

ORIGINAL PAPERS

HBsAg seroclearance or seroconversion induced by peg-interferon alpha and lamivudine or adefovir combination therapy in chronic hepatitis B treatment: a meta-analysis and systematic review

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ABSTRACT

Background and aims: Seroclearance or seroconversion of hepatitis B surface antigen (HBsAg) is generally considered as a clinical endpoint. The purpose of the present meta-analysis was to evaluate the effect of combined therapy with pegylated interferon alpha (PEG-IFN α) with or without lamivudine (LAM) or adefovir (ADV) on HBsAg seroclearance or seroconversion in subjects with chronic hepatitis B (CHB).

Methods: Randomized controlled trials performed through May 30th 2015 in adults with CHB receiving PEG-IFN α and LAM or ADV combination therapy or monotherapy for 48-52 weeks were included. The Review Manager Software 5.2.0 was used for the meta-analysis.

Results: No statistical differences in HBsAg seroclearance (9.9% vs. 7.1%, OR = 1.47, 95% CI: 0.75, 2.90; $p = 0.26$) or HBsAg seroconversion (4.2% vs. 3.7%, OR = 1.17, 95% CI: 0.57, 2.37; $p = 0.67$) rates were noticed between PEG-IFN α + LAM and PEG-IFN α + placebo during post-treatment follow-up for 24-26-weeks in subjects with hepatitis Be antigen (HBeAg)-positive CHB.

No statistical differences in HBsAg clearance (10.5% vs. 6.4%, OR = 1.68, 95% CI: 0.75, 3.76; $p = 0.21$) were seen, but statistical differences in HBsAg seroconversion (6.3% vs. 0%, OR = 7.22, 95% CI: 1.23, 42.40; $p = 0.03$) were observed, between PEG-IFN α + ADV and PEG-IFN α for 48-52 weeks of treatment in subjects with HBeAg-positive CHB.

A systematic evaluation showed no differences in HBsAg disappearance and seroconversion rates between PEG-IFN α + placebo and PEG-IFN α + LAM for 48-52 weeks in subjects with HBeAg-positive CHB.

A systematic assessment found no differences in HBsAg disappearance and seroconversion rates between PEG-IFN α + placebo and PEG-IFN α + LAM during 24 weeks' to 3 years' follow-up after treatment in subjects with HBeAg-negative CHB.

Conclusion: Combined therapy with PEG-IFN α and LAM or ADV was not superior to monotherapy with PEG-IFN α in terms of HBsAg seroclearance or seroconversion

Key words: Adefovir. Chronic hepatitis B. Combination therapy. Hepatitis B surface antigen. Peginterferon alpha. Lamivudine.

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INTRODUCTION

Approximately 350 million individuals are infected with hepatitis B virus (HBV) in the whole world. Chronic HBV infection remains a health problem worldwide, which is closely associated with hepatic fibrosis, compensated or decompensated liver cirrhosis, and liver cancer, especially in the Asia-Pacific region (1). Antiviral therapy may be crucial to prevent the progression of liver cirrhosis, and to reduce end-stage liver disease and hepatocellular carcinoma (HCC). Antiviral agents for the treatment of chronic hepatitis B (CHB) include nucleoside/nucleotide analogues (NAs) and interferon-alpha (standard or pegylated). Seroclearance or seroconversion of hepatitis B surface antigen (HBsAg) is generally considered as the ideal clinical goal of antiviral therapy (2,3). Meanwhile, an increasing number of research studies have emphasized the prognostic role of serum HBsAg levels in CHB patients treated with peg-interferon alpha (PEG-IFN α) and/or NAs, especially in HBeAg-positive individuals. However, few systematic reviews of the ideal endpoint have been thus far proposed to provide a scientific basis for the evaluation of these different antiviral therapies.

One meta-analysis (including nine trials) published in 2011 tried to analyze the efficacy of lamivudine (LAM) monotherapy and PEG-IFN α + LAM combination therapy focusing on HBsAg seroclearance or seroconversion, and the result indicated that PEG-IFN α + LAM combination therapy was more effective than LAM monotherapy for HBsAg clearance or seroconversion. However, the result of this study was poorly accepted by doctors in clinical practices because the meta-analysis had ignored either treatment duration (24 weeks or 52 weeks) or HBeAg status (positive or negative). Moreover, among the nine original trials included, combination therapy had followed a simultaneous or a sequential approach, and treatment-free follow-up exhibited different durations (4). Another

Zhang Y, Chen B, Wang L, Chi J, Song S, Liu M, Zhao Z. HBsAg seroclearance or seroconversion induced by peg-interferon alpha and lamivudine or adefovir combination therapy in chronic hepatitis B treatment: a meta-analysis and systematic review. *Rev Esp Enferm Dig* 2016;108:263-270.

meta-analysis (three trials included) published in 2013 showed no difference in HBsAg clearance between combined interferon plus adefovir (ADV) therapy and interferon monotherapy, but no reference was made to HBsAg seroconversion (5).

In our present meta-analysis, we aimed to determine the efficacy of PEG-IFN α + NA combination therapy and PEG-IFN α monotherapy on HBsAg seroclearance or seroconversion in HBeAg-positive/negative CHB patients.

METHODS

Inclusion and exclusion criteria

Studies that met all the following inclusion criteria were included: a) study type: clinical randomized controlled trial (RCT); b) study population: HBsAg-positive and HBeAg-positive/negative CHB patients; c) intervention type: PEG-IFN α combined with LAM or ADV therapy vs. PEG-IFN α monotherapy for at least 48 weeks; d) outcomes: HBsAg clearance or HBsAg seroconversion measured at the end of treatment or follow-up.

Studies that met any of the following exclusion criteria were excluded: a) non-clinical studies; b) non-adult patients; pregnant or breast-feeding women; patients co-infected with hepatitis C virus, hepatitis D virus, or human immunodeficiency virus; patients with a history of alcohol or drug abuse, hepatocellular carcinoma, decompensated liver disease, serious medical or psychiatric illness, or liver transplantation; c) therapy period less than 48 weeks; concurrent use of corticosteroids, immunosuppressive agents, tyrosine alpha-1 or Chinese herbal medicine; d) not reporting any of the efficacy measures of HBsAg clearance or HBsAg seroconversion as defined by the authors; e) republished studies or full text not available; and f) abstracts in English with full text in a language other than Chinese or English.

Literature search strategy

Two researchers independently performed a literature retrieval by electronic searches of The Cochrane Central Register of Controlled Trials (CENTRAL) in The Cochrane Library, MEDLINE (via Ovid), EMBASE (via Ovid), and the Chinese Biomedical CD Database (CBM), China Network Knowledge Information (CNKI), Chinese Science Journal Database (CQVIP), and Wanfang Database. All the above databases were searched from their date of inception onwards through May 30th 2015. The following search terms were used: "hepatitis B", "interferon", "nucleoside/nucleotide analogs", "lamivudine", "adefovir", and "combination therapy". In addition, conference proceedings in Chinese and citations in previous publications were searched.

Selection of studies

Two authors independently screened the studies according to the prespecified selection criteria. Disagreements were resolved by their conferring with each other.

Efficacy measures and definitions

HBsAg clearance was defined as the disappearance of HBsAg from the serum. HBsAg seroconversion was defined as HBsAg disappearance plus anti-HBs antibody appearance.

Data extraction and management

Two researchers extracted data independently using the same data extraction form, and reached consensus by discussion with each other when discrepancies developed. The following key characteristics and data were extracted from each trial: study design, sample size calculations, methods of combination, HBeAg positivity or negativity, country, interventions (species, dosage, duration of therapy, length of follow-up), outcomes (events and total number).

Assessment of methodological quality in included studies

Two authors independently assessed the *methodological quality* of each randomized trial included according to the Jadad quality scale (6). We examined randomization, concealment, blinding, and reporting of patient withdrawal and dropout rates, and a score greater than or equal to 3 was defined as high-quality. Disagreements were resolved by further conferring with each other.

Assessment of heterogeneity and statistical analyses

Quantitative meta-analyses were performed to assess the difference in effectiveness between the PEG-IFN α plus LAM or ADV combination group and PEG-IFN α monotherapy group. In order to further distinguish the difference between ADV and LAM, we performed a subgroup analysis and also took HBeAg status into account. A data synthesis was performed and forest plots were generated using Review Manager Software 5.2 (Cochrane Collaboration, Oxford, England). Due to the dichotomous data, odds ratios (ORs) and their 95% confidence intervals (CIs) were chosen together to evaluate the strength of the association. Heterogeneity was assessed using a Chi-squared test. Heterogeneity was measured using the I^2 statistic, and statistical significance was set at $p < 0.10$. The fixed-effect method (the Peto OR was calculated if "zero" was present in the 2×2 table, otherwise the Mantel-Haenszel OR was used) or the random-effect method (D-L OR) ($p < 0.10$, $I^2 > 50\%$) was used to combine the results with different weights in the absence of statistically significant heterogeneity ($p \geq 0.10$, $I^2 \leq 50\%$). The overall effect was judged by the Z-test, and $p < 0.05$ was deemed significant.

RESULTS

Search results and study characteristics

We identified a total of 5,472 publications through electronic searches. Firstly, we excluded 5,071 from these databases, as they were duplicates, clearly irrelevant articles,

or non-randomized clinical trials. Then we rejected 390 trials from additional 401 full texts because of treatment duration < 48 weeks, lack of information on HBsAg or no events in both groups, ongoing studies, or only conference abstracts. Additionally, three studies meeting our inclusion criteria were included by hand-searching. A total of fourteen (7-20) clinical randomized controlled trials were included in the meta-analysis. The flow diagram of the literature search was displayed in figure 1. The fourteen studies (7 high-quality ones included) analyzed came from five countries (8 from China, 2 from the Netherlands, 2 from France, 1 from Turkey, 1 from Italy). All studies were published as full publications, eight in English (9,14-20) and six in Chinese (7,8,10-13). Fourteen trials involving a total of 2,818 patients (1,858 HBeAg-positive cases and 960 HBeAg-negative cases) were analyzed in our study; eight trials (7-13,17) and six trials (14-16,18-20), respectively, used PEG-IFN α + ADV and PEG-IFN α + LAM as simultaneous combination therapy for 48-52 weeks; four trials used PEG-IFN α 2b (9,14,16,20), and the remaining ten trials used PEG-IFN α 2a. Treatment-free follow-up results showed that two of six PEG-IFN α + LAM trials were conducted for an average 3 years (16,19), and the remaining four for 24-26 weeks; two (7,17) of eight PEG-IFN α + ADV trials were followed up for 24-48 weeks, and the remaining six had no follow-up. Of all these trials, three showed both HBsAg clearance and seroconversion (8,14,17), three showed HBsAg seroconversion (9,10,15), and eight showed HBsAg clearance. The characteristics of RCTs included in the meta-analysis are shown in table I.

HBsAg disappearance and seroconversion in the PEG-IFN α + LAM vs. PEG-IFN α + placebo groups for 48-52 weeks (HBeAg-positive patients)

Only a high-quality research was retrieved and analyzed in the study. Janssen HLA et al. (14) showed no difference in the proportion of patients with HBsAg clearance between PEG-IFN α plus LAM (7% [9/130]) and PEG-IFN α plus placebo (5% [7/136], $p = 0.54$) at the end of treatment (week 48-52). In addition, no differences in the ratio of patients with HBsAg seroconversion was seen between PEG-IFN α plus LAM (6% [8/130]) and PEG-IFN α plus placebo (4% [6/136], $p = 0.53$).

HBsAg disappearance and seroconversion in the PEG-IFN α + LAM vs. PEG-IFN α + placebo groups for 24-26 weeks of treatment-free follow-up (HBeAg-positive patients)

HBeAg-positive patients were followed for 24-26 weeks, and the pooled rates of HBsAg clearance and seroconversion were 9.9% and 4.2% in the PEG-IFN α + LAM group, and 7.1% and 3.7% in PEG-IFN α + placebo group, respectively. No evidences of inter-trial heterogeneity were identified ($\chi^2 = 0.96$, d.f. = 1, $p = 0.33$, $I^2 = 0\%$; $\chi^2 = 0.19$, d.f. = 1, $p = 0.66$, $I^2 = 0\%$, respectively), and the pooled ORs in our meta-analysis, using a fixed-effects model, showed no significant differences in HBsAg clearance rate (M-H OR = 1.47, 95% CI 0.75, 2.90; $p = 0.26$) and HBsAg seroconversion rate (M-H OR = 1.17, 95% CI 0.57, 2.37; $p = 0.67$) (Fig. 2).

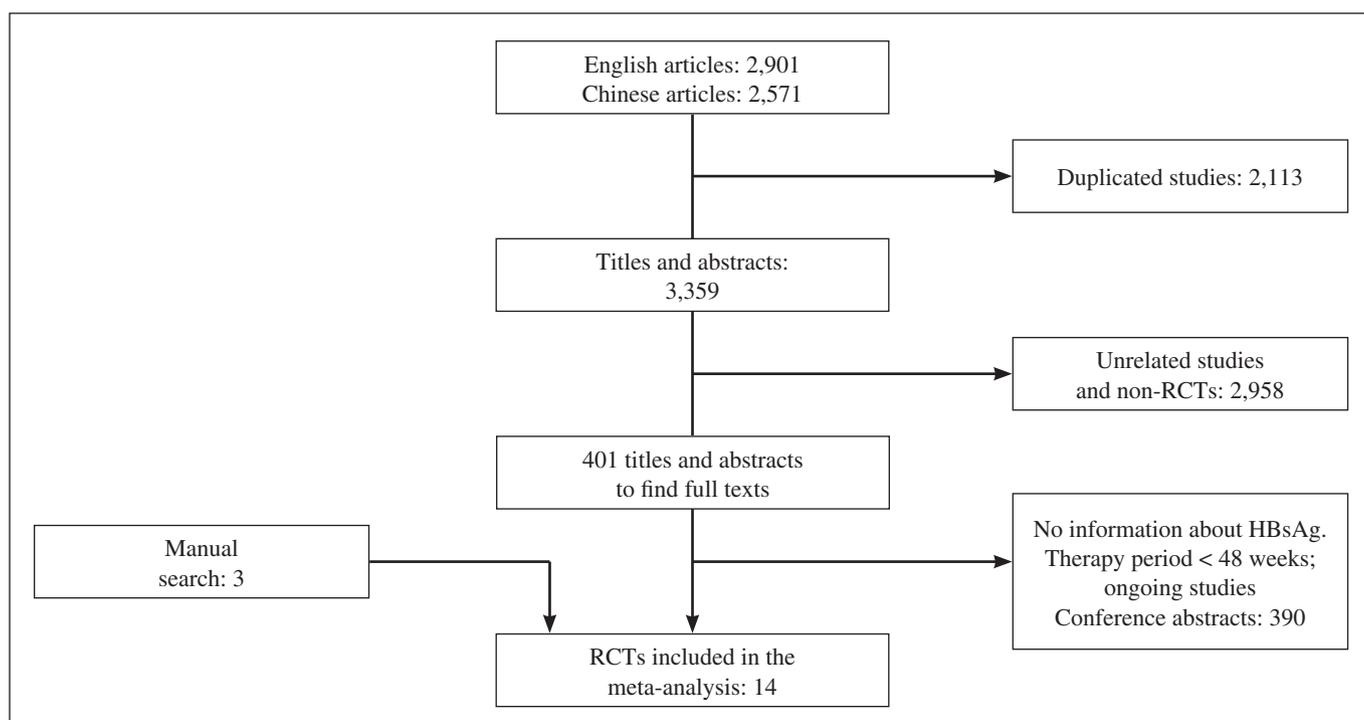


Fig. 1. Flow diagram of the literature search.

Table I. Characteristics of the randomized controlled trials included in the meta-analysis

Study	Country	n	Combination	PEG-IFN- α monotherapy	Jadad score	HBeAg	Follow-up
Ao FJ, 2010 (7)	China	113	PEG-IFN- α 2a 135 ug/w 1/w + ADV10 mg 48 w	PEG-IFN- α 2a 135 ug/w 1/w 48 w	2	+	48 w
Ding WM, 2010 (8)	China	65	PEG-IFN- α 2a 180 ug/w 1/w + ADV10 mg 48 w	PEG-IFN- α 2a 180 ug/w 1/w 48 w	2	+	0 w
Liu YH, 2014 (9)	China	61	PEG-IFN α -2b 1.5 ug/kg 1/w + ADV 10 mg 1/d 52 w	PEG-IFN α -2b 1.5 ug/kg 1/w 52 w	3	+	0 w
Chen HO, 2013 (10)	China	73	PEG-IFN- α 2a 180 ug/w 1/w + ADV10 mg 48 w	PEG-IFN- α 2a 180 ug/w 1/w 48 w	1	+	0 w
Li WB, 2013 (11)	China	60	PEG-IFN- α 2a 180 ug/w 1/w + ADV10 mg 48 w	PEG-IFN- α 2a 180 ug/w 1/w 48 w	2	+	0 w
Liang JP, 2013 (12)	China	90	PEG-IFN- α 2a 180 ug/w 1/w + ADV10 mg 48 w	PEG-IFN- α 2a 180 ug/w 1/w 48 w	2	+	0 w
Han SY, 2014 (13)	China	147	PEG-IFN- α 2a 180 ug/w 1/w + ADV10 mg 48 w	PEG-IFN- α 2a 180 ug/w 1/w 48 w	1	+	0 w
Janssen HLA, 2005 (14)	Netherlands	266	PEG-IFN α -2b 100 ug/w + LAM 100 mg/d 52 w	PEG-IFN α -2b 100 ug/w + placebo 52 w	5	+	26 w
Lau GKK, 2005 (15)	Hong Kong/China	814	PEG-IFN- α 2a 180 ug/w 1/w + LAM 100 mg/d 48 w	PEG-IFN- α 2a 180 ug/w 1/w + placebo 48w	4	+	24 w
Buster EH, 2008 (16)	Netherlands	172	PEG-IFN α -2b 100 ug/w + LAM 100 mg/d 52 w	PEG-IFN α -2b 100 ug/w + placebo 52 w	5	+	3 \pm 0.8 y
Piccolo P, 2009 (17)	Italy	60	PEG-IFN- α 2a 180 ug/w 1/w + ADV10 mg 48 w	PEG-IFN- α 2a 180 ug/w 1/w 48 w	4	-	24 w
Marcellin P, 2004 (18)	France	537	PEG-IFN- α 2a 180 ug/w 1/w + LAM 100 mg/d 48 w	PEG-IFN- α 2a 180 ug/w 1/w + placebo 48 w	4	-	24 w
Marcellin P, 2009 (19)	France	315	PEG-IFN- α 2a 180 ug/w 1/w + LAM 100 mg/d 48 w	PEG-IFN- α 2a 180 ug/w 1/w + placebo 48 w	4	-	3 y
Kaymakoglu S, 2007 (20)	Turkey	48	PEG-IFN α -2b 1.5 ug/kg 1/w + LAM 100 mg/d 48 w	PEG-IFN α -2b 1.5 ug/kg 1/w 48 w	2	-	24 w

§LAM: Lamivudine; ADV: Adefovir; PEG-IFN- α : Pegylated interferon alpha; w: Week ; y: Years; HBeAg: Hepatitis B "e" antigen.

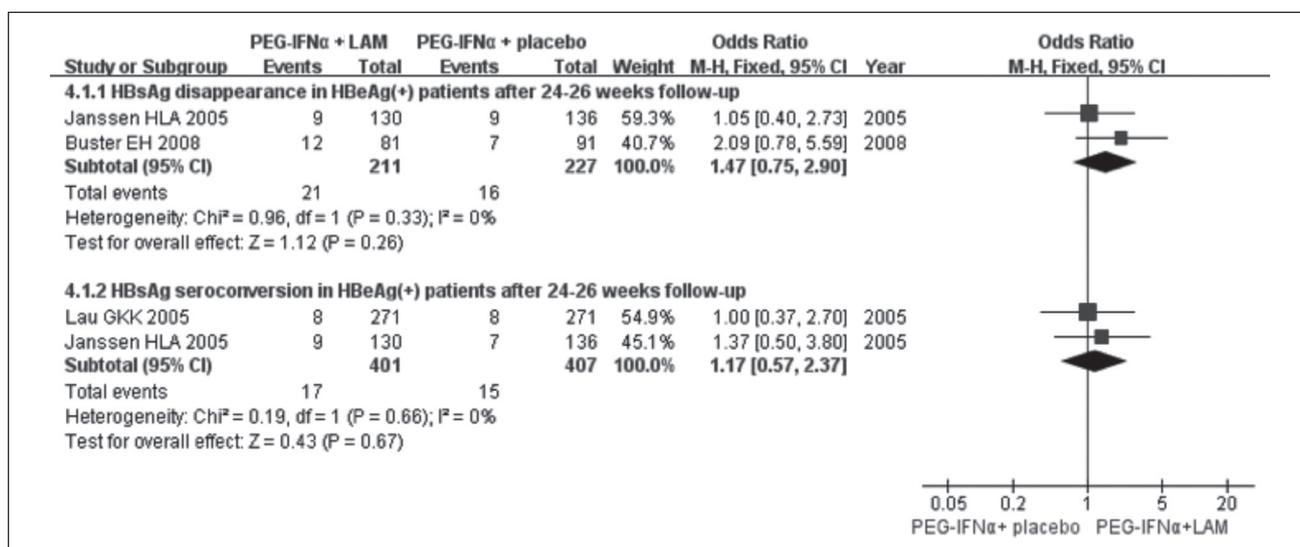


Fig. 2. Analysis of hepatitis B surface antigen (HBsAg) disappearance and seroconversion for pegylated interferon alpha (PEG-IFN α) plus lamivudine (LAM) vs. PEG-IFN- α plus placebo during 24-26 weeks of treatment-free follow-up in subjects with HBeAg-positive CHB.

HBsAg disappearance and seroconversion in the PEG-IFN α + LAM vs. PEG-IFN α + placebo groups for 24 weeks-3 years of treatment-free follow-up (HBeAg-negative patients)

We noticed a highly valuable study for the PEG-IFN α -2a HBeAg-negative chronic hepatitis B study group (18,19), which reported that the HBsAg seroconversion rate of HBeAg-negative patients treated with PEG-IFN α -2a + LAM was 1.7% (3/179), and 2.8% (5/177) for those treated with PEG-IFN α -2a + placebo for 24 weeks of treatment-free follow-up, with no statistical difference detected ($p = 0.47$). Interestingly, the 3-year follow-up study showed that the HBsAg clearance rates of two treatment groups at the 24th week, 48th week, 2nd year, and 3rd year of follow-up had a general ascending trend; however, there was no significant statistical difference between the PEG-IFN α -2a + LAM group (2.8% [5/179] at week 24, 4.4% [5/114] at week 48, 5.3% [6/114] at year 2, 7.9% [9/114] at year 3) and PEG-IFN α -2a + placebo group (3.9% [7/177] and $p = 0.55$ at week 24, 3.4% [4/116] and $p = 0.71$ at week 48, 6.0% [7/116] and $p = 0.80$ at year 2, 7.8% [9/116] and $p = 0.97$ at year 3) (Fig. 3). Meanwhile, no statistical difference ($p = 0.35$) was detected at week 24 of follow-up when the PEG-IFN α + LAM group (3.4% [1/29]) was compared with the PEG-IFN α group (10.5% [2/19]) in a relatively low-quality RCT (without placebo) from Turkey (20).

HBsAg disappearance and seroconversion in the PEG-IFN α + ADV vs. PEG-IFN α groups during 48-52 weeks of treatment (HBeAg-positive patients)

The pooled rates of HBsAg disappearance and seroconversion were 10.5% and 6.4% in the PEG-IFN α + ADV group, and 6.3% and 0% in the PEG-IFN α group, respectively. No evidences of inter-trial heterogeneity were identified ($\chi^2 = 1.11$, d.f. = 4, $p = 0.89$, $I^2 = 0\%$; $\chi^2 = 0.00$, d.f. = 2, $p = 1.0$, $I^2 = 0\%$, respectively); the combined ORs in our meta-analysis using a fixed-effects model demonstrated no significant differences (Peto OR = 1.68, 95% CI 0.75, 3.76; $p = 0.21$) in terms of HBsAg clearance, while a statistically significant difference was seen in HBsAg seroconversion (Peto OR = 7.22, 95% CI 1.23, 42.40; $p = 0.03$) (Fig. 4).

HBsAg disappearance in the PEG-IFN α + ADV vs. PEG-IFN α groups during 24-48 weeks of treatment-free follow-up (HBeAg-negative/positive subjects)

Piccolo et al. (17) reported that the HBsAg clearance rate in HBeAg-negative patients treated with PEG-IFN α + ADV was 3.3% (1/30), whereas with PEG-IFN α it was 0% (0/30) after 24 weeks of treatment-free follow-up; no statistical difference was detected between the two treat-

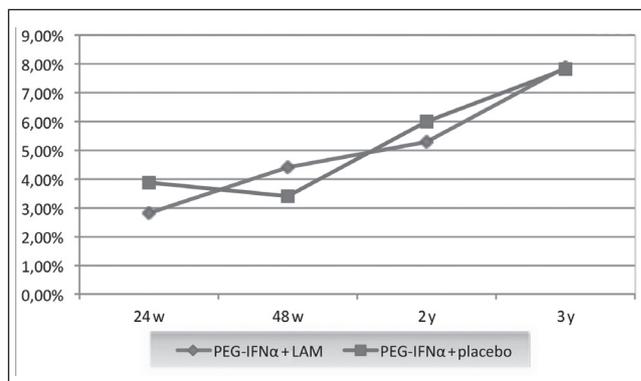


Fig. 3. Line chart of hepatitis B surface antigen (HBsAg) disappearance rates for pegylated interferon alpha (PEG-IFN α) plus lamivudine (LAM) vs. PEG-IFN α plus placebo during 24 w-3 y of treatment-free follow-up in subjects with HBeAg-negative CHB.

ment groups ($p = 0.32$). Ao FJ et al. (7) reported a HBsAg eradication rate of 2.8% (1/35) in the PEG-IFN α and ADV combined treatment group for HBeAg-positive patients, and of 2.6% (1/38) in the PEG-IFN α group after 48 weeks of treatment-free follow-up, and no statistical differences were seen ($p = 0.95$).

Sensitivity analysis and publication bias

A sensitivity analysis was conducted using the M-H OR method instead of the Peto OR when evaluating HBsAg clearance rate in the PEG-IFN α + ADV combination therapy group vs. the PEG-IFN α monotherapy group during 48-52 weeks of treatment. Results showed no statistically significant differences (M-H OR = 1.71, 95% CI 0.74, 3.91; $p = 0.21$); without inter-trial heterogeneity ($\chi^2 = 1.09$, d.f. = 4, $p = 0.90$, $I^2 = 0\%$) stable meta-analysis results were noted. Asymmetric funnel plots were produced based on HBsAg clearance and seroconversion rates between the PEG-IFN α + ADV group and the PEG-IFN α group, which indicated a moderate possibility of publication bias.

DISCUSSION

Currently, two kinds of antiviral drugs (interferon alpha and NAs) have been used in clinical practice to treat HBV infection. NAs have proved to effectively suppress HBV DNA replication, but HBsAg seroclearance or seroconversion, which is considered to be the ideal endpoint of therapy, is rarely obtained and long-term use of NAs frequently results in an increased risk of drug resistance. On the other hand, the advantages of IFN over NAs include absence of resistance, limited duration, and higher HBeAg seroconversion rate; however, a long-lasting response occurs only in a small proportion of patients. Therefore, an identification of novel strategies is needed

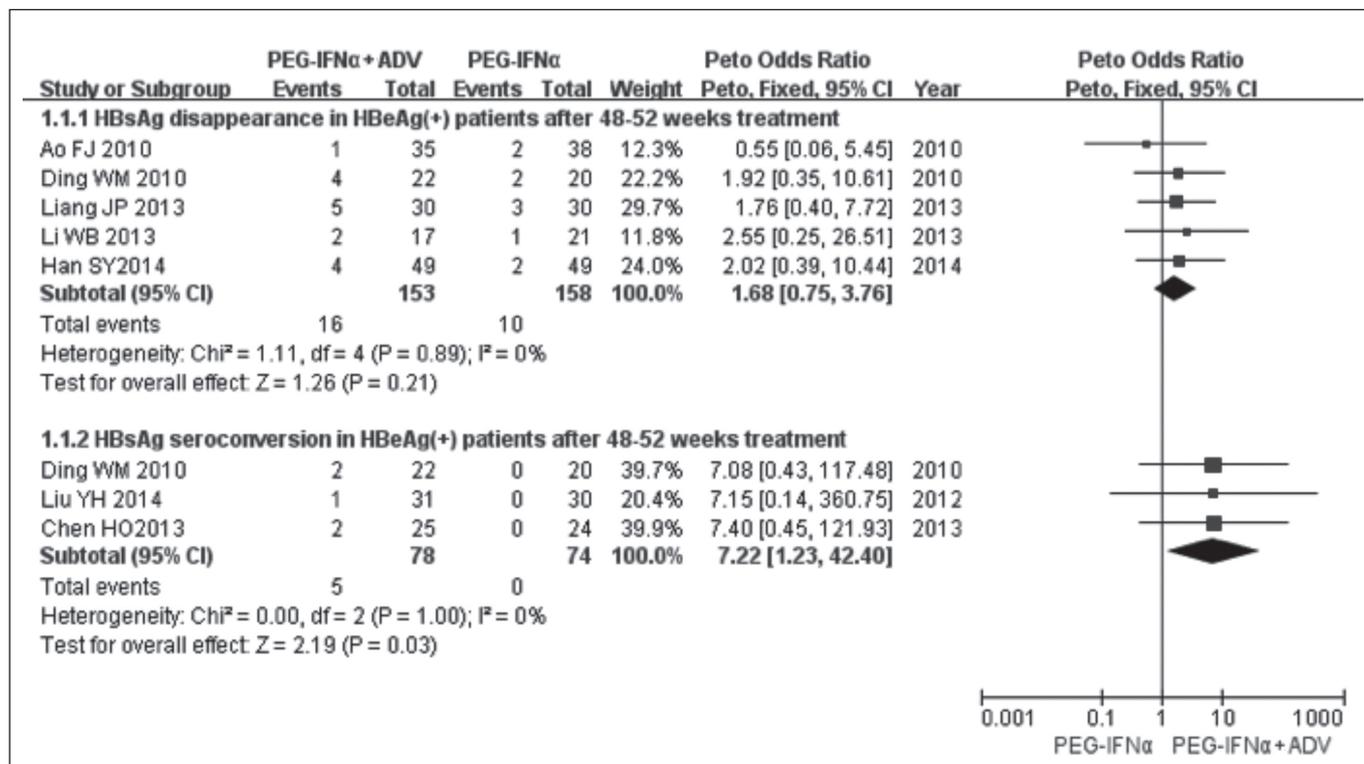


Fig. 4. Analysis of hepatitis B surface antigen (HBsAg) disappearance and seroconversion during treatment with pegylated interferon alpha (PEG-IFN α) plus adefovir (ADV) vs. PEG-IFN α for 48-52 weeks in subjects with HBeAg-positive CHB.

to induce durable responses in a larger subset of patients. Recent studies have suggested that combination therapy with PEG-IFN α and NAs (LAM, ADV) may enhance therapeutic efficacy. However, it remains controversial whether this combination therapy is more efficient than PEG-IFN α monotherapy when focusing on HBsAg seroclearance or seroconversion.

It was reported that HBsAg seroclearance was 7% among HBeAg-positive patients after 52 weeks on pegylated interferon (14); 5% to 8% after 2-5 years of entecavir monotherapy (21,22); and 1.4% to 10% after 3-4 years of tenofovir monotherapy (23,24). LAM or ADV are no longer used as monotherapy regimens since the advent of tenofovir or entecavir; therefore, in this study, therapeutic efficacy was compared between PEG-IFN α combined with LAM or ADV and PEG-IFN α monotherapy using a meta-analysis of clinical data from 14 eligible trials. Since HBeAg positivity or negativity could reflect a cellular immune response against hepatocytes infected with HBV, we set up subgroups according to HBeAg positivity or negativity in our meta-analysis. Pooled rates of HBsAg seroclearance and seroconversion in our meta-analysis were 10.5% and 6.3% in the PEG-IFN α plus ADV combination treatment group, as compared with 6.4% and 0% in the PEG-IFN α monotherapy group (HBeAg-positive subjects) after 48-52 weeks, respectively; and 9.9% and 4.2% in the PEG-IFN α plus LAM group, as compared with 7.1% and 3.7% in the PEG-IFN α combined with placebo treat-

ment group (HBeAg-positive subjects) after 24-26 weeks of treatment-free follow-up, respectively. Consistently, we also noticed the possibility of a publication bias according to the asymmetric funnel plots. Certainly, our analysis showed that in HBeAg-positive and HBeAg-negative CHB patients higher rates of HBsAg clearance and seroconversion were observed in the PEG-IFN α plus NAs (ADV or LAM) combination group as compared with those in the PEG-IFN α (with or without placebo) monotherapy group. No significant difference was detected in HBsAg clearance and seroconversion. And, interestingly, we found that, with increased follow-up time, HBsAg clearance rates in the two groups (PEG-IFN α + LAM group and PEG-IFN α + placebo group) were enhanced for HBeAg-negative patients. However, there was no significant difference at each follow-up time point.

HBV DNA replication cannot be completely eliminated because covalently closed circular DNA (cccDNA) persists in the nucleus of infected hepatocytes. HBsAg production is related to HBV whole virions, and HBsAg seroclearance would reflect a reduction of cccDNA. So, HBsAg seroclearance or seroconversion was developed as an indicator to evaluate the efficiency of antiviral treatment. Since the antiviral mechanisms of PEG-IFN α and NAs are different, higher HBsAg clearance and seroconversion rates are expected in the combination group when compared to the PEG-IFN α monotherapy group. The immune mechanisms of HBsAg clearance and seroconversion remain unclear, and progress

is affected by many factors. Firstly, HBV-specific T-cells are functionally impaired in CHB. It has been reported that NAs may indirectly promote a reduction of PD-1 level and restore HBV-specific T-cell responsiveness by efficiently suppressing HBV DNA replication, even though NA treatment has no remarkable effects on the survival and function of immune cells (25). Secondly, regulatory T-cells (Tregs) and dendritic cells (DCs) play key roles in immune response regulation. The numbers and functions of Tregs and DCs are restored to normal levels after NA treatment (26,27). Thirdly, natural killer cell characteristics in CHB patients are associated with HBsAg clearance (28). Combination treatment with PEG-IFN α and ADV significantly influences NK-cell function (29,30). In addition, B-cells play an important role in the clearance of HBV and in protection against reinfection. IFN α may reverse B-cell hyperactivation and functional impairment, which is related to HBsAg seroconversion in CHB patients (31).

Admittedly, our meta-analysis suggested that the combination of PEG-IFN α and NAs was not more efficient than PEG-IFN α (with or without placebo) monotherapy according to HBsAg clearance and seroconversion, but our study contained several limitations. On the one hand, sample sizes were inadequate and most of the studies included were low-quality studies, which led to difficulties in classifying the risk of bias, which might weaken our meta-analysis conclusions in this regard. On the other hand, we did not take serum HBsAg levels and HBV genotypes into account as we focused on a meta-analysis (32) published in *Hepatology Research*, which concluded that lower serum HBsAg levels (≤ 100 IU/ml) and HBV genotype B or C were associated with a higher rate of spontaneous HBsAg seroclearance (51.4% vs. 11.7%, $p < 0.05$). Therefore, most importantly, the decreases in serum HBsAg levels induced by antiviral drugs in the later meta-analysis are also welcomed if they meet the purpose of directing clinical medication for the better, rather than just focusing on HBsAg seroclearance or seroconversion. In addition, this study included a short follow-up period. As it may take longer for the host to enhance their immune response and induce a decline in HBsAg level, it is necessary that observation occurs during a long follow-up period, such as over 3 years. Finally, our study did not analyze ALT levels, as it is generally believed that serum ALT levels reflect the host's immune response to chronic HBV infection and the intensive immune response is quickly restored after antiviral treatment.

In conclusion, after treating patients with CHB for 48-52 weeks, and after a treatment-free follow-up of 24 weeks to 3 years, the combination of PEG-IFN α and LAM or ADV was not superior to monotherapy with PEG-IFN α in terms of HBsAg seroclearance or seroconversion. More homogeneous original researches are needed to clarify therapeutic efficiency in HBeAg-negative CHB patients, since our results are based only on a clinical report of patients with HBeAg negativity. In future studies, subgroup analyses should mainly be conducted according to

HBsAg levels, ALT levels, follow-up duration, and HBV genotype when more homogeneous reports are published.

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