Author's response to reviews

Title: Seropositivity among HPV-16/18 DNA positive young women

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Version: 2 Date: 20 July 2010

Author's response to reviews: see over
July 20, 2010

Editor-in-Chief

Melissa Norton, MD

Manuscript title: Determinants of seropositivity among HPV-16/18 DNA positive young women

Dear Editor,

We have revised our manuscript according to the reviewers’ comments and the editorial comments. Here we are including our answers to their concerns.

Thanks in advance for considering this manuscript,

Sincerely,

Dr. Carolina Porras, MSc.
Answers to the Editorial comments

* Please provide a copy of reference #16 that is in press (Coseo S et al: Seroprevalence and Determinants of Human Papillomavirus 16/18 Seropositivity among Young Women in Costa Rica, Sex Transm Dis 2009 In Press). This should be uploaded as an additional file.

We uploaded the article in press in STD of Coseo S et al, as additional file

* Tables can be inserted into the manuscript after the References section; they do not need to be included as an additional file.

We inserted the tables into the manuscript after the references section.

Answers to Reviewer's comments

Referee #1.

Reviewer's report

Title: Seropositivity among HPV-16/18 DNA positive young women

Version: 1 Date: 1 July 2010

Reviewer: Carina Eklund

Reviewer's report:

Review of "Seropositivity among HPV 16/18 DNA positive young women"

The authors are investigating factors associated with HPV seropositivity in HPV DNA positive young women. The seroprevalence found in these women are as expected compared with previous studies.

Minor Essential Revisions

1. When describing the results of HPV DNA detection by HC2 the authors use RLU/CO, in one section this is specified as relative light units / cutoff, in the statistical analysis of viral load RLU/CO are specified as the ratio of relative light unit values to positive control. This indicates that the cut-off and positive control are the same value, is this correct?

RLU/CO represents the ratio of the relative light units/cutoff. We thank the reviewer for pointing out the incorrect language used in the Methods section. We have corrected this error. To further clarify this point, we have added the following description to Page 9 of the manuscript:

With each assay a cut-off RLU (relative light units) value is calculated as the mean RLU value of three positive calibrators. Specimens with relative light units/cut-off (RLU/CO) ratios <1.00 were considered negative; specimens with RLU/CO ratios ≥ 1.00 were considered positive for one or more of the carcinogenic HPV types detected by the assay.

Discretionary Revisions

2. The title "Seropositivity among HPV-16/18 DNA positive young women" are not indicating that the focus of the article are on different determinants of seropositivity. The title "Determinants of seropositivity among HPV-16/18 DNA positive young women" would better describe the content and the new data in the article.

We agree with the reviewer that the title "Determinants of seropositivity among HPV-16/18 DNA positive young women" describes the content of the article better. We have changed the title accordingly.
3. In the Abstract the authors are describing that "anti-HPV 16 antibodies increased with lifetime number of sexual partners. It should be clarified if it was an increase in procentage or in antibody titer, this is not clear at the moment. 

We report the increase in percentage seropositive. To clarify this, we included the following change in the abstract “seropositivity increased with lifetime number of sexual partners”.

4. It would be interesting to see more details on how the different cut-off in the ELISA was established; 8EU/ml for HPV 16 and 7EU/ml for HPV 18, there are no description of the calculations in the reference given. Since the authors in the discussion are mentioning the difference in ELISA performance when explaining difference in determinants for seropositivity between HPV 16 and HPV 18 it would be interesting to see more information on the validation of the assays.

The assay cut-off was defined to be a value above three standard deviations of the geometric mean titer taken from a group of HPV-negative individuals.

On page 9, we added the statement above and referenced the paper that provides more details.


**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**
I declare that I have no competing interests
The authors evaluate the determinants for HPV 16 and 18 seroconversion in concomitant infected women.

The Authors underlines that the study is a cross-sectional because the cervical and blood samples were taken at enrollment, anyway women with HPV positive test underwent follow-up so probably could be interesting to evaluate the result of seropositivity at enrolment and the results (HPV, Pap-test and/or colposcopy) in the follow-up; so to understand if there is a difference in clearance, persistence or progression between seropositive and seronegative women.

Among this group of women HPV16/18 DNA positive at baseline, we have not analyzed if there is a difference in clearance, persistence or progression of the infection between seropositive and seronegative. We agree that this analysis would be interesting and have plans to conduct such an analysis in the future, once the initial follow-up phase of our study is completed and sufficient follow-up information is available.

The authors underlines that in natural history of HPV infection, the antibody levels became evident several months after initially infections; the study evaluate prevalence infection and all HPV infections are considered similar, but each women will have a difference time of persistence of the infection; so for this reason women with very recently infection will not have seroconversion and is not possible to consider surrogate misures to estimate time of persistence, like age of sexual debut or time with most recent partner.

We agree that “left censoring” is a limitation of our study. This fact is discussed in the 4th paragraph of the Discussion.

Results: Women with abnormal..... had 1.63-2.79 fold increased risk of seropositivity: why the authors wrote risk (?), it is not a risk (!) to became seropositivity.

Thank you for noting this mistake. We have corrected the text in the abstract (page 5) and in the results (page 13).

HPV method: Authors have to better explain also in this part that all HPV type specific results come from PCR_LIPa while HC2 was used only to evaluate semi-quantitative viral load

We added more details in the text to clarify these points. Page 8 and 9.

Laboratory methods: please specify better the quality assurance program applied to HPV DNA test and Elisa test, and if they used WHO international standard.
We added in the methods another reference that provides more details about the ELISA assay used for the detection and quantitative determination of IgG antibodies against HPV-16 or 18.


The article of van Doorn LJ, et al provides more details about the SPF$_{10}$/LiPA$_{25}$ and how the HPV testing algorithm was established.


Pag 11
The authors could be better explained why they choose 30 RLU/CO as discriminant between high and low viral load and why they didn’t us a Real time method;
As specified on page 11, we used the median RLU/CO value among participants (RLU/CO=30) to classify women as having low viral load (RLU/CO value $\leq$30) or high viral load (RLU/CO value $>$30).

Results:
The authors should comment better some of their results:
- Not association with smoke: several studies demonstrated that hpv infections are more persistence in smoking women; how it is possible to explain the discondance results?
- association with increasing lifetime partners: also this point is well known as a risk factor for HPV infection. Could this association correlate with the possibility that a women can be re-infected in life-time with the same type of HPV, i.e immuno-system was already stimulated in the past with the same HPV virus, or do contacts in the past with other HPV types increase the possibility for a seroconversion?
- No significant association with condom use: it is in contrast with the knolegme that women using condom have high proportion of clearance.

We have chosen not to speculate about the points raised above in the Discussion, but would be glad to reconsider if the Editor feels this is important to do.

- Hc2 is able to identify 13 HR types so in co-infections it is difficult to evaluate if viral load by hc2 is associated to HPV 16/HPV 18 or to other HR types.

We agree with the reviewer that it is difficult to evaluate if viral load by HC2 test is associated to HPV16/HPV18 or other high risk types detected by the assay. Given that when we restricted the analysis to women with single HPV-16 (170/484, 35%) and HPV-18 (55/186, 30%) infections, the results were comparable to those seen overall, we present the results for all women HPV16/18 DNA positive. On page 11-paragraph 4$^{th}$ of the Statistical analysis we included a statement regarding our sensitivity analysis restricted to single infections.
- PCR (innolipa and in house) has higher sensitivity for HPVs compared to HC2: how many PCR positive samples were not positive in HC2? How the authors resolved the viral load in these samples?

The vast majority of HPV-16 DNA (89%) and HPV-18 DNA (86%) positives by PCR were also positive by HC2.

The small fraction of women who were PCR+ and HC- were considered to have a low viral load.

- How many samples were Innolipa neg, but HPV16/HPV18 positive by pcr with type specific primers? And how many of these samples were positive to HC2?

For HPV-16 DNA positive women the results of the DNA testing were as follow:

- 484 women
  - 417 (86%) positive only by LiPA
  - 67 (14%) positive only by type specific PCR HPV-16
  - 7 (10%) negative by HC2
  - 59 (88%) positive by HC2
  - 1 (2%) missing results for HC2

For HPV-18 DNA positive women the results of the DNA testing were as follow:

- 179 women
  - 152 (85%) positive only by LiPA
  - 27 (15%) positive only by type specific PCR HPV-18
  - 6 (22%) negative by HC2
  - 20 (74%) positive by HC2
  - 1 (4%) missing results for HC2

- the authors reports results combining cytology and viral load, but what the most strong factor: cito or semiquantitative viral load? It's well known that LSIL are associated with High viral loads. The reported results reported could be report more in details the results in the corrispective table.

We chose to report results on cytology and viral load combined to highlight the fact that both are important and that either increased viral load or presence of SIL is associated with seroprevalence. One effect is not more important/stronger than the other, as suggested by the reviewer; rather, they both contribute.

Discussion:

Page 15: hormonal contraceptive use is a risk co-factor for HPV infections and in the same time it will stimulate immune-response, so it acts on the acquisition and on clearance?

We have included our thoughts regarding the hormonal contraceptive findings in the 3rd paragraph of the Discussion.

Cross-sectional study: for specimens collection, but probably the authors have longitudinal data based on follow-up of positive women so it is better to include some analysis comparing hpv seropostivity at enrolment and follow-up data.

Please refer to our response to this reviewer’s 1st comment.
The authors have to explain better the differences observed for seropositivity to HPV 16 compared to seropositivity for HPV 18, it is difficult to understand differences in location of infections caused by these 2 viruses.

To clarify this point we added the following text at the end of the discussion

“Possible explanations include differences in the performance of the ELISA assays designed to measure antibodies against HPV-16 and HPV-18, lower power for the HPV-18 analysis since the number of HPV-18 infected women (n=179) was smaller than that of HPV-16 infected women (n=484), or true biological differences resultant from differences in patterns/location of infections caused by these two viruses. The later statement is supported by reports that HPV-18 infections are often under-represented in precancers, and that they are preferentially associated with the development of cervical adenocarcinomas that often arise deep in the endocervical canal”.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I attended occasionally in Abbott, GSK and Sanofi advisory board