Author’s response to reviews

Title: Persistence of Cervical High-Risk Human Papillomavirus in Women Living with HIV in Denmark – the SHADE

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Persistence of Cervical High-Risk Human Papillomavirus in Women Living with HIV in Denmark – the SHADE

Copenhagen, July 4th, 2019

To the Editor, BMC Infectious Diseases
Enclosed, please find a point-by-point rebuttal and edits as per the reviewer’s comments to our manuscript “Persistence of Cervical High-Risk Human Papillomavirus in Women Living with HIV in Denmark – the SHADE”, INFD-D-19-00849.

The changes are marked in blue in the resubmitted manuscript. We thank the reviewers for their diligent work on our manuscript and have addressed the comments. Should the editor or the reviewers upon a 2nd review have additional comments please do not hesitate to return the manuscript for further review.

On behalf of the authors, sincerely yours,

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Reviewer #1:

Overall comments:

The authors present a prospective study of HPV natural history in HIV-positive women in Denmark, based upon the linkage of various medical databases that is possible in few places outside Denmark. This includes linkage with the national cervical cancer screening database, although this database does not contribute to the main analysis of HPV persistence.

Principal findings are the confirmation, in Denmark, that HPV persistence (with CD4 count) and detection of cervical HSIL (with AIDS diagnosis) are linked to severity of immunosuppression in this population.

1: I don't think it is appropriate to claim that "most frequent persistent genotypes were HPV52, 33 and 31" in the abstract. This suggests that these types were more persistent than others, which was not shown (nor even statistically tested given the small samples size).

Reply: Thank you for pointing out this linguistic misinterpretation of the text. In this study HPV52, 33 and 31 where the most often observed persistent genotypes. We have changed the abstract accordingly to reflect this:

Page 3, line 65: "most frequently observed persistent genotypes were HPV52, 33 and 31".
2. "a high rate of persistent hrHPV infections with predominantly non-16/18 hrHPV genotypes" also suggests some particular dangerousness of these types in HIV-positive, but that was also not shown in the paper, as numbers were too small to link even with HSIL outcomes. Maybe linking HPV persistence to outcomes is a future objective of this study? But the data is not there yet, and we know from larger studies and meta-analyses that HPV16 and 18 remain the most carcinogenic types in cervical cancer even in HIV-positive persons - but this message has been somewhat lost in the tendency to focus on the nonHPV16/18.

Reply: In the HIV/HPV literature a higher rate of infections with non-16/18 hrHPV genotypes is repeatedly highlighted, which is in line with our findings and therefore reported in this study – regardless of pathology findings. However, we agree that this probably does not mean that these genotypes have another pathogenicity in people living with HIV.

3. Also in the abstract, it seems that the conclusion "With the current HIV guidelines aiming at early identification of PLWH and initiation of ART upon diagnosis, numbers of HPV-related cancers may decrease in WLWH.", has little link to the data presented in the current study

Reply: The mentioned sentence has been deleted.

Page 3, line 72-73: Sentence deleted.

4. Results, line 233 - perhaps replace "showed up" with "participated" ?

Reply: Thank you for this comment.

Page 8, line 218: “showed up” was replaced by “participated”.

5. Table 4 - The meaning of the p-values is unclear. What are the comparison groups?

Reply: We agree that the small study size means that the p-values have limited value. To accommodate this, the p-values column has been removed and a sentence has been added in the “Results section” stating that there was no significant difference between the HPV persistence and the HPV clearance group and their cytological and histological outcomes.

Page 9, line 239-240: “There was no significant difference between the HPV persistence and the HPV clearance group and their cytological and histological outcomes.”

6. Discussion - Whilst there is a lot of valuable discussion about the issue of HPV natural history and cervical cancer prevention in HIV-positive women, there needs to be a stronger link to how this study informs the picture.

Reply: Thank you. We summarized the main findings stating that ASCUS and LSIL was predicted by short duration of ART, whereas HSIL was predicted by prior AIDS, and that non-
HPV16/18 genotypes predominated. These findings are vital for further evidence development towards a national cervical cancer strategy in high income countries. We also take the liberty to refer to Reviewer 2 who states, “This manuscript is well-written, clear and will add significantly to the available literature in the topic”. However, as we also note, evidence is scarce and diverse across the globe. We could elaborate at length on how our data could be transformed into clinical management, but we feel that the study limitations, especially the study size, merits caution on interpretation and description of impact.

Reviewer #2:

Overall comments:

Women infected with HIV are at increased risk of HPV infection, cervical precancerous lesions and cervical cancer. Cervical cancer screening has improved dramatically over the last decade with the introduction of HPV testing. Yet there are a number of issues that need to be addressed in women infected with HIV. One of these issues is the identification of the women at need of treatment among those detected with high-risk HPV (hr-HPV). One way to address this problem is through the identification of persistent hr-HPV infection. The authors of this manuscript examined the factors associated with persistent HPV infection (in terms of HPV types and patient characteristics) through a secondary analysis of data collected in a prospective cohort in Denmark. This manuscript is well-written, clear and will add significantly to the available literature in the topic. Its main limitation is related to a lack of detail on the selection of the study population and a possible selection bias. Please find below few comments to each of the sections of the manuscript for consideration:

We thank the reviewer for this very positive and gratifying comment.

1. Introduction

While the author introduced clearly the topic, the rational for the paper is restricted to one sentence on the lack of data on hrHPV persistence. The introduction would therefore benefit from a more detailed presentation of this (lack of) evidence.

Reply: We chose to present this matter at length in the discussion, with a detailed rundown of the relatively few studies focusing on the topic, emphasizing the heterogeneity of the studies and the lack of available data.

Please see Discussion: Page 10, line 271-284:

2. Minor: line 124 page 5: last sentence is a repetition of the first sentence.

Reply: The mentioned last sentence has been deleted.
Page 5, line 105-107: Sentence deleted.

3. Method

The manuscript should provide a more detailed presentation of the selection of the population at each evaluation: was HPV testing systematically proposed? If not, what were the criteria for being tested? Was cytology performed only in participants who were HPV positive?

Reply: A sentence has been added for clarification.

Page 6, line 143-144: At each visit all participants underwent a gynecological examination including an HPV test and a cervical cytology sample.

4. Page 8: the authors used different labels for the CD4 variable ("latest CD4" and "CD4 at inclusion"), which may result in a lack of clarity.

Reply: Thank you for pointing this out. Latest CD4 has replaced CD4 at inclusion.

5. Was the nadir CD4 available?

Reply: Nadir CD4 was available, but we chose prior AIDS as a proxy for immunodeficiency.

6. Page 8: Instead of excluding observations with missing explanatory variables, the authors could have considered using multiple imputation (if missingness could be considered as random);

Reply: Thank you for noting this. We did have substantial discussions on the choice of statistical methodology, and we consulted a senior statistician who is also a co-author of the paper. We chose the statistical strategy as we believe this is the best way to present the data in a transparent fashion and as we cannot rule out that missingness of explanatory variables are non-random.

7. A number of investigations have shown that viral load suppression is an important predictor of persistent HPV infection in this population, were they able to address this characteristic.

Reply: We did not look for HPV viral loads, nor did we correlate HIV viral load to HPV persistence. For the former, HPV viral load is a much-debated issue as no firm methodology is at present recognized internationally, for the latter most patients on ART in Denmark are fully suppressed (including patients in this study – please see Table 1), and therefore we chose to use ART duration as a predictor of persistence/clearance.
8. Results

Related to the previous comment: how many participants were tested for HPV at each time point?

Reply: All patients were tested for HPV at each time point. Please see Reviewer 2, point 3.

9. What are the characteristics of the participants (and of HPV) who were HPV+ but did not have a second test?

Reply: Thank you for this very good question. Since our focus and scope was on patients with time-spatial HPV tests at the time of analysis, women with an initial HPV positive test but no subsequent follow up was not included.

10. Factors associated to the presence of HSIL: is there any association between this outcome and the HPV type?

Reply: Only 8 patients presented with HSIL and therefore this analysis could not be performed.

11. Discussion

Related to the previous comment: a possible selection bias should be discussed if all participants did not receive an HPV testing; the relatively high proportion of participants who were excluded because they did not have a follow-up test (25%) should also be discussed.

Reply:

Regarding point 1: All included patients had an HPV test performed at each visit.

Regarding point 2: A sentence regarding this matter has been added to the limitations section:

Page 11, line 329-330: The study has limitations including the lack of a control group, the fact that some patients did not participate in all planned visits, etc.

12. Page 10, line 307: it is only HPV 33 outside HPV 16

Reply: The sentence: “The finding of non-HPV16 genotypes in high-grade CIN and CC in WLWH...” refers to the prior four lines, where other than HPV33 non-HPV16 genotypes have been mentioned. Please see below:

In the SHADE study, the predominant hrHPV genotypes were HPV58, 52, 51, and 35 [14]. A worldwide review of almost 20,000 WLWH found that in the African region HPV16, 18 and 45
positivity increased consistently with severity of cervical diagnosis compared to normal cytological samples as well as in confirmed CC [4]. Among European WLWH only HPV33 positivity increased by severity [4]. The finding of non-HPV16 genotypes in high-grade CIN and CC in WLWH….

13. The fact that HPV infection was not associated with any of the cytology outcomes should be further discussed.

Reply: The presence of HPV infection prior to cytological abnormalities, especially high grade, is implicit. The statements in the conclusion are aimed at describing our findings in the context of the people living with HIV. We do not entertain that HIV causes cytological abnormalities without previous or present HPV infection.