

July 30, 2018

Dr., MD
Deputy Editor, WORLD JOURNAL OF HEPATOLOGY

Pr., MD, PhD
Editor, WORLD JOURNAL HEPATOLOGY

Manuscript NO: 39428 - "Factors associated with DAA virological failure and RAS description in HIV/HCV patients"

Dear Dr.

Thank you for giving us the opportunity to revise our manuscript according to very helpful questions and comments of the referees.

We provide you a point-by-point response to referees comments and have the pleasure to send you a revised version of our manuscript. To facilitate the lecture of the reviewers, all modifications are in bold and color

Reviewer 03729295

- 1) Title: the title reflects the main subject and reader may readily understand the key concepts. However, we noticed proofreading for instance: "resistance" instead "resistant"; we also noticed in the title 19 words. It should be no more than 12 words. I would like to propose a title: "Factors associated with DAA virological failure and RAS description in HIV/HCV patients". Running title: "DAA failure in HIV/HCV coinfecting patients"

We agree with your proposals and changed the title as well as the running title as suggested, also taking into account the suggestions of reviewer 03764321

The definite title is then "Factors associated with DAA virological treatment failure and RAS description in HIV/HCV patients".

- 2) The Abstract summarizes and reflects the work described in the manuscript. However, we noticed the study Aim is written as Background that contents "30 words" instead "20", because Aim requires no more than 20 words. The "method" does not describe sequencing analysis and adjustment for factors associated with risk of failure, while these informations are noticed in the "results"; we also noticed 60 words, while "method" requires no less than 80 words. In the "results" we noticed "HIV-RNA <50 cop/mL" that should be written "HIV-RNA <50 copies/mL". We noticed equally

“conclusion” contents “36 words” more than “26 words as required by the Journal”. I would have wished you consider these observations.

We have made the requested changes in the Abstract

- i. Addition of a paragraph Aim with 20 words
- ii. The “method” section has been enlarged by adding a reference to sequencing analysis and adjustment for factors associated with risk of failure (80 words)
- iii. The results section has been changed accordingly to the request
- iv. The conclusion has been shortened to 26 words

- 3) Key words: the key words reflect the focus of the manuscript to a degree. We noticed 4 keywords, while the Journal requires [5-10]; “HIV/HCV coinfection” is missing; the type of failure is not specified (like virological failure). I would have wished you consider these observations.

We have added 2 key words. Key words are now 6: HIV, HCV, HIV/HCV coinfection, DAA, treatment virological failure, resistant associated mutations (RAS)

4) In the “introduction or Background section”: sufficient background informations to provide the rational for the study are included, for instance: “Cure rates of over 90%, similar to those in HCV monoinfected patients, are described in clinical trial and real-life cohorts”, “failures are often associated with the development of resistance-associated substitutions (RAS); However, emerging resistant strains appearing at viral rebound are a consequence rather than a cause of failure”; “In rare circumstances, especially for genotype 1a viruses, baseline mutations in the (NS5A) gene can preexist in the viral species before treatment introduction and may have a potentially deleterious impact on (SVR)”, “Authors aimed to describe the characteristics of patients failing first-line DAA treatment in the real-life French nationwide ANRS CO13 HEPAVIH cohort of HIV/HCV patients”, etc.

We have simplified the sentence in the introduction to:

“For the few patients failing treatment, resistance-associated substitutions (RAS) can emerge and emerging resistant strains appearing at viral rebound are a consequence rather than a cause of failure [11, 12].”

Indeed, among the objectives of our project was the description of emergent as indicated in the title and at the end of the background section: “Furthermore, we described the emergence of clinically relevant RAS to DAA classes at DAA treatment failure.”

5) Method section: Experimental procedure enough well explained. The manuscript describes basic study design (ARRIVE check-list), adequate data collection and measurements described [data were collected prospectively by each participating center, using an eCRF; Virological failures were categorized; liver biopsy (METAVIR fibrosis stage F4), liver stiffness ≥ 12.5 kPa (FibroScan[®]; Echosens, France) and FibroTest[®] value ≥ 0.75 (Biopredictive, France); HCV resistance testing using automated sequencer (ABI-3500xL Dx) when HCV-RNA >1000 IU/mL at sequencing time point], and Statistical data describing and analysis are done, etc. However, we noticed Sample size (or number of patients treated with DAA and included in the study is missing, whereas the results describe “877 patients treated with DAA-combination among them 559 subjects included in the analysis”. In addition, the statement regarding “biostatistics review” is not noticed in “method section”, but contributors’ list related statistics analysis is noticed in “Acknowledgement section”. I would have wished you consider these informations.

We added to the statistical analysis section:

“We included all patients who met the inclusion criteria as described in the study population section.”

The statistical analysis was realized by Camille Gilbert a biostatistician of the Inserm team Morph3eus/clinical trial unit CMG-EC and the statistical methods of this study were reviewed by Linda Wittkop (methodologist and head of the Inserm team Morph3eus-Bordeaux population health UMR1219 and head of a ISO:2009 version 2015 certified clinical trial unit for “clinical research, design and conduct of clinical trials in infectious diseases and vaccine research”). Furthermore, the statistical methods have been approved by the working group and the scientific committee of the French nationwide ANRS CO13 HEPAVIH cohort.

A biostatistics review certificate has been submitted along with this review as requested.

6) Results section: the research objectives are achieved by the experiments used in this study to a degree. Data related to the “characteristics of patients” and “viral response” are summarized in Table1; Adjusted logistic regression for factors associated with virological failure (Table2); RAS results in 14 patients with virological failure for whom sequencing was performed in routine (Table3). We remarked “Mean treatment duration” noticed in the “Abstract results” is missing in the “core results content” and the “method section” does not notice “mean” calculation.

We added the mean DAA treatment duration to the core results content (plus table 1) and clarified the method section:

Method section

“Variables are described as number and percentages, or median and IQR (or mean (standard deviation)), as appropriate.”

General characteristics at DAA initiation:

“Mean treatment duration was 16 weeks overall (15 weeks in patients who failed DAA therapy and 16 weeks in those with sustained virological response).”

7) Discussion section: Finding enough well interpreted and discussed with relevant literature. Study limitation enough well described, like “low number of subjects with virological failure, and thus, probably has limited power to identify all potential risk factors”. Future direction and implications: “Among 7 patients with failure and interpretable pharmacological data, suboptimal blood concentrations of DAA were measured in 2 of them; these results could reflect different situations (drug interactions, suboptimal dosing errors, and suboptimal adherence) and warrant further investigation and wider-scale assessment of pharmacological data”, “high prevalence of non-structural-5A and -3 RAS at failure in the study confirms the EASL recommendation to evaluate, if resistance testing is available, HCV resistance to NS5A inhibitors (spanning amino acids 24 to 93) as these analyses can guide decisions for a further treatment”, etc.

We thank the referees for this comment.

8) Illustrations and tables: no figure is noticed, tables sufficient, good quality and appropriately illustrative of the paper contents.

We thank the referees for this comment.

9) Biostatistics: the manuscript describes Statistical data analysis in “Method section”, but the “method section” does not notice “mean” information, while “Mean treatment duration” noticed in the “Abstract results” is missing in the “core results content”. Statement regarding biostatistics review not noticed there, but contributors’ list related statistics analysis is noticed in “Acknowledgement section”.

We added the mean DAA treatment duration to the core results content (plus table 1) and clarified the method section:

Method section

“Variables are described as number and percentages, or median and IQR (or mean (standard deviation)), as appropriate.”

The statistical analysis was realized by Camille Gilbert a biostatistician of the Inserm team Morph3eus/clinical trial unit CMG-EC and the statistical methods of this study were reviewed by Linda Wittkop (methodologist and head of the Inserm team Morph3eus-Bordeaux population health UMR1219 and head of a ISO:2009 version 2015 certified clinical trial unit for “clinical research, design and conduct of clinical trials in infectious diseases and vaccine research”). Furthermore, the statistical methods have been approved

by the working group and the scientific committee of the French nationwide ANRS CO13 HEPAVIH cohort.

A biostatistics review certificate has been submitted along with this review as requested.

10) Units: HCV-RNA : “copy or copies/mL” instead “cop/mL”

We have changed all pages of the manuscript accordingly.

11) References: latest, important and authoritative references are cited in the manuscript. However, we noticed:

- In “introduction section, line 4”: citation with more than 5 ref. [1-6] noticed; reference numbers regarding “superscript” and “no space”: line 5 “X^[7-10]” instead “X [7-10]”,...
- Manuscript Reference style does not meet journal requirement regarding “first authors’ name and volume number that should be typed in bold letters, journal title in abbreviated form and italic police. It should be matched with Journal format requirement.

We have made the requested changes

12) Quality of manuscript organization and presentation: manuscript enough well, concisely and coherently organized and presented. However, we noticed that:

- Police format and line spacing do not meet journal requirement: “Book Antiqua” instead “Arial”; Line spacing: “1.5” instead “2” and for References section “1.5” instead “1”

We have corrected according to your request.

- ORCID number and ARRIVE guideline statement are not noticed in title page of the manuscript.

We added to the title page that the ARRIVE guidelines have been adopted.

- Supported foundation (or funding) is stated after Acknowledgement. It should be stated in the first section of writing requirement. I would have wished you consider these observations.

We added the funding section to the title page.

13) Author prepared the manuscript according to the appropriate research methods and reporting, to a degree.

We thank the referees for this comment.

14) The ANRS CO13 HEPAVIH cohort, national multi-centre prospective hospital-based observational study of HIV/HCV patients, received approval by an Institutional Review board;

Patients included in the cohort gave their consent for study participation. However, related formal ethics documents not provided by the Authors.

We submitted the related formal ethics document alongside with the reviewed version of our manuscript.

Specific Comments To Authors

Beside the above I would like to make important Specific Comments related your work as follows:

1. First: We noticed in your work: “no study to date has focused on HIV coinfection in the field”; “Similarities with previous studies like “low virological failure rate of 3.9%; low platelet count associated with a higher probability of DAA failure”.
2. Second: “high prevalence of non-structural-5A and -3 RAS at failure in the study confirms the EASL recommendation.....”

Conclusions appropriately summarize the main findings that this study identified.

3. Third: Study limitations are enough well described; Future direction “suboptimal blood concentrations of DAA were measured in 2 patients among 7; these results could reflect different situations (drug interactions, suboptimal dosing errors, and suboptimal adherence) and warrant further investigation and wider-scale assessment of pharmacological data”.

We thank the referees for these comments.

Reviewer 03764321

1. The title: it is better to write virological treatment failure instead of virological failure and this should be corrected in the whole manuscript.

We agree with your proposal and changed the title and running title as suggested, also taking into account the suggestions of reviewer 03729295

The definite title is then “Factors associated with DAA virological failure and RAS description in HIV/HCV patients”.

2. In page 6 the words patients failing DAA replaced by with DAA treatment failure.

We have corrected according to your request

3. Key words: HIV, HCV, DAA, failure. To: HIV, HCV, DAA, treatment failure.

We have taken into account the suggestions and increased the number of key words to 6. Key words are now: HIV, HCV, HIV/HCV coinfection, DAA, treatment virological failure, resistant associated mutations (RAS)

4. Page 8 introduction: has replaced by had. ; same page line 19: non virological treatment failure non should be removed virological treatment failure

We have corrected according to your request

5. Page 9: virological failure to virological treatment failure or just DAA treatment failure.

We have corrected according to your request

6. Page 10 : Data collection and definitions : You didn't mention what are the risk factors for both HIV & HCV infections

We have added that the "risk factors for HIV and HCV infections" were among the data collected

7. Page 10 : what do you mean by unknown at EOT? How it was unknown?

The term EOT means "at end of treatment" and we have detailed that in the text.

Undefined failure at EOT is defined by an unknown result of HCV-RNA at end of treatment (if the test was not performed for example) but a test that became positive thereafter, without premature discontinuation of treatment.

8. Page 13 : Factors associated with failure to Factors associated with treatment failure.

We have corrected according to your request

9. You said we found that a low platelet count was significantly associated with a higher rate of virological treatment failure. Then you said that low platelet count is a surrogate marker of cirrhosis and then you said we failed to observe a significant relationship between cirrhosis and failure. Are the patients with low platelet count in your study had cirrhosis or the lowered number is due to something else?

Effectively the only factor associated with treatment failure was a low platelet count ($p < 0.007$)

Although there was a tendency in the univariate analysis, the other markers of cirrhosis such as FIB-4 > 3.25 ($p=0.132$), low albumin levels ($p=0.146$) or low prothrombin time ($p=0.087$) were not significantly associated with treatment failure.

In the same way, in the multivariate analysis, severe cirrhosis was not significantly associated with treatment failure (OR=2.1, CI95: 0.4-10.3).

However, when we compared in our study patients with severe cirrhosis (child Pugh B or C or elastometry > 20 Kpa) to the patients without severe cirrhosis, we found that patients with severe cirrhosis had lower platelets count than those with non severe cirrhosis.

We have reformulated the corresponding sentences in the discussion section.

10. In Table 1: Patients' characteristics at treatment initiation according to virological response Platelets < 100 Giga/L (n=408) 57 (14) 51 (13) 6 (43) 0.007 This calculations and others in your manuscript are not understandable what dose the in between () means is it percentage? if it is of what?

To make the table clearer, we have added after the title of the 1st table the following sentence:

Results are presented as number (percentages in brackets) or median (IQR in brackets as) unless stated otherwise

September 06, 2018

Dr Fang-Fang Ji, Science Editor, Editorial Office **WORLD JOURNAL
HEPATOLOGY**

39428-Review file: Manuscript NO: 39428 - "Factors associated with DAA virological failure and RAS description in HIV/HCV patients"

Dear Mr Fang-Fang Ji,

Thank you for giving us the opportunity to improve one more our manuscript according to for helpful recommendations and comments.

We provide you a point-by-point response and have the pleasure to send you a revised version of the **39428-Review file**. To facilitate the lecture of the reviewers, all modifications are in bold and color

Answers to Reviewer 03644796 comments

- 1- The paper is well-written, but like many other real-life studies, has several limitations, including the use of multiple DAA regimens with no randomization, patients of multiple genotypes, low number of patients with virologic failure, the absence of data for baseline RAS, most of which were discussed by the authors. Sanger sequencing assay was used for the detection of RAS, which may not be sensitive enough to detect minor populations of RAS (<15%).

We thank the authors for these comments and have detailed the paragraph on the limitations of the study accordingly (p13)

- 2- MELD (or MELD-Na) should be reported in Table 1 and evaluated as an independent variable in Table 2.

The International Normalized Ratio is not a variable that we have collected in the cohort. This does not allow us to calculate MELD score in our cirrhotic patients.

- 3- Finally, some of the DAA regimens studied were from the previous generation (Daclatasvir, Simeprevir), and the results may not be entirely applicable to the newer, pangenotypic regimens.

Those results were obtained with 2nd generation DAA (ledipasvir, daclatasvir, elbasvir/grazoprevir) but we excluded from the analysis the 1st generation DAA (simeprevir and boceprevir).

However, we agree that those results may not be entirely applicable to the newer, pangenotypic regimens such as velpatasvir/sofosbuvir or pibentavir/glecaprevir and have added a sentence in the discussion section (p13)

4- Use of short forms without full in the title: HIV, HCV, DAA.

We have changed the title accordingly to the request for “Factors associated with DAA virological treatment failure and resistance-associated substitutions description in HIV/HCV coinfecting patients”

5- P5 line11 cop/mL – Is it copies/mL? Please present in IU/mL.

HIV viral load is usually presented as copies/mL and not in UI/mL

6- P6 line 21 non virological – non-virological

We focused our study on the virological treatment failures

7- P7 line 13 sub study – substudy 5

We made the requested change

8- P8 line 21 sub-type-specific – subtype-specific

We made the requested change

9- P10 line 3 Child Pugh – Child-Pugh 7

We made the requested change

10- P12 line 5 inhibitor based – inhibitor-based

We made the requested change

Special comments from the editor:

1. About the audio core tip, please reoffer it. Acceptable file formats: **.mp3, .wav**, or **.aiff**.

No audio core tip : There is no need of a specific consent because this kind of study about treatment are covered by the inclusion consent form)

2. There are similar sentences (highlighted in the report) with other articles, please rewrote these sentences.

Most of these sentences (found similar to other articles and highlighted in the report) come from the method section of our manuscript and are standardized through

articles of our team ANRS CO13-HEPAVIH. It is logical to find in this article a similar description of the methodology, of the variables and end of points definitions than in the previous ones of our cohort.

We have however reformulated the sentences that were found similar with other articles in the discussion section.

2. Write the 4 Strobe statements

We have written the 4 following statements p 4

Institutional review board statement: the study was approved by the Institutional Review Board Ile de France III, Paris, France

Informed consent statement: a written informed consent was obtained from each participant to the study:

Biostatistics: The statistical methods of this study were reviewed by Linda Wittkop from Bordeaux Population Health Research Center, Bordeaux. SAS software version 9.4 (SAS Institute Inc., Cary, North Carolina) was used for all analyses.

Conflict-of-interest statement:

Dominique Salmon has been speaker and received invitation to conferences by Gilead, Abott, and MSD)

Laurent Alric received grant and personal fees from MSD, Gilead, Abbvie, Janssen and BMS outside the submitted work.

Christine Katlama received consultancy fees and/or travel grants from MSD, Janssen, ViiV outside the submitted work.

Karine Lacombe personal fees from Gilead, personal fees from Janssen, personal fees from Abbvie, personal fees from Merck outside the submitted work.

Philippe Morlat received personal fees and non-financial support from GILEAD, Janssen, MSD and ViiV Health Care outside the submitted work.

Gilles Peytavin received travel grants, consultancy fees or study grants from pharmaceutical companies including Abbvie, Bristol-Myers Squibb, Gilead sciences, Janssen, Merck and ViiV Healthcare outside the submitted work.

Eric Rosenthal received personal fees from Gilead and Abbvie and travel grants, consultancy fees from Gilead, Abbvie, MSD and BMS outside the submitted work.

Philippe Sogni received personal fees and non-financial support from Gilead, BMS, MSD Abvie outside the submitted work

Caroline Solas received personal fees from Gilead, Abbvie, Janssen, MSD and ViiV Healthcare outside the submitted work.

Linda Wittkop reports grants from ANRS, during the conduct of the study; personal fees from Janssen, Gilead, MSD, outside the submitted work.

Other authors had nothing to declare.

4- Delate form the abstract, the background section

We have deleted the following sentence p 4

“Background: In HIV/HCV coinfection, all-oral DAA (direct-acting antiviral) regimens achieve virological cure in more than 95%”

5- Add articles highlights

Research background

In HIV/HCV coinfection, all-oral DAA (direct-acting antiviral) regimens achieve virological cure in more than 95% of the patients.

Research motivation

Risk factors for failure are mainly related to the severity of cirrhosis HCV monoinfected patients but are unknown in the population of HIV HCV coinfectd patients. We wanted to know if other factors related to non-adherence or to HIV status could be involved in the occurrence of failures. We thought that knowing risk factors for failure would allow to adapt the treatment to the patients with higher risk for failure.

Research objectives

The main objectives were to determine the risk factors for virological treatment failure to DAA in HIV/HCV coinfectd patients and to describe the frequency of RAS.

Research methods

HIV/HCV coinfectd patients starting a first DAA regimen before February 2016 and included in the French ANRS CO13 HEPAVIH cohort were eligible. Failure was defined as: i) non-response (HCV-RNA remained detectable during treatment, at end of treatment (EOT)), ii) relapse (HCV-RNA suppressed at EOT but detectable thereafter). Sequencing analysis was performed to describe prevalence of drug class

specific RAS. Factors associated with failure were determined using logistic regression models.

Research results

Research findings: Among 559 patients, 77% had suppressed plasma HIV-RNA <50 copies/mL at DAA treatment initiation, 41% were cirrhotic, and 68% were HCV treatment-experienced. Virological treatment failures occurred in 22 patients and were mainly relapses (17, 77%) then undefined failure (3, 14%) and non-responses (2, 9%). Mean treatment duration was 16 weeks overall. Post-treatment NS3, NS5A or NS5B RAS were detected in 10/14 patients with samples available for sequencing analysis. After adjustment for age, sex, ribavirin use, HCV genotype and treatment duration, low platelet count was the only factor significantly associated with a higher risk of failure (OR: 6.5; 95%CI: 1.8-22.6).

Contributions to the field: In HIV/HCV coinfecting patients, the risk factors of failure seem to be related more to the severity of cirrhosis than to HIV immunovirological status or non-adherence issues.

Problems that remain to be solved: It remains to be proven that the low platelet count associated with a higher probability of failure reflects the severity of cirrhosis.

Research conclusions

In our study of HIV/HCV patients receiving all oral DAA, only 3.9% HIV-HCV coinfecting patients failed DAA regimens. RAS were found in 70% of those failing. Low platelet count was independently associated with virological failure. We think that this low platelet count reflects the severity of cirrhosis.

Research perspectives

As the number of treatment failure is low, it would be useful to build international collaborations and gather the data of several cohorts in order to gain power.

Those results obtained with all oral DAA of 1st generation could be checked with the newer, pangenotypic drug regimen.

We hope that these complements will answer your requests

Best regards

Dominique Salmon