Variables	PegIFNα + Nucleoside Analogues (n = 18)	PegIFNα + Nucleotide Analogues (n = 77)		*P*	PegIFNα + Nucleoside Analogues (n = 18)	PegIFNα + Nucleotide Analogues (n = 36)		*P*
	PegIFNα + ETV (n = 18)	PegIFNα + ADV (n = 40)	PegIFNα + TDF (n = 37)		PegIFNα + ETV (n = 18)	PegIFNα + ADV (n = 18)	PegIFNα + TDF (n = 18)	
NAs experienced[c]	10 (55.6)	30 (39.0)		0.199	10 (55.6)	13 (36.1)		0.173
	10 (55.6)	13 (32.5)	17 (45.9)	0.215	10 (55.6)	6 (33.3)	7 (38.9)	0.374
Weeks of NAs before combined PegIFNα (wk)[b]	96 (42–168)	48 (14–192)		0.397	96 (42–168)	96 (10–384)		0.948
	96 (42–168)	96 (24–384)	48 (11–60)	0.085	96 (42–168)	384 (170–456)	32 (9–96)	0.105
Weeks of adding on (wk)[a]	10.33 ± 13.90	10.94 ± 12.49		0.857	10.33 ± 13.90	11.67 ± 11.88		0.715
	10.33 ± 13.90	12.03 ± 11.59	9.76 ± 13.45	0.728	10.33 ± 13.90	13.33 ± 11.56	10.00 ± 12.29	0.685
Total weeks of combination (wk)[a]	36.5 ± 13.86	36.6 ± 12.74		0.977	36.5 ± 13.86	36.3 ± 13.86		0.964
	36.5 ± 13.86	35.1 ± 12.02	38.2 ± 13.44	0.564	36.5 ± 13.86	34.7 ± 11.56	38.0 ± 12.29	0.731
age (yr)[a]	37 ± 6.3	35 ± 7.7		0.222	37 ± 6.3	35 ± 6.4		0.154
	37 ± 6.3	36 ± 13.9	34 ± 8.5	0.332	37 ± 6.3	35 ± 6.0	34 ± 6.9	0.260
male[c]	17 (94.4)	59 (76.6)		0.169	17 (94.4)	29 (80.6)		0.343
	17 (94.4)	31 (77.5)	28 (75.7)	0.230	17 (94.4)	15 (83.3)	14 (77.8)	0.318
HBeAg positive[c]	13 (72.2)	65 (84.4)		0.382	13 (72.2)	29 (80.6)		0.728
	13 (72.2)	33 (82.5)	32 (86.5)	0.431	13 (72.2)	14 (77.8)	15 (83.3)	0.725
BMI	23.7 ± 2.0	22.5 ± 2.6		0.205	23.7 ± 2.0	22.4 ± 1.8		0.072

(kg/cm^2)[a]								
	23.7 ± 2.0	22.7 ± 2.6	22.4 ± 2.7	0.887	23.7 ± 2.0	22.3 ± 1.9	22.5 ± 1.7	0.195
HGb (g/L)[a]	156 ± 9.8	152 ± 14.4		0.260	156 ± 9.8	152 ± 14.3		0.244
	156 ± 9.8	152 ± 14.2	153 ± 14.8	0.504	156 ± 9.8	152 ± 14.8	152 ± 14.3	0.510
PLT (x10^9/L)[a]	198 ± 46.6	193 ± 42.1		0.671	198 ± 46.6	191 ± 42.5		0.582
	198 ± 46.6	185 ± 44.3	202 ± 38.2	0.223	198 ± 46.6	182 ± 43.4	201 ± 40.5	0.392
ALB (U/L)[a]	48 ± 2.9	46 ± 3.6		0.169	48 ± 2.9	46 ± 3.7		0.091
	48 ± 2.9	46 ± 3.4	47 ± 3.6	0.109	48 ± 2.9	46 ± 3.4	46 ± 4.1	0.203
ALT (U/L)[b]	48 (32–153)	97 (34–209)		0.269	48 (32–153)	99 (34–234)		0.210
	48 (32–153)	111 (34–263)	90 (35–183)	0.340	48 (32–153)	97 (34–279)	101 (35–202)	0.407
ALT > ULN[c]	11 (61.1)	50 (69.4)		0.499	11 (61.1)	24 (70.6)		0.488
	11 (61.1)	26 (72.2)	24 (66.7)	0.700	11 (61.1)	12 (70.6)	12 (70.6)	0.786
AST (U/L)[b]	27 (23–75)	48 (23–94)		0.214	27 (23–75)	53 (25–102)		0.136
	27 (23–75)	48 (22–98)	47 (24–91)	0.415	27 (23–75)	42 (22–111)	57 (26–99)	0.314
GGT (U/L)[b]	23 (18–63)	18 (26–47)		0.980	23 (18–63)	33 (20–53)		0.610
	23 (18–63)	29 (17–57)	24 (20–35)	0.958	23 (18–63)	36 (18–58)	32 (22–45)	0.860
TBIL (μmol/L)[a]	13.1 ± 7.5	13.1 ± 7.0		0.980	13.1 ± 7.5	14.1 ± 9.0		0.677
	13.1 ± 7.5	12.4 ± 4.5	13.9 ± 8.9	0.676	13.1 ± 7.5	13.2 ± 4.7	15.1 ± 12.2	0.745
HBsAg (log_{10} IU/mL)[a]	3.25 ± 1.1	3.64 ± 0.9		0.116	3.25 ± 1.1	3.71 ± 0.8		0.091
	3.25 ± 1.1	3.67 ± 0.9	3.61 ± 0.9	0.282	3.25 ± 1.1	3.77 ± 1.0	3.66 ± 0.7	0.228
HBsAg≥250 IU/mL[c]	15 (83.3)	71 (92.2)		0.477	15 (83.3)	33 (91.7)		0.388

	15 (83.3)	36 (90.0)	35 (94.6)	0.417	15 (83.3)	16 (88.9)	17 (94.4)	0.557
HBeAg (s/co)[a]	411.38 ± 517.81	510.20 ± 617.34		0.552	411.38 ± 517.81	449.20 ± 616.64		0.832
	411.38 ± 517.81	606.30 ± 95.87	406.40 ± 621.28	0.303	411.38 ± 517.81	568.45 ± 639.86	329.96 ± 585.93	0.469
HBV DNA (log_{10} IU/ml)[a]	4.68 ± 2.30	5.41 ± 2.21		0.241	4.68 ± 2.30	5.48 ± 2.20		0.249
	4.68 ± 2.30	5.78 ± 2.13	4.68 ± 2.29	0.214	4.68 ± 2.30	5.66 ± 2.18	5.33 ± 2.28	0.479

Notes:

a. Variables were expressed as $\bar{x}\pm s$

b. Variables were expressed as median (IQR)

c. Variables were expressed as n (%)

Abbreviations: 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; PegIFNα, pegylated interferon alpha; PLT, platelet; TBIL, total bilirubin; TDF, tenofovir disoproxil fumarate; ULN, upper limit of normal

Primary endpoint before and after PSM

HBsAg level gradually decreased during treatment. After 48 weeks, patients in the PegIFNα + NTs therapy 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 PegIFNα + ADV group (−3.47 vs −2.33 log_{10} IU/mL, P = 0.029) and PegIFNα + TDF group (−3.44 vs −2.33 log_{10} IU/mL, P = 0.046) reduced significantly more HBsAg levels than 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 (Fig.2 A, D). In subgroup comparison, both PegIFNα + ADV group (−3.55 vs −2.33 log_{10} IU/mL, P = 0.035) and PegIFNα + TDF group (−3.49 vs −2.33 log_{10} IU/mL, P = 0.039) reduced HBsAg more than PegIFNα + ETV group (Table 3).

Serological response

Before matching, 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 (98.7% vs 72.2%, P = 0.001). This difference was still present after matching (100% vs 72.2%, P = 0.003) (Fig. 3). Similarly, both PegIFNα + ADV group and PegIFNα + TDF group had a higher rate in HBsAg reduction > 1 log_{10} IU/ml at 48 weeks than PegIFNα + ETV group before and after PSM (Table 2, 3) (Fig. 3).

We further analyzed patients with HBsAg loss after receiving different 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 the same, but the difference was not statistically significant (P = 0.109) (Table 3). After PSM, patients achieving HBsAg loss in the PegIFNα + NTs and PegIFNα + NSs group were three (8.3%) and four (22.2%), respectively, without significant statistical difference (P = 0.205) (Fig. 3). Subgroup analysis did not show a statistically significant difference (Table 2, 3).

At 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 (Before PSM: −532.27 vs − -394.33 s/co, P = 0.447; after PSM: − -478.72 vs −394.33 s/co, P = 0.667) (Table 2, 3) (Fig. 2 B, E). HBeAg loss at 48 weeks occurred in 11 patients (16.9%) treated with PegIFNα + NTs and in three patients (23.1%) with PegIFNα + NSs therapy before matching (P = 0.895) (Table 2); meanwhile, eight (12.3%) and two (15.4%) patients from each group achieved HBeAg seroconversion, respectively (P = 1.000) (Fig. 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 difference between the two groups(Fig. 3). No differences were observed among subgroups (Table 2, 3).

Virological response

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) (Fig. 2 C, F). Meanwhile, the number of patients who reached HBV DNA below the lower detection limit (< 500 IU/mL) at 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) (Fig. 3). No differences were observed among subgroups (Table 2, 3).

Interestingly, the proportion of patients who 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) (Fig. 3). PegIFNα + TDF group had a significantly much higher rate than PegIFNα + ETV group (97.3% vs 72.2%, P = 0.012) After PSM, however, the proportion in the PegIFNα + NTs group was not significantly higher (91.7% vs 72.2%, P = 0.205) compared with the group treated with PegIFNα + NSs, still PegIFNα + TDF group remained a significantly higher proportion than PegIFNα + ETV group (100.0% vs 72.2%, P = 0.045) (Table 3) (Fig. 3).

Biochemical response

For patients with elevated baseline ALT, the proportion of those who returned to normal levels at 48 weeks also differed between the two groups, although 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%) and nine patients (52.9%) in the PegIFNα + NTs and PegIFNα + NSs groups had biochemical responses, respectively (P = 0.494) (Fig. 3). Biochemical responses did not vary substantially by subgroups (Table 2, 3).

TABLE 2. Efficacy Results at Weeks 48 before PSM

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

TABLE 3. Efficacy Results at Weeks 48 after PSM

Response	PegIFNα + NSs (n = 18)	PegIFNα + NTs (n = 36)		*P* (total)	*P* (ETV vs ADV)	*P* (ETV vs TDF)	*P* (ADV vs TDF)
	PegIFNα + ETV (n = 18)	PegIFNα + ADV (n = 18)	PegIFNα + TDF (n = 18)				
HBsAg reduction from baseline at wk 48, log_{10} IU/mL	-2.33	-3.52		0.032			
	-2.33	-3.55	-3.49	0.092	0.035	0.039	0.853
HBeAg reduction from baseline at wk 48, s/co	-394.33	-478.72		0.667			
	-394.33	-618.26	-356.63	0.417	0.301	0.862	0.236
HBV DNA reduction from baseline at wk 48, log_{10} IU/mL	-3.32	-4.72		0.194			
	-3.32	-4.85	-4.60	0.426	0.240	0.311	0.840
HBsAg loss, n (%)	4 (22.2)	3 (8.3)		0.205			
	4 (22.2)	1 (5.6)	2 (11.1)	0.316	0.338	0.658	1.000
HBeAg loss, n (%)	3 (23.1)	4 (13.8)		0.657			
	3 (23.1)	2 (14.3)	2 (13.3)	0.764	0.648	0.639	1.000
HBeAg seroconversion, n (%)	2 (15.4)	3 (10.3)		0.637			
	2 (15.4)	1 (7.1)	2 (13.3)	0.773	0.596	1.000	1.000
HBV DNA undetactable, n (%)	17 (94.4)	33 (94.3)		1.000			
	17 (94.4)	15 (88.2)	18 (100.0)	0.221	0.603	1.000	0.229
HBsAg reduction > 1 log_{10} from baseline, n (%)	13 (72.2)	36 (100.0)		0.003			
	13 (72.2)	18 (100.0)	18 (100.0)	0.002	0.045	0.045	/
HBsAg reduction > 1 log_{10} and DNA undetactable, n (%)	13 (72.2)	33 (91.7)		0.205			
	13 (72.2)	15 (83.3)	18 (100.0)	0.042	0.691	0.045	0.229
ALT normalization, n (%)	9 (52.9)	15 (42.9)		0.494			
	9 (52.9)	7 (38.9)	8 (47.1)	0.704	0.404	0.732	0.625

Predictors associated with HBsAg reduction > 1 log10 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 at 48 weeks. Univariate analysis was performed to analyze the effect of clinical data and laboratory tests. Factors with clinical and statistical significance were jointly included in multivariate regression analysis. As a result, we found that higher platelet counts (OR = 1.043, 95%CI = 1.002–1.085) and treatment with PegIFNα plus nucleotide analogues (OR = 77.861, 95%CI = 3.923–1545.273) (Table 4) were independent predictors contributing to HBsAg reduction > 1 $\log_{10}$ IU/mL at 48 weeks.

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* value	OR (95% CI)	*P* value
Age (yr)	0.858 (0.780–0.944)	0.002		
BMI (kg/cm^2)	0.753 (0.479–1.183)	0.218		
HBeAg positive	0.405 (0.068–2.418)	0.322		
PegIFNα add-on NAs	2.341 (0.408–13.445)	0.340		
NAs add-on PegIFNα	0.340 (0.059–1.953)	0.226		
PegIFNα plus nucleotide analogues	29.231 (3.155–270.801)	0.003	77.861 (3.923–1545.273)	0.004
PegIFNα plus ADV	1.490 (0.259–8.563)	0.655		
PegIFNα plus ETV	1.181 (0.129–10.774)	0.883		
PegIFNα plus TDF	0.618 (0.118–3.240)	0.569		
Week of PegIFNα adding NAs (wk)	0.992 (0.931–1.058)	0.813		
Weeks of NAs before adding PegIFNα (wk)	1.003 (0.992–1.015)	0.565		
Total weeks of combination (wk)	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		
qHBsAg at baseline (IU/ml)	3.338 (1.479–7.533)	0.004		
qHBsAg > 250 IU/ml at baseline	5.857 (0.908–37.798)	0.063		
qHBsAg at week 12 (IU/ml)	1.000 (1.000–1.001)	0.362		
qHBsAg decline at week 12 (log_{10} IU/ml)	0.813 (0.507–1.303)	0.390		
qHBsAg decline at week 24 (log_{10} IU/ml)	0.538 (0.310–0.932)	0.027		
HBV DNA at baseline (IU/ml)	1.317 (0.837–2.074)	0.234		
HBV DNA at week 12 (IU/ml)	1.000 (1.000–1.000)	0.543		
HBV DNA decline at week 12 (log_{10}	0.905 (0.749–1.093)	0.300		

IU/ml)				
HGb (g/L)	0.966 (0.898–1.039)	0.350		
PLT (x10^9/L)	1.024 (1.001–1.048)	0.037	1.043 (1.002–1.085)	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		

Abbreviations: ADV, adefovir dipivoxil; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate transaminase; BMI, body mass index; CI, confidence interval; ETV, entecavir; GGT, gamma glutamyl transferase; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HGb, hemoglobin; PegIFNα, pegylated interferon alpha; PLT, platelet; TBIL, total bilirubin; TDF, tenofovir disoproxil fumarate; ULN, upper limit of normal

Discussion

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

There have been a number of 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 rate (9.1% vs 2.8%)[24]. 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 obvious that combination therapy has prominent advantages over monotherapy, but the baseline conditions, optimal treatment duration, and sustained response rate of combination therapy require further exploration.

At the same time, it remains controversial whether efficacy differs between nucleotide analogues and nucleoside analogues when combined with PegIFNα. The two types of oral drugs have been found to be 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 naïve patients[14]. Furthermore, HBeAg negative patients whose HBsAg had not been reduced in 48

weeks during 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 researches have proven that TDF treatment was associated with lower risk of HCC than ETV therapy. A large retrospective analysis in China found that over a median follow-up time of 3.6 years, 4.9% ETV-treated patients developed HCC while it occurred in only 0.6% TDF-treated patients[19]. Similarly, a research in Korea had a consistent finding that the annual incidence rate of HCC was significantly lower in the TDF group than ETV group (0.64 vs 1.06 per 100 person-year)[18]. Notably, studies have indicated that patients treated with nucleotide analogues, especially ADV, have higher serum IFN-λ3 levels than those treated with nucleoside analogues[26, 27]. The ability of IFN-λ3 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 IFN-α[26]. ISGs can encode antiviral proteins through complex intracellular signaling pathways, indicating that IFN-λ3 may have a more durable antiviral effect. Recombinant IFN-λ3 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. In a study by Ahn *et al*., after 48 weeks of therapy combining PegIFNα and TDF followed by TDF monotherapy until 120 weeks, the HBsAg clearance rate could reach 10.4%. 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 patients were followed-up with up to 96 weeks, only 0.8% 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 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]. Lin *et al*. recently found that 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 addition of ETV to Peg-IFNα-2b (−1.799 $\log_{10}$ IU/mL vs −1.078 $\log_{10}$ IU/mL, $P = 0.0491$)[30]. 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 slightly lack universality and a larger sample size study is required to verify the results. Therefore, it is presently still no so clear whether PegIFNα combined with different NAs influences HBsAg reduction and clearance. The loss rate of HBeAg after 48 weeks was similar between PegIFNα + TDF and PegIFNα + ETV (29.0% vs 31.0%)[31]. 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[32]. But Lin *et al.* showed than the HBeAg loss rate was significantly higher in TDF add-on group than that in ETV add-on group at week 48 (40% vs 10%, $P = 0.028$)[30]. Interestingly, these studies suggested a possibility that PegIFNα combined with different NAs could have different efficacies, but direct evidence was demanded and mechanism behind the differences need to be discussed. Based on these findings, we conducted this retrospective study to provide this evidence. TAF has only been launched in recent years, and with insufficient studies discussing the efficacy of PegIFNα plus TAF, we therefore did not include patients who received TAF in the current study. Meanwhile, no patients in our cohort used LAM, so the only nucleoside analogue analyzed was ETV. To our knowledge, our 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 be helpful to 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 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 the reductions in Lin's study (Lin *et al*. 2020). This might be because our study had a longer combination course and some patients had a prior treatment of NAs. 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 between the two groups were found in the following indicators: HBsAg loss rate, HBV DNA reduction, HBeAg reduction, HBeAg loss rate, HBeAg seroconversion rate, HBV DNA undetectable rate, and ALT normalization rate. The observation end point 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-naïve patients. The ability to maintain HBsAg clearance steadily after combination therapy also cannot be confirmed. Another reason for the significant differences in decline levels, but not in HBsAg loss rates, may be the small sample size. Based on the results of our study, we believe that nucleotide analogues can significantly reduce more HBsAg than nucleoside analogues when combined with PegIFNα. This reduction will contribute to achieving HBsAg clearance and even 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 PegIFNα + ETV group and PegIFNα + TDF group after PSM (100.0% vs 72.2%, P = 0.045). This result exemplifies the dual effectiveness of combination therapy with TDF over combination therapy with ETV in inhibiting viral replication and reducing HBsAg levels simultaneously.

Furthermore, multivariate logistic regression showed that treatment with PegIFNα plus nucleotide analogues was an independent predictor for HBsAg decline > 1 $\log_{10}$ IU/mL at 48 weeks, suggesting that the combination of PegIFNα and nucleotide analogues can increase HBsAg decline. Higher platelet count was also an independent predictor for HBsAg reduction > 1 $\log_{10}$ IU/mL.

Combination strategies been studied include "De novo", "NA-experienced", "add-on", and "switch-to". Several studies have shown that the "NA-experienced" strategy seemed to be the best. The "switch-to" strategy was particularly effective and improved HBsAg clearance[13, 29, 33]. This may be 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. Among the patients included in this study, the number of NA-experienced patients was relatively small and was prone to bias, so no statistical analysis of this sub-population was conducted.

Limitations of our study include that it is a retrospective study with a small sample size and short therapy duration without a long-term follow-up. Furthermore, the combination strategy was not precisely uniform although the duration of combination therapy had been guaranteed to be at least 24 weeks. Even though, 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 so the following analysis was considered reliable. Further randomized controlled trials are required for verification, and patients who are NAs-experienced for at least 48 weeks before the initiation of PegIFNα add-on need to be particularly examined.

Conclusion

In conclusion, reduction of HBsAg was more pronounced in PegIFNα therapy combined with nucleotide analogues than nucleoside analogues, a finding that will be beneficial for promoting further HBsAg clearance and functional cure. This result can provide a basis for clinical decision-making. Similar results and related mechanisms need to be further confirmed.

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; IFN-λ3, interferon lambda 3; IQR, interquartile range; ISGs, interferon-stimulated genes; LAM, lamivudine; NAs, nucleos(t)die analogues; NSs, nucleoside analogues; NTs, nucleotide analogues; PegIFNα, pegylated interferon alpha; PLT, platelet; PSM, propensity score matching; SD, standard deviation; TAF, tenofovir alafenamide; TBIL, total bilirubin; TDF, tenofovir disoproxil fumarate; ULN, upper limit of normal

Declarations

Acknowledgments

We thank Accdon (www.accdon.com) for its linguistic assistance during the preparation of this manuscript.

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.

Funding

The present study was supported by the Shanghai Pujiang Program (17PJD005) , the National Natural Science Foundation of China (81670528, 81672009, and 81871640), Scientific Research Fund of Huashan Hospital Fudan University grant number 2016QD073 and the Shanghai Municipal Health Funds (201840024 to FY).

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Ethics approval and consent to participate

This study was approved by the Institutional Ethics Committee of Huashan Hospital, Fudan University, China (KY2018–251). Informed consent was obtained from all patients.

Consent for publication

Not applicable.

Authors' information

1Department of Infectious Diseases, Huashan Hospital of Fudan University, Shanghai, China; (Address: 12 Middle Wulumuqi Road, Shanghai 20040, China)

2Shanghai Public Health Center of Fudan University, Shanghai, China; (Address: No. 2901, Caolang Road, Jinshan District, Shanghai 201508, China)

3Department of Infectious Diseases, Jing'An District Centre Hospital of Shanghai (Huashan Hospital, Fudan University Jing'An Branch), Shanghai, China; (Address: No. 259, Xikang Road (Xinzha Intersection), Shanghai, China)

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Figures

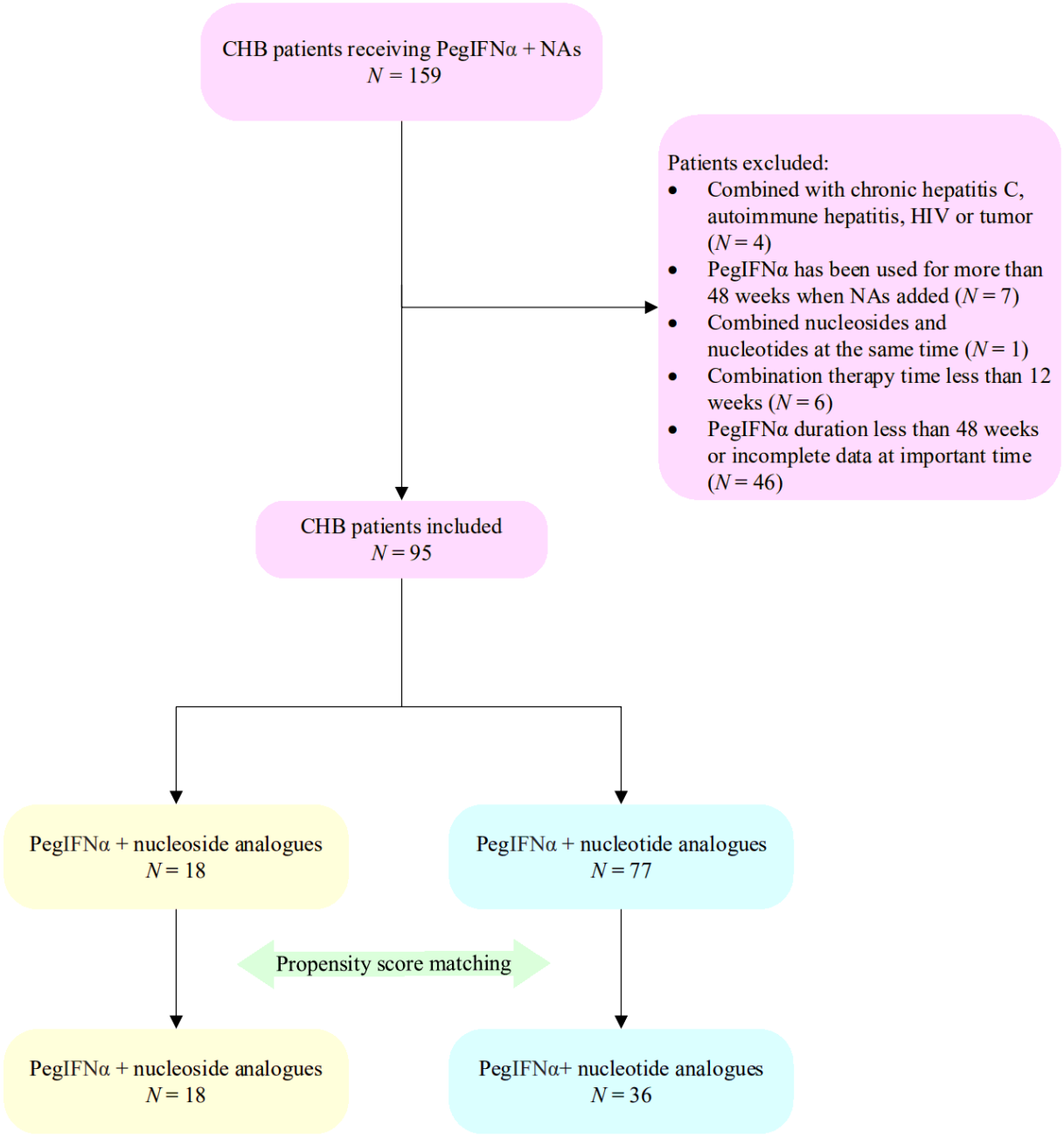

Figure 1

Flow diagram describing the selection of the study population. CHB, chronic hepatitis B; HIV, human immunodeficiency virus; NAs, nucleos(t)ides; PegIFNα: Pegylated interferon alpha;

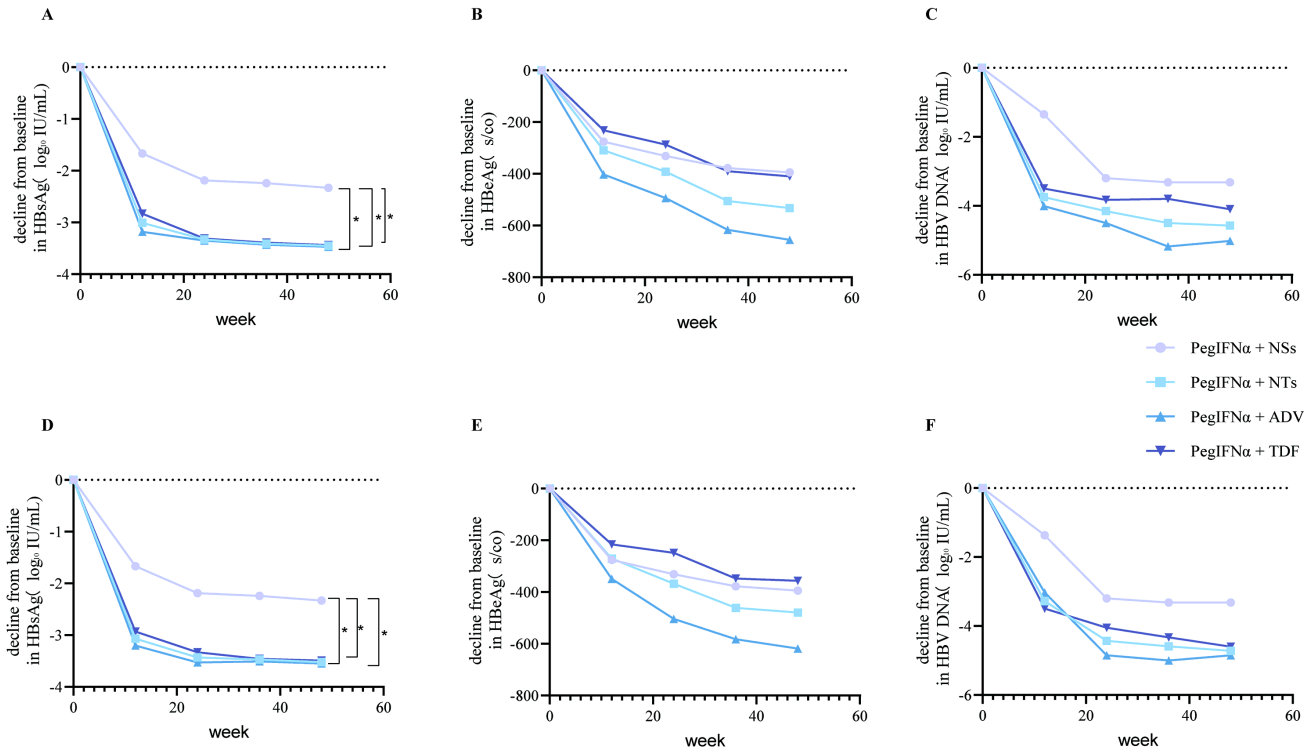

Figure 2

Mean reductions from baseline in HBsAg, HBeAg, and HBV DNA at the end of therapy before and after propensity score matching 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. ADV, adefovir dipivoxil; ETV, enticavir; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; PegIFNα, pegylated interferon α; TDF, tenofovir

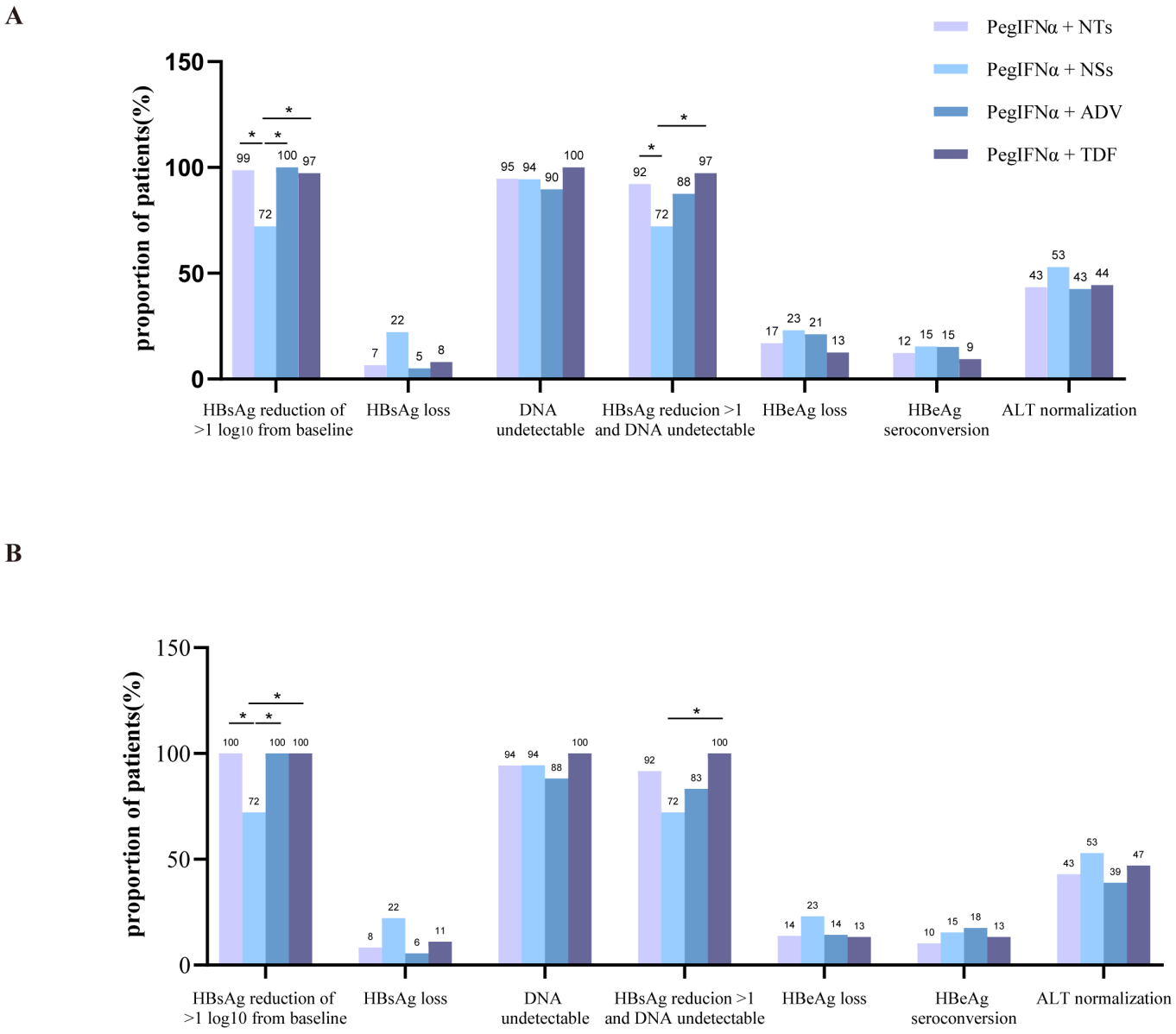

Figure 3

The rate of HBsAg reduction > 1 log10IU/mL, HBsAg loss, DNA undetectable, HBsAg reduction > 1 log10 IU/mL and DNA undetectable, HBeAg loss, HBeAg seroconversion, and ALT normalization at the end of therapy. A: efficacy index before propensity score matching B: efficacy index after propensity score matching *: P < 0.05 ALT: alanine aminotransferase; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; NS, nucleoside analogues; NT, nucleotide analogues; PegIFNα, pegylated interferon α