Author's response to reviews

**Title:** Prevalence of naturally occurring NS5A resistance-associated substitutions in patients infected with hepatitis C virus subtype 1a, 1b, and 3a, co-infected or not with HIV in Brazil

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To The Editor

BMC Infectious Diseases

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Dear Editor Ming Zhang,

We have received the editorial letter with the comments of the expert reviewers and we appreciate them. We have checked out the expert reviewer’s questions to improve the manuscript. As suggested by reviewer 4, the expression “resistance-associated variant (RAV)” was replaced to “resistance-associated substitution (RAS)” throughout the text, including the title. “Title: Prevalence of naturally occurring NS5A resistance-associated substitutions in patients infected with hepatitis C virus subtypes 1a, 1b and 3a, co-infected or not with HIV in Brazil” We have changed the tables, and the original table 1 was excluded. The manuscript was edited by a native English speaker. We appreciate all of reviewer’s suggestions, and based in your comments several changes were made throughout the text.

Reviewer 1:

Minor Issues:

1. I would suggest a little more introduction into the HCV nonstructural proteins and why they are important enough to target in this context. I felt this was lacking in the introduction.

A: Information on the HCV nonstructural proteins was included in this issue. “Currently, HCV-infected patients are treated with direct-acting antiviral agents (DAAs) using effective and well-tolerated completely oral regimens. DAAs target HCV non-structural proteins essential for genome replication and virion assembly, i.e. NS3/4A, NS5B, and NS5A. In addition, NS5A has also been implicated in host immune evasion [3].”

2. Line 124; delete “of”

A: We have done it.

3. Line 142: “groups” to replace group

A: We have done it.

4. Lines 228- 233: This sentence needs to be structured. As is, it is a run-on.

A: A native English speaker revised the text.

5. Lines 236-237: The authors speak as though they know that these mutations confer resistance in their current cohort. This patient outcome data was not presented in the manuscript and most resistance discussed in the manuscript is a result of previously published works from other groups. I would suggest rewording this sentence to reflect that this is the theoretical outcome of these mutations.

A:
We agree, and this was done as suggested.6. Line 253-256. Need to restructure this sentence. As is, it is a run-on.A: The discussion topic was almost totally rewritten.7. Abbreviations: PEG-IFNa, AASLD, and RBV are used scarcely throughout the manuscript and do not warrant abbreviation.A: We agree. Reviewer 2:1. I would omit the historical perspective on interferon-treatments from the introduction, I believe that combinations of DAAs with interferon are not in use anymore. It would be more interesting to present the currently available DAA treatment regimens and information on the particular DAAs that are currently used in Brazil? Moreover, what are the important RAVs in NS5A in the different genotypes?A: We agree. The historical information about interferon-treatments was exclude from the introduction and the currently DAAs regimen used in Brazil was added to this topic.2. Please highlight also somewhere in the manuscript (introduction or discussion) the evidence for the clinical benefit of resistance testing and also the controversy, as most guidelines do not recommend testing or recommend testing only in certain cases.A: We agree. This information was included in the discussion. “NS5A RASs appear to have an impact on patient response to treatment, especially in those infected with HCV-1a and HCV-3a. Testing for baseline RASs is recommended for determining treatment duration in HCV GT-1a-infected patients who are being considered for therapy with elbasvir/grazoprevir. This testing is also recommended for patients infected with HCV GT-1a and GT-3a with cirrhosis (American Association for the Study of Liver Diseases guidelines) and in all patients before retreatment following a failed therapeutic regimen with DAAs [30-32].”3. The manuscript should be edited by a native English speaker. Some sentences seem incomplete or are quite confusing. Here are a few examples:Line 174: RAV were observed in subtype 1a sequences. Line 274: "Special attention must be driven to NS5A resistance".Line 288: ..., once NS5A profile resistance is dependent to HCV subtype and geographic origin.A: A native English speaker revised the text.4. The first part of the discussion is a repetition of the results section. Overall, the discussion is overly long and could be shortened. It is sufficient to highlight substantial differences between RAV frequencies in Brazil and reported frequencies in the literature.A: We agree. The discussion topic was almost totally rewritten.5. To my knowledge R30Q in GT1b is no RAV (see your Citation 7+20)A: It was changed and analysis included only RAS clinically relevant (see Table 1).6. Did the authors look for minor populations at RAV positions?A: We have done it (see Table 1).7. Were the HIV co-infected patients under treatment?A: Yes, all of them.8. What was the viral load of the patients in the cohort (median; range)?A: We included the plasma viral load in method section.MethodsStudy Population“...HCV viral load of monoinfected patients was 6.17 Log UI/mL (IQR 5.78-6.51) and 6.31 Log UI/mL (IQR 6.02-6.71) for coinfected patients...”9. Please include a phylogenetic analysis of the mono vs the co-infected to rule out that your higher prevalence of RAVs in mono infected is due to a founder effect.A: This will be taking in account in another manuscript of our research group.10. The absence of RAVs in HIV co-infected 1b is most likely a consequence of the small number. Please discuss A: We have found RAS in HIV co-infected 1b patients (Table 1).11. In the chapter" Resistance-associated variants in HCV/HIV co-infected patients" the Y93H mutation is not listed for GT1b.A: It is there. “The RAS frequency in HCV/HIV-coinfected patients was 4% (4/101). Grouping by HCV subtypes, RASs were detected in 3.9% of GT-1a sequences (M28T and Q30H/R) and 11.1% of GT-1b sequences (Y93H) (Table 1).”12. Page 10 line 230-234: The authors state that the discrepancies between the reported RAV frequencies and the frequencies in this study may be the result of different classifications of RAVs. Can the authors give an example?A: It was included. “In the present study, grouping NS5A HCV sequences from mono-infected and HCV/HIV co-infected patients, RASs were more frequent in HCV GT-1a sequences (7.6%; 9/118) compared to GT-1b (6.5%; 6/93), this observation is