Point-by-point response to reviewers' comments

Dear Prof. Cui,

Thank you for considering our manuscript (manuscript number 39199) for publication in *World Journal of Hepatology*. We would also like to thank the reviewers for their helpful comments.We have now carefully considered each of the reviewers' comments and have now revised our manuscript accordingly. All changes are highlighted in the revised manuscript. In addition, all authors have approved the changes and the copyright agreement and Conflict of Interest statement are valid.

Below, we respond to the comments, starting with some general points and then moving onto a detailed point-by-point response.

Two reviewers and the Editor commented on the manuscript title. To address these concerns, we have now changed the title of our manuscript to '*Hepatitis B virus subgenotype F3 reactivation with vaccine escape mutations: A case report and review of the literature*'.

Reviewer #1 asked that the manuscript be shortened by 40%. We have made every effort to remove non-essential information and to delete all redundant phrases in the manuscript. The manuscript was given to an editing service for editing and shortages. However, the request from Reviewer #3 to provide more detailed information on certain points means that new text was introduced to the revised manuscript and for this reason, we have not been able to reduce the manuscript by the requested amount. We believe that the information within the manuscript is important for the reader to understand our study.

In the following, we respond to each of the Reviewers'comments in turn.

Reviewer #1 (02514886)

"Manuscript NO: 39199 Title "Hepatitis B virus subgenotype F3 reactivation with severe vaccine escape: A case report." By Dr. Stefan Schlabe, and colleagues.In this

reports, the authors describe a patient infected with a rare, subgenotyp F3 of HBV, which has the ability to escape eradication by vaccines. They have undertaken very extensive investigations to generate clinically relevant understanding most valuable in therapeutic settings. Therefore, I have no hard words for the authors. However, I believe that minor language editing throughout should very significantly benefit the impact of your manuscript. Starting from the title. The present title reads: "Hepatitis B virus subgenotype F3 reactivation with severe vaccine escape: A case report" I hope you notice that the words "with severe" is not appropriate. Please revise/edit the title."

Response: The title has been revised accordingly.

In Figure 3, the entry in red "Germany patient." I guess you could write "German patient"

Response: The red entry in Figure 3 has been edited to "German patient".

Additionally, the overall text volume for one case is too large. Please condense your manuscript text to about 60% of its' current size.

Response: We have shortened the text by removing redundant phrases and deleting non-essential information. However, we were not able to shorten the text by 40%because Reviewer #2 asked for additional information to be included (see below). We believe that further deletions will hinder the reader's understanding of our study and will eliminate the essential details required for a literature review.

Reviewer #2 (00722239)

SPECIFIC COMMENTS TO AUTHORS

This case report describes the case of HBV subgenotype F3 reactivation combined with several vaccine escape mutations. The manuscript is very interesting and well-written. I have only two minor comments.

1. Title: What is the "severe vaccine escape"? I wonder that is "several vaccine escape mutations".

Response: The title has been modified accordingly.

2. In Fig.1, The date on the horizontal axis is wrong. What is "01.11.95"?

Response: We have now changed the horizontal axis. Initially, we chose a "day-month-year" format for the time scale. We have changed it to a "month-year" format for a more accurate and clearer representation. Starting point is November 1995, when Hepatitis B was initially detected.

Reviewer #3 (03538158)

SPECIFIC COMMENTS TO AUTHORS

1. In this case report, it is unclear why they used HBV vaccine in patients with HBV DNA-positive. Authors should explain this in detail.

Response: Our HBV-positive patient was vaccinated in an attempt to induce cellular immunity and neutralizing antibody production. In the 1980s and 1990s, several pilot trials and animal studies tried to induce an immune response in patients with chronic HBV infection. Animal studies and studies of other chronic viral infections (e.g.HSV) had suggested a positive effect on the immune response and some experimental clinical trials were undertaken in patients. The first therapeutic vaccine trials used prophylactic recombinant vaccines. This was regarded as a logical choice because HBV treatment aimed to clear HBsAg and induce anti-HBs antibody production. The production of these antibodies is impaired in non-responders because of defects in cellular immunity. Therefore, different immunization schemes, delivery routes, and adjuvants were tested to induce antibody production. Most trials were done with pre-S2-/S-containing vaccines. However, in several controlled studies, immunization schemes and vaccines showed no benefit. Therapeutic vaccination never became a clinical routine and was a purely experimental measure.

We have now added a short passage to describe these experimental attempts at therapeutic vaccination and have provided two publications which illustrate the heterogeneity of therapeutic vaccination of HBV-positive patients.

2. Authors should describe the amino acid mutations authors found, compared to the sequences, which has been reported.

Response: We have provided details about the most important vaccine escape mutations at positions 120, 129, and 145. The most important mutation is G145R. While the P120T and P120S mutations at position 120 are well known, the P120Q was found in one Korean child (Lee 2001) and the effect of this amino acid exchange was assumed to be similar to those known mutations. Changes in position 120 disrupt the structure of the "a" domain. The same is true for exchanges in positions 129 and 142. Q129H and P142S mutations were described in the 1990s as escape variants (Review Torresi 2002). F134V revealed no differences in antigenicity (Hossain 2017). A128V was described in an Italian blood donor with occult HBV infection (Capezzuto 2010). P142L was described in a liver transplant patient with recurrence and L109I was documented in a HIV-coinfected patient with HBV reactivation; these samples were tested in commercial assays and the mutated variants were difficult to detect by antibody-based tests (Ly et al. 2006). Most published mutations were in the HBV genotypes A–D and most have not been clearly documented in genotype F. Owing to overlapping reading frames, mutations in the S domain correspond with the polymerase gene that influences the replication fitness of the variants. Albeit very interesting, a detailed discussion on all the mutations would be far beyond the scope of a case report. We have now cited a recent review for further reading (Coppola 2015). Furthermore, we have assembled these details in a table which can be provided as a supplemental file, which we attached to the revised manuscript.

References

Capezzuto C, Franchi E, Urbani S, Romano L, Franchini M. Occult hepatitis B in blood donors: a description of two cases. Blood Transfus 2010;8(4):297-302. [PMID: 20967173 DOI: 10.2450/2010.0119-09]

Coppola N, Onorato L, Minichini C, Di Caprio G, Starace M, Sagnelli C, et al. Clinical significance of hepatitis B surface antigen mutants. World J Hepatol 2015;7(27):2729-39.[PMID: 26644816 DOI: 10.4254/wjh.v7.i27.2729]

Hossain MG, Ueda K. Investigation of a Novel Hepatitis B Virus Surface Antigen (HBsAg) Escape Mutant Affecting Immunogenicity. PLoS One 2017;12(1):e0167871.[PMID: 28045894 DOI: 10.1371/journal.pone.0167871]

Lee KM, Kim YS, Ko YY, Yoo BM, Lee KJ, Kim JH, et al. Emergence of vaccine-induced escape mutant of hepatitis B virus with multiple surface gene mutations in a Korean child. J Korean Med Sci 2001;16(3):359-62.[PMID: 11410701 DOI: 10.3346/jkms.2001.16.3.359]

Ly TD, Servant-Delmas A, Bagot S, Gonzalo S, Ferey MP, Ebel A, et al. Sensitivities of four new commercial hepatitis B virus surface antigen (HBsAg) assays in detection of HBsAg mutant forms. J Clin Microbiol 2006;44(7):2321-6. [PMID: 16825343 DOI: 10.1128/JCM.00121-06]

Torresi J (2002) The virological and clinical significance of mutations in the overlapping envelope and polymerase genes of hepatitis B virus. J Clin Virol 25: 97-106 [PMID:12367644]