The Efficacy and Safety of Entecavir and Interferon Combination Therapy for Chronic Hepatitis B Virus Infection: A Meta-Analysis

Qiao-Ling Xie, Ying Zhu *, Ling-Hong Wu, Lin-Lin Fu, Yan Xiang

Department of Infectious Diseases. The First Affiliated Hospital of Dalian Medical University, Dalian, Liaoning, China

* zhuyingsh52@126.com

Abstract

The objective of this study was to evaluate the effectiveness and safety of entecavir (ETV) and interferon (IFN) combination therapy in the treatment of chronic hepatitis B (CHB) mono-infection via a meta-analysis of randomized controlled trials (RCTs). All eligible RCTs evaluating combination therapy for treating CHB were identified from nine electronic databases. A meta-analysis was performed in accordance with the Cochrane Systemic Review handbook. Eleven trials encompassing 1010 participants were included in this meta-analysis. It showed that at 12 and ≥ 96 weeks of therapy, the combination of ETV and IFN was not better than ETV in improving the undetectable HBV DNA (12 weeks: RR=1.12, 95% CI=0.88-1.42; ≥ 96 weeks: RR = 0.64, 95% CI=0.21-1.98, respectively) and HBeAg seroconversion rates (12 weeks: RR=1.35, 95% CI=0.60-3.04; ≥ 96 weeks: RR=1.36, 95% CI=0.75-2.64, respectively). But at 48 weeks of therapy and approximately 2 years of follow up, combination therapy was superior to ETV in improving the undetectable HBV DNA (48 weeks: RR=1.46, 95% CI=1.13-1.90; follow up: RR=2.20, 95% CI=1.26-3.81, respectively) and HBeAg seroconversion rates (48 weeks: RR=1.82, 95% CI=1.44-2.30; follow up: RR=1.92, 95% CI=1.19-3.11, respectively). When compared to IFN group, at 24 and 48 weeks of therapy, combination group showed a greater undetectable HBV DNA (24 weeks: RR=2.14, 95% CI=1.59-2.89; 48 weeks: RR=2.28, 95% CI=1.54-3.37, respectively) and ALT normalization rate (24 weeks: RR=1.56, 95% CI=1.24-1.96; 48 weeks: RR=1.55, 95% CI = 1.16-2.07, respectively). At 48 weeks of therapy, combination group achieved a greater HBeAg seroconversion rate than IFN (48 weeks: RR=1.58, 95% CI=1.24-2.00). No significant differences were observed in the side effects of the three therapies. So we can conclude that ETV and IFN combination therapy is more effective than ETV or IFN monotherapy in CHB treatment. ETV, IFN, and the combination of the two are safe in CHB treatment.
Introduction
Liver disease associated with persistent hepatitis B virus (HBV) infection represents a major health problem with global impact. Approximately 2 billion people have been infected with HBV at one point, and over 350 million people suffer from chronic hepatitis B (CHB) worldwide [1]. The progression of HBV-related liver disease to cirrhosis, liver failure and hepatocellular carcinoma (HCC) is estimated to result in 0.5–1.2 million annual deaths [2]. Antiviral therapy is an effective way of preventing disease progression and even reversing liver fibrosis and cirrhosis [3–6]. The currently available treatments for CHB include two kinds of therapeutic agents: nucleoside and nucleotide analogues (NAs) and interferon (IFN) [7]. The NAs include lamivudine (LAM), telbivudine (LDT), entecavir (ETV), emtricitabine (FTC), adefovir dipivoxil (ADV) and tenofovir (TDF) [8]. IFN is divided into conventional IFN and pegylated IFN. The major advantages of the NAs are their high tolerability, effective suppression of HBV DNA replication and a high rate of on-treatment response. However, the drawbacks of NAs are also noteworthy; patients suffer from an indefinite treatment duration and drug resistance triggered by long-term therapy. In contrast, IFN is an immunomodulatory drug with a low rate of resistance, a finite course of treatment and potential long-term post-treatment responses. However, responses to IFN are only attained in a minority of patients, and severe adverse reactions make IFN poorly tolerated [9–11].

Currently, mono-therapy approaches with NAs or IFN cannot produce satisfactory antiviral effects. One theoretically viable strategy is the combination of NAs and IFN. The Japanese Guidelines for the treatment of HBV recommend that young HBeAg-negative patients with high HBV DNA level be treated sequentially with ETV followed by IFN as a first-line therapy [12]. At present, a combination therapy of NAs and IFN is not recommended in the guidelines proposed by the Asian-Pacific Association (updated in 2012) [13], the American Association (updated in 2009) [4] or the European Association for the Study of the Liver (updated in July 2012) [14]. Until now, the efficacy and safety of combination therapy has not been evaluated [15–16]. Recently, some systematic reviews have examined the combination of IFN and LAM or ADV, but the results are controversial [17–18]. Marcellin and colleagues have investigated LDT in combination with IFN, and concluded that for increased risk of peripheral neuropathy, combination therapy of the two should not be used [19].

ETV and TDF are similarly effective and safe in CHB treatment [20]. Both of them are more potent than LAM and ADV in suppressing HBV DNA and have a lower rate of resistance. However, TDF is more expensive than ETV. The effectiveness and safety of ETV and IFN combination therapy for CHB is uncertain. Several recent RCTs showed that a combination of ETV and IFN was superior to mono-therapy; however, other reports claimed that mono-therapies and combination therapy had similar results [21–31]. Because the sample sizes of the present RCTs are small and the consequences of each are incompatible, a more definitive conclusion is elusive. Since, HBeAg-positive CHB is characterized by high levels of HBV DNA, high risks of complications, high relapse rates, and a more pronounced need for efficacious therapy [13,32–33]. We conducted this meta-analysis to evaluate the efficacy and safety of ETV and IFN combination therapy in HBeAg-positive patients and to ultimately provide evidence for clinical decisions.

Materials and Methods
Literature search strategy
This systematic search was conducted independently by two researchers (Qiao-Ling Xie and Ling-Hong Wu). We searched Pubmed/Medline, the Cochrane Central Register of Controlled
Trials, the Cochrane Database of Systematic Review databases, EMBASE, The Wiley Online library, Web of Science, The Chinese Journal of Science and Technology of VIP, The China National Knowledge Infrastructure (CNKI), and The Wanfang database for relevant literature. The latest article was published in October 2014. The publication language of each RCT was not restricted. The search strategy was based on a combination of the key words "hepatitis B or HBV or CHB," "entecavir or ETV," and "interferon or interferons or IFN". We also searched reference lists and relevant reviews for additional articles.

Inclusion and exclusion criteria
The articles included in this meta-analysis met the following inclusion criteria: 1) they were RCTs; 2) all patients infected with HBV had the following clinical indicators: HBsAg and HBeAg in the serum for more than 6 months, HBV DNA levels \( \geq 10^5 \) copies/ml and ALT levels \( > 2 \) times the upper normal limit; 3) the patients received initial treatment; 4) the studies compared the combination of ETV and IFN to ETV or IFN mono-therapy. Studies meeting the following exclusion criteria were excluded in this study: 1) non-RCTs; 2) co-infection with hepatitis A, C, D, or E, cytomegalovirus, or HIV; 3) anti-viral therapy was not performed initially; 4) patients had liver cirrhosis, liver failure, HCC, or other liver related complications caused by autoimmune diseases, drugs or alcoholism.

Efficacy measures
Efficacy was evaluated based on the following criteria: undetectable HBV DNA: HBV DNA levels \(< 1,000 \) copies/ml; ALT normalization: ALT levels \(< 40 \) IU/ml; HBeAg seroconversion: HBeAg loss and occurrence of HBeAb. Drug safety was evaluated according to side effects, laboratory abnormalities, hepatitis flares, or death.

Data extraction
Data extraction was carried out by two reviewers independently (Qiao-Ling Xie and Lin-Lin Fu). We recorded the following for each study: 1) trial characteristics (the first author’s name, published year, country of study, sum of each group, and quality of RCT); 2) patient characteristics (mean age, ethnicity of patients); 3) the details of each regimen (i.e., the antiviral drug used and treatment duration); and 4) observation time and outcomes. We contacted the authors of the eligible publications that had inadequate information; if effective data were still not obtained, those papers were excluded. All the data were reviewed to eliminate duplicate reports of the same trial.

Assessment for risk of bias in the included studies
Methodological quality was defined as the confidence that the design and the report of the RCT would restrict bias in the comparison of the interventions [34]. According to empirical evidence [35–37], the methodological quality of the trials was assessed based on sequence generation, allocation concealment, blinding (of participants, personnel, and outcome assessors), incomplete outcome data, selective outcome reporting, and other sources of bias. The risk of each bias was defined (Table 1). We also used the Jadad scale to evaluate the quality of the RCTs [38]. Discrepancies were resolved by discussion with a third person (Ying Zhu).

Statistical analysis
This meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement [39]. STATE V 12.0 (Intercooled
state software 12.0) was used for the data analysis. All P values were two-tailed with a significance level of 0.05. For prospective studies and dichotomous data, we used relative risk (RR) as an effect measure and reported its 95% CI. This meta-analysis was performed using a random-effects model or fixed-effects model based on significant heterogeneity. Heterogeneity was evaluated via the I² and P values. The fixed-effects method was used when the value of I² was < 50% or when 50% < the value of I² < 60%, but P > 0.1. The random-effects method was used when the value of I² was > 60% or when 50% < the value of I² < 60%, but P < 0.1 [40–41]. Sensitivity analysis was carried out to estimate the stability of the results. Begg’s rank correlation test and Egger’s linear regression test were used to assess publication biases.

### Results

#### Study selection and baseline characteristics

We initially identified 2918 papers. By evaluating the titles and abstracts, those that were not RCTs, were duplicated, not involved with ETV and IFN, studied other NAs (e.g., LAM, ADV, LDT or TDF), were co-infection with other viruses, and ETV and IFN not used jointly were excluded, leaving 25 studies. By reviewing the full texts of these articles, finally, 11 trials (7 in Chinese and 4 in English) [21–31] were included in this meta-analysis (Fig 1). These studies include a total of 1010 patients: 439 were treated with combination therapy, 300 were treated with ETV mono-therapy, and 271 were treated with IFN mono-therapy. All studies reported the baseline characteristics of the 2 groups in detail. There were no significant differences in gender, age or duration of treatment between the two groups in these papers (Table 2).

#### Risk of bias of studies included

Eleven eligible studies were RCTs [21–31]. Five studies received Jadad scores of 5, and the others received scores of 2 or 3 (Table 2). 7 trials reported the randomization performed in the study, 5 of which described the method of randomization in detail [22,24,27,29,31].

| Components | Sequence generation | Allocation concealment | Blinding | Incomplete outcomes | Selective reporting | Other bias |
|------------|---------------------|------------------------|----------|---------------------|---------------------|-----------|
| Low risk of bias | The method used is right (i.e., computer generated random numbers, table of random numbers). | The method used (i.e., central allocation) is unlikely to induce biases on the final observed effect. | Blinding was performed adequately, or the outcome measurement is not likely to be affected by lack of blinding. | The numbers and reasons for dropouts and withdrawals in all intervention groups were described or if it was specified that there were no dropouts or withdrawals. | Pre-defined, or clinically relevant and reasonably expected outcomes are reported on. | The trial appears to be free of other sources of bias. |
| Unclear risk of bias | The trial is described as randomized, but the method of sequence generation is not specified. | There is inadequate information to assess whether the method used is likely to induce biases. | There is inadequate information to assess whether the method used is likely to induce bias on the estimate of effect. | The report gave the impression that there had been no dropouts or withdrawals, but this was not stated in detail. | It is unclear whether data on these outcomes were recorded or not. | There is inadequate information to assess whether other sources of bias are present. |
| High risk of bias | The method used is improper and likely to introduce confounding. | The method used is likely to induce biases. | There is no blinding or the outcome measurement is likely to be affected by lack of blinding. | The number or reasons for dropouts and withdrawals were not described. | Not all of the trial’s pre-specified primary outcomes have been reported or similar. | There are other factors in the trial that could put it at risk of bias (i.e., lack of sample size or power calculation). |

Table 1. The definition of the risk of each bias.

doi:10.1371/journal.pone.0132219.t001
randomization methods in the other 4 studies were not appropriate [21,25–26,30]. All trials were open-labelled, but we do not expect this to have caused bias. The primary outcomes assessed (i.e., undetectable HBV DNA, ALT normalization, and HBeAg seroconversion) were unlikely to have been affected by knowledge of the intervention. All trials reported sample size calculations and complete data, and none of the patients dropped out of the trial. Four papers reported the results of approximately 2 years of follow up [22,26–27,31]. All trials reported at least one interested outcome parameters. All trials were likely to be free of selective reporting (Fig 2A and 2B).
Combination therapy with ETV and IFN was used in the trial group. ETV or IFN treatment was used in the control group, so we performed this meta-analysis to compare the differences between combination therapy and ETV or IFN mono-therapy.

**Undetectable HBV DNA**

1. **ETV+IFN vs. ETV.** All the trials reported the rate of undetectable HBV DNA [21–24,28–31]. Because no significant heterogeneity was found across the studies at 12 weeks of treatment and approximately 2 years of follow up, we chose a fixed-effects model (12 weeks: $I^2 = 0.0\%$, $P = 0.545$; follow up: $I^2 = 0.0\%$, $P = 0.771$, respectively). Significant heterogeneity existed among studies at 24, 48 and $\geq 96$ weeks of therapy, so we chose a random-effects model (24 weeks: $I^2 = 62.9\%$, $P = 0.068$; 48 weeks: $I^2 = 78.9\%$, $P = 0.000$; $\geq 96$ weeks: $I^2 = 90.5\%$, $P = 0.004$, respectively). At 12, 24 and $\geq 96$ weeks of therapy, the rate of undetectable HBV DNA was similar between the two groups (12 weeks: $RR = 1.12$, 95% CI = 0.88–1.42; 24 week: $RR = 1.17$, 95% CI = 0.93–1.48; $\geq 96$ weeks: $RR = 0.64$, 95% CI = 0.21–1.98,

| Num | Trial | Yr  | Location | Ethnicity | Sample size | Mean age | Regimen | Time(wk) | Observation | Outcomes | Jadad |
|-----|-------|-----|----------|-----------|-------------|----------|---------|----------|-------------|----------|-------|
| 1   | Li [21]| 2012| China    | Asian     | 51/43/44   | 43.9±12.8 | ETV48wk/α-IFN48wk/ (ETV +α-IFN)48wk | 12,24,48 | A,B,C,D     | 2        |
| 2   | Mao[22]| 2012| China    | Asian     | 40/40/40   | 44.8±5.30, 43.2±6.4, 7±5.9 | ETV52wk/IFNα1b52wk/ ETV24wk+(IFNα1b + ETV)4wk+IFNα1b24wk | 52,F      | A,B,C,      | 5        |
| 3   | Zeng [23]| 2013| China    | Asian     | 20/20/20   | 31.0±6.8, 30.9±6.3 | ETV96wk/Peg-IFN48wk/ (ETV + Peg-IFN)12wk +Peg-IFN36wk | 4,12,24   | A,B,C,D     | 3        |
| 4   | Cui[24]| 2013| China    | Asian     | 36/36/36   | 41.7±5.9  | ETV48wk/IFNα2b48wk/ ETV24wk+(ETV + IFNα2b)4wk+IFNα2b24wk | 48        | A,B,C,D     | 5        |
| 5   | Fan[25]| 2012| China    | Asian     | 00/40/40   | 48.9±0.4, 49.7±0.6 | IFNNo-2b48wk/ETV12wk +IFNNo-2b36wk | 12,24,48 | A,B,C       | 2        |
| 6   | L.B[26]| 2013| Italy    | White     | 00/20/20   | 39.0±7.5, 33.5±7.5 | Peg-IFN48wk/ETV12wk+(ETV + Peg-IFN)12wk +Peg-IFN36wk | 48,F      | A,B,C       | 2        |
| 7   | Xie[27]| 2014| China    | Asian     | 00/72/73   | 29.5±8.1, 29.2±6.9 | peg-IFN48wk/peg-IFN13wk+(peg-IFN +ETV)24wk+peg-IFN11wk | 48,F      | A,B,C,D     | 5        |
| 8   | Wei[28]| 2012| China    | Asian     | 12/00/13   | 35.4±11.0 | ETV48wk/ETV24wk+(ETV + Peg-IFN)24wk | 48        | A,B,C,D     | 3        |
| 9   | Chen [29]| 2013| China    | Asian     | 32/00/33   | 36.7±8.1, 38.4±11.6 | ETV48wk/(ETV + IFNα1b)48wk | 12,24,48 | A,B,C,D     | 5        |
| 10  | Chen-C [30]| 2012| Taiwan  | Asian     | 19/00/35   | 25.6±43.5 | ETV(72–96)wk/(ETV + Peg-IFN)24wk+Peg-IFN24wk | 24,96     | A,B,C,D     | 2        |
| 11  | Brouwer [31]| 2014| Global  | Asian/ Other | 90/00/85 | 31±9, 32±10 | ETV96wk/ETV24wk+(ETV + Peg-IFN)24 wk + ETV (24–48)wk | 48,72,96,F | A,B,C,D     | 5        |

Note: ETV, entecavir; IFN, interferon; Peg-IFN, peginterferon α-2a; SD, standard deviation; A, undetectable HBV-DNA; B, ALT normalization; C, HBcAg seroconversion; D, adverse events; F, follow up; wk, weeks;
Fig 2. Risk of Bias in the Studies that were included in this Meta-analysis. (A) Review judgments of authors on each methodological quality item presented as percentages across all studies. (B) Review judgments of authors on each methodological quality item for each included study.

doi:10.1371/journal.pone.0132219.g002
respectively). However, at 48 weeks of therapy and approximately 2 years of follow up, a greater undetectable HBV DNA rate was observed in the combination group compared to the ETV group (48 weeks: RR = 1.46, 95% CI = 1.13–1.90; follow up: RR = 2.20, 95% CI = 1.26–3.81, respectively; Fig 3A and 3B).

2. **ETV+IFN vs. IFN.** All trials reported the rate of undetectable HBV DNA [21–27]. No heterogeneity was observed across the studies at 24 weeks of treatment and approximately 2 years of follow up; therefore, we chose a fixed-effects model (24 weeks: I² = 15.4%, P = 0.307; follow up: I² = 14.3%, P = 0.280, respectively). Heterogeneity was detected across the studies at 12 and 48 weeks of treatment, so we chose a random-effects model (12 weeks: I² = 62.9%, P = 0.068; 48 weeks: I² = 70.2%, P = 0.009, respectively). At 12, 24 and 48 weeks of therapy and approximately 2 years of follow up, a greater rate of undetectable HBV DNA was observed in the combination therapy group compared to the IFN group (12 weeks: RR = 1.98, 95% CI = 1.04–3.77; 24 weeks: RR = 2.14, 95% CI = 1.59–2.89; 48 weeks: RR = 2.28, 95% CI = 1.54–3.37; follow up: RR = 3.30, 95% CI = 1.79–6.09; Fig 4A and 4B).

**ALT normalization**

1. **ETV+IFN vs. ETV.** All trials reported the rate of ALT normalization [21–24,28–31]. Because no significant heterogeneity was detected across the studies at 12 and 24 weeks of treatment, we chose a fixed-effects model (12 weeks: I² = 0.0%, P = 0.869; 24 weeks: I² = 53%, P = 0.119, respectively). Significant heterogeneity existed among studies at 48 and ≥ 96 weeks of treatment and approximately 2 years of follow up, so we chose a random-effects model (48weeks: I² = 90.4%, P = 0.000; ≥96 weeks: I² = 80.7%, P = 0.023; follow up: I² = 93.1%, P = 0.000, respectively). At 12, 24, 48 and ≥ 96 weeks of therapy and approximately 2 years of follow up, the rates of ALT normalization were similar between the two groups (12 weeks: RR = 0.95, 95% CI = 0.73–1.25; 24 weeks: RR = 1.19, 95% CI = 0.99–1.43; 48 weeks: RR = 1.33, 95% CI = 0.91–1.94; ≥ 96 weeks: RR = 0.76, 95% CI = 0.46–1.27; follow up: RR = 1.57, 95% CI = 0.56–4.34, respectively; Fig 5A and 5B).

2. **ETV+IFN vs. IFN.** All trials reported the rate of ALT normalization [21–27]. Because no significant heterogeneity was found across the studies at 12 and 24 weeks of treatment, we chose a fixed-effects model (12 weeks: I² = 7.4%, P = 0.339; 24 weeks: I² = 0.0%, P = 0.784, respectively). Heterogeneity was observed among studies at 48 weeks of treatment and approximately 2 years of follow up; we therefore chose a random-effects model (48 weeks: I² = 70.5%, P = 0.017; follow up: I² = 77.0%, P = 0.013, respectively). The results showed that at 12 weeks and approximately 2 years of follow up, the rate of ALT normalization was similar between the two groups (12 weeks: RR = 1.34, 95% CI = 0.97–1.86; follow up: RR = 1.57, 95% CI = 0.91–2.70, respectively). However, at 24 and 48 weeks of therapy, combination therapy achieved higher ALT normalization rates than the IFN group (24 weeks: RR = 1.56, 95% CI = 1.24–1.96; 48 weeks: RR = 1.55, 95% CI = 1.16–2.07, respectively; Fig 6A and 6B).

**HBeAg seroconversion**

1. **ETV+IFN vs. ETV.** All trials reported the rate of HBeAg seroconversion [21–24,28–31]. No significant heterogeneity was observed; we therefore chose a fixed-effects model (12 weeks: I² = 58.1%, P = 0.123; 24 weeks: I² = 16.6%, P = 0.309; 48 weeks: I² = 0.0%, P = 0.453; ≥ 96
Fig 3. Forest Plot for Undetectable HBV DNA when ETV was used as the Control Group. (A) Forest plot for undetectable HBV DNA based on a fixed-effects model. (B) Forest plot for undetectable HBV DNA based on a random-effects model.

doi:10.1371/journal.pone.0132219.g003
Fig 4. Forest Plot for Undetectable HBV DNA when IFN was used as the Control Group. (A) Forest plot for undetectable HBV DNA based on a random-effects model. (B) Forest plot for undetectable HBV DNA based on a fixed-effects model.
Fig 5. Forest Plot for ALT Normalization when ETV was used as the Control Group. (A) Forest plot for ALT normalization based on a random-effects model. (B) Forest plot for ALT normalization based on a fixed-effects model.

doi:10.1371/journal.pone.0132219.g005
Fig 6. Forest Plot for ALT Normalization when IFN was used as the Control Group. (A) Forest plot for ALT normalization based on a random-effects model. (B) Forest plot for ALT normalization based on a fixed-effects model.

doi:10.1371/journal.pone.0132219.g006
The results showed that at 12 and ≥ 96 weeks of therapy, the rate of HBeAg seroconversion was similar in the two groups (12 weeks: RR = 1.35, 95% CI = 0.60–3.04; ≥ 96 weeks: RR = 1.36, 95% CI = 0.75–2.46, respectively). However, at 24 and 48 weeks of therapy and approximately 2 years of follow up, the combination therapy group achieved greater HBeAg seroconversion rates than the ETV group (24 weeks: RR = 2.23, 95% CI = 1.42–3.49; 48 weeks: RR = 1.82, 95% CI = 1.44–2.30; follow up: RR = 1.92, 95% CI = 1.19–3.11, respectively; Fig 7).

2. ETV+IFN vs. IFN. Six trials reported the rates of HBeAg seroconversion [21–24,26–27]. Because no significant heterogeneity was found, we chose a fixed-effects model (12 weeks: I² = .%, P = .; 24 weeks: I² = 0.0%, P = 0.387; 48 weeks: I² = 48.5%, P = 0.101, respectively) The results showed that at 12 and 24 weeks of therapy, the rate of HBeAg seroconversion was similar in the two groups (12 weeks: RR = 1.86, 95% CI = 0.34–2.15; 24 weeks: RR = 1.49, 95% CI = 0.79–2.82, respectively). However, at 48 weeks of therapy combination therapy achieved greater HBeAg seroconversion rates than the IFN group (48 weeks: RR = 1.58, 95% CI: 1.24–2.00; Fig 8).

Adverse reactions
Eight trials reported adverse reactions [21,23–24,27–31]. Cui reported no significant differences in adverse events between the combination group and the IFN group (p > 0.05, 69.44%, 77.78%) [24]. Li Jin and Xie et al. reported that there were some common side effects including influenza-like illness, fatigue, gastrointestinal symptoms, weight loss, hair loss, myelosuppression and neuropsychiatric problems in both the combination group and IFN group [21,27]. Xie et al., Wei et al. and Chen C-C et al. observed hyperthyroidism in the combination group [27–28,30]. Wei et al. estimated that there were no differences in the rate of glomerular filtration decrease between the combination group and ETV group. Additionally, there were no severe hepatitis flares or decompensation reported. The mild side effects of combination therapy were tolerable and did not necessitate early termination or dose reduction.

Sensitivity analysis
Some of the I² heterogeneity values were large, so we performed sensitivity analysis with a random-effects model. Sensitivity analysis was performed for all models with high heterogeneity. This analysis showed that the pooled RRs were similar before and after removal of each trial, and no single trial significantly altered the pooled RRs. This suggests that these results are stable (Table 3).

Publication bias
We performed Begg’s test and Egger’s test to evaluate the occurrence of publication bias. The results were listed in Table 4. There was no evidence of publication biases except for the outcome parameter of undetectable HBV-DNA (ETV+IFN vs. IFN). Thus, we cautiously concluded that the biases in this meta-analysis were not obvious.

Discussion
HBV is a small, partially double-stranded DNA virus that belongs to the hepadnaviridae family [42]. The natural course of chronic HBV infection consists of four phases: immune tolerance, immune reactive, inactive carrier and reactivation phase [7, 14]. To date, the efficacy and safety
of mono-therapy with IFN or NAs has been unsatisfactory [43]. The suboptimal outcomes of the current treatment options for CHB prompt the exploration of their use in combination to achieve synergistic efficacy and decreased mutagenicity [44]. In our study, we focused on the effectiveness and safety of ETV and IFN combination therapy.

This meta-analysis analyzed four outcome parameters: the undetectable HBV DNA rate, ALT normalization rate, HBeAg seroconversion rate and adverse reactions. The HBV DNA level is a primary marker for appraising the treatment responses of patients with CHB [45–46]. The early and sustained suppression of HBV DNA replication produces long-term virological, biochemical and serological responses [47]. HBeAg is a protein expressed by the pre-C gene. HBeAg seroconversion is a key point of treatment responses and is a necessary condition for halting drug therapy for HBeAg-positive patients [48].
Table 3. Sensitivity analysis for all outcomes with high heterogeneity.

| Outcomes          | Times   | Study omitted      | RR      | 95% CI      |
|-------------------|---------|--------------------|---------|-------------|
| HBV DNA (A)       | 24 weeks| Li (2012)          | 1.08    | 0.95–1.24   |
|                   |         | Chen (2013)        | 1.31    | 0.75–2.29   |
|                   |         | Zeng (2013)        | 1.27    | 0.77–2.08   |
|                   |         | Combined           | 1.17    | 0.93–1.48   |
|                   | 48 weeks| Cui (2013)         | 1.35    | 1.06–1.71   |
|                   |         | Li (2012)          | 1.48    | 1.06–2.05   |
|                   |         | Wei (2013)         | 1.50    | 1.13–2.00   |
|                   |         | Chen (2013)        | 1.60    | 1.17–2.18   |
|                   |         | Mao (2012)         | 1.33    | 1.06–1.65   |
|                   |         | Brouwer (2014)     | 1.59    | 1.08–2.32   |
|                   |         | Combined           | 1.46    | 1.13–1.90   |
|                   | 96 weeks| Brouwer (2014)     | 0.37    | 0.19–0.72   |
|                   |         | Chen-c (2012)      | 1.03    | 0.87–1.22   |
|                   |         | Combined           | 0.64    | 0.21–1.98   |
| HBV DNA (B)       | 12 weeks| Li (2012)          | 2.62    | 1.32–5.18   |
|                   |         | Zeng (2013)        | 1.47    | 0.94–2.30   |
|                   |         | Fan (2012)         | 2.08    | 0.67–6.44   |
|                   |         | Combined           | 1.98    | 1.04–3.77   |
|                   | 48 weeks| Cui (2013)         | 2.10    | 1.39–3.18   |
|                   |         | L. Boglione (2013) | 2.10    | 1.46–3.01   |
|                   |         | Li (2012)          | 2.62    | 1.75–3.92   |
|                   |         | Fan (2012)         | 2.61    | 1.42–4.77   |
|                   |         | Mao (2012)         | 2.13    | 1.38–3.28   |
|                   |         | Combined           | 2.28    | 1.54–3.37   |
| ALT normalization (A) | 48 weeks| Cui (2013)        | 1.19    | 0.81–1.75   |
|                   |         | Li (2012)          | 1.31    | 0.81–2.11   |
|                   |         | Wei (2013)         | 1.36    | 0.88–2.09   |
|                   |         | Brouwer (2014)     | 1.55    | 1.09–2.20   |
|                   |         | Mao (2012)         | 1.19    | 0.81–1.75   |
|                   |         | Combined           | 1.33    | 0.91–1.94   |
|                   | 96 weeks| Brouwer (2014)     | 0.60    | 0.38–0.93   |
|                   |         | Chen-C (2012)      | 0.91    | 0.82–1.00   |
|                   |         | Combined           | 0.76    | 0.46–1.27   |
|                   |         | follow up          | 1.06    | 0.79–1.43   |
|                   |         | Brouwer (2014)     | 2.36    | 1.51–3.68   |
|                   |         | Combined           | 1.57    | 0.56–4.34   |
| ALT normalization (ALT) | 48 weeks| Cui (2013)      | 1.43    | 1.05–1.96   |
|                   |         | Li (2012)          | 1.66    | 1.09–2.53   |
|                   |         | Xie (2014)         | 1.74    | 1.23–2.45   |
|                   |         | Mac (2012)         | 1.42    | 1.05–1.94   |
|                   |         | Combined           | 1.55    | 1.16–2.07   |
|                   |         | follow up          | 1.30    | 0.70–2.43   |
|                   |         | Mac (2012)         | 1.46    | 0.67–3.21   |
|                   |         | L. Boglione (2013) | 2.08    | 1.48–2.94   |
|                   |         | Combined           | 1.57    | 0.91–2.70   |

Note: A, using ETV as a control group; B, using IFN as a control group; RR, Relative Risk; CI, Confidence interval.

doi:10.1371/journal.pone.0132219.t003
When ETV mono-therapy was used as the control group, this meta-analysis estimated that combination therapy achieved greater HBV DNA undetectable rates (at 48 weeks of therapy) and HBeAg seroconversion rates (at 24 and 48 weeks of therapy) than the control group. However, at ≥ 96 weeks of therapy, we found that the rate of HBV DNA undetectable and HBeAg seroconversion was similar between the two groups. This suggested that, when compared with ETV, combination therapy achieved transient superiority; however, at the late stage of treatment, the efficacy of the two groups was similar. Maybe, IFN and ETV were combined transiently at an early stage of treatment in some trials. However, it seemed contradictory that significant differences were observed once again at 2 years of follow up. It is possible that only few outcomes were investigated at 2 years of follow up [22,31]; therefore, this result may not be reliable, and a much bigger sample size RCT is needed. We can only conclude that combination therapy can achieve a superior response than ETV mono-therapy at an early stage of treatment.

When IFN was used as the control group, this meta-analysis showed that at 48 weeks of therapy, combination therapy achieved greater virological, biochemical and serological response rates than IFN mono-therapy. There are several potential reasons for this result: 1) ETV may reinforce the antiviral effect in the combination group by suppressing HBV DNA replication; 2) A high HBV DNA load is related to an inefficient T cell response to HBV-related antigens [49]; and 3) It has been hypothesized that the inhibition of viral replication by ETV can decrease the HBV-related protein synthesis on the surface of hepatocytes, which may restore the immune response and contribute to the immunomodulatory activity of IFN for infected cells clearance. Boni and his colleagues have also reported that a decreased viral load induced by NAs therapy can lead to the subsequent restoration of CD8 and CD4 cellular immune responses against HBV [50–51]. We can therefore conclude that combination therapy consisting of ETV and IFN is more rapid and effective than IFN mono-therapy in HBeAg-positive CHB.

There were eight articles reported adverse effects. Side effects with ETV therapy have rarely been reported. When compared to IFN, the adverse reactions of the two groups were similar. The emergence of new and different adverse events was not observed in the combination group. Therefore, we can cautiously suggest that combination therapy is safe and tolerable, but long-term observation is needed.

Some limits merit consideration. First, the differences between conventional IFN and pegylated IFN were not further evaluated in subgroup analysis. Second, the differences between the initial combination therapy and sequential combination therapy were not further discussed in subgroup analysis because of the small number of relevant articles. Third, the quality of some

| Model | Outcomes | Begg's Test | Egger's test |
|-------|----------|-------------|--------------|
|       |          | Z | P<z | T | Pt | 95% CI |
| A     | HBV DNA  | 1.04 | 0.30 | 1.40 | 0.18 | -0.66, 3.11 |
| B     | HBV DNA  | 2.56 | 0.01 | 3.93 | 0.00 | 1.08, 3.87 |
| A     | ALT      | 0.77 | 0.44 | 1.72 | 0.11 | -0.50, 4.50 |
| B     | ALT      | 0.43 | 0.67 | 0.92 | 0.38 | -1.61, 3.91 |
| A     | HBeAg    | 1.52 | 0.13 | 1.74 | 0.10 | -0.19, 1.84 |
| B     | HBeAg    | -0.12 | 1.00 | 0.34 | 0.74 | -2.26, 3.01 |

Note: A, ETV+IFN vs. ETV; B, ETV+IFN vs. IFN

doi:10.1371/journal.pone.0132219.t004
of the included trials was not high because details about the methods of randomization, allocation, concealment, and blinding were unclear.

Our meta-analysis indicated that ETV and IFN combination therapy is more effective than ETV or IFN mono-therapy in HBeAg-positive CHB treatment. The combination of the two is also safe in the treatment of CHB. However, there are still some limits to combination therapy: first, combination therapy is very expensive; second, a definite duration for combination therapy is unclear; and third, it is uncertain that whether an initial combination therapy approach or a sequential therapy approach is more suitable. Therefore, studies with much larger sample sizes are needed to explore the advantages of combination therapy.

Supporting Information

S1 Checklist. PRISMA checklist. (DOC)

S1 Table. Characteristics of studies excluded. (DOC)

Acknowledgments

We are grateful to Z.F. Zhang for specific advice and C. Wang for partial data extraction for the meta-analysis.

Author Contributions

Conceived and designed the experiments: QLX YZ. Performed the experiments: QLX LHW LLF YX. Analyzed the data: QLX YZ. Contributed reagents/materials/analysis tools: QLX LHW LLF YX. Wrote the paper: QLX.

References

1. Safioleas M, Lygidakis NJ, Manti C. Hepatitis B today. Hepatogastroenterology. 2007; 54: 545–548. PMID: 17523319
2. Liaw YF, Chu CM. Hepatitis B virus infection. Lancet. 2009; 373: 582–592. doi: 10.1016/S0140-6736(09)60207-5 PMID: 19217993
3. Liaw YF, Leung N, Guan R, Lau GK, Merican I. Asian Pacific consensus statement on the management of chronic hepatitis B: an update. J Gastroenterol Hepatol. 2003; 18: 239–245. PMID: 12603522
4. Lok ASF, McMahon BJ. Chronic hepatitis B: update 2009. Hepatology. 2009; 50: 661–662. doi: 10.1002/hep.23190 PMID: 19714720
5. European Association For The Study Of The Liver. Clinical practice guidelines: management of chronic hepatitis B. J Hepatol. 2009; 50: 227–242. doi: 10.1016/j.jhep.2008.10.001 PMID: 19054588
6. Marcellin P, Gane E, Buti M, Afdhal N, Sievert W, Jacobson IM, et al. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. Lancet. 2013; 381: 468–475. doi: 10.1016/S0140-6736(12)61425-1 PMID: 23234725
7. Hynicka LM, Yunker N, Patel PH. A review of oral antiretroviral therapy for the treatment of chronic hepatitis B. Ann Pharmacother. 2010; 44: 1271–1286. doi: 10.1345/aph.1M590 PMID: 20587747
8. Lam YF, Yuen MF, Seto WK, and Lai CL. Current antiviral therapy of chronic hepatitis B: efficacy and safety. Curr Hepat Rep. 2011; 10: 235–243. PMID: 22131901
9. Buti M. HBeAg-positive chronic hepatitis B: Why do I treat my patients with Nucleos(t)ide analogs? Liver Int. 34(Suppl 1) 2014; 108–111. doi: 10.1111/liv.12392 PMID: 24373086
10. Brunetto MR, Bonino F. Interferon therapy of chronic hepatitis B. Intervirology. 2014; 57: 163–170. doi: 10.1159/000360941 PMID: 25034484
11. Kim SR, Yang J, Kudo M, Hino O. Recent advances in the management of chronic hepatitis B. Hepat Mon. 2011; 11: 601–611. PMID: 22140383
Entecavir and Interferon Combination Therapy for CHB

12. Kumada H, Okanoue T, Onji M, Moriwaki H, Izumi N, Tanaka E, et al. Guidelines for the treatment of chronic hepatitis and cirrhosis due to hepatitis B virus infection for the fiscal year 2008 in Japan. Hepatol Res. 2010; 40: 1–7. doi: 10.1111/j.1872-034X.2009.00633.x PMID: 20156295

13. Yun-Fan Liaw, Jia-Horng Kao, Teerha Piratvisuth, Henry Lik Yuen Chan, Rong-Nan Chie, Chun-Jen Liu, et al. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2012 update. Hepatol Int. 2012; 6:531–561.

14. European Association for the Study of the Liver. EASL clinical practice guidelines: management of chronic hepatitis B virus infection. J Hepatol. 2012; 57: 167–185. doi: 10.1016/j.jhep.2012.02.010 PMID: 22436845

15. Enomoto M, Tamori A, Nishiguchi S, Kawada N. Combination therapy with a nucleos(t)ide analogue and interferon for chronic hepatitis B: simultaneous or sequential. J Gastroenterol. 2013; 48: 999–1005. doi: 10.1007/s00535-012-0742-5 PMID: 23338486

16. Tang C-M, Yau TO, Yu J. Management of chronic hepatitis B infection: current treatment guidelines, challenges, and new developments. World J Gastroenterol. 2014; 20: 6262–6278. doi: 10.3748/wjg.v20.i20.6262 PMID: 24876747

17. Shi Y, Wu YH, Shu ZY, Zhang WJ, Yang J, Chen Z. Interferon and lamivudine combination therapy versus lamivudine mono-therapy for hepatitis B and antigen-negative hepatitis B treatment: a meta-analysis of randomized controlled trials. Hepatobiliary Pancreat Dis Int. 2010; 5: 462–472.

18. Huang R, Yao Y, Zhang J, Wu C. Interferon-alpha plus adefovir combination therapy versus interferon-alpha mono-therapy for chronic hepatitis B treatment: a meta-analysis. Hepatol Res. 2013; 43: 1040–1051. doi: 10.1111/hepr.12058 PMID: 23356962

19. Marcellin P, Wursthorn K, Wedemeyer H, Chuang W, Lau G, Avila C, et al. Telbivudine plus pegylated interferon alfa-2a in a randomized study in chronic hepatitis B is associated with an unexpected high rate of peripheral neuropathy. J Hepatol. 2015; 62: 41–47. doi: 10.1016/j.jhep.2014.08.021 PMID: 25152207

20. Weixia Ke, Liu Li, Zhang Chi, Xiaohua Ye, Yanhui Gao, Shudong Zhou, et al. Comparison of efficacy and safety of tenofovir and entecavir in chronic hepatitis B virus infection: a systematic review and meta-analysis. PLoS One. 2014; 9(6): e98865. doi: 10.1371/journal.pone.0098865 PMID: 24905092

21. Jin L. Early curative effect of alpha interferon combination entecavir treatment for chronic hepatitis B. Chin Hepatol. 2012; 1(10):714–717.

22. Huiqou M. Efficacy analysis of sequential entecavir and interferon combination therapy for HBeAg positive chronic hepatitis B. Morden Practice Medicine. 2012; 24(5): 550–551.

23. Dong Zeng, Jing Yuan, Ying Xia Liu, Ying Zhang, Sha Xi Li, Si Min Yao, et al. Peg-interferon α-2a joint entecavir treatment high viral load with HBeAg positive: a clinical study of chronic hepatitis B.Chinese J Exp Clin Virol. 2013; 27(2):115–118.

24. Cui J, Zheng CL, Liu AQ. Effect and safety analysis of entecavir combination interferon sequential therapy for hepatitis B. Chinese J Prim Med Pharm. 2013; 20(17): 2616–2618.

25. Fan H, He P. Clinical effection of entecavir sequential treatment with interferon for high viral load of chronic hepatitis B. Chin Med Sci. 2012; 2(10): 75–76.

26. Boglione L, D’Avolio, Cariti G, Milia MG, Simiele M, De Nicolo A, et al. Sequential therapy with entecavir and PEG-INF in patients affected by chronic hepatitis B and high levels of HBV-DNA with non-D genotypes. J Viral Hepat. 2012; 20: e11–e19

27. Xun W. Efficacy and safety of entecavir superposition of peglated interferon for the treatment of chronic hepatitis b patients with 48 weeks. Chin J Infect Dis. 2013; 31(8):181–182.

28. Chen Y. Interferon α—β joint entecavir in the treatment of HBeAg positive chronic hepatitis b clinical observation on 33 cases of 48 weeks. Journal of Clinical Medical. 2013; 30: 42–43.

29. Qiong Xie, Huijuan Zhou, Xuefan Bai, Shuhuan Wu, Jian-Jie Chen, Jifang Sheng, et al. A randomized, open-label clinical study of combined pegylated Interferon Alfa-2a (40KD) and Entecavir treatment for Hepatitis B’e– Antigen–Positive Chronic Hepatitis B. Clinical Infectious Diseases. 2014; 59(12):1714–23. doi: 10.1093/cid/ciu702 PMID: 25190434

30. Chen CC, Wang PC, Chang HW, Chen CF. Safety and efficacy of two-step peginterferon α-2a treatment in patients of chronic hepatitis B with acute exacerbation. J Viral Hepat. 2012; 19: 161–172. doi: 10.1111/j.1365-2893.2011.01469.x PMID: 22329370

31. Brouwer WP, Xie Q, Sonneveld MJ, Zhang NP, Zhang Q, Fehmi Tabak, et al. Adding peginterferon to entecavir for hepatitis B e antigen positive chronic hepatitis B: a multicenter randomised trial (ARES Study). J Hepatol. 2014; 00(00):1–11.

32. Ridruejo E, Marciano S, Galdame O, Reggiardo MV, Muñoz AE, Adrovor R, et al. Relapse rates in chronic hepatitis B naïve patients after discontinuation of antiviral therapy with entecavir. J Viral Hepat. 2014; 21: 590–596. doi: 10.1111/jvh.12200 PMID: 24188363
33. Sohn HR, Min BY, Song JC, Seong MH, Lee SS, Jang ES, et al. Off-treatment virologic relapse and outcomes of re-treatment in chronic hepatitis B patients who achieved complete viral suppression with oral nucleos(t)ide analogs. BMC Infect Dis. 2014; 14: 439. doi:10.1186/1471-2334-14-439 PMID: 25125320

34. Moher D, Pham B, Jones A, Cook DJ, Jadad AR, Moher M, et al. Does quality of reports of randomised trials affect estimates of intervention efficacy reported in meta-analyses. Lancet. 1998; 352: 609–613. PMID: 9746022

35. Schulz KF, Chalmers I, Hayes RJ, Altman DG. Empirical evidence of bias. Dimensions of methodological quality associated with estimates of treatment effects in controlled trials. JAMA. 1995; 273: 408–412. PMID: 7823387

36. Kjaergard LL, Villumsen J, Gluud C. Reported methodologic quality and discrepancies between large and small randomized trials in meta-analyses. Ann Intern Med. 2011; 135: 982–989.

37. Wood L, Egger M, Gluud LL, Schulz KF, Jüni P, Altman DG, et al. Empirical evidence of bias in treatment effect estimates in controlled trials with different interventions and outcomes: meta-epidemiological study. BMJ Clin Res Ed. 2008; 336: 601–605.

38. Jadad AR, Moore RA, Carroll D, Jenkinson C, Reynolds DJ, Gavaghan DJ, et al. Assessing the quality of reports of randomized clinical trials: is blinding necessary? Control Clin Trials. 1996; 17: 1–12. PMID: 8721797

39. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and Meta-analyses: the PRISMA statement. PLOS Med. 2009; 6(6): e1000097.

40. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst. 1959; 22: 719–748. PMID: 13655060

41. Higgins JPT, Green S, eds. Cochrane Handbook for Systematic Reviews of Interventions, Version 5.1.0 [updated March 2011]. The Cochrane Collaboration. Available: http://www.cochrane-handbook.org.

42. Robinson WS, Lutwick LI. The virus of hepatitis, type B (first of two parts). N Engl J Med. 1976; 295: 1168–1175. PMID: 62280

43. Yu DX. Optimized antiviral therapy strategy of chronic hepatitis B: combination therapy. Hepatology. 2010; 13: 377–379.

44. Lai CL, Ratziu V, Yuen MF, Poynard T. Viral hepatitis B. Lancet. 2003; 362: 2089–2094. PMID: 14697813

45. Keeffe EB, Dieterich DT, Han SH, Jacobson IM, Martin P, et al. A treatment algorithm for the management of chronic hepatitis B virus infection in the United States: an update. Clin Gastroenterol Hepatol. 2006; 4: 936–962. PMID: 16844425

46. Sherman M. Predicting survival in hepatitis B. Gut. 2005; 54: 1521–1523. PMID: 16227355

47. Chen CJ, Yang Hl, Su J, Jen CL, You SL, Lu SN, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA. 2006; 295: 65–73. PMID: 16391218

48. Keeffe EB, Zeuzem S, Koff RS, Dieterich DT, Esteban-Mur R, Gane EJ, et al. Report of an international workshop: Roadmap for management of patients receiving oral therapy for chronic hepatitis B. Clin Gastroenterol Hepatol. 2007; 5: 890–897. PMID: 17632041

49. Chisari FV, Ferrari C. Hepatitis B virus immunopathogenesis. Annu Rev Immunol. 1995; 13: 29–60. PMID: 7612225

50. Boni C, Bertoletti A, Penna A, Cavalli A, Pili M, Urbani S, et al. Lamivudine treatment can restore T cell responsiveness in chronic hepatitis B. J Clin Invest. 1998; 102: 968–975. PMID: 9727065

51. Boni C, Penna A, Oggi GS, Bertoletti A, Pili M, Cavalli A, et al. Lamivudine treatment can overcome cytotoxic T-cell hyporesponsiveness in chronic hepatitis B: new perspectives for immune therapy. Hepatology. 2001; 33: 963–971. PMID: 11283861