Author's response to reviews

Title: Human papillomavirus (HPV) types 16, 18, 31, 45 DNA loads and HPV-16 integration in persistent and transient infections in young women.

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Author's response to reviews:

Dear Ms Roxane Rajabi,

Thank you for procuring two in-depth reviews for our manuscript. We greatly appreciate the opportunity you gave us to improve our manuscript using the suggestions in these critiques. You will find attached a revised manuscript with associated tables and figures. We have revised the paper by taking into account the reviewers’ suggestions. All revised text has been marked which includes an abstract, a text, and a set of tables and graphs (highlighted in yellow).

In addition, we include below detailed responses following each of the relevant comments received from the reviewers (marked in italics).

We trust that you will find these revisions satisfactory.

Eduardo Franco
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Response to critiques:

Reviewer #1

“In the HPV-16 integration section, last sentence of the first paragraph, the authors discuss HPV 6 E6/E2, rather than HPV 16 E6/E2”

Answer: We corrected this error and also rewrote the sentence for clarity.

“In the last paragraph of the results section, the authors go a long way to describe how they have ensured that they were able to measure E2 and E6 quantitatively. I was wondering however, if it would not be possible to have integration in the presence of episomal HPV DNA (as has been described in the
literature). Since these young women frequently have high viral loads, the effect of integration on E6/E2 ratio might easily be obscured. Maybe the authors can comment on this.”

Answer: We have amended and expanded two paragraphs in the discussion (pages 13 and 14) to respond to this excellent question and provide additional details.

“In the discussion, page 12, last paragraph there is clearly a chicken or egg paradox. Does disruption of E2 lead to integration, or does integration lead to disruption of E2?”

Answer: Although the question cannot be answered unequivocally, we believe that our response to the previous comment provided more insights to the Discussion section. We have presented all available evidence from the literature in the context of our results.

“On the next page, the authors quote Brown et al [46], on disruption of E2 in 25% of infections, however, the paper in the ref list under 46 does not discuss HPV integration, surely there must have been a mix up.”

Answer: We regret the error. We have deleted this reference (Brown et al) and replaced with that of Collins et al. We have amended the list of references in the current version.

“In refs 5 and 35, it is not Snidjers but Snijders, like in ref 40. Update ref 39, since this paper has been published now.”

These corrections have been made.

“It took me quite a while to understand figure 2. This could of course be a personal problem, but I would like to suggest helping readers by giving an example, for instance: upper left box, viral load at entry versus viral load at 6 months, lower left box, viral load at entry versus viral load at 24 months. This way, it will be easier to understand. Furthermore, it would be helpful to give the correlation coefficient for each box (e.g., in the upper left corner, except for 18 vs 24)”

Answer: We took the suggestion and revised figure 2 labels and legend to make the interpretation clearer to the readers. Since most of the correlations between visits were already presented in the table 1, we need not present correlation coefficients with each graph.

“Again, figure 3 puzzled me. In the text, page 9, first sentence I read: ”infection clearance was inversely associated with viral load” [high viral load, low clearance]. In table 3 I read: “third viral load tertile (highest) is taken as reference”, and indeed values for second and first tertiles are higher [suggesting higher clearance rate]. In the legend to the figure (incidentally, where you use lower, middle and upper tertile, why not use the same terms in table and figure consistently) I read that the upper tertile (= third? = highest?) is the solid line. All
women start with an infection [1.00], and than the line goes down every time a woman clears the infection. But in that case, shouldn’t the solid line end up on top and not as the lowest? I am afraid I’m lost and need some help.”

Answer: Table 3 and figure 3 provide complementary information. We revised figure 3 legend to make it clearer. Higher quantities of viral load take longer to clear and only few infections clear. In table 3, we presented that the infections with low quantities of viral loads (tertile 1) clears 2 to 5 times faster than the infections with high quantities of viral load (tertile 3). Consistent with these data, figure 3 shows that because of faster clearance the solid line (tertile 1) ended up lower than lines for tertiles 2 and 3, which correspond to higher viral loads.

Reviewer #2
1. “A table to present ….with respect to their HPV status”.
2. “A figure to show cumulative % of HPV positive subjects over time”.

Answer: Regarding both of the above suggestions, we did not mention the characteristics explicitly as we did not use most of them in our current analyses. In addition, we presented detailed tables and the suggested figure in our previous publications (references 22 and 23). However, as per the reviewer’s suggestion, we added some description of characteristics to the first paragraph in the results section.

3. “Figure 1, can the author separate the incident infections from…incident from non-incident?”

Answer: In figure 1, first visit represents all prevalent cases and their respective viral loads. Subsequent visits include both new and old cases. Splitting these cases between prevalent and incident cases would have affected statistical precision, particularly for HPVs 18 and 45, and cluttered the graph. The correlations and associations between viral load and persistent infection were presented in table 1 as well as in figure 3. In table 1, we present the correlations between viral loads in the subsequent visits and in figure 3, we present time to clearance of these infections from the time of onset. We thus believe that the reader is provided with detailed information.

4. “HPV integration is rare and only detected in one ….were any reference cell lines tested?”

Answer: We added further information about sensitivity analysis in the page 14, last paragraph. Additional references were also mentioned.

5. On page 9, presenting the mean HPV 16 E6/E2 ratio is not informative….e.g. #1.0, 1-1.5, #2.0

Answer: We added exact numbers and details about the range of ratios in page 10, paragraph 1.

6. TS-PCR?, though specific, is not .....Consider deleting this session.
The test allowed investigating if specimens with ratios below 2 but above 1.5 could contain integrated forms and also confirm integration in samples with higher ratios. Several publications have utilized this technique to confirm integration suspected with high HPV E6-E2 ratios, and confirmed the presence of integration. Although sensitivity of these assays needs to be formally evaluated, we do not have any reference on the clinical sensitivity of RS PCR. Since we mentioned the limitation in our text, we ask that the text should be kept as is (now revised).