Author's response to reviews

**Title:** Anal cytological abnormalities and epidemiological correlates among men who have sex with men at risk for HIV-1 infection

**Authors:**

Maria Gabriella Dona' (dona@ifo.it)
Maria Benevolo (benevolo@ifo.it)
Amina Vocaturo (vocaturo@ifo.it)
Guido Palamara (palamara@ifo.it)
Alessandra Latini (a.latini@ifo.it)
Amalia Giglio (agiglio@ifo.it)
Francesca Rollo (rollo@ifo.it)
Giampaolo Impara (giampaoloimpara@yahoo.it)
Fabrizio Ensoli (ensoli@ifo.it)
Fulvia Pimpinelli (pimpinelli@ifo.it)
Aldo Di Carlo (dirsci.isg@ifo.it)
Massimo Giuliani (giuliani@ifo.it)

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**Author's response to reviews:** see over
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Prof. Paolo Bruzzi  
Editor  
BMC Cancer

Dear Prof. Bruzzi,

We are pleased to submit the revised version of our manuscript (MS 8212551977603166), now entitled “Anal cytological abnormalities and epidemiological correlates among men who have sex with men at risk for HIV-1 infection”. In this version, a new author has been added to the authorship list.

We hope that this new version of the manuscript, revised according to the comments and suggestions of the referees, will be considered for publication in BMC Cancer.

In this letter we have addressed point-by-point the specific issues raised by the referees. Our responses to their suggestions are indicated below. All changes in the revised manuscript have been highlighted in red.

Sincerely,

Maria Gabriella Donà
Editorial points

Ethical Approval - Research involving human subjects (including human material or human data) that is reported in the manuscript must have been performed with the approval of an appropriate ethics committee. Research carried out on humans must be in compliance with the Helsinki Declaration. A statement to this effect must appear in the Methods section of the manuscript, including the name of the body which gave approval, with a reference number where appropriate.

The statement about the compliance with the Helsinki Declaration was already included in the manuscript. The name of the body which gave approval has now been included in the Methods section, together with the reference number (page 7).

Answers to Referee #1 (Alcina Nicol)

Questions and comments to the authors:

There is any information regarding previous HPV vaccination by any participants; please could the authors inform something regarding it on the Methods.

The information about HPV vaccination has been included in the Methods, “Participants recruitment and questionnaires” section (page 6), that now reads as follows: “Participants, ≥18-year-olds, that had not been previously vaccinated against HPV, were considered eligible according to the following criteria…”

In spite of the authors pointed out the weaknesses of their work. On the last paragraph – discussion section, the authors claimed that additional investigations, including HRA with biopsy of abnormal findings are needed in individuals with anal cytological changes to assess the actual rate of high grade AIN. Then, Have the authors any plans to follow up those individuals in further study?

Following the suggestions of the other referee, the last paragraph has been deleted. However, to address your comment, the following sentence has been added at the end of the Discussion (page 14): “All patients included in this study are currently in follow-up in order to monitor the possible
development of anal dysplasia in cytologically negative participants and to evaluate the actual presence of AIN in individuals with abnormal anal cytology.”
The findings of the follow-up study will be the focus of future reports.

Answers to Referee #2 (Elisabeth A Stier)

This manuscript does provide valuable data by adding to the available information on prevalence of anal HPV and abnl anal cytology in HIV-MSM.
However, the title is misleading – as the term “correlates” is not correct. Would rename.
The term “correlates” is used in clinical epidemiology studies to indicate those variables (clinical, biological, demographic, behavioral, social, etc.) proper to a population of cases, to be assessed as possibly associated with the clinical condition (or infection) under study. The term “correlates” is commonly used in cross-sectional or prevalence studies. The term is not synonymous with “risk factors” or "determinants", which are properly assessed by longitudinal or incidence studies. Unfortunately, to our knowledge, there is not a synonym of the term “correlates” suitable for the title of our manuscript. However, in order to make the term “correlates” less vague, we added the adjective "epidemiological” in the title, that now reads as follows: “Anal cytological abnormalities and epidemiological correlates among men who have sex with men at risk for HIV-1 infection”.

Abstract:
Correlates is a vague term—typically would suggest histologic correlates—not demographic as is used here. would find a different word.
Please, see answer to previous comment. In addition, the term refers not only to demographic variables, but also to the behavioral and clinical variables investigated in the present study. However, in order to make the term less vague, we added the adjective "epidemiological” (page 3): “This study aimed to evaluate the prevalence and epidemiological correlates of anal cytological abnormalities among relatively young…”

The following statements do not make sense unless the “higher proportion” was not statistically significant. “None of the socio-demographic and behavioral factors analyzed showed a significant association with abnormal cytological findings. However, a higher
proportion of ASC-US+ cases was found in older MSM, in those with a higher number of lifetime sexual partners and in those with a history of ano-genital warts.”

For clarity, these statements have been modified, and now read as follows (page 3): “A higher proportion of ASC-US+ was found in older MSM, in those with a higher number of lifetime partners and in those with a history of ano-genital warts. However, neither these variables nor the others analyzed showed a significant association with abnormal cytological findings.”

Intro—needs to be more focused on what this study does—ie, prevalence data for HPV and anal cytology for young MSM. Would delete the second paragraph, and re: the third, even if histologic HGAIN is found in this population, that in of itself is not a reason to screen. Rather if treatment of the HGAIN in this patient population results in decreased anal cancer rates, then screening may be appropriate. Again, focus on what this manuscript shows.

We agree with the reviewer that the presence of histological HGAIN is not a sufficient reason to screen and that screening is appropriate only if treatment of HGAIN results in decreased anal cancer rates. We have now modified the introduction, mostly deleting the second paragraph and focusing the third one on what the study aims are (pages 5-6).

Results—
Is 38.5% different from 36% different from 43.5%?? “With respect to HPV-negative individuals, increased proportions of anal cytological abnormalities were evidenced both in patients infected by LR types only (12.0% vs. 38.5%, COR=4.56, 95% CI: 1.78-11.90) and in those with any HR type (12.0% vs. 36.0%, COR=4.10, 95% CI: 1.88-9.17). Importantly, MSM with HPV 16 and/or 18 anal infection showed a proportion of abnormal cytology more than three times higher than that found among HPV-negative participants (43.5% vs. 12.0%, COR=5.62, 95% CI: 2.33-13.81).”

Comparison between the prevalence of abnormal cytology in individuals infected by LR-HPV only (38.5%), those infected by any HR-HPV (36.0%) and those infected by HPV16 and/or 18 (43.5%) was performed, showing a non-significant difference (please, see changes below). However, the comparison between the prevalence of abnormal cytology in individuals infected by any HR-HPV (36.0%) and in those infected by HPV16 and/or 18 (43.5%) was not performed, since HPV16 and/or 18-infected cases are included in the former group.
The paragraph now reads as follows (pages 9-10): “The prevalence of cytological abnormalities found among individuals infected by LR-HPV only was not significantly different from either that observed in MSM infected by any HR-HPV (38.5% vs. 36.0%, COR=0.90, 95% CI: 0.45-1.80) or that observed in MSM infected by HPV16 and/or 18 (38.5% vs. 43.5%, COR=1.23, 95% CI: 0.55-2.74).”

If anything, what is more surprising is that abnormal cytology is associated with HPV infection of any kind……

The association found between abnormal cytology and infection with any type of HPV originates from the use of HPV-negative individuals as a reference group. Therefore, this finding only highlights that, in comparison with MSM not infected by HPV, those positive for HPV show a significantly higher proportion of cytological abnormalities. This is not surprising, given that the group “any HPV type” includes LR and HR types, for which a significant association has been found.

Need to be clear on what is TREND data vs. that which is statistically significant. “However, a tendency was observed for some variables. In detail, a higher proportion of ASC-US+ cases was found in older MSM, in those with 20-49 lifetime sexual partners and in those with a history of ano-genital warts than in the respective reference groups.”

For clarity, the first sentence has been deleted and the second statement has been modified as follows (page 10, last paragraph of Results): “Although the proportion of ASC-US+ cases found in older MSM, in those with 20-49 lifetime sexual partners and in those with a history of ano-genital warts was higher than in the respective reference groups, in none of these cases was the increase statistically significant.”

In addition, the following sentence has been added to the Discussion (page 13): “Finally, it is possible that our ability to identify statistically significant associations for some of the variables analyzed may have been limited by the small number of patients included in each group after stratification.”

Discussion—

Would comment on the 14% with an inadequate specimen for cytology? Yet you were able to get HPV results. Seems surprising.
This observation has now been discussed, also including an appropriate reference (page 12): “Notably, a valid HPV test result was obtained for all samples, independently of the adequacy for the morphological evaluation, a fact that has also been observed in other studies [Darwich et al, 2012]. This finding suggests that, although some samples lacked a sufficient number of nucleated cells to allow the cytological interpretation, the material was enough to obtain a valid HPV test result. This is probably due to the high sensitivity of PCR-based methods, which are able to detect virtually one copy of DNA.”

In addition, a sentence has been added to the Methods section (page 8) to clarify that HPV test results that did not show any amplification of the β-globin control were considered valid whenever one or more HPV-specific hybridization bands were observed: “Results were considered valid whenever the amplification of the β-globin control and/or at least one HPV hybridization band were observed.”

**Suggested edits:**

**First 3 sentences not needed. Would delete.**
The first three sentences have been deleted (page 10).

**Below should reference 18 and 19. “Notably, this is one of the few studies conducted on relatively young MSM (median age 32 years), while most of the previous studies focused on older MSM.”**
The references have been added (page 11). They are now references 17-18.

**Final paragraph in the conclusion not needed.**
The final paragraph has been deleted (page 14). However, in order to address the comments of the other referee, the following sentence has been added: “All patients included in this study are currently in follow-up in order to monitor the possible development of anal dysplasia in cytologically negative participants and to evaluate the actual presence of AIN in individuals with abnormal anal cytology.”

**Table 2—**

**Would add a line with data on HPV 16 and 18 prevalence**
Data about HPV16/18 prevalence have been included in this table.
Would delete table 4, especially as it really does not show a tendency for increased risk of abnormal anal cytology with increased age.

Table 4 shows all the variables analyzed in this study, which do not include age only, but also a number of socio-demographic (education, income), behavioral (age at first intercourse, lifetime and recent number of partners, sexual practices, condom use) and clinical (STD history, including ano-genital warts, genital herpes, syphilis, gonorrhea) characteristics. Although it is true that none of these variables was associated with abnormal anal cytology, only Table 4 shows how the data were stratified and that an appropriate and careful analysis was performed to evaluate the possible associations with the selected variables. Most importantly, as we have now specified at the end of the Discussion, it is possible that for some variables the low number of patients in each group may have limited our ability to identify statistically significant associations. These detailed data, necessary for a correct interpretation of our findings, are only provided by this Table. Therefore, we believe the deletion of Table 4 may hinder the correct interpretation of our observations.

Quality of written English: Needs some language corrections before being published

The manuscript has been revised by a native speaker, now acknowledged in the Acknowledgments section.