

Reviewer #1:

It is a great investigation related to serum HBV-RNA quasispecies establishment with a NGS method. Authors found the none significant difference between HBC-RNA and HBC-DNA in the first place and established a practical NGS method to analyze HBV-RNA. This HBV-RNA quasispecies has been produced and well studied in the variability/conservation and complexity. HBV genetic test in clinical is quite important and benefit lots of chronic hepatitis B infection. HBV-RNA is a little-studied subfield in the corresponding research area. A new method to analyze serum HBV-RNA quasispecies without HBV-DNA interference is needed. Well, there're some minor revisions authors should addressed before the publication.

- 1. Authors did not list any limitations of the study and its findings clearly while the future directions of the topic had been described in the last paragraph of Discussion.**

Thank you for your positive comments about our manuscript and for your suggestions. In our view, the main limitation of our study was that the present comparison of complexity and conservation of circulating HBV-DNA and RNA quasispecies with this small and heterogeneous group of patients just enabled to take a preliminary picture of HBV-DNA and RNA quasispecies, which needs to be complemented with further studies exploring both of them in larger and more homogeneous groups of patients in terms of severity of liver disease and CHB clinical stage, in order to verify whether the present results could be extrapolated to any stage of the disease. In addition, the implications of HBV quasispecies parameters analyzed in the context of both treatment and prognosis of chronic hepatitis B are still uncertain. Thus, longitudinal follow-up studies of circulating HBV-RNA quasispecies evolution are necessary to assess its adaptation to different evolutive pressures and to compare with that of HBV-DNA. We added a note explaining this point of view in the last paragraph of discussion, lines 538 to 549.

- 2. When author showed the results with statistical analysis, such as Line 370-374, the exact test method should be mentioned for better understanding of the study.**

We would like to thank the reviewer for these suggestions. As indicated, the exact statistical tests used have been mentioned in the parts of results section with statistical analyses, in order to help understanding of the present study. Please see “Comparison of HBV-DNA and RNA Quasispecies Using NGS” section in Results, lines 373 (the same statistical analysis is mentioned in the legend of figure 2, where the statistical test used was also mentioned), 377 and 379.

Moreover, by reviewing the statistical tests used we noticed that forgot to comment the two-proportions z-test with Yates continuity correction, which we used to compare the percentages of positions with some variability, i.e. information content (IC) < 2 bits, between HBV-DNA and RNA quasispecies. This previously unmentioned test has been added to the “Statistical Analysis” section of Material and methods (line 342). Thus, statistical comparisons between HBV-RNA and DNA quasispecies complexity were performed using the Kruskal-Wallis test, t test and two-proportions z-test with Yates continuity correction where appropriate. This test has also been cited in “Comparison of HBV-DNA and RNA Quasispecies Using NGS” section in Results, where it has been

used (lines 397-398). Thank you for helping us to clarify the statistical analysis of the study.