Round1

Reviewer #1: This is a prospective multicentric study to assess subclinical proximal tubular dysfunction in HBV-patients undergoing TDF or ETV or naïve patients. The results are inconclusive, as the trend in favor of ETV did not reach significance, most likely due to underpowered size or relatively short F-U (24 months).

Of course, we agree with the limitations of this study due essentially to insufficient sample size. However, the HR for the cumulative incidence of SPT associated with TDF versus naive group was 2.28, showing a trend towards significance in favor of TDF tubular toxicity (please see page 15, adjusted analysis and page 16 discussion section).

Finally, the median survival without SPT, evaluable only in the TDF group, was 5.9 months. The occurrence of SPT was significantly different for TDF group compared to the others (log-rank test p=0.0283) p 15.

I would like the authors further clarify some key aspects:

1. How was the sample size calculated?

The number of subjects was calculated on the basis of two comparisons under the main objective with a power of 80% and an alpha risk of 0.025 for each comparison (i.e. 0.05 for the entire analysis). The difference in the prevalence of SPT at 24 months between groups was hypothesized as follows:

- 5% of SPT prevalence in naïve patients compared to 15% in patients treated with TDF on one hand,

- 5% of SPT prevalence in naive patients compared to 15% in patients treated with ETV on the other hand.

It was necessary to include 200 patients per group (naive untreated / treated with ETV / treated with TDF). When taking into account the patients lost to follow-up, a total of 700 patients should have been included.

The basis of our calculation assumptions was probably wrong in particular we could have anticipated a much higher prevalence of SPT in the tenofovir arm than in the naïve arm at

M24, and a prbably comparable prevalence in the entecavir and naive arms. But the main problem was the number of patients lost to follow-up or with incomplete and/or unusable data in this labile population.

How were the patients assigned to each one of the three options?

This is a prospective real-life study, HBV-patients being treated or not, according to EASL recommendations[15]. When they were treated, the therapeutic choice depended on the decision of the investigator. We clarify this in the methods section page 11.

2. There could be a selection bias as the baseline parameters that could influence renal function were not well balanced regarding drug assignation.

We completely agree with the reviewer. (please see corrections page 20 of the discussion)
In addition, we were able to limit the role of confounding factors in the interpretation of the prevalence or incidence of SPT, as the whole population was homogeneous, characterized by a young age (median 37.5 years old) and very few renal comorbidities. All the patients had an eGFR above 50mL/min/1.73m^2 at inclusion. In addition, all analyzes were subsequently adjusted for factors that were not comparable at baseline.

Reviewer #2: The authors investigated the prevalence and incidence of proximal tubulopathy in patients with chronic hepatitis B, particularly in patients treated with ETV or TDF. The study is well performed and the statistical evaluations seems to be completely correct. However, I have some questions. 1. The authors state that there are no data about the prevalence of SPT in the general population. If that is true, where does the cut-off originate to judge one of the markers as indicator of SPT? 2. It is difficult to understand why the authors haven't included a matched study population without HBV infection. This could be done without problems. 3. The main study criterium was the prevalence of SPT at month 24. Why didn't they investigate markers of early tubulotoxicity, this means early after start of therapy? 4. How was the course of SPT during antiviral therapy? Early occurrence and attenuation after one year (or later)? 5. Is there a dose dependency of tubulotoxicity? Cumulative dose of antivirals? Dose-independent tubular damage ("Idiosyncratic

renal damage")? 6. Is the choice of the two markers really ideal? The authors should explain, why they have not used more specific markers of tubulotoxicity?

1. The authors state that there are no data about the prevalence of SPT in the general population. If that is true, where does the cut-off originate to judge one of the markers as indicator of SPT?

This comment from the reviewer is absolutely correct. In fact, the markers we chose in this study have been in use for many decades and validated in populations of healthy subjects. Moreover these markers have been widely and classically used in articles dealing with the toxicity of nucleotide analogues, for instance in a very recent article in the HIV population (PMID: 32757496).

The adult 95% reference range is between 0.80-1.35 mmol/L for TmP/eGFR. Independently of age, normal values are above 0.8 mmol / L in healthy subjects[28]. The normal value of FEUA is about 8% and values above 10% are chosen to diagnose a reabsorption defect[29]. We made the correction in the text and added the bibliographical references. (please see discussion page 18)

2. It is difficult to understand why the authors haven't included a matched study population without HBV infection.

The absence of a matched control population not infected with HBV could be considered a limitation of the study and we cannot definitively conclude that there is a direct link between SPT and HBV-infection. However, we can strongly suggest this hypothesis as the tubular markers we chose have been in use for many decades and validated in populations of healthy subjects. Please see the comment above and discussion P 18.

3. The main study criterium was the prevalence of SPT at month 24. Why didn't they investigate markers of early tubulotoxicity, this means early after start of therapy?

Studying the onset of SPT, we evaluated SPT every 3 months up to M24. In the three groups, the incidence of SPT over time was described by the Kaplan Meyer for free SPT survival

among the different groups. The curve shows an early onset of SPT before the 6th month for tenofovir and indeed median SPT-free survival only assessable in the TDF group was 5.9 months.

Please see Figure 2. (The SPT in the naive group seems to occur progressively over time and the ETV group seems to be little affected, suggesting a possible protective effect of ETV on potential HBV tubular toxicity, but no statistical conclusion can be given)

4. How was the course of SPT during antiviral therapy? Early occurrence and attenuation after one year (or later)?

   Please see the comment above.

5. Is there a dose dependency of tubulotoxicity?

Nucleot(s)ides analogues dosage, especially TDF, could have been useful to explore the dose dependency of tubulotoxicity. Polymorphisms at genes coding for transporter proteins involved in TDF elimination (ABCC2 or ABCC4 genes) have been associated with tubular renal damage suggesting that overexposure to TDF could cause kidney tubular cell damage. In a recent study in HIV-infected patients, Rodriguez-Novoa et al[37] showed that median TDF plasma trough concentration was higher in patients with SPT defined with the same early tools as ours. Even if this finding could suggest cumulative toxicity, the causative role for elevated TDF plasma concentration in the development of SPT could not be demonstrated in this cross-sectional analysis.

Unfortunately, dosages of TDF were not available and were not recommended at the time of the study in our country. Please see discussion Page 21.

6. Cumulative dose of antivirals? Dose-independent tubular damage ("Idiosyncratic renal damage")?

The overexposure of tenofovir has so been suggested but not proven in the mechanisms of toxicity. Finally, the mechanism of tubular toxicity is probably not idiosyncratic and could be due to a cumulative dose effect: indeed, a recent paper suggested a progressive mitochondrial dysfunction as a mechanism of TDF tubular toxicity[38] Please see discussion Page 21.

7. Is the choice of the two markers really ideal? The authors should explain, why they have not used more specific markers of tubulotoxicity?

Periodic screening of tubular function parameters should be recommended to patients receiving TDF : they must be inexpensive and easy to use

Other more sophisticated markers like RBP and Beta2 microglobulin are interesting but their dosage is expensive and not widely used.

Kim1 and NGal are rather markers of acute tubular injury which is far too late for the diagnosis[19].

(1) Science editor: 1 Scientific quality: The manuscript describes a prospective study of the subclinical proximal tubulopathy in hepatitis B. The topic is within the scope of the WJH. (1) Classification: Grade C, Grade C, and Grade E; (2) Summary of the Peer-Review Report: The study is well performed and the statistical evaluations seems to be completely correct, but the results are inconclusive. **The questions raised by the reviewers should be carefully answered**; and (3) Format: There are 4 tables and 2 figures. A total of 34 references are cited, including 5 references published in the last 3 years. There are no self-citations. 2 Language evaluation: Classification: Grade B, Grade B, and Grade C. 3 Academic norms and rules: The authors provided the Biostatistics Review Certificate, the Clinical Trial Registration Statement, the signed Conflict-of-Interest Disclosure Form and Copyright License Agreement, and the Institutional Review Board Approval Form, the Written informed consent, and the CONSORT 2010 Statement. No academic misconduct was found in the CrossCheck detection and Bing search. 4 Supplementary comments: This is an invited manuscript. The topic has not previously been published in the WJH. 5 Issues raised: (1) **The language classification is Grade C. Please visit the following website for the professional English language editing companies we recommend: https://www.wjgnet.com/bpg/gerinfo/240;** (2) **The authors did not provide original pictures. Please provide the original figure documents**. **Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor**; and (3) **The "Article Highlights" section is missing. Please add the "Article Highlights" section at the end of the main text.** 6 Re-Review: Required. 7 Recommendation: Conditional acceptance.

(2) *Editorial office director:* I have checked the comments written by the science editor. **This study was supported by Bristol Myers Squibb, France. Please upload the approved grant application form(s) or funding agency copy of any approval document(s).**

(3) *Company editor-in-chief*: I have reviewed the Peer-Review Report, the full text of the manuscript and the relevant ethics documents, all of which have met the basic publishing requirements, and the manuscript is conditionally accepted with major revisions. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report and the Criteria for Manuscript Revision by Authors. Before final acceptance, authors need to correct the issues raised by the editor to meet the publishing requirements. Re-Review: Required.

4.4 Requirements for figures: Please provide the decomposable Figures, whose parts are all movable and editable, organize them into a PowerPoint file, and submit as "**56366-Figures.ppt**" on the system. The figures should be uploaded to the file destination of "Image File".

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4.7 Requirements for article highlights: If your manuscript is an original study (basic study or clinical study), meta-analysis, or systemic review, the "Article Highlights" section should be provided. Detailed writing requirements for "Article Highlights" can be found in the Guidelines and Requirements for Manuscript Revision.

4.8 Language quality: Please resolve all language issues in the manuscript based on the peer review report. Please be sure to have a native-English speaker edit the manuscript for grammar, sentence structure, word usage, spelling, capitalization, punctuation, format, and general readability, so that the manuscript's language will meet our direct publishing needs.

Round2

Editor's comments (02541859) I do not understand 'The median survival time without SPT in the tenofovir disoproxil-treated group was 5.9 mo'. Thank you. Otherwise looks good.

Response : The median survival time, corresponded to the time during which more than 50% of the patients remained SPT-free. Please see page 15 and correction of the abstract page 7, and the core tip page 9