Author's response to reviews

Title: Drug resistance in HIV patients with virological failure or slow virological response to antiretroviral therapy in Ethiopia

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Version: 2
Date: 17 March 2014

Author's response to reviews: see over
Re: Revisions to Manuscript ID: 1378018623117292

Thank you for the opportunity given to revise this manuscript “Drug resistance in HIV patients with virological failure or slow virological response to antiretroviral therapy in Ethiopia” We appreciated the thoughtful comments on our manuscript from BMC infectious diseases external peers and editorial committee.

A detailed list of how we addressed each of the points raised by the editors and reviewers is included in the following pages. Our revised manuscript is attached.

I am grateful to the journal for considering publication of our paper and hope that our revisions have provided the necessary clarity for your readership.

Kind regards

Alemseged Abdissa
Corresponding author

Response to referee and editorial comments

Version: Date:10 February 2014
Reviewer: Mariza Morgado

Reviewer's report:

Major Compulsory Revisions:

1) Concerning the evaluation of transmitted drug resistance, in the abstract the authors state that 6/256 patients harbored resistance mutations at baseline. In the results, however, they only refer to 6 of 12 failing patients. Indeed, in order to assess the prevalence of transmitted drug resistance mutations (TDRM) the authors should have tested the total of samples at baseline and not only those from patients presenting virological failure at 6 months of follow-up. Although these ones might have more chance of failure, among the 12 individuals analyzed 7 had mutations at baseline, one of them not conferring resistance by itself, but not the other 5. For two of them, one from each group, virological failure was not associated with the presence of drug resistance mutations at 6 moths of follow-up. Although in the discussion (page 14) the authors stated that the data presented document the existence of transmitted resistance, and that a larger sentinel study would be necessary, they did not present an explanation justifying why they did not sequence the remaining samples. In my opinion, this is
a strong limitation of this manuscript to be published as full paper. The paper is too long for the amount of the results presented and should be shortened.

Response: It is true that to determine the prevalence of transmitted drug resistance we should have genotyped all the 265 samples. However, we did not genotype samples from patients with virological success because of the limited resource. However, we still think that the mutations in failing patients provide important information in setting where there is paucity of data. We have corrected the proportion 6/265 as 6/12 in the abstract section.

To make this point clear, we have therefore added the following sentence “The drug resistance mutation analysis was done only on virological failures because of limited resource. Thus, the actual rate of transmitted drug resistance was not estimated” (line: 312-314)

2) For drug resistance analyses the authors used the same algorithm for assessing both transmitted and acquired DRM, while they should have used the CPR Tool for TDRM and the HIVdb for acquiring DRM.

We have investigated the request made by the reviewer, and found that the CPR algorithm implemented at Stanford currently uses the WHO 2009 SDRM list (See: http://cpr.stanford.edu/pages/releaseNotes.html#appendix19), whereas the HIVdb algorithm (used in this study) is frequently updated with new resistance mutation calculations (http://hivdb.stanford.edu/DR/asi/releaseNotes/updates.html#Ver6.2.0_20120529). The suggested use of CPR on one part of the dataset and of HIVdb on another part, will therefore make a meaningful comparison of data difficult and we have therefore decided to keep the HIVdb predictions which was made with the current HIVdb algorithm (at the time of submission version 6.3.1).

Minor Essential Revisions
There is no information about the submission of the sequences to the Genbank.

Sequences have been submitted to GenBank and the accession numbers are now indicated under the heading “Sequences submitted to GenBank: “in the main manuscript. (line: 216-219).

Version:1 Date:3 February 2014
Reviewer: Valeria Micheli
Reviewer’s report:
Dear authors:

the article is well written but I’d like to suggest the following modifications:

• Major Compulsory Revisions

1. Results: the authors mentioned a total of 318 with 6 dropped out resulting in 312 eligible patients. In addition 7 died and 10 were lost to follow up: the total is seventeen (5.5%) instead of twenty seven (8.6%), as reported. Please modify or better explain the cohort.

Response: We have edited this paragraph to simplify and better clarify. (line: 223-226)
2. Virological outcomes and immunological criteria: to better define the three patients with good viral suppression at 6 months but slow response at 3 months, please mention their baseline viral load.

Response: It was mentioned in the table 2. In this version we have provided the viral load in the text as well (line: 237-239)

3. Virological outcomes and immunological criteria: “only five of the 14 participants with virological failure 4/14 (28.6%)”; this sentence is not clear, I mean: are the failures 4 or 5? In table 2 there are only four immunological failure.

Response: This has been corrected only 4/14 (28.6%) had immunological failure (line: 243)

4. HIVDR mutations at baseline: the authors mentioned 2 patients presenting combination of NNRTIs and PIs mutations (1 patient: T74S associated with a reduction of Nelfinavir susceptibility and 1 patient: A71T). A71T/V are polymorphisms that occur in 2-3% of untreated persons, so it could be better not to include them in a HIV drug resistance analysis at baseline.

Response: We agree the A71T is polymorphic and less important. T74S is also polymorphic, occurring in up to 10% of subtype C viruses from untreated patients (Nucleic Acids Res. 2003 Jan 1;31(1):298-303.). Thus we excluded the PI mutations to focus only on the resistance to first line drugs and shorten the manuscript as well.

5. HIVDR mutations at 6 months: the sentence “nine of 11 participants with virological failure received ART regimens that were not or only partially active” is wrong, as you showed on table 3 (only 5 patients received a partially active regimen due to the resistance to the NNRTI at baseline). The other four became resistant to their regimen during the therapy in spite of a fully active antiretroviral regimen at baseline.

Response: We agree, it has been corrected. (line:284-287)

6. HIVDR mutations at 6 months: the sentence “four out of nine participants with resistance mutations at 6 months had a drug concentration below the limit of quantification” is wrong, because only two patients presenting <0.01 microg/mL had drug resistance mutations at 6 months (#213 and #269); #357 is wild-type and #373 misses drug resistance genotyping.

Response: It is true that #357 is wild type and #373 misses drug resistance data. The statement is now corrected. (line: 292-296)

7. Discussion: the authors mentioned 12% of immunological failure in presence of virological suppression; the rate of immunological failures was 8.7% (22/251) as reported in the results. Please explain the discrepancy. In addition it isn’t clear the percentage 29% of correct identification of patients experiencing immunological failure. Please better speficy.
Response: We have shortened the statement about immunological failures in the discussion section and the discrepancy is corrected. (line 355-356)

8. Discussion: the patient presenting slow virological response with a high level resistance to all three drugs; it isn’t strange that the viral load is low, due to the impact of the mutation K65R on the viral fitness. Please add this comment.

Response: Thanks well teased out, the explanation is included in the discussion section. However, we are modest in our expression as virus from one of the failing patient also carried K65R. May be mutational interactions dictate the virological outcome. (line:362-365)

9. A warning for the conclusion: don’t underestimate the immunological failure; patients with virological suppression but immunological failure are considered “discordant patients” that deserve a strict follow-up in order to avoid the progression of the infection.

Response: We have down played the statement about immunological failures. But, if we don’t have access to viral load testing we are not able to know whether they are discordant or not. That is why it is imperative to improve access to viral load testing in resource limited settings.

• Minor Essential Revisions

1. Abstract/Results: please modify “harboring resistance mutations in 6/265 patients” into “resulting in 2.2% of acquired drug resistance mutations prevalence”

Response: Corrected; however we are hesitant to put in percentage as we did not genotyped all the samples

2. Background: please modify “the challenge of potential emergence” into “the risk of potential emergence”

Response: corrected (line: 94)

3. Background: where NNRTIs or NRTIs or PIs are mentioned use the plural form NNRTIs, NRTIs and PIs instead of NNRTI, NRTI and PI in whole paper, tables and legends too.

Response: corrected

4. Background: please modify “to have full historical knowledge about primary and secondary resistance mutations” into “to define the pattern of both primary and secondary resistance mutations”

Response: corrected (line: 104-105)

5. Study participants: please specify the unit of CD4 count (cells/ microL)

Response: corrected
6. Specimen collection and processing: please modify “CD4 count and viral loads were done” into “CD4 count and viral loads were measured”

Response: corrected (line: 154)

7. Resistance genotyping: please modify “final extension at; 66°C” into “final extension at: 66°C” as previously indicated

Response: corrected (line: 186)

8. Baseline characteristics of the study participants: after Table 1 please modify “all participants were prescribed an ART regimen as per” into “according to the 2008 Ethiopian ART guidelines”

Response: we have deleted this statement as it was redundant: it has been already stated in the methods section (line: 143)

9. Baseline characteristics of the study participants: please uniform the unit for viral load using copies/mL instead of copies/ml

Response: corrected

10. HIVDR mutations at 6 months: please specify the technical difficulties for 3 participants missing HIVDR both at baseline and at failure (viral load significantly high!)

Response: Now, the technical difficulties are specified (line: 271-272)

11. HIVDR mutations at 6 months: Please modify “resistance to all the three ART” into “resistance to all the three antiretroviral drugs”

Response: corrected

12. List of abbreviations: please correct some editing errors.

Kind regards

Response: corrected

Other changes

Ethics statement is now integrated in the sub-section “study participants” (line: 138-139)
05-Dec-2013,

MS: 1378018623117292
Drug resistance in HIV patients with virological failure or slow virological response to antiretroviral therapy in Ethiopia
Alemseged Abdissa, Daniel Yilma, Jannik Fonager, Anne M Audelin, Lone H Christensen, Mette F Olsen, Markos Tesfaye, Pernille Kaestel, Tsinuel Girma, Abraham Aseffa, Henrik Friis, Court Pedersen and Aase B Andersen

Dear Mr Abdissa,

Your manuscript has now been peer reviewed and the comments are accessible in PDF format from the links below. Do let us know if you have any problems opening the files.

Referee 1: http://www.biomedcentral.com/imedia/5774309161204651_comment.pdf
Referee 2: http://www.biomedcentral.com/imedia/1197730498120986_comment.pdf

Editor's Comment:

This manuscript has been reviewed and the reviewers' comments are below. In the present form the paper is not suitable for publication.

Many major compulsory revisions are necessary, and the parer must undergo a thorough rewrite. The authors can respond to the major compulsory revisions listed in the comments. The associate editor adds more comments:

In agreement with a reviewer, the authors did not present an explanation justifying why they did not sequence all the samples, so that the real prevalence of TDRMs remains unknown. This assessment is probably beyond the scope of the work, and it would be difficult to address; the parer reports a valuable experience in a limited resource setting; but this is a strong limitation of this manuscript to be published as full paper, and it should be shortened.
Please consider these comments carefully and submit a revised manuscript.

With best wishes,

Miss Sheryl Ramos

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