PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Hepatitis B virus infection among Chinese patients with hepatitis C virus infection: prevalence, clinical characteristics, viral interactions and host genotypes, a nationwide cross-sectional study
AUTHORS	Yan, LiBo; Rao, HuiYing; Ma, YuanJi; Bai, Lang; Chen, En-Qiang; Du, LingYao; Yang, RuiFeng; Wei, Lai; Tang, Hong

VERSION 1 - REVIEW

REVIEWER	Coppola Second university of NAples
REVIEW RETURNED	03-May-2016

GENERAL COMMENTS	The manuscript “Hepatitis B virus infection among Chinese patients with hepatitis C virus infection: prevalence, clinical characteristics, viral interactions and host genotypes” evaluates the prevalence and the demographic and clinical characteristics of HBV infection in 997 Chinese anti-HCV positive patients. The prevalence of HBV coinfection was low (4.11%); the HBsAg positive patients had similar epidemiologic characteristics of those HBsAg negative but a higher prevalence of cirrhosis. The manuscript has some limitations 1. The study seems to have a low interest, since the results presented are already extensively known in literature. 2. As reported by the authors, the data HBV viral load were not available 3. The data on HBeAg/anti-HBe status were not reported 4. The authors should more clearly report the criteria of diagnosis of cirrhosis 5. The histological data were not available 6. The diagnostic criteria for fatty liver seems to be not standardized Minor Points: 1. The authors should report the serological status for HIV infection 2. The tables 2, 3 and 4 may be organized in one table 3. Some sentences are not clear to the reviewer a. Page 11, lines 14-16 b. Figre 1, page 25: “number of patients with minor deviation”

REVIEWER	Fokam, J UTV
REVIEW RETURNED	10-Jun-2016

GENERAL COMMENTS	The study by Yan et al. describes an important public health issue in

	the people republic of China: HCV and HBV infections, and potential pathogenesis. The study objectives, methods and results are cohorent. However, there are issues to be addressed in the abstract, the discuss and conclusion sections. I. Essential comments: page 3: Abstract: the conlcusion in the bastract is not supported by the findings. See suggested revision in the attached file. Page 4: Strengths and limitations: Several limitataions were left out by the authors, for instance: - No available data on occult HBV due to HBV DNA not performed. Tthis leads to possible under-estimateion of the real burden of HBV in this study population; - No sequencing of HBV genes which could provide insights on molecular epidemiology, escape-mutations and drug resistance variants in this study population; - No quantitative measurement of Ag HBs, which could provide insights on the cccDNA metabolic effect at the liver level. - Page 7: Methods: start with study design (as re-arranged in the revised paper attached), and followed other revisions suggested; - Page 8: Diagnosis of fatty liver could be affected by bias due to varying techniques used. Could authors clearly addressed how these were mitigated. Otherwise related results might be less relevant to aggregate and interprete together; - Page 8: Include a sub-section on Data quality control and validation - Page 8 statistical analysis: Log are better expressed as mean and not median, since normally distributed; - Page 13: Discussion: Delete lines 1-3. Delete the sentence in lines 20-22 Line 26: Add a sentence to explain that the huge statistcal difference between the two stratified groups could account for the observed disparities, thus suggesting a lower statistical power; Line 30: Start a new paragraph with the new sentence. - Page 14: Lines 1-5: Replace completely the statements, by discussing the effective public health implications of the findings in the frame of DAA usage nowadays; Lines 10-25: revise as indicated in the attached file. - Page 15: Revise as indicated in the attached file. In the conclusion, Give the epidemiological interpretation of the HBV burden found 8low, moderate, high?) in this HCV-infected Chinese populations. Highlight the subgroup in need of closer monitoring and the related lessons for global perspectives. - Pages 17-20: References should be revised for conveneience with the journal. Add references on occult hepatitis B and utility of quantitative AgHBs, to better showcase limitations and areas for further investigations. II. Minor comments: - Revise the english language and some grammatical errors (many

	indciated in the attached file). - Add missing statistcial values to support significance in some results. The reviewer also provided a marked copy with additional comments. Please contact the publisher for full details.

REVIEWER	Ivana Lazarevic Institute of Microbiology and Immunology, Faculty of Medicine, University of Belgrade, Serbia
REVIEW RETURNED	21-Jul-2016

GENERAL COMMENTS	The manuscript titled “Hepatitis B virus infection among Chinese patients with hepatitis C virus infection: prevalence, clinical characteristics, viral interactions and host genotypes” is addressing an interesting subject of dual HCV/HBV infection. Although this topic is wildly investigated in recent years, data from nation-wide, multicenter study are new and useful. Points to be regarded: The language is a significant drawback of the manuscript and therefore it should be re-written with help of native speaking scientist. In Patients and Methods: The method for detection of SNPs in IL28 region was not at all mentioned. The method HBsAg detection was just mentioned as ELISA without any specificities (like the name and manufacturer). In Discussion: The Discussion is thorough but has a lot of repetitions and could be shorter. There are some sentences which are incomprehensible due to language problems: Page 12, line 10; Page 13, line 2 and line 15; Page 14, line 19

VERSION 1 – AUTHOR RESPONSE

Reviewer 1.

1. The study seems to have a low interest, since the results presented are already extensively known in literature.

Author Response: Thank you very much for this kindly suggestion.

Despite the previous study showed that 2-10% HCV patients might be infected with HBV in some regions, such conclusion can not be simply applied to other regions such as China because the geographic distribution of these two viruses was different. In this study, HCV patients were enrolled during a defined period of time at 28 representative large hospitals in provinces across China. Though the research results for other countries might be well known, this is the first such research on Chinese population, as far we knew.

Those patients were representative of the current situation in China, making the analysis of clinical characteristics, viral interactions and host genotypes associated with HBV/HCV dual infection more useful. (line 12-19 of page 5 , line 2-26 of page 12 in the marked manuscript)

2. As reported by the authors, the data HBV viral load were not available

Author Response: Thank you for this kindly advice. HBV viral load is important for evaluating prevalence of occult HBV infection and viral interactions of HBV/HCV. Unfortunately we didn’t have the data of HBV DNA due to the shortage of serum. New research designed with a new cohort of HBV/HCV dual infected patients is underway. In the new cohort, we will collect the data of HBV viral

load, which will be published later separately. We add a new section about the limitations about HBV DNA and occult HBV in “Strengths and limitations of this study” on page 4. (lines 12-16 of page 4 in the marked manuscript)

3. The data on HBeAg/anti-HBe status were not reported
Author Response: Thanks for this kindly suggestion.
We agree that HBeAg/anti-HBe status is important for the evaluation of HBV infected patients. The main aim of this study is to evaluate the prevalence of HBV infection among Chinese HCV patients. The diagnosis of HBV infection is based on HBsAg. The status of HBsAg can provide the most important information of HBV. As indicated above, no data of HBeAg/anti-HBe status were included in this manuscript due to the shortage of serum. Our center is working on a new cohort of HBV/HCV dual infected patients. In the new cohort, we will collect the data of HBeAg/anti-HBe status for future analysis. We added the limitations about HBeAg/anti-HBe status in “Strengths and limitations of this study” on page.4. (lines 12-13 of page 4 in the marked manuscript)

4. The authors should more clearly report the criteria of diagnosis of cirrhosis
Author Response: Thank you very much for this kindly suggestion.
We added the criteria of cirrhosis diagnosis in the material and method section. Cirrhosis was diagnosed by liver biopsy, or by a Fibroscan (Echosens, Paris, France) score of more than 13 kPa, or radiologic imaging showing nodular liver or splenomegaly combined with platelet count <100,000. Decompensated cirrhosis was defined as cirrhosis with sequelae such as ascites, variceal bleeding, and hepatic encephalopathy or hepatorenal syndrome. (lines 22-27 of page 8 in the marked manuscript)

5. The histological data were not available
Author Response: Thanks for this kindly advice.
We agreed with you that histology data is important. Due to the features of invasiveness, high cost and potential complications of biopsy procedure, the acceptance of this method by patients was limited in China. Only a small group of patients were performed liver biopsy in this study. It’s a limitation of this study.

6. The diagnostic criteria for fatty liver seems to be not standardized
Author Response: Thank you very much for this kindly suggestion.
We apologized for did not verify the clear diagnostic criteria with Peking University People's hospital. After verification with the Peking University People's hospital staff, we added this diagnostic criteria for fatty liver. Fatty liver was diagnosed by liver biopsy, or by hepatic imaging (hepatic ultrasound, computerized tomography, magnetic resonance imaging). (lines 27-29 of page 8 in the marked manuscript)

Minor Points:
1. The authors should report the serological status for HIV infection
Author Response: Thank you very much for this kindly suggestion.
This study did not identify patients with possible HCV/HIV dual infection. It was due to a concern of potential impact on patient enrollment. Due to the shortage of serum, we can’t retest the HIV antibody.

2. The tables 2, 3 and 4 may be organized in one table
Author Response: Thanks for this kindly suggestion. We have organized Table.2 and Table.4 in one table (Table 3) now. Table.3 was adjusted the order into Table.2.

3. Some sentences are not clear to the reviewer

Author Response: We already revised the manuscript accordingly and the new version has been improved.
a. Page 11, lines 14-16
The sentences “Similar distributions of genotypes of other IL28B SNPs in response to peginterferon/ribavirin treatments were observed” was changed into “The frequency distribution of IL28B host genotypes for other 12 SNPs based on HBV infection is shown in Table 4. However, no IL28B host genotypes showed evidenced strong statistical association with HBV /HCV dual infection.” (Lines 14-17 of page 11 in the marked manuscript)
b. Figure 1, page 25: “number of patients with minor deviation”
Thanks for this kindly suggestion. It was change into “Numbers of patients with minor protocol deviation, but it didn’t impact the subject eligibility”. (Figure.1)

Reviewer: 2
Reviewer Name

page 3: Abstract: the conlcusion in the abstract is not supported by the findings. See suggested revision in the attached file.
Author Response: Thanks for this kindly suggestion.
The conclusion is modified. The conclusion is “The HBV burden was moderate among HCV-infected patients in China. Liver cirrhosis was more common in patients with HBV/HCV dual infection, suggesting a closer monitoring for dual-infected individuals.” (Lines 23-25 of page 3 in the marked manuscript)

Page 4: Strengths and limitations: Several limitations were left out by the authors, for instance:
- No available data on occult HBV due to HBV DNA not performed. This leads to possible under-estimation of the real burden of HBV in this study population;
Author Response: Thank you very much for this kindly useful suggestion.
We have added the limitations in Page 4. (line12-24 of page 4 in the marked manuscript). The limitations are also mentioned in the discussion.
- The data in this study did not provide HBV DNA levels and HBeAg/anti-HBe status due to the shortage of serum in the dual infection group
- No available data on occult HBV due to HBV DNA not performed. This leads to possible under-estimateion of the real burden of HBV in this study population.
- No sequencing of HBV genes which could provide insights on molecular epidemiology, escape-mutations and drug resistance variants in this study population;
- No quantitative measurement of HBsAg, which could provide insights on the cccDNA metabolic effect at the liver level.

Page 7: Methods: start with study design (as re-arranged in the revised paper attached), and followed other revisions suggested;
Author Response: Thanks for this kindly advice.
We have re-arranged the methods, including starting with study design, adding the enrollment procedure and separating a paragraph for the ethical consideration (line 2-25 in page 7 in the marked manuscript). Other suggested revisions also re-corrected in the revised manuscript.

Page 8: Diagnosis of fatty liver could be affected by bias due to varying techniques used. Could authors clearly addressed how these were mitigated. Otherwise related results might be less relevant to aggregate and interprete together;
Author Response: Thanks for this kindly suggestion.
We apologized for did not verify the clear diagnostic criteria with Peking University People's hospital. After verification with the Peking University People's hospital staff, fatty liver was diagnosed using by liver biopsy, or by hepatic imaging (hepatic ultrasound, computerized tomography, magnetic

resonance imaging). (lines 27-29 of page 8 in the marked manuscript)

- Page 8: Include a sub-section on Data quality control and validation
Author Response: Thank you very much for this kindly suggestion.
Peking University People's Hospital and Bristol-Myers Squibb designed the protocol. All the data were inputted in Electronic Data Capture System by each center, which checked by a clinical research associate. The contract research organization controlled and validated the data quality. (lines 1-4 of page 9 in the marked manuscript)

- Page 8 statistical analysis: Log are better expressed as mean and not median, since normally distributed;
Author Response: Thank you very much for this kindly suggestion. We have added "HCV RNA levels were log-transformed, which expressed as mean values and examined using the Student t-test" in the statistical analysis (line 8-10 in page 9 in the marked manuscript). We also change HCV RNA into mean values in Table 1 and Table 4.

- Page 13: Discussion: Delete lines 1-3. Delete the sentence in lines 20-22
Author Response: Thank you very much for this kindly suggestion. We already revised the manuscript accordingly.

- Page 13 Line 26: Add a sentence to explain that the huge statistcal difference between the two stratified groups could account for the observed disparities, thus suggesting a lower statistical power;
Author Response: Thanks for this kindly advice.
We have added a sentence to explain the reasons for the observed disparities. Due to the lack of HBV DNA data, We did not perform statistical comparison between stratified groups based on HBV DNA. The observed disparities might be accounted by huge statistical difference between the stratified groups based on HBV DNA. (Lines 24-26 of page 13 in the marked copy)

Line 30: Start a new paragraph with the new sentence.
Author Response: Thank you very much for this kindly suggestion. We already revised the manuscript accordingly.

- Page 14: Lines 1-5: Replace completely the statements, by discussing the effective public health implications of the findings in the frame of DAA usage nowadays;
Author Response: Thank you very much for this kindly advice. We have discussed implications of IL-28B genotypes in the frame of DAA usage nowadays. The common therapy is now gradually shifting to an era of direct-acting antivirals (DAAs). Overall sustained virological response rates (SVR) of DAAs are equally high and above 90% in numerous patient cohorts. Overall SVR of ledipasvir and sofosbuvir can still above 90% for untreated HCV genotype 1 infected patients, most of them with non-CC IL-28B genotype. While others studies proved that all the patients with genotype 4 and 5 who did not achieve SVR12 treated with ledipasvir and sofosbuvir had non-CC IL-28B genotype. A head-to-head study was needed to explore the effect of IL-28B on DAAs treatment. So far there was no study focus on the efficacy of DAAs in HBV/HCV coinfection patients. Our study has established a good foundation for generating hypotheses for future studies. (Lines 6-14 of page 14 in the marked copy)

Lines 10-25: revise as indicated in the attached file.
Author Response: Thank you very much for your careful modification. We have modified the sentences that were marked in red color.

- Page 15: Revise as indicated in the attached file.
In the conclusion, Give the epidemiological interpretation of the HBV burden found 8low, moderate,

high?) in this HCV-infected Chinese populations. Highlight the subgroup in need of closer monitoring and the related lessons for global perspectives.
Author Response: Thank you very much for this kindly advice. We have added the HBV burden was moderate among HCV-infected patient in the conclusion. Because previous studies showed that 2-10% HCV patients might also be infected with HBV in some regions. The estimated prevalence of HBV infection was 1.3%-5.8% among HCV infection patients in United States. Therefore, the HBV burden was moderate among HCV-infected patient.
Liver cirrhosis was more common in patients with HBV/HCV dual infection than in patients with HCV mono-infection, suggesting a closer monitoring for dual-infected individuals in China. (Lines 20-25 of page 15 in the marked copy)

- Pages 17-20: References should be revised for convenience with the journal. Add references on occult hepatitis B and utility of quantitative HBsAg, to better showcase limitations and areas for further investigations.
Author Response: Thank you very much for this kindly advice. We have revised the references according to the journal requirement. We have added references on occult hepatitis B and utility of quantitative HBsAg, to better showcase limitations of this study and new areas for further investigations. (Lines 11-17 and 22-26 of page 20 in the marked copy)

II. Minor comments:

- Revise the english language and some grammatical errors (many indicated in the attached file).
Author Response: We already had the manuscript revised by an American scientist who is a native-English speaker and is doing research in related scientific field.

- Add missing statistcial values to support significance in some results.
Author Response: Thanks for this kindly suggestion. We have added the statistical values. The HBsAg prevalence by age was 0.70%, 3.97%, and 5.85% for the age groups of 18-30, 30-50,and >50 years, respectively (P=0.057). The difference of HCV genotypes between the two groups was not statistically significant (23/41 versus 559/956, P=0.493)。(Lines 9-11 of page 10, Lines 6-7 of page 11 in the marked copy)

Reviewer: 3

1. The language is a significant drawback of the manuscript and therefore it should be re-written with help of native speaking scientist.
Author Response: We already had the manuscript revised by an American scientist who is a native-English speaker and is doing research in related scientific field.

In Patients and Methods:
2. The method for detection of SNPs in IL28 region was not at all mentioned.
Author Response: Thanks for this kindly suggestion.
We have supplemented the method for detection of SNPs in IL28 region. Thirteen single nucleotide polymorphisms (SNPs) within the IL28B genomic region were genotyped. The host genotype was identified by iPLEX Gold (Sequenom,San Diego, CA, USA) conducted centrally (CapitalBio, Beijing,China), a platform that could map SNP validation. For genetic markers, we applied the following quality control criteria: call rate< 90%, Hardy–Weinberg P-value < 0.005 or low-quality genotype clustering. No individuals were excluded due to high genotyping call rate. (Lines 17-21 of page 8 in the marked copy)

3. The method HBsAg detection was just mentioned as ELISA without any specificities (like the name and manufacturer).

Author Response: Thanks for this kindly suggestion.
We have added name and manufacturer of HBsAg ELISA kit (Abbott Laboratories, Abbott Park, IL, USA). (Lines 8-9 of page 8 in the marked copy)

In Discussion:
The Discussion is thorough but has a lot of repetitions and could be shorter.
Author Response: Thank you very much for kindly suggestion.
We have deleted all repetitions and rewrote the discussion section.

There are some sentences which are incomprehensible due to language problems:
Page 12, line 10; Page 13, line 2 and line 15; Page 14, line 19
Author Response: We already had the manuscript revised by an American scientist who is a native-English speaker and is doing research in related scientific field.

Lastly, we apologized for the typo in the enrollment criteria. We confirmed with Peking University People's hospital staff that HCV infection was confirmed or reconfirmed (anti-HCV antibody and HCV RNA positive) within 90 days prior to the enrollment.

I hope these modifications and my responses to the reviewer's comments are satisfactory. I have also submitted the entire revised manuscript that has the modified parts marked as red color through submission site. The sentences changed the order were marked as green color.
Thank you for your help in this matter.