Dear editors and reviewers,

On behalf of my co-authors, we thank you very much for giving us an opportunity to revise our manuscript. We appreciated editor and reviewers very much for their positive and constructive comments and suggestions on our manuscript. These comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our study. We have studied comments carefully and have made corrections ***in red*** in the manuscript which we hope meet with approval.

The responds to the reviewer's comments are as follows:

Reviewer(s)' Comments to Author:

Reviewer: 1

Comments to the Author

1) The phrasing of the results in the abstract, in the second sentence onward, is very long and difficult to understand. Two variables (HBsAg clearance rate and seroconversion rate) are presented simultaneously in the add-on group at two separate different time points, and then this is repeated for the monotherapy group, then all of this is repeated in an intention-to-treat analysis. I propose that the abstract only present the figures for intention-to-treat analysis, omit the time point of week 48 (which was never a primary endpoint); and present the data for HbsAg clearance rate first between both groups, then the data for seroconversion rate between both groups. This maintains the overall message of the study without extraneous information that overfills the abstract.

Response:

Special thanks to you for your good comment. We have re-written this part according to your suggestion.

RESULTS

Demographic and baseline characteristics were comparable between the two groups. Intention-to-treatment analysis (ITT analysis) showed that the HBsAg clearance rate in the add-on group and monotherapy group was 37.4% (34/91) and 1.9% (2/104) at week 72, respectively. The HBsAg seroconversion rate in the add-on group was 29.7% (27/91) at week 72, and no patient in the monotherapy group achieved HBsAg seroconversion at week 72. The HBsAg clearance and seroconversion rates in the add-on group were significantly higher than in the monotherapy group at week 72 ($P < 0.001$, respectively). Younger patients, lower baseline HBsAg concentration, lower HBsAg concentrations at weeks 12 and 24, greater HBsAg decline from baseline to weeks 12 and 24, and the alanine aminotransferase (ALT) ≥ 2×ULN (upper limit of normal) during the first 12 weeks of therapy were strong predictors of HBsAg clearance in patients with peg-IFN α-2a add-on treatment.

2) In the abstract and throughout the manuscript (e.g. in the core tip, in the results), predictors of HbsAg clearance in the add-on group are mentioned such as age, baseline HBsAg concentration, HBsAg concentrations at weeks 12 and 24, and HBsAg changes from baseline to weeks 12 and 24. Frustratingly, however, it is not mentioned which direction of magnitude is predictive of HbsAg clearance for each of these predictors. Is it younger age? Or older age? Is it lower baseline HBsAg concentration or higher baseline HBsAg concentration? Is it HBsAg decline or elevation from baseline? This should be made explicit to the casual reader.

Response:

Special thanks to you for your good comment. We have re-written this part according to your suggestion.

Core tip: Despite promising results with the combination therapy of Peg-IFN

and NA, the best combination therapeutic strategy of Peg-IFN and NA to the treatment of CHB remains unclear. This prospective study was to evaluate the efficacy and safety of add-on 48-week of peg-IFN α-2a to an ongoing NA regime in CHB patients with HBsAg levels ≤ 1500 IU/mL, HBeAg-negative and HBV DNA < 1.0×10^2 IU/mL after over one year of NA therapy. The results suggest that high rates of HBsAg clearance and seroconversion could be achieved by add-on peg-IFNα-2a to an ongoing NA regime for HBeAg-negative CHB patients with HBsAg levels ≤ 1500 IU/mL and HBV DNA <1.0×10^2 IU/mL after long-term NA treatment, and the treatment were relatively safe. Besides, Younger patients, lower HBsAg concentrations at baseline, weeks 12 and 24, greater HBsAg decline from baseline to weeks 12 and 24, and ALT ≥ 2×ULN during the first 12 weeks of therapy were strong predictors of HBsAg clearance for patients with peg-IFN α-2a add-on treatment.

3) In the abstract, the percentage of patients in the add-on group with adverse events should be mentioned.

Response:

Regarding the safety of the treatment, 4.4% (4/91) of patients in the add-on group discontinued peg-IFN α-2a due to adverse events. No severe adverse events were noted.

4) In the abstract conclusion, it should be mentioned that this study was only in HBeAg- patients.

Response:

CONCLUSION

Peg-IFN-α as an add-on therapy augments HBsAg clearance in HBeAg-negative CHB patients with HBsAg ≤ 1500 IU/mL after over one year of NA therapy.

5) In the section entitled "Study Design", it should be mentioned that HBsAg

clearance at week 48 was also measured- I assume it was a secondary endpoint.

Response:

The primary endpoint was HBsAg clearance at week 72 and the secondary endpoints included the rate of HBsAg clearance at week 48, the rates of HBsAg seroconversion at weeks 48 and 72, HBsAg, ALT and AST dynamics over time, and the safety during treatment.

6) The statement "A patient was considered as a responder if HBsAg was cleared in 72 weeks" is redundant and should be deleted. The terminology "responder" is never again used by the researchers in the manuscript.

Response:

Special thanks to you for your good comment. The statement "A patient was considered as a responder if HBsAg was cleared in 72 weeks" has been deleted.

7) The statement "The statistical methods of this study were reviewed by Lei-Lei Pei from Institute of Public Health Xi'an Jiaotong University" is not appropriate. It is scientifically unprofessional to name assistants by their full names in the methods of a manuscript. Include this person instead in the thanks/acknowledgement section, or better yet include him/her in the author list as he/she made a significant contribution.

Response:

Special thanks to you for your good comment. We have included this person in the acknowledgement section.

ACKNOWLEDGEMENTS

The authors would like to thank Lei-Lei Pei from Institute of Public Health Xi'an Jiaotong University for reviewing the statistical methods of this study. The authors also would like to thank the patients and their families for their contribution to this study.

8) The description of ROC curves "which plot sensitivity by 1-specifity" should

be omitted as ROC curves are commonly understood by readers.

Response:

Special thanks to you for your good comment. We have omitted this part according to your suggestion.

9) The sentence "Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were used to predict the possibility of HBsAg clearance based on weeks 12 and 24 HBsAg change from the baseline" is completely wrong and must be deleted. The authors never present calculations of the positive predictive value or negative predictive value of any test.

Response:

Special thanks to you for your good comment. We have deleted this part according to your suggestion.

10) The description of per protocol analysis "analyses were restricted to subjects who finished the scheduled treatment or follow-up" should be omitted, as this concept is commonly understood by readers.

Response:

Special thanks to you for your good comment. We have omitted this part according to your suggestion.

11) The description of intention-to-treat analysis "all subjects who were enrolled in the study were included in the analysis" should be omitted, as this concept is commonly understood by readers.

Response:

Special thanks to you for your good comment. We have omitted this part according to your suggestion.

12) Figure 2 is confusing as it presents multiple different outcomes in two different analyses. I propose it be split into two separate figures- one simply presenting data about HBsAg clearance, and the other presenting data about

HBsAg seroconversion.

Response:

We have split the Figure 2 into Figure 2 and Figure 3 according to your suggestion. The Figure 2 and Figure 3 were showed in the revised manuscript.

13) In the section titled "Primary endpoint", data is presented about HBsAg clearance rate at week 48. However，this was not a primary endpoint. Only HBsAg clearance rate at week 72 was listed in the abstract and the methods section as a primary endpoint, further confusing the reader.

Response:

Special thanks to you for your good comment. We have re-written this part according to your suggestion.

Primary endpoint

At week 72, per protocol analysis（PP analysis）showed that 40.0% (34/85) of patients in the add-on group had HBsAg clearance compared with 2.1% (2/96) of patients in the monotherapy group. The HBsAg clearance rate in the add-on group was significantly higher than in the monotherapy group at week 72 (Fig.2A) ($P < 0.001$). Furthermore, we also did an intention-to-treatment analysis (ITT analysis) and the results showed that the HBsAg clearance rate was 37.4% (34/91) in the add-on group and 1.9% (2/104) in the monotherapy group at week 72. Similar to the PP analysis, the HBsAg clearance rate in the add-on group was significantly higher than that in the monotherapy group at week 72 (Fig. 2B) ($P < 0.001$).

Secondary endpoints

HBsAg clearance rate at week 48

PP analysis showed that the HBsAg clearance rate in the add-on group was 28.2% (24/85) at week 48, significantly higher than 1.04% (1/96) in the

monotherapy group (Fig. 2A) ($P < 0.001$). ITT analysis also showed the HBsAg clearance rate was significantly higher in the add-on group than in the monotherapy group (26.4% [24/91] vs 0.96% [1/104], $P < 0.001$) (Fig. 2B).

14) In the section entitled "HBsAg dynamics", serum HBsAg levels are expressed as medians, and the decline is presented as baseline values and values at weeks 48 and 72. However later in this section, HBsAg elevation is expressed as the mean of the quantitative elevation values. Thus, there are two conflicting ways of presenting HBsAg dynamics, which is confusing. I suggest the authors standardise it throughout the manuscript. They should decide whether medians or means are more appropriate for HBsAg levels at baseline (depending on tests of the normality of the distribution of these values, e.g. the Shapiro-Wilk or Kolmogorov–Smirnov test) and present this accordingly. They should then present any HBsAg change as the median or mean of the quantitative elevation/decline values, whichever is more appropriate according to normality of distribution of these values. Then, the appropriate statistical testing for significance of the HBsAg change between add-on and monotherapy groups can be applied, and p-values herein should be stated.

Response:

Special thanks to you for your good comment. We have re-written this part according to your suggestion.

Median serum HBsAg level increased from 2.77 $\log_{10}$ IU/mL at baseline to 2.87 $\log_{10}$ IU/mL after 4 weeks of peg-IFNα-2a add-on therapy, gradually decreased to 2.76 $\log_{10}$ IU/mL at week 8, to 1.99 $\log_{10}$ IU/mL at week 48, and to 1.81 $\log_{10}$ IU/mL at week 72.

15) Figure 3 is confusing as it presents multiple different outcomes. I propose it be split into two separate figures- one simply presenting data about HBsAg changes, and the other presenting data about ALT and AST changes.

Response:

We have split the Figure 3 into Figure 4 and Figure 5 according to your

suggestion. The Figure 4 and Figure 5 were showed in the revised manuscript.

16) In the paragraph beginning with "Furthermore, more patients in the add-on group had low levels of HBsAg at the end of follow-up than the monotherapy group", HBsAg levels are presented not as log10 values, but as absolute values (1000, 100 and 10). This is confusing. The authors should standardise their presentation of data to log10 values.

Response:

Special thanks to you for your good comment. We have re-written this part according to your suggestion.

Furthermore, more patients in the add-on group had low levels of HBsAg at the end of follow-up than the monotherapy group. Most patients (97.8%, 89/91) in the add-on group demonstrated HBsAg levels < $3\log_{10}$ IU/mL (*vs.* monotherapy group 82.7%, 86/104; $P = 0.001$). Moreover, 71.4% (65/91) patients in the add-on group showed HBsAg levels < $2\log_{10}$ IU/mL (*vs.* monotherapy group 35.6%, 37/104; $P < 0.001$). Meanwhile, 52.7% (48/91) patients in the add-on group showed HBsAg levels < $1\log_{10}$ IU/mL (*vs.* monotherapy group 10.6%, 11/104; $P < 0.001$) (Fig. 4B).

17) ADV sequential combination is not defined in the manuscript, and it is unclear to readers why these patients are specifically highlighted in the paragraph entitled "Efficacy of add-on peg-IFN α-2a to ongoing low-genetic barrier NA (ADV)", and why their mean HBsAg decline is highlighted. I would suggest deleting this paragraph for brevity.

Response:

Special thanks to you for your good comment. We have deleted this part according to your suggestion.

18) It is unclear what "virological breakthrough" is defined as. Furthermore, the phrase "(No.69 and No.91)" to describe two patients with breakthrough is

meaningless to the reader and should be deleted.

19) It is unclear why a discussion of one patient who developed HCC is significant enough to warrant its own paragraph. It is not relevant to this overall study. I strongly suggest, for brevity, both virological breakthrough and HCC development are instead briefly included in the analysis of adverse outcomes/adverse events, rather than taking up their own unnecessarily long paragraphs.

Response:

Special thanks to you for your good comment. We have deleted these two parts and re-written these parts according to your suggestion of 18) and 19).

Safety

AEs were analyzed in the study population up to 72 weeks. 90.1% of the patients in the add-on group experienced AEs, significantly higher than 9.6% in the NA alone group (Table 4). The most common AEs were thrombocytopenia (90.1%), followed by neutropenia (87.9%) and pyrexia (82.4%) in the add-on group. 4.4% (4/91) of patients in the add-on group discontinued peg-IFNα-2a due to AEs, including two patients suffered from hyperthyroidism at week 24, one patient with thrombocytopenia (25×10^9/L) and one with coronary heart disease deterioration at week 12. ALT flares (>5×ULN) occurred in 7.7% of the add-on group. No patient in the monotherapy group discontinued treatment due to safety reasons.

Besides, one patient in the monotherapy group developed into HCC. Two patients treated with ADV in the monotherapy group experienced virological breakthrough (For patient adherent with NA therapy, serum HBV DNA converted to positivity following sustained negativity, and as confirmed 1 month later using the same regent) and were rescued by TDF and ETV therapy, respectively. Considering the risk of virological breakthrough with ADV monotherapy, ADV was replaced by TDF in patients with HBsAg positive at week 72.

20) In the paragraph entitled "Baseline HBsAg level and age for HBsAg clearance at week 72", baseline characteristics include "NA". It is not defined what NA means in this context. Does it mean the type of nucleot(s)ide analogue used? Furthermore, the presentation of univariate analysis and multivariate analysis is poorly done. Firstly, the authors should list which variables were assessed in univariate analysis. Ideally, this should be presented with the odds ratios in a table with appropriate p values. Then, those variables that were significant or approaching significance (P <0.10) should be combined in a multivariable logistic regression analysis with the odds ratios and p values presented in the same table, in a different column.

Response:

Special thanks to you for your good comment. We have re-written this part according to your suggestion. The results were showed in Table 2.

21) Furthermore, the authors have failed to define which units are being used for each of these variables in univariate and multivariate analysis. Is the unit of age months or years? Is the unit of baseline HBsAg level 1 log10 IU/mL, 0.1 log 10 IU/mL, or 1 IU/mL? Without these units, the odds ratios are impossible to interpret.

Response:

Special thanks to you for your good comment. We have re-written this part according to your suggestion.

To evaluate the baseline characteristics (gender, age [years], baseline HBsAg level [in log_{10} scale, IU/mL], ALT [IU/L], mode of HBV transmission, BMI [kg/cm^2], Fibro Scan value [kPa], NA [nucleoside analogue or nucleotide analog]) and on-treatment factors (HBsAg levels [log_{10} IU/mL] at weeks 12 and 24, HBsAg decline at weeks 12 and 24 versus baseline, and increase of ALT ≥ 2×ULN during the first 12 weeks of therapy) in predicting HBsAg clearance at week 72, univariable logistic regression analysis was performed.

22) the term "cut point" should be "cut-off point", and the cut-off points should be "33 years" and "2.25 $\log_{10}$ IU/mL", without the "<" symbol.

Response:

Special thanks to you for your good comment. We have re-written this part according to your suggestion.

The AUROC of age was 0.699, and the optimal cut-off point was 33 years. The AUROCs of HBsAg level were 0.689, 0.877, 0.921, and the optimal cut-off points were 2.25 $\log_{10}$ IU/mL for baseline, 1.89 $\log_{10}$IU/mL for week 12 and 1.46 $\log_{10}$ IU/mL for week 24, respectively. The AUROCs of HBsAg decline from baseline to week 12 and week 24 were 0.901 and 0.924, respectively, and the optimal cut-off points were 0.5 $\log_{10}$ IU/mL and 1.0 $\log_{10}$ IU/mL, respectively.

23) In the paragraph entitled "ALT elevation, HBsAg levels and changes of HBsAg for HBsAg clearance at week 72", the presentation of univariate analysis and multivariate analysis is again poorly done. They should follow the same advice as per my point 20 above. I am most interested to know if these univariate and multivariate analyses have also included the same baseline demographic variables and HBsAg levels as in the previous analyses referred to in point 20. If they have not, I believe they must be. These on-treatment dynamic changes of ALT and HBsAg may actually be confounded by baseline variables.

24) Furthermore, the units in this second univariate analysis and multivariate analysis are not defined, just like in my point 22 above. Most importantly, what are the units of HBsAg change at week 12 and 24? Without these units, the odds ratios are impossible to interpret.

Response:

Special thanks to you for your good comment. We have re-written this part according to your suggestion of 23) and 24). The results were showed in Table 2.

46.2% (42/91) of the patients in the peg-IFNα-2a add-on group showed ALT

≥2×ULN during the first 12 weeks of therapy, with a maximum of 414 IU/L. To evaluate the ALT change on HBsAg clearance, we dichotomized the patients into two groups (ALT ≥2×ULN or <2×ULN) at week 12 and two groups of patients followed the same treatment. To evaluate the baseline characteristics (gender, age [years], baseline HBsAg level [in $\log_{10}$ scale, IU/mL], ALT [IU/mL], mode of HBV transmission, BMI [kg/cm^2], Fibro Scan value [kPa], NA [nucleoside analogue or nucleotide analog]) and on-treatment factors (HBsAg levels [$\log_{10}$ IU/mL] at weeks 12 and 24, HBsAg decline at weeks 12 and 24 versus baseline, and increase of ALT ≥ 2×ULN during the first 12 weeks of therapy) in predicting HBsAg clearance at week 72, univariable logistic regression analysis was performed. The results showed that age (P = 0.002, OR=0.924; 95% confidence interval [CI], 0.878-0.972), baseline HBsAg level (P = 0.003, OR= 0.371; 95% CI, 0.194-0.711), HBsAg level at week 12 (P < 0.001, OR = 0.273, 95% CI: 0.157-0.474), HBsAg level at week 24 (P < 0.001, OR = 0.218, 95% CI: 0.117-0.405), HBsAg decline from baseline to week 12 (P < 0.001, OR= 10.646, 95% CI: 3.776-25.018), HBsAg decline from baseline to week 24 (P < 0.001, OR = 7.045, 95% CI: 3.223-15.400), and ALT elevation ≥ 2×ULN during the first 12 weeks of therapy (P =0.002, OR= 4.182, 95% CI: 1.691-10.340) were strong predictors for HBsAg clearance at week 72. Baseline gender, ALT, mode of HBV transmission, BMI, Fibro Scan value, NA [nucleoside analogue or nucleotide analog]) were not statistically significant.

In order to further evaluate age, baseline and decline of HBsAg in early treatment and ALT elevation in early treatment in predicting HBsAg clearance at week 72, multivariable logistic regressions were conducted for age, HBsAg levels at baseline, week 12, and week 24 as well as the week 12 and week 24 HBsAg decline from baseline adjusted for gender and NA. Similar to the univariable regression analysis results, all variables were significantly related to HBsAg clearance at week 72: age (P = 0.025, OR= 0.946; 95% CI, 0.833-0.981), baseline HBsAg level (P = 0.019, OR= 0.557; 95% CI, 0.206-0.827), HBsAg level at week 12 (P = 0.002, OR = 0.542, 95% CI: 0.194-0.792), HBsAg level at week 24

(P = 0.004, OR = 0.188, 95% CI: 0.058-0.410), HBsAg decline from baseline to week 12 (P < 0.001, OR= 8.925, 95% CI: 3.376-17.226), HBsAg decline from baseline to week 24 (P <0.001, OR = 8.830, 95% CI: 4.553-18.213), and ALT elevation ≥ 2×ULN during the first 12 weeks of therapy (P = 0.014, OR= 5.275, 95% CI: 3.324-11.823). The results were showed in Table 3.

25) It is unclear why the paragraph entitled "The effect of HBsAg levels at weeks 12 and 24 and the changes of HBsAg from baseline to weeks 12 and 24" is a separate paragraph. It should be combined with the paragraph above as it is describing the ROC curves of those variables. Similarly, it is unclear why the paragraph entitled "The effect of early ALT elevation for HBsAg clearance" is a separate paragraph. It should be combined with the paragraph above.

Response:

Special thanks to you for your good comment. We have re-written this part according to your suggestion.

ROC curves were used to evaluate the performance of the above significant variables for HBsAg clearance. The AUROC of age was 0.699, and the optimal cut-off point was 33 years. The AUROCs of HBsAg level were 0.689, 0.877, 0.921, and the optimal cut-off points were 2.25 $\log_{10}$ IU/mL for baseline, 1.89 $\log_{10}$IU/mL for week 12 and 1.46 $\log_{10}$ IU/mL for week 24, respectively. The AUROCs of HBsAg decline from baseline to week 12 and week 24 were 0.901 and 0.924, respectively, and the optimal cut-off points were 0.5 $\log_{10}$ IU/mL and 1.0 $\log_{10}$ IU/mL, respectively.

Based on the optimal cut-off values, our data showed that the rates of HBsAg clearance were 58.1% (18/31) for patients younger than 33 years old and 62.1% (18/29) for patients with baseline HBsAg <2.25 $\log_{10}$ IU/mL. Patients with HBsAg < 1.89 $\log_{10}$ IU/mL at week 12 had an HBsAg clearance rate of 73.7% (28/38). Patients with HBsAg < 1.46 $\log_{10}$ IU/mL at week 24 had an HBsAg clearance rete of 72.7% (32/44). The rates of HBsAg clearance were 80.0% (28/35) and 77.5% (31/40) for patients with HBsAg decline > 0.5$\log_{10}$

IU/mL from baseline to week 12 and > 1.0$\log_{10}$ IU/mL to week 24, respectively. Patients with ALT ≥2×ULN during the first 12 weeks of therapy demonstrated 54.8% (23/42) of HBsAg clearance.

26) In the paragraph entitled "Safety", p values should be presented for the difference in adverse events.

Response:

Special thanks to you for your good comment. We have added p values according to your suggestion. The results were showed in Table 4.

27) In the discussion, the sentence "Several reasons including higher baseline HBsAg titer and poor compliance to full treatment in that study could well explained the discrepancy between our and Marc Bourlière's results" is unprofessional. Full names of previously uncited authors are not to be used in scientific writing. Furthermore, the quantitative differences in compliance between the current study and Bourliere et al's study should be made clear.

Response:

Special thanks to you for your good comment. We have re-written this part according to your suggestion.

Several reasons could well explain the discrepancy between our results and results in that randomized controlled trial. Firstly, 93.4% (85/91) of patients in our study finished the scheduled peg-IFN α-2a treatment and follow-up, while only 76% (65/85) of patients received the full dose and duration of peg-IFN α-2a treatment in that randomized controlled trial. Good compliance and tolerance to full dose and duration of peg-IFN α-2a treatment maybe the main reason for significantly higher rates of HBsAg clearance and seroconversion in our study. Secondly, lower baseline HBsAg titer of patients in our study maybe another important reason. Furthermore, all patients in our study were CHB and the Fibro Scan value <7.1 kPa, while about 35.0% (31/90) of patients with liver fibrosis and even cirrhosis in that randomized controlled trial. Lower baseline

degree of liver fibrosis of patients in our study could well explain the discrepancy between these two studies.

28) The statement "Therefore, we believe that extension of the time with peg-IFN α-2a therapy may further improve HBsAg clearance in patients with HBsAg <100 IU/ml at week 72" is not backed up by any reasoning and should be omitted. There is no data in this study, where IFN durations were fixed, suggesting that longer IFN treatment results in greater HBsAg clearance.

Response:

Special thanks to you for your good comment. We have omitted the statement "Therefore, we believe that extension of the time with peg-IFN α-2a therapy may further improve HBsAg clearance in patients with HBsAg <100 IU/ml at week 72" according to your suggestion.

29) The statement "The patients in the treatment group were not randomized. This may lead to bias that potentially impact the follow-up results" is unclear. What exact types of bias are the authors referring to? After all, demographic and baseline characteristics between treatment groups were not statistically different.

Response:

Special thanks to you for your good comment.

This study has several limitations. First, the patients in the treatment groups were not randomized. This may lead to confounder bias induced by unknown confounding factors, and potentially impacted the follow-up results, although demographic and baseline characteristics between treatment groups were not statistically different.

30) Table 4 should include p values.

Response:

p values have been added in Table 4.

31) Table 2 uses the term "cut point" instead of "cut-off point"

Response:

We have corrected this error in Table 3.

32) Fibro Scan is not the technical term for a test. Authors should use the term "transient elastography" instead, and state in the methods that the Fibroscan technology was used (FibroScan; EchoSens, Paris, France).

Response:

Liver stiffness measurement was performed by transient elastography (Fibroscan, EchoSens, Paris, France).

33) Transient elastography/Fibroscan values in Table 1 need a unit (I assume kPA).

Response:

We have added the unit Fibroscan values "kPA " in Table 1.

34) In Tables 2 and 3, units for every measurement must be given, and the direction of HBsAg change (elevation or decline) must be made clear, rather than just using the term "HBsAg change".

Response:

We have added all units of these variables in Table 2 and 3.

35) The authors have presented very provocative 'cut-off points' on ROC curves for variables predicting the primary outcome. However， in their discussion, they should discuss the implications of these cut-off points. Do they, at present, believe that add-on therapy should be denied to patients who have unfavourable characteristics based on one of these cut-off points (e.g. a 34 year old person)? Or on several of these cut-off points (e.g. a 34 year old person with HBsAg 2.26 log10 at baseline)? Or do the authors propose, as future research,

combining these characteristics into a mathematically modelled and weighted scoring system which can be retrospectively and prospectively validated?

Response:

Special thanks to you for your good comments. We have re-written these parts according to all of your suggestion.

All the above variables and their 'cut-off points' on ROC curves are meaningful for predicting the HBsAg clearance. However, this study had a relatively small number of patients receiving peg-IFN α-2a add-on therapy (n=91). Exploratory analyses into baseline and on-treatment predictors of HBsAg clearance should be interpreted with caution. As future research, more patients in multiple centers will be enrolled and these meaningful characteristics will be combined into a mathematically modelled and weighted scoring system which can be retrospectively and prospectively validated.

Reviewer: 2

Comments to the Author

1) it is well known that the response to first-line anti-HBV treatment Entecavir and Adefovir is much better compared to second-line treatment like adefovir. So did the patient looked to the difference in the response between patients who were on ETV and TDF versus those who were on ADV in the group that had add on peg INF.

Response:

Special thanks to you for your good comments. We have considered the efficacy difference between first-line antiviral drugs and second-line antiviral drugs combination with peg-IFN α. However, there were only 10 patients treated with ADV combination with peg-IFN α in this study. Considering the small sample size, we did not specially compare the difference in efficacy between ADV combination with peg-IFN α and EDV / TDF combination with peg-IFN α. We will further expand the sample size in future study to compare

the difference in efficacy between ADV combination with peg-IFN α and EDV / TDF combination with peg-IFN α.

2) Another point since all those patients were assessed for hepatic fibrosis using transient elastography (fibroscan) did the author found any difference in response to treatment in the two groups across the different stages of fibrosis.

Response:

Special thanks to you for your good comments. We have re-written this part according to your suggestion. The result was showed in the univariate logistic regression analysis and Table 2.

3) some parts of the discussion section, the authors seem repeating the result rather than discussing their findings.

Response:

Special thanks to you for your good comments. We have re-written the discussion according to your suggestion.

4) With regards to the development of HCC longer follow-up might be needed to evaluate the effect of peg INF add on therapy in reducing the risk of HCC. This can be addressed in the discussion section.

Response:

Thank you for your recommendation. We have addressed the effect of peg INF add on therapy in reducing the risk of HCC in discussion section according to your suggestion.

5) Additionally, the authors can use their findings to add recommendations at the end of the discussion section.

Response:

Considering your suggestion, we added the following paragraphs to the discussion:

All the above variables and their 'cut-off points' on ROC curves are meaningful for predicting the HBsAg clearance. However, this study had a relatively small number of patients receiving peg-IFN α-2a add-on therapy (n=91). Exploratory analyses into baseline and on-treatment predictors of HBsAg clearance should

be interpreted with caution. As future research, more patients in multiple centers will be enrolled and these meaningful characteristics will be combined into a mathematically modelled and weighted scoring system which can be retrospectively and prospectively validated.

Reviewer: 3

Comments to the Author

1) Please explain in more the limitation of a non-randomised study when answering a research question

Response:

Thank you for your good comments. The patients in the treatment groups were not randomized. This may lead to confounder bias induced by unknown confounding factors, and potentially impacted the follow-up results, although demographic and baseline characteristics between treatment groups were not statistically different.

2) 1537 patients were screened and 1342 were excluded because of their DNA and HBsAg levels etc. This suggests that this highly selected group is very hard to find in clinical practice - please comment on this.

Response:

Thank you for your good comments. Date from the Chinese Center for Disease Control and Prevention (CDC) showed that there are about 70 million patients with chronic HBV infection in China, of which about 20 to 30 million patients are CHB. Preliminary statistical analysis in our center indicates that 75.5% (3677/4870) of CHB patients received NA treatment, including lamivudine (LAM), adefovir dipivoxil (ADV), telbivudine (LdT), ETV, and tenofovir fumarate (TDF). In those patients with NA treatment, 37.7% (1386/3677) patients were hepatitis B e antigen (HBeAg)-negative and HBsAg ≤1500 IU/mL after over one year of NA treatment (unpublished data). Based on these data, we estimate that approximately 5.69-8.53 million CHB patients in China are HBsAg ≤1500IU/mL after over one year of NA treatment. This suggests that

patients with HBsAg levels ≤1500 IU/mL, HBeAg-negative and HBV DNA <1.0×10^2 IU/mL after over one year of NA therapy are very easy to find in clinical practice.

In the past years, many CHB patients could not afford combination therapy with Peg-IFN and NA due to economic reasons. However, with the improvement of medical insurance, NA and Peg-IFN have become quite cheap in China now. Thus, we believe that more and more CHB patients with HBsAg ≤1500IU/mL after over one year of NA treatment will be willing to receive peg-IFN αadd-on therapy to an ongoing NA regime in pursuit of clinical cure of CHB.

3) The baseline fibrosis stage seems to be lower in Dr Wu's study than Dr Bourliere's study and this may explain the results - please discuss this.

Response:

Thank you for the valuable suggestion. We have added a paragraph in discussion section according your suggestion.

However, a randomized controlled trial reported that the addition of 48 weeks of peg-IFN α-2a to NA therapy resulted in a small proportion of HBsAg clearance and HBs seroconversion[18]. Several reasons could well explain the discrepancy between our results and results in that randomized controlled trial. Firstly, 93.4% (85/91) of patients in our study finished the scheduled peg-IFN α-2a treatment and follow-up, while only 76% (65/85) of patients received the full dose and duration of peg-IFN α-2a treatment in that randomized controlled trial. Good compliance and tolerance to full dose and duration of peg-IFN α-2a treatment maybe the main reason for significantly higher rates of HBsAg clearance and seroconversion in our study. Secondly, lower baseline HBsAg titer of patients in our study maybe another important reason. Furthermore, all patients in our study were CHB and the Fibro Scan value <7.1 kPa, while about 35.0% (31/90) of patients with liver fibrosis and even cirrhosis in that randomized controlled trial. Lower baseline degree of liver fibrosis of patients in our study could well explain the discrepancy between these two studies.

4) Dr Bourliere's study did not include any data on HBV genotype and this was considered a weakness. Can Dr Wu describe the genotype mix in his study?

Response:

Thank you for the valuable suggestion. In our study, it is uncertain whether different HBV genotypes play a role in the response to the same therapeutic strategy due to HBV genotypes were not available in patients with long-term HBV suppression at entry to the study.

5) The core tip is very poorly written and this should be revised.

Thank you for the valuable suggestion. We have re-written this part according to your suggestion

Core tip:

Despite promising results with the combination therapy of Peg-IFN and NA, the best combination therapeutic strategy of Peg-IFN and NA to the treatment of CHB remains unclear. This prospective study was to evaluate the efficacy and safety of add-on 48-week of peg-IFN α-2a to an ongoing NA regime in CHB patients with HBsAg levels ≤ 1500 IU/mL, HBeAg-negative and HBV DNA < 1.0×10^2 IU/mL after over one year of NA therapy. The results suggest that high rates of HBsAg clearance and seroconversion could be achieved by add-on peg-IFNα-2a to an ongoing NA regime for HBeAg-negative CHB patients with HBsAg levels ≤ 1500 IU/mL and HBV DNA <1.0×10^2 IU/mL after long-term NA treatment, and the treatment were relatively safe. Besides, Younger patients, lower HBsAg concentrations at baseline, weeks 12 and 24, greater HBsAg decline from baseline to weeks 12 and 24, and ALT ≥ 2×ULN during the first 12 weeks of therapy were strong predictors of HBsAg clearance for patients with peg-IFN α-2a add-on treatment.

6) The second paragraph in the introduction does not make sense and is not clear- please revise this.

Response:

Thank you for your good comments. We have re-written the second paragraph in the introduction.

Although the rate of HBsAg clearance is very low, it demonstrated that CHB is a disease that can be 'cured' through effective treatment, which may not completely clear HBV, but close to the status of complete eradication of HBV, including covalently closed circular DNA (cccDNA).

7) The word 'debated' is not used in the correct sense in the core tip and discussion.

Thank you for the valuable suggestion. We have re-written the Core tip.

8) I think Dr Wu's data and Dr Bourliere's study data are more similar than the authors suggest - please add a paragraph in the discussion about this.

Response:

Thank you for the valuable suggestion. We have re-written this part according to your suggestion

However, a randomized controlled trial reported that the addition of 48 weeks of peg-IFN α-2a to NA therapy resulted in a small proportion of HBsAg clearance and HBs seroconversion[18]. Several reasons could well explain the discrepancy between our results and results in that randomized controlled trial. Firstly, 93.4% (85/91) of patients in our study finished the scheduled peg-IFN α-2a treatment and follow-up, while only 76% (65/85) of patients received the full dose and duration of peg-IFN α-2a treatment in that randomized controlled trial. Good compliance and tolerance to full dose and duration of peg-IFN α-2a treatment maybe the main reason for significantly higher rates of HBsAg clearance and seroconversion in our study. Secondly, lower baseline HBsAg titer of patients in our study maybe another important reason. Furthermore, all patients in our study were CHB and the Fibro Scan value <7.1 kPa, while about 35.0% (31/90) of patients with liver fibrosis and even cirrhosis in that

randomized controlled trial. Lower baseline degree of liver fibrosis of patients in our study could well explain the discrepancy between these two studies.

We would like to express our great appreciation to you and reviewers for their positive and constructive comments and suggestions on our manuscript. We have studied comments carefully and have made corrections which we hope meet with approval. Thank you!