Dev 20, 2020

Lian-Sheng Ma

Company Editor-in-Chief

Dear Prof. Ma,

Thank you very much for your decision letter and advice on our manuscript (NO.: 59733, Case Report) entitled “Clinical cure and liver fibrosis reversal after postoperative antiviral combination therapy in HBV-associated non-cirrhotic hepatocellular carcinoma: Case report.” We also thank the reviewers and editors for the constructive and insightful comments and suggestions. Accordingly, we have revised the manuscript in accordance with the reviewers’ suggestions (shown in red). In addition, point-by-point responses to the comments are listed below this letter.

We hope that the revision is acceptable for the publication in your journal.

Look forward to hearing from you soon.

With best wishes,

Yours sincerely,

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First of all, we would like to express our sincere gratitude to the reviewers' editors for the constructive and positive comments and suggestions.

**Replies to Reviewer #1**

1. The summary statement states that lamivudine was administered two years after treatment. It is unclear what treatment took place two years prior to lamivudine.

**Response:** Thank you for your meticulous job. The correct statement is that he discontinued the antiviral therapy after 2 years. We have added the sentence in the Case Presentation section (page 6, line 5).

2. The relationship between HBsAg titers and ALT values is not clearly explained. Also, the concept of ALT elevation by host immunity would not be obvious to potential readers of the article. This should be better addressed. The authors also state that: "Acute elevation of ALT levels is mainly immune-mediated". What does this mean? No context is provided for such statement.

**Response**: The course of HBV infection is a dynamic process. An acute elevation of ALT can occur in different stages of the disease. It is also considered to be immune-mediated. On the one hand, it represents the enhancement of the host immune response, indicating the decline in HBV DNA, HBeAg, and HBsAg; on the other, it may lead to progressive liver injury, which may result in liver decompensation and even death. An acute elevation of ALT during PEG IFN treatment was associated with a subsequent decline in HBsAg and HBV RNA and HBsAg clearance, suggesting a strong antiviral immune response [1].

3. Another unclear point is provided by the following statement: "Several factors can affect the postoperative HCC recurrence, with HBsAg as the main factor". Does this imply a cause-effect relationship? Is so, how? How does HBsAg itself drives HCC recurrence?

**Response:** Thank you for your kind reminder. There are several factors influencing the postoperative recurrence of liver cancer, including the host, tumor, and virus factors, among which HBsAg is the most important virus factor. There is no causal relationship between HBsAg titer and HCC recurrence, although studies have shown that a higher HBsAg level

was associated with late recurrence of HCC (P=0.032) in HBeAg-negative and non-cirrhotic patients with low viral load [2].

4. The paper could benefit of a graphical depiction of the patient's timeline of illness and treatment.

**Response:** Thank you for your kind suggestion. We have modified it. Please see Figure 1A.

Reviewer #2

1. The resolution of fibrosis after antiviral therapy in HBV-infected patients has been reported previously and not so rare observation (Chang T et al, Hepatology 2010; 52:886, Marcellin P et al, Lancet 2013; 381:463 etc). Previous reports should be cited adequately.

**Response:** Thank you for your kind reminder. I have quoted the literature on the reversal of liver fibrosis in patients infected with HBV after antiviral treatment. The details are as follows: Previous studies have shown that the resolution of fibrosis occurred in HBV-infected patients after NAs or IFNα treatment [3, 4]. After 60 weeks of a combination therapy of ETV+PEG IFNα-2b, the pathological re-examination results of the liver tissue showed that liver fibrosis was significantly reversed (S3→S1). We have added the sentences in the Discussion section (page 10, lines 2-6).

2. In Fig 3B, the picture of liver puncture biopsy is shown but the picture does not include portal area of liver lobule. It is difficult to evaluate the presence of fibrosis from the picture. The picture including portal region should be provided.

**Response:** Thank you for your meticulous job. I have found a suitable picture and modified it accordingly (Figure 2).

3. The changes of biological marker indicating liver fibrosis should be provided, for example by calculating Fib-4 index

**Response:** Thank you for your kind suggestion. We calculated two recognized noninvasive liver fibrosis indices, FIB-4 and APRI, based on the reported formula. After 60 weeks of

combination therapy, the FIB-4 decreased from 2.39 to 1.13, and the APRI decreased from 24.36 to 14.45. Therefore, both hepatic pathology and noninvasive hepatic fibrosis index results demonstrated that liver fibrosis was significantly reversed after combination therapy. The FIB-4 and APRI were calculated as previously described: FIB-4=age (year)×AST (IU/L)/(PLT (× $10^9$/L)×ALT (IU/L)1/2); APRI=AST (IU/L)×100/PLT (×$10^9$/L).

4. As shown in the report, the development of HBs Ab in chronic HBV infection is rare, however, the HBV Ag/Ab seroconversion after antiviral treatment has been reported even in immunosuppressive state such as patients coinfected with HIV/HBV and patients receiving hemodialysis. What is the incidence of HBs Ag/Ab seroconversion in the natural course of chronic HBV infection?

**Response:** That is a good question. The occurrence probability of spontaneous HBsAg clearance or serological conversion is very low. A total of 289 inactive HBsAg carriers were followed up in You'an Hospital affiliated to Capital Medical University for 3 years. The results showed that 17 patients (2.60%) had spontaneous HBsAg serologic clearance, with an annual incidence of 0.87% [5]. Also, the annual rate of HBsAg seroclearance for NA-treated patients is very low (0.8%) [6], and it is assumed that approximately 52 years of NA therapy is required for most patients to achieve HBsAg seroclearance considering HBsAg kinetics [7]. In another follow-up study of 1,767 patients with HBeAg-positive chronic hepatitis B who were initially treated with nucleoside (acid) analogues (NAs) in You'an Hospital affiliated to Capital Medical University, the total annual HBsAg clearance rate was 0.46% in all patients. The annual clearance rates of HBsAg in the adefovir, entecavir, telbivudine, and interferon groups were 0.52%, 0.47%, 0.45%, and 1.18%, respectively [8].

5. The reported case received IFN, oral antivirals and HBV vaccination. What is the treatment recommendation of authors for these kind of chronic HBV patients?

**Response:** That is a very good question. Pegylated interferon (Peg-IFN) alfa provides a higher chance for HBsAg loss at the rate of 3%–7% after 1 year of Peg-IFN treatment compared with NAs [9, 10]. Recent randomized controlled trials investigated the effect of

adding or switching to Peg-IFN in patients with suppressed HBV in response to NA treatment rather than first-line Peg-IFN monotherapy in NA-naive patients [11]. HBV vaccination may also have a role in HBsAg seroclearance. In a human trial, recombinant HBV vaccination achieved an approximately 10% HBsAg seroconversion rate in the inactive carrier phase of chronic hepatitis B [12]. More importantly, a study in South Korea suggests that entecavir plus Peg-IFN alfa-2a treatment followed by sequential HBV vaccination on an intensified schedule significantly increased the chance for HBsAg seroclearance compared with entecavir alone [13].

6. Fig 2 and Table 1 include same data. Table 1 is not necessary if Fig 2 is provided.

**Response:** Thank you very much for your good suggestion. I have deleted Table 1.

Reviewer #3

1. In the discussion, it is written: ...patient with HBeAg-negative chronic hepatitis B, however, in the description of the case it is written HBeAg-positive. Despite it is obvious that it is the evolution of the HBeAg through the time, as described, it is difficult to follow the case.

**Response:** Thank you for your kind reminder. The patient was HBeAg-positive in 2000 and treated with lamivudine. In 2006, he was administered entecavir antiviral therapy, and HBeAg seroconversion was achieved in 2009. He has been HBeAg-negative since 2009.

2. The authors state that there was a reversal of over fibrosis (from G1S3 to G2S1), however, they did not explain why there is an increase in inflammation despite seroconversion, and apparently no other risk factors for other liver diseases (such as NAFLD).

**Response:** That is a very good question. This patient had an early elevation of ALT, which was thought to be associated with Peg-IFNα, which mediated immune inflammation. After 64 weeks of combined therapy, liver inflammation (G2) was still present, which was considered to be related to nonalcoholic fatty liver disease (NAFLD). Because the first liver pathology indicated approximately 15% steatosis, the second liver pathology increased to 25%. At the same time, the patient had better nutritional conditions after surgery and gained

significant weight in recent years. In addition, the patient was not taking other drugs with liver damage or drinking alcohol

3. It is not clear why in the presentation of the case the findings in the US are mentioned, but the image actually corresponds to MRI.

**Response:** Thank you for your careful work. I have made corresponding modifications, as follows: In October 2016, color Doppler ultrasonography showed a space-occupying hepatic lesion, and it was confirmed to be HCC (5.3×5×5cm) by enhanced magnetic resonance imaging (MRI) of the liver in Zhongshan Hospital affiliated to Fudan University (Figure 1). We have added the sentences in the Case Presentation section (page 6, lines 7-10).

4. The figures regarding histology don't show the described changes, and also there is no special staining for evaluating liver fibrosis.

**Response:** Thank you for your meticulous job. I have found a suitable picture and modified it accordingly (Figure 2).

## Reference


1 Ghany MG, Feld JJ, Chang KM, Chan HLY, Lok ASF, Visvanathan K, Janssen HLA. Serum alanine aminotransferase flares in chronic hepatitis B infection: the good and the bad. *The lancet Gastroenterology & hepatology* 2020; 5(4): 406-417 [PMID: 32057301 PMCID: PMC7158588 DOI: 10.1016/s2468-1253(19)30344-9]

2 Zhou HB, Li QM, Zhong ZR, Hu JY, Jiang XL, Wang H, Wang H, Yang B, Hu HP. Level of hepatitis B surface antigen might serve as a new marker to predict hepatocellular carcinoma recurrence following curative resection in patients with low viral load. *American journal of cancer research* 2015; 5(2): 756-771 [PMID: 25973313 PMCID: PMC4396049]

3 Chang TT, Liaw YF, Wu SS, Schiff E, Han KH, Lai CL, Safadi R, Lee SS, Halota W, Goodman Z, Chi YC, Zhang H, Hindes R, Iloeje U, Beebe S, Kreter B. Long-term entecavir therapy results in the reversal of fibrosis/cirrhosis and continued histological improvement in patients with chronic hepatitis B. *Hepatology* 2010; 52(3): 886-893 [PMID: 20683932 DOI: 10.1002/hep.23785]

4 Marcellin P, Gane E, Buti M, Afdhal N, Sievert W, Jacobson IM, Washington MK, Germanidis G, Flaherty JF, Aguilar Schall R, Bornstein JD, Kitrinos KM, Subramanian GM, McHutchison JG, Heathcote EJ. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. *Lancet* 2013; 381(9865): 468-475 [PMID: 23234725 DOI: 10.1016/s0140-6736(12)61425-1]

5 韦新焕，张晶，张卫，柳雅立. 非活动性 HBsAg 携带者临床特征及自发性 HBsAg 血清学

清除相关因素分析. *肝脏* 2020; 25(02): 128-130+154

6 Nagata N, Kagawa T, Hirose S, Arase Y, Tsuruya K, Anzai K, Shiraishi K, Mine T. Off-treatment durability of antiviral response to nucleoside analogues in patients with chronic hepatitis B. *BMC gastroenterology* 2016; 16: 38 [PMID: 26987437 PMCID: PMC4794926 DOI: 10.1186/s12876-016-0454-z]

7 Chevaliez S, Hézode C, Bahrami S, Grare M, Pawlotsky JM. Long-term hepatitis B surface antigen (HBsAg) kinetics during nucleoside/nucleotide analogue therapy: finite treatment duration unlikely. *Journal of hepatology* 2013; 58(4): 676-683 [PMID: 23219442 DOI: 10.1016/j.jhep.2012.11.039]

8 程凯亮, 徐曼曼, 邱倩, 李雁, 杨丽坤, 段小宛, 陈煜, 段钟平, 王丽. HBeAg 阳性慢性乙型肝炎患者 HBsAg 清除率:基于真实世界的比较效果研究. *中华肝脏病杂志* 2016; 24(05): 335-340

9 Lau GK, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, Cooksley G, Gane E, Fried MW, Chow WC, Paik SW, Chang WY, Berg T, Flisiak R, McCloud P, Pluck N. Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. *The New England journal of medicine* 2005; 352(26): 2682-2695 [PMID: 15987917 DOI: 10.1056/NEJMoa043470]

10 Janssen HL, van Zonneveld M, Senturk H, Zeuzem S, Akarca US, Cakaloglu Y, Simon C, So TM, Gerken G, de Man RA, Niesters HG, Zondervan P, Hansen B, Schalm SW. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. *Lancet* 2005; 365(9454): 123-129 [PMID: 15639293 DOI: 10.1016/s0140-6736(05)17701-0]

11 Zhang W, Xie Q, Ning Q, Dou X, Chen X, Jia J, Xie Y, Ren H. The role of peginterferon in nucleos(t)ide-analogue-treated chronic hepatitis B patients: A review of published literature. *Journal of viral hepatitis* 2017; 24(8): 618-623 [PMID: 28211135 DOI: 10.1111/jvh.12695]

12 Yalcin K, Acar M, Degertekin H. Specific hepatitis B vaccine therapy in inactive HBsAg carriers: a randomized controlled trial. *Infection* 2003; 31(4): 221-225 [PMID: 14562945 DOI: 10.1007/s15010-003-3187-1]

13 Lee JH, Lee YB, Cho EJ, Yu SJ, Yoon JH, Kim YJ. Entecavir Plus Pegylated Interferon and Sequential HBV Vaccination Increases HBsAg Seroclearance: A Randomized Controlled Proof-of-Concept Study. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2020 [PMID: 32556157 DOI: 10.1093/cid/ciaa807]