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COMMENTS TO AUTHORS

Title: HBsAg clearance in inactive HBsAg carriers treated by peginterferon alfa-2a The paper submitted by Li et al. examined the effect of peginterferon alfa-2a in inactive HBsAg carriers with serum HBsAg level <100 IU/ml. The authors found that HBsAg clearance was achieved in 13 out of 20 patients (65%) after 72 weeks of peginterferon alfa-2a therapy, while no patients in control group experienced HBsAg clearance. Although it has demonstrated a clear link between peginterferon therapy and HBsAg loss, the benefit of treatment for inactive carriers is still questionable in the real clinical practice. The following comments are the reasons why I think so.

(Major comments)QUESTION 1. The result of this paper is substantial value as HBsAg clearance is suggested as optimal endpoint of antiviral therapy. However, antiviral therapy is not recommended for inactive carriers, since the inactive HBV carrier status confers a favorable long-term outcome with a very low risk of cirrhosis or HCC in the majority of patients. So far, there has been lack of evidence whether interferon-induced HBsAg loss in inactive carriers is beneficial in the real clinical practice or not. In addition, I think the possibility of spontaneous HBsAg clearance should have been taken into account.

Although in generally speaking, inactive HBsAg carriers is associated with a good outcome compared with hepatitis patients. There is a lifetime risk of hepatitis, cirrhosis and hepatocellular carcinoma associated with HBV for inactive HBsAg carriers. A long-term follow up study showed cumulative probabilities of hepatitis relapse in inactive HBsAg carriers were of 10.2%, 17.4%, 19.3%, 20.2%, and 20.2% after 5, 10, 15, 20, and 25 years'follow up, respectively, with an annual rate of 1.55%. Another long-term longitudinal study (up to 23 years) showed that 1–17% of inactive carriers reverted back to HBeAg-positive chronic hepatitis. Cirrhosis and HCC may still develop in some inactive hepatitis B surface antigen (HBsAg) carriers.

However, the facts that no cirrhosis or HCC occurred in patients with HBsAg loss after IFN treatment indicate that HBsAg clearance is currently the only parameter associated with an excellent long-term prognosis. Therefore, inactive HBsAg carriers could still get benefits from interferon treatment, if HBsAg loss could be achieved after treatment.

QUESTION 2: According to Tseng et al (Hepatology 2012; 55:68-76), HBsAg levels<10 IU/mL at baseline is the strongest predictor of HBsAg loss.

ANSWER: According to Tseng et al (Hepatology 2012; 55:68-76), although HBsAg<10 IU / ml can be used to predict the occurrence of HBsAg loss, the incidence rate was only 7.4 per 100 P-yers.The duration of disappearance of HBsAg was 5.8±4.2years. In this paper, all treated patients after 72 weeks of treatment and 24 weeks of follow-up, the HBsAg loss rate was increased to 65%, while the control group there was no HBsAg loss. Therefore, inactive HBsAg carriers with low HBsAg level could still get benefits from interferon treatment if HBsAg loss could be achieved after treatment, and the rate of HBsAg loss can be increased and the duration of treatment can be shortened.

QUESTION: But, half of the subjects included in this study already had HBsAg<10 IU/mL and

undetectable HBV DNA. It is recommended for authors to comment on these facts in the discussion section.

ANSWER: In 10 cases with HBsAg levels<10 IU/mL and undetectable HBV DNA <100 IU/Ml, 8 (80%) cases obtained HBsAg loss. It was suggested that peginterferon α-2a treatment can make inactive carriers achieve HBsAg clearence in a short-term time compared with spontaneous HBsAg loss occurred in nature history.

2. The comparison of clinical characteristics between patients with HBsAg loss and patients without HBsAg loss is recommended in the results section.

ANSWER: However, in treatment group, the patients achieved HBsAg loss had lower HBsAg baseline of 8.09 (3.81~22.50) IU/mL and were younger(age of 31.46±12.16 yrs) than those(HBsAg baseline 18.95 (2.85~83.00) IU/mL and age of 38.24±9.25 yrs),but without statistical significance, of patients without HBsAg loss after treatment.

(Specific comments) A specific correction on its use of English language can improve this manuscript. Abstract In the results section, "HBsAg levels decreased from 25.72±25.58 IU/ml at baseline to 17.11±21.62 IU/ml at week 96 (p=0.108)" is unclear. Specific description of which patients' group it refers is needed. Introduction This manuscript defined inactive carriers as patients who are HBsAg positive, HBeAg-negative, anti HBe-positive with undetectable HBV DNA level and normal ALT levels. Others may be okay, but, in case of HBV DNA, the practice guidelines include not only undetectable HBV DNA but also low HBV DNA (<2,000 IU/mL).

Answer: Already supplement. In the control group, HBsAg levels of the control group decreased from 25.72±25.58 IU/ml at baseline to 17.11±21.62 IU/ml at week 96 (p=0.108). In the manuscript, undetectable HBV DNA means HBV DNA<100 IU/mL.

Methods 1. The definition of normal ALT is missing (<40/mL for all patients? 19 IU/mL for females and <30 IU/mL for males?)

ANSWER: The definition of normal ALT is 19 IU/mL for females and <30 IU/mL for males.

2. Based on what reference was the treatment duration set as 72 weeks?

Answer: Although the recommended treatment for chronic hepatitis B patients with PEG-IFN is 48 weeks, but the extension of treatment can significantly improve the rate of disappearance of HBsAg[32]. So, the course of treatment was prolonged to 72 weeks.

3. Please define the 'clinical evidence cirrhosis' in the exclusion criteria.

ANSWER: Diagnosed as patients with liver stiffness >9kPa or performance of portal hypertension(spleen enlargement with reduction in counts of PLT), by FibroScan and Ultrasonic examination

4. Please describe the information about the HBV genotypes of the subjects.

Answer: It is a pity that the HBV genotype can not be get for reason of undetectable HBV DNA level

in the subjects. But epidemiological studies showed the HBV genotypes in China were mainly genotype B and C .

5. Please define 'hepatitis reactivation'.

ANSWER: Hepatitis B recurrence was defined as the condition that HBV DNA becomes positive again different from undetectable stage before and abnormal ALT continued more than three months, and exclude other factors for elevated ALT.

QUESTION: Results In patients with HBsAg loss, what was the mean/median time of achieving HBsAg loss after the initiation of treatment?

Answer: Mean time of HBsAg loss refer to the average months or years for achieving HBsAg loss after the initiation of treatment. Median time of achieving HBsAg loss refer to the months or years in the middle position for achieving HBsAg loss after the initiation of treatment.

QUESTION: Discussion As a retrospective study, it carries the limit that every retrospective study does. It is recommended to comment about the limitation of this study in the discussion section.

ANSWER:Retrospective studies may have a retrospective bias, but all of the data in this article are from the database which would make up the shortcomings at the maximum extent.

Table Since the manuscript can give enough information on the patient selection criteria in the methods section, Table 1 seems unnecessary. Please describe it in the method section in more detail.

Answer: delete table 1.

QUESTION: Reference The international guidelines are recently updated (Reference 1 and 3). But it has not been applied to this manuscript. Please check them and update the citations.

Answer: Reference 1 and 3 have updated.

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COMMENTS TO AUTHORS

The manuscript entitled "HBsAg clearance in inactive HBsAg carriers treated by peginterferon alfa-2a" discusses a possible application of an interferon therapy in inactive HBsAg carriers with a very low HBsAg level. The authors report that in their study the HBsAg disappeared in 65% of treated patients. This result seems to be very good, taking into account that usually HBsAg clearance is rarely observed. However, it seems to me that a treatment by peginterferon alfa-2a 180 μg/week for such a long period as 72 weeks is not justified for inactive HBsAg carriers. For such patients the risks of complication development outweigh the possible benefits. As a practical doctor I know that it is very difficult for patients to receive this therapy for a long time. Therefore I can not recommend this manuscript for publication.

ANSWER: Please see discussion. Firstly, inactive HBsAg carriers should get further improvement in outcomes if HBsAg loss could be achieved after interferon treatment. However, this inactive state was not always sustained. A long-term follow up study showed cumulative probabilities of hepatitis relapse in inactive HBsAg carriers of 10.2%, 17.4%, 19.3%, 20.2%, and 20.2% after 5, 10, 15, 20, and 25 years of follow up, respectively, with an annual rate of 1.55%. Another long-term longitudinal study (up to 23 years) showed that 1–17% of inactive carriers reverted back to HBeAg-positive chronic hepatitis. Cirrhosis and HCC may still develop in some inactive hepatitis B surface antigen (HBsAg) carriers. There was no cirrhosis or HCC occurred in patients with HBsAg loss after IFN treatment, indicating that HBsAg clearance is currently the only parameter associated with an excellent long-term prognosis, and the strongest factor predicting excellent long-term outcome in life time of HBV infected individuals is HBsAg loss spontaneous or after treatment. Secondly, Effects, including the probability of HBsAg clearance, can be enhanced by extended therapy of peginterferon α-2a.

Reviewer's code: 03473435

COMMENTS TO AUTHORS

In this manuscript by Li et al. entitled "HBsAg clearance in inactive HBsAg carriers treated by peginterferon alfa-2a," the authors evaluated the effectiveness of 72 weeks of peginterferon alfa-2a in inducing HBsAg loss and seroconversion in a subset of treatment-na?ve, chronic HBV inactive carriers with completely suppressed HBV DNA and low HBsAg (<100 IU/ml) at baseline. The authors retrospectively evaluated 20 patients treated with 72 weeks of peginterferon-alfa-2a and followed off treatment for 24 weeks (for a total observational time of 96 weeks) and compared outcome and safety measures to 40 untreated control chronic HBV patients matched for age, sex, baseline HBsAg and HBV DNA levels. Pertinent findings included a high HBsAg loss and anti-HBs seroconversion rates in the treatment arm compared to the control arm, where HBsAg loss was not observed. The followings are comments and questions:

1. As this study is really focused on a subpopulation of CHB inactive carriers with virally suppressed HBV DNA and HBsAg < 100 IU/ml who are treatment-naive, this needs to be made clear throughout the abstract and manuscript. The findings cannot be generalizable to all CHB inactive carriers, only to this subpopulation.

ANSWER: Content has been added throughout the abstract and manuscript.The object of observation were inactive HBsAg carriers with a serum HBsAg level <100 IU/ml and persistently undetectable HBV DNA levels(<100 IU/mL).

2. As response to peginterferon-alfa-2a is HBV genotype-dependent, this needs to be addressed in the manuscript. If patient data are not available (given prolonged history of undetectable viral load), then additional epidemiologic HBV genotype breakdown of the surrounding region would still be useful.

Answer: It is a pity that the HBV genotype can be not get for reason of undetectable HBV DNA level in the subjects. But epidemiological studies showed the HBV genotypes in China were mainly genotype B and C.

3. It would be useful to elaborate further on the clinical course of the treatment patients and expand on the Result Section. What was the timing of HBsAg loss for these treatment patients? Was it on-therapy or during the follow-up period? At what week did HBsAg loss occur? Was there HBsAg loss and sero-revision observed?

ANSWER: Of the 20 treated patients, 13 achieved HBsAg loss,12 occurred during treatment and 1 at follow time,with mean of 40.62±22.74months after the initiation of treatment, in which 12 achieved HBsAg seroconversion.

What were the baseline anti-HBs levels (as some patients will have detectable anti-HBs levels but < 10IU/ml)? Did anti-HBs relate to clinical outcome?

Answer: See section of Selection of patients. Patients who were treatment-naïve with HBsAg positive, anti-HBs-negative and HBeAg negative for more than 6 months, were included in the study.

What was the age and gender breakdown of those who achieved HBsAg loss and did these parameters have a bearing on the outcome?

Answer: However, in treatment group, the patients achieved HBsAg loss had lower HBsAg baseline of 8.09 (3.81~22.50) IU/mL and were younger(age of 31.46±12.16 yrs) than those(HBsAg baseline 18.95 (2.85~83.00) IU/mL and age of 38.24±9.25 yrs),but without statistical significance, of patients

without HBsAg loss after treatment.

4. Please expand on the safety data. Immunomodulator therapy is associated with increased risk of ALT flares both on and off-treatment. ALT flares, particularly when immune-induced, are associated with good clinical outcome. What was observed on and off-treatment and how did these findings impact clinical outcomes?

Answer: ALT levels increased during treatment in 18 of 20 (90.0%) treated patients, and 9 (45.0%) individuals experienced ALT >80 IU/L. However, bilirubin levels remained within normal limits throughout treatment and follow-up in all treated patients. Normalization of ALT levels coincided with HBsAg loss and/or the end of treatment, and was maintained during follow-up.

5. Please use local lab conversion factor to convert HBV DNA to IU/ml. Please provide upper limit of normal for ALT used in this manuscript.

ANSWER: We have convert HBV DNA to IU/ml. There was 19 IU/mL for females and <30 IU/mL for males.