Author’s response to reviews

Title: The impact of HBV or HCV infection in a cohort of HIV-infected pregnant women receiving a nevirapine-based antiretroviral regimen in Malawi

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Version: 2  Date: 16 January 2014

Author’s response to reviews: see over
Dear Dr. Castelnuovo,

we are pleased to submit the revised version of our article entitled “The impact of HBV or HCV infection in a cohort of HIV-infected pregnant women receiving a nevirapine-based antiretroviral regimen in Malawi”.

These are our replies to the reviewers’ comments:

Reviewer N. 1 (Claudia Hawkins)

Major comments

1. We agree that the number of coinfected patients is small (now included as a limitation in a dedicated paragraph in the discussion section). The new analyses that we have now performed (see answer N. 2) have slightly changed some values for significance making, perhaps, the results clearer (P value for the association of coinfection with mild hepatoxicity is now = 0.035 compared to a previous value of 0.049). However, we have now toned down our conclusions and removed the interpretation of a lower trend in complete viral suppression in coinfected patients.

2. We have now the data on the presence of HCV-RNA in HCV-antibody positive patients (previously not available). All patients were tested at baseline and at least twice during follow-up. Among the 9 HCV antibodies positive only one had presence of HCV-RNA (Pag. 6, “Laboratory procedures”). We therefore included only this patient among the coinfected group and re-analyzed the data according to these new classification. The results were mainly unchanged apart from slightly different values for significance, as reported above.

3. We did not have direct measures of treatment adherence. However, following the reviewer’s suggestion we tried to evaluate if the attendance to the clinical visits (as a possible marker of adherence to the treatment program) could have an impact. No correlation was found with the number of missed clinical visits and the emergence of toxicity. We decided not to include these data in the paper and included the lack of direct measures of adherence among the limitations of the study. In the methods section (and in the abstract) we had reported that women who stopped
therapy at 6 months were those not meeting the criteria for treatment (CD4+ > 350/mm³ at baseline). However, we have now reported the exact number of those who stopped in the results section (Pag. 7, second paragraph). We have also included a paragraph in the discussion to report that in the new (2013) WHO guidelines for the prevention of mother to child transmission of HIV life-long antiretroviral treatment is recommended for all pregnant women, eliminating the possible risks of viral rebound at treatment interruption (Pag. 10, second paragraph).

4. Longitudinal measurement of HBV-DNA were not available for all HBV-infected women and therefore we had decided not to include them. However, following the reviewer’s suggestion we have analyzed the available (partial) data and included them in the manuscript. Indeed, we found that detectable levels of HBV-DNA at 6 months were significantly correlated with higher ALT values further supporting the role of actively replicating HBV infection in determining liver toxicity. A paragraph has been included in the results section (Pag. 8, last paragraph) and one in the discussion section (Pag. 10, second paragraph).

Minor comments:

1. Background of the abstract has been modified to clearly report the objectives of the study.

   We have now included the effect of HBV-DNA in the results section. Conclusions have been rephrased to clarify that a greater incidence of liver toxicity ≥ grade 2 was associated to baseline higher CD4+ counts.

2. We have rephrased second sentence of background.

3. AST was also run (reported among baseline characteristics) but the analysis of liver toxicity was based on ALT measurements.

4. Second paragraph of “Viroimmunological response” referred to HBV-infected women only (excluding HCV-positive women). It has now been deleted, and all the paragraph has been re-organized according to the suggestion of reviewer N. 2.

5. Infrequent corrected.

6. Third paragraph of the discussion removed.

7. We agree with the reviewer that the second paragraph of the conclusions could not be related to the findings of the study and we decided to remove it.

8. A limitation section has now been included in the discussion

9. Reference to the paper from Chasela et al was already included.

Reviewer N. 2 (Francois Rouet)
1. See answer N.2 to reviewer N. 1

2. Information on ALT measurement and CD4+ count assessment has now been included in the methods section (Pag. 5).

3. The definition of the grades of hepatotoxicity according to the ACTG classification has now been included in the methods section (Pag. 5-6).

4. We have now included 2 figures for the Kaplan-Meier analysis for the development of mild or moderate-severe liver toxicity in mono and coinfected women (Figure 1 A and B). We have also included a table for the Cox model (new Table 2).

5. We have now re-organized Table 1 (new title “Patient characteristics”) specifically indicating those measured at baseline. We have reported in the first paragraph of the results section that baseline meant before drug administration. We have also reported in the methods that women enrolled in the study were naïve to antiretrovirals before drug administration. We have also deleted the “All” column in Table 1 and included the P values. We agree that the difference in ALT values at baseline is not clinical significant and now specifically stated it in the text. The proportion with a grade 1 toxicity in the 2 groups was reported in the text.

6. We have changed “Safety” with “Hepatotoxicity”. As reported above we have now included one Figure for the Kaplan-Meier analysis and a Table for the Cox model and described them in the results section. We have also deleted Table 2 (replacing it with a new Table 2 with the Cox model).

7. There was no difference in mortality in the 2 groups and we have now reported clearly in the manuscript (Pag. 9) and included the Kaplan-Meier analysis as Figure 2.

8. We have also added 2 new figures (Figure 3 and 4) to report the CD4+ count changes over the follow-up and the virological response.
9. We have now used the thresholds of 50 and 1,000 copies/ml of HIV-RNA to assess the virological response and deleted the 400 copies/ml.

10. We have now moved the first paragraph of the discussion later in the discussion.

11. Overall, the discussion has been slightly shortened.

12. Reference of Mbogua et al now cited in the background and mentioned in a paragraph of the discussion (Pag. 11, first paragraph). The other two papers were mainly on HCV that was really rare in our cohort.

Looking forward to hearing from you

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