Author's response to reviews

Title: Herpes simplex virus type 2 seroprevalence and risk factors among men who have sex with men from Rio de Janeiro, Brazil: a cross-sectional study

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Author's response to reviews: see over
**Reply to reviewers:**

**Reviewer 1: Javier Lama**

**Reviewer:**

This is a well written and elegant manuscript describing the epidemiology of HSV-2 infection in high risk men who have sex with men in Rio de Janeiro who participated in a HIV-negative cohort designed to assess the incidence of HIV infection among other secondary endpoints.

However, the old nature of the data and mainly, methodological concerns regarding how the reported study population was chosen (a subset of participants of a high risk HIV-negative cohort), attempt with the extrapolation of the results and totally bias any of the conclusions in the way the manuscript is being reported by the authors.

First of all, given the nature of the study population (a subset of participants of a high risk HIV-negative cohort), the relationship of HIV infection, a well recognized associated factor HSV-2 seropositivity in many other epidemiological studies (as well is reported by the authors in the Background section), and its potential influence in other potential covariates is neither being addressed in the study nor commented as a main limitation in the Discussion section in order to generalize the results to most of the MSM population in Rio de Janeiro.

It had been worthy to analyze HSV-2 seropositivity not among those qualifying to enter in this HIV negative cohort, but also among those 1165 men who were screened. If the authors desire to publish their manuscript they must change the title and the approximation and analyses of the manuscript, including the description of the described main limitation in the Discussion section.

**Authors:**

We agree with Dr Lama that the main limitation of the study is our selective group of high-risk men who have sex with men (MSM). Indeed, we need to make it clear to the reader that the population is a subgroup of participants of a high-risk HIV-negative cohort. It was not our intent to suggest that our population is a representative sample of MSM from Rio de Janeiro. In the new version of the manuscript, we have specifically added sentences to the text in order to make the reader aware of possible biases that our population is subject to. We take this opportunity to thank Dr Lama for the suggestions made.

**Reviewer’s specific comments and our responses:**

1. The manuscript may be shortened in length. There is no word count for the manuscript text.

We have shortened the text and tables. The abstract and text word counts are 276 and 2,813, respectively. This information is given in the title page.

2. The manuscript must recognize, starting in the title, that this is a high risk population. The study population it is not just “a sample of MSM from Rio de Janeiro” as stated in the first sentence of the Discussion. It is a sample of high risk MSM previously filtered and qualifying for a HIV negative cohort.
We have re-stated in the title and in the text that our population is a sample of the high-risk MSM population from Rio de Janeiro. Sentences were changed in the Abstract and throughout the text.

3. It would be of help to shortly describe in the Methods section, "the Projeto Rio" entry criteria to better know how this study population was selected. This reviewer was directed by himself to review the entry criteria in a previously published manuscript. Further readers may not do the same.

We have added the entry criteria of ‘Projeto Rio’ to the text. See methods section, first paragraph.

4. No explanation is provided to know why only the first 403 participants (86.3%) out of the total 467 cohort participants underwent for HSV-2 testing. Based on this authors’ self-selection, baseline HSV-2 prevalence among cohort participants would be overestimated in the context that people at higher risk in need of health care services would be enrolled first than later. This limitation must be also addressed in the Discussion section.

Yes, it is possible that the first 403 men are different from the remaining participants. We have addressed this potential bias in the discussion section, paragraph number eight. The argument that the first 403 men belong to a higher risk group and thus overestimate HSV-2 seroprevalence would make sense in a scenario where health care is not available to everyone and/or these men were recruited in a hospital/clinic setting. This is not the case in our study. Although our health care is not optimal, basic health care is universally provided free of charge, including access to STD diagnosis and management. Thus, an individual with symptoms does not need to participate in a study to receive health care. We also argue in the discussion section that indeed an opposite selection bias could have occurred given that men were not recruited at hospital/clinic setting. It is possible that since recruitment happened outside hospital/clinic setting and among healthy individuals, the healthiest would be more willing to participate. If this were true, we could be underestimating the HSV-2 seroprevalence.

5. Every time the Hepatitis B diagnosis is referred in the manuscript based on the seropositivity for anti-HBc, the authors must address that this represents past or current evidence of Hepatitis B infection and not active Hepatitis B infection at all.

We have indicated throughout the text that the serology testing for Hepatitis B indicates previous infection.

6. The statistical analyses subsection at the Methods section states “potential risk factors for HSV-2 seropositivity were...” The authors must agree that through this study design, they cannot evaluate risk factors. They would evaluate “potential associated factors” for HSV-2 seropositivity”. Other sections of the manuscript made this mistake as well. I encourage authors to carefully review the manuscript wording for this item.

We have changed the wording from “risk factor for HSV-2 seropositivity” to “factors associated with HSV-2 seropositivity” throughout the text. Thanks for the suggestion.
7. The second paragraph of the Result section, states “One hundred and eighty one men (46%) were defined as MSM”. This must be corrected. By definition, the entire population is MSM. What the authors are referring is to men self identifying themselves as homosexual according to Table 2.

We have re-worded the passage referring to the participants self identifying themselves as MSM.

8. Given that they are not described in Table 2, it would be of help to provide IQR and ranges for age of first sexual intercourse with men and women.

We have added to the text the IQR and the range for age of first sexual intercourse: 12-16 years.

9. Table 2 must be shortened in length. For two-option variables (yes/no), only positive (yes) results with their correspondent totals must be reported. That would decrease the length of the table by its half.

We have shortened Table 2 by removing the “no” category for the “yes/no” dichotomous variables.

10. As for syphilis conditions, to avoid confusions, Hepatitis B definition must be recalled in the table footnote for Tables 2, 3 and 4.

We have added the Hepatitis B definition to the footnotes of Tables 2, 3 and 4.

11. Point prevalences for variables of interests must include 95% confidence intervals.

We have included the 95% confidence intervals for the variables of interest: proportion of men seropositive for HSV-2: 39.3-52.7%.

12. Table 3 would be decreased in length. Only most important variables should be reported in the table. Others would be referred in the text as not shown data.

Given that we are not repeating in the text some of the findings present in Table 3, we would prefer to keep Table 3 as it is if there is no requirement from the Journal’s office to reduce its size.

13. PR abbreviation is not defined in the article text.

We have added the definition of PR to the text.

14. Have the authors evaluated age as a continuous variable and its relation with HSV-2 seropositivity? It is not clear why the 26 year-old cut off was taken.
Age was dichotomized with respect to the median. We did also try the model with age as a continuous variable and the results were similar. Thus, we opted to leave it as a categorical variable.

15. Discussion section is elegant, but would be abbreviated.

Given that the discussion is elegant and that there are no strict rules on manuscript length, we would prefer to leave it to the editorial office to decide if the text needs to be shortened, if applicable.

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Reviewer 2: Andreas Sauerbrei

Reviewer’s specific comments and our responses:

1. Information about the subjects’ consent obtained prior to processing the samples and the subjects’ histories must be provided.

Because the cohort is no longer active, we could not re-consent the study subjects with regards to the use of the stored serum samples. We submitted the study to the IRB. The IRB analyzed the project and granted the investigator permission to use the stored samples obtained at enrollment of ‘Projeto Rio’. This procedure complies with the Brazilian ethical regulation. The IRB approval is attached.

2. Page 5, paragraph 2, last sentence: The authors should explain which results were included in this study when re-testing of first results was carried out.

Unfortunately, as we proceeded to address this point we noted an error in the manuscript. Actually, no re-testing was necessary. The re-testing was done in our initial analysis when assuming a positive test cut-off of 1.1. In this analysis, we chose to use a more stringent cut-off of 3.5. When assuming this cut-off, no sample needed re-testing. This makes sense since the cut-off of 3.5 is more strict thus reduces false-positives. We have removed the re-testing sentence from the manuscript. Thanks for bringing our attention to this very relevant matter.

3. Page 5, Statistical analysis: How did the authors define the prevalence ratio?

Prevalence ratio is the ratio of the prevalence in the exposed group to the prevalence in the non-exposed group. Prevalence in the exposed group is the proportion of individuals seropositive for HSV-2 among the exposed. Prevalence in the non-exposed group is the proportion of individuals seropositive for HSV-2 among the non-exposed. The Poisson model was used to estimate the association between factors and HSV-2 seropositivity. The prevalence ratio was obtained by taking the exponential of the coefficients estimated with the Poisson model. For further information, we refer the reviewer to Barros and Hirakata (2003). Alternatives for logistic regression in cross-sectional studies: an empirical comparison of models that directly estimate the prevalence ratio. BMC Medical Research Methodology 3:21.
4. There are several repetitions that should be omitted (e.g. page 4, paragraph 2, last sentence; page 5, paragraph 1).

These repetitions were removed.

5. All abbreviations should be defined exactly and all percentages should be given precisely.

We have defined all abbreviations and give the percentages to the nearest decimal.

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**Reviewer 3: Loredana Sarmati**

**Reviewer:**
Authors’ objective was to determine with a cross sectional study the seroprevalence and to evaluate the correlates of HSV -2 infection on stored sera obtained from men who have sex with men from Rio de Janeiro. The results showed an high prevalence (higher than 40%) in this subjects population that resulted correlated with other sexual transmitted infections (HBV and syphilis), with older age and black race, with unprotected sex and with have a stable partner from no more than 6 month.

On the whole the work don’t add new informations to what it is just known about the transmission of HSV and in particularly HSV type 2. There are many published articles which demonstrated that HSV-2 infection is significantly and independently associated with years of sexual activity, history of previous STDs, number of sex partners, number of pregnancies, number of induced abortions, and the percentage of sex acts involving receptive anal intercourse. Otherwise studies on HSV 1 and 2 transmission in European population from many countries (Sex Transm Infect 2004;80:185–191) showed an HSV 2 seroprevalence of at most 20 %. Higher was the percentage of HSV-2 seropositive sex workers (79.0%) in Singapore (International Journal of STD & AIDS 2006; 17: 395–399). Some data are also known about the HSV-2 seroprevalence in Brazil (The Journal of Infectious Diseases 2002;186(Suppl1):S3–28) that resulted higher than in Europe but very variable from a place to another.

Little is known on HSV2 infection in the gay HIV-seronegative subjects. A recent study on an Australian cohort of HIV-negative gay men (JID 2006:194, 1 561-70) showed a low seroprevalence of HSV 2 infection.

**Authors:**
Indeed, our work adds information regarding the HSV-2 seropositivity among the high-risk HIV-negative MSM community of Rio de Janeiro.

**Reviewer’s specific comments and our responses:**

1. The HSV 1 seroprevalence of the study population.
Our study did not aim to estimate the HSV-1 seroprevalence and we did not perform the test.

2. The HSV 1 and 2 seroprevalence of the female population of Rio de Janeiro (HSV 2 is usually prevalent in female).

Our study population was composed of high-risk MSM from Rio de Janeiro. We refer the reviewer to studies conducted with Brazilian women:


3. If there was the possibility to know if there were ethnic differences in this group of subject that could have different HSV 2 seroprevalence.

The Brazilian Census subdivides the population into three groups: whites (50%), mixed (Pardo, 43%) and blacks (7%). In our study, we collected information relating to the participant’s race in the same fashion. In our study population, 52.4% were whites and 47.6% were non-whites, this subgroup included blacks and Pardos. Pardos composed the majority of the non-white population. HSV-2 seroprevalence did not differ among blacks and Pardos. Thus, we combined the two into the non-whites category. Non-white race was significantly associated with HSV-2 seropositivity in the bivariate and multivariate analyses as shown in Tables 3 and 4.

4. Pag 4 1° phrase: new test don’t permit to evaluate the presence of HSV in absence of clinical symptoms (this fact is true in any case) but to differentiate antibodies to HSV 2 from those to HSV1.

Yes, this is correct, the new test allows for the differentiation of the antibodies to HSV-2 from HSV-1. We have corrected the sentence in the text. We thank the reviewer for pointing this out.