Author's response to reviews

**Title:** Genotypic Distribution and Hepatic Fibrosis Among HIV/HCV Co-infected Individuals in Southern China: A Retrospective Cross-sectional Study

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**Author's response to reviews:** see over
Dear BMC Infectious Diseases Editors:

I am pleased to resubmit the MS: 1055366814156401 “Genotypic Distribution and Hepatic Fibrosis Among HIV/HCV Co-infected Individuals in Southern China: A Retrospective Cross-sectional Study.” We thank the reviewers for their comments and believe the changes we made have resulted in a stronger manuscript. I have addressed each of their concerns as outlined below. Find attached a tracked changes version, a clean version, and a detailed list of responses to reviewer comments.

**Reviewer #1 Comments:**

**Major Compulsory Revisions**

1. This should be a cross-sectional study on HCV epidemiology, but however, the authors did include only ART-naive patients. This exclusion criteria seems to introduce an important bias that could easily be avoided. Also the authors excluded patients with decompensated cirrhosis, severe cytopenias, etc… since this is a non-interventional study, there is no reason to exclude those patients – especially when the study aims to give a perspective on HCV epidemiology.

   We agree with the reviewer that the manuscript is unclear about sampling and who was included in the study. There are two clarifications. The first is that some co-infected patients were on ART (n=37). This error in the methods section has been revised on page 5.

   The second is that this study is a secondary analysis of a clinical HCV co-infection treatment study. The original study excluded individuals with severe cytopenias and decompensated cirrhosis. The mono-infected cohort was not recruited for the HCV treatment study but were chosen based on the same exclusion criteria to minimize bias. Excluding patients with the most severe disease, which certainly would impact fibrosis scores, likely has a smaller effect on genotypic distribution. We have clarified the methods section on page 5 to reflect this secondary analysis. We have also addressed this limitation in the discussion. Despite this limitation, we still believe this study has value in extending current literature on co-infection in Southern China.

2. The authors report results only for FIB-4 scores and not for APRI. Although the discuss it in the methods. In general, since the authors exclude patients with cirrhosis and cytopenias (thrombocytopenia as a marker of portal hypertension), this analyses does not really reflect the actual severity of fibrosis in this HCV cohort, since patients with most pronounced liver disease were excluded.

   We agree with the reviewer that the exclusion of HCV-infected individuals limits the inferences that can be made from this data. This limitation has been added to the discussion in paragraph 4 on page 10.

   We agree that adding the APRI data is useful in order to interpret this data. APRI was not associated with xyz. The lack of association between increased APRI score and genotype 3 is also an interesting negative finding given low to moderate concordance between the two non-invasive fibrosis scores in previous studies (Mendeni 2011, Clinical Infectious Disease; Dumea 2014, BMC ID). Our findings regarding the APRI score have now been added to the text as well as a footnote for Table 3. A brief comment on concordance has also been added to the discussion on page 10.
3. Data presentation, tables is poor. Table 1 should at least list HCV-mono and HIV/HCV coinfected patients separately. And compare their characteristics. It is also clear that information is missing for some categories, e.g. marriage status and education status. This should be added as “unknown”.

We have changed Table 1 to reflect the above comment. The table now separates mono-infection and co-infection and provides associated p-values for each of the variables. Missing data has been added in as “unknown” in appropriate categories.

4. Statistical analyses: It is not clear how missing data was handled in multivariate analyses – especially since patients with missing data are usually excluded for MVA, but the authors seem to include all “n=175” in the logistic regression model. The adjusted OR generated by their model seems extraordinary high with 46 !!! and 17”” fold increased risk for HIV/HCV co-infection in case of IVDA as transmission route and no highschool graduation, respectively. This should be assessed by an biostatistician!!

We agree with the reviewer’s first point that the handling of missing data is unclear. The logistic regression model excluded missing values. We removed the confusing sample size from the title and added a footnote stating exclusion of missing values.

   With regard to the second point on high AOR for both IDU and high school education, this was re-assessed with the assistance of a biostatistician and determined to be accurate. The analysis was done using the Aikaike Information Criteria (AIC) method. The best-fit model had the lowest AIC and produced the high AORs. The high number of missing values, all from the mono-infected cohort, contributes to the high OR. For example, only 3 patients in mono-infection reported IDU as route of transmission (with 25 missing) as compared to 72 in co-infection (1 missing). The very wide confidence interval is attributable to low sample size. However, our conclusions based on these results are still consistent with reports from the literature. We have outlined the selection bias due to missing data in our limitations section on page 11 and provided evidence for the association between HIV/HCV co-infection, IDU, and lower education in the text.

MINOR ESSENTIAL REVISIONS

Abstract
1. This is not really a “molecular epidemiology” study. Determination of HCV genotype is a standard procedure and the term molecular should probably be reserved to experimental/mechanistic studies.

The word “molecular” has been removed from the abstract on page 2.

Introduction
1. Age of successful HAART should be changed to “era of…”

This correction has been made to the introduction on page 3, line 50.

2. HAART should be changed to ART, this terminology has been proposed by international guidelines

This correction has been made throughout the document.
3. The number HCV patients, HIV patients, and HIV/HCV coinfected in China should be given.
The section on HIV and HCV epidemiology in the introduction was changed to reflect the above reviewer’s statement. Accurate prevalence of HIV/HCV co-infection has not yet been established and thus a total number was not cited. “Total numbers of HIV-positive and HCV-positive individuals in China is estimated at 780,000 [5] and 8.9 million [6], respectively. In HIV patients currently on ART, approximately 18.2% are co-infected with HCV [7].”

4. “underlies” should probably read as “underlines”
Word choice changed from “underlies” to “underscores.”

5. I would suggest to change the sentence to “HIV coinfection accelerates HCVassociated fibrosis progression…, especially when CD4+ cell counts are low…” compare reference: Reiberger et al, Journal of Viral Hepatitis 2010
Sentence edited to read: “Studies have demonstrated that HIV co-infection accelerates HCV-associated liver fibrosis progression, a phenomenon possibly mediated by effects of HIV infection on fibrogenesis in the setting of immunosuppression [11, 12]”
Reiberger reference added (reference #12).

6. References for recent advanced in HCV therapeutics should also include those studies for HIV/HCV coinfection, please cite
- Photon-2 study: SOF/RBV, Molina et al, Lancet 2015
- Turquoise-1 study: 3D/RBV, Sulkowski et al, JAMA 2015
These two studies were both cited in the introduction at the top of page 4.

Reference has been added (reference #20).

Methods
1. Now the authors refer to ART (before they use HAART), please just use ART from the beginning.
This has been corrected; please see “Introduction Point 2”.

2. The methods again state, that there is a high prevalence of HIV and HCV infections in China, but again they don’t give actual number – this should be done in the introduction. Please refer to Introduction Point 3.

3. Did the authors mean “IFN-free” when they state “providing free HCV therapy” ??
This sentence was written in reference to the previous one, which states that HIV therapy is provided for free to HIV-positive patients. A similar system unfortunately does not exist for HCV therapy here. Wording changed to “free of charge” on page 5 for clarification.

4. What is a “convenience sample of treatment naïve …”?
This refers to the process of patient recruitment for the original treatment study, which
was non-random and dependent on clinic visitation and consent by individual participants.

5. Why were only anti-retroviral naïve patients included?
Please refer to Major Revision point 1, in which we revised this statement, as ART patients were in fact included.

6. The authors should give the APRI and FIB-4 formulas they used for calculation of the respective scores. Not only refer to class1, class2, and class3m but give actual values that were actually used for their cutoffs in their study.
Section edited with addition of formulas and cutoffs:
“The equation for APRI is as follows: (AST / platelet count) x 100 [25]. FIB-4 was calculated using the following formula: age (years) x AST / (platelet count x ALT^{0.5}) [26]. Standardized APRI and FIB-4 cutoff values were used to classify fibrosis as “no significant fibrosis” (Class 1: APRI ≤0.5; FIB-4 <1.45), “intermediate status” (Class 2: APRI 0.5-1.5; FIB-4 1.45-3.25), or “significant fibrosis” (Class 3: APRI > 1.5; FIB-4 >3.25) [25, 26].”

Results
1. The authors conclude that “most” patients had not completed high school and were married, however, the percentage were 49.1% and 35.4%, respectively. The formulation should be changed to reflect that these were not the majority in the respective category.
This sentence has been removed and re-written to reflect our changes in Table 1. Please see text on page 7 paragraph 1.

Discussion
1.1 It is not clear to the reader what the others mean by “separate health system in China allow mono-infected indiviudals to persist …???
To clarify, the infrastructure in China maintains two separate non-integrated health services for HIV patients and HCV patients, in such that HCV patients may not get the same education or support on high-risk behaviors as HIV patients do, and thus be susceptible to co-infection. The sentence has been changed to the following on page 8: “HCV mono-infected individuals in China do not have access to the same education and preventative services as HIV-infected individuals, leading to continued high-risk behaviors and acquisition of co-infection.”

References
1. Ref 31 is not necessary. This should just be added to the methods section.
Reference 31 has been removed.

**REVIEWER #2 COMMENTS:**
*Point 1: the introduction section my be reduced of the 30%*
We removed several non-essential sentences and worded more concisely to shorten the introduction.

*Point 2: in the methods section are poor detail the definition of the HCV illness (Child*
Pugh classification, HCC definition, cirrhosis). The method to make the phylogenetic tree arte poor explain.

Patients were not recruited based on Child-Pugh classification (only on basis of positive seromarkers) and as patients with decompensated cirrhosis were excluded, HCC was not defined.

We have added details to the methodology of the phylogenetic tree on page 6.

Point 3. The result section may be extended. The data on patients underwent to liver biopsy are not mentioned. The supplement data may be added in the results section.

The result section has been extended with more descriptive details provided in the text. Patients did not undergo liver biopsy (mentioned in the text) and use of and consent to liver biopsy is much less frequent here in China.

Point 4. In the discussion section may be mentioned the more recent anti-HCV drug.

We agree with the reviewer on the importance of the most recent anti-HCV drugs. We have mentioned several throughout the discussion with regards to genotype 3 and 6 and also have updated our introduction with mention of the most recent studies pertaining to HCV treatment in HIV/HCV co-infection.

REVIEWER #3 COMMENTS:

1. The question is well defined. Concerning methodology, my major concern is the patient number: it is not clear why only 188 patients were selected out of patient data from 3 years and an outpatient service in which 3000 patients are seen monthly. For an epidemiological study and the potentially available patient data, this is a low number of patients. In addition, the authors mention in the inclusion criteria that only treatment-naïve patients were included, while table 3 shows also 37 patients on ART. For an epidemiological study on genotype distribution and fibrosis progression, it is not appropriate to exclude patients with signs of advanced disease like decompensated cirrhosis or severe cytopenia. This might be an important bias regarding the results concerning the interaction between genotype and fibrosis progression.

The reviewer has brought up many good points. 1) 3000 HIV patients are seen monthly, but only a proportion of these are also HCV-positive and met the eligibility criteria specified. 2) We made an error in stating that only ART-naïve patients were included, and this has been corrected. 3) We have clarified the sampling and mentioned the bias introduced by excluding patients with advanced disease in our discussion. Co-infected patients were enrolled concurrently in an HCV treatment study and either decompensated disease or severe cytopenias would have been a contraindication to treatment. Please see the discussion of this above in response to reviewer #1.

2. The authors compare mono- to coinfected patients. Therefore, they should show patients characteristics for both cohorts separately in table 1. The difference in genotype distribution should be shown in a table, so that the numbers can be compared by the reader. Concerning fibrosis progression, it might be better to substitute this term by degree of fibrosis or prevalence of advanced fibrosis, because the authors do not show progression, but prevalence. Analysis of FIB-4 and APRI calculation should be mentioned in the text, not only FIB-4.
1) Table 1 has been revised to show patient characteristics separately for mono- and co-infected groups. 2) We agree that our study does not show progression, but rather prevalence. There are no assertions from our study that we are showing the former. 3) APRI results are now mentioned in the text.