PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (see an example) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

This paper was submitted to the STI but declined for publication following peer review. The authors addressed the reviewers' comments and submitted the revised paper to BMJ Open. The paper was subsequently accepted for publication at BMJ Open.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Antiretroviral therapy is not associated with reduced herpes simplex virus shedding in HIV co-infected adults: An observational cohort study
AUTHORS	Tan, Darrell; Raboud, Janet; Kaul, Rupert; Walmsley, Sharon

VERSION 1 - REVIEW

REVIEWER	NAGOT, Nicolas University of Montpellier, France
REVIEW RETURNED	05-Aug-2013

GENERAL COMMENTS	This paper addresses the question of a potential impact of ART on HSV shedding in a view of documenting future clinical trials of HSV-2 suppressive therapy among ART-treated patients. The underlying hypothesis is that such antiviral treatment could much reduce the negative impacts of HSV-2 on immune activation, on HIV-1 shedding and on low-level systemic HIV-1 RNA, and their potential consequences on disease progression and ART efficacy. Based on the assumption that both HSV-2 and HIV-1 share the same negative impact, the authors decided to consider HSV-1 and HSV-2 altogether, at the oral and genital sites. The results are interesting and add important knowledge in this field. However, several points may be discussed further by the authors. 1. With respect to the research question, the comparison of the proportion of patients with any HSV shedding (i.e. HSV shedding at least once over the 28 days) between the two groups would be very informative. After the works from the Seattle group, we know that HSV-2 shedding episodes leads to the influx of T-CD8+ lymphocytes at the site of shedding, which will persist for weeks. It is therefore reasonable to hypothesise that just one episode of shedding can impact the local immunity for weeks. Although two or more episodes during this period may increase these alterations, assessing whether any shedding occur or not is probably important. In addition, for comparison purposes with other studies and in line with the results suggesting that HSV-2 certainly drives the HSV-HIV interactions, it would be of interest to show a sub-group analysis among HSV-2 co-infected patients considering genital samples only. 2. The choice to censor patients who will develop GUD during follow-up is arguable. A GUD is primarily the clinical manifestation of a high and exacerbated HSV shedding episode. Since HSV

	assessment is qualitative, I think censoring these patients introduce a bias that minimise HSV shedding. Although the decision not to include patients who would benefit from a HSV suppressive therapy makes sense, censoring participants because of GUD occurrence deserves more explanation from the authors 3. The study results and observed impact of ART on HSV shedding could be further compared to previous reports (e.g Mayaud et al. STI 2008). Previous work with their limitations and remaining knowledge gaps may also be added in the introduction section. 4. In the section on study limitations, the authors may discuss the relatively high HSV detection threshold of their technique which may have contributed to the relatively low rates of HSV shedding. Several other groups used a quantitative HSV-2 DNA PCR with detection threshold of about 500 DNA copies/ml. In addition, the authors mention that their study was, retrospectively, underpowered to show a significant difference because of the lower than expected HSV shedding rate. 5. The study title may appear a bit misleading; the main finding of this study is that ART is not associated with a reduced HSV shedding, but not that some HSV shedding does persists on ART, which has been reported previously by several teams.

- The manuscript received two reviews at The STI but the other reviewer declined to make the reviews public. Please contact BMJ Open editorial office for any further information.

VERSION 1 – AUTHOR RESPONSE

REVIEWER #1

1. Provide a comparison of the proportion of patients with any HSV shedding, and a subgroup analysis among HSV-2 co-infected patients considering genital samples only.

Response: We agree with the reviewer that a comparison regarding the proportion of patients with any HSV shedding is of interest, and have added these results to the section entitled, “HSV shedding by HAART status”. A formal comparison among HSV-2 co-infected patients of genital samples only is already provided in Table 2 (in the row labelled “anogenital HSV-2 shedding rate” under “HSV-2 shedding rate among HSV-2 seropositive participants”).

2. Provide further explanation regarding the choice to censor patients who develop GUD during follow-up.

Response: Data from participants who developed herpes symptoms during the observation period were censored at the time of diagnosis for two main reasons. First, asymptomatic shedding would be expected to persist after a symptomatic HSV outbreak, but for an unclear amount of time; further, such asymptomatic shedding might actually constitute continuation of the symptomatic reactivation episode rather than distinct subsequent episodes of asymptomatic shedding. As such, it was unclear when the optimal time for reinitiating sampling after a symptomatic outbreak would be, and we considered it most straightforward to censor at the time of diagnosis. Second, we reasoned that symptomatic herpes episodes represent times when individuals might be expected to benefit clinically from antiviral therapy, whereas we were most interested in quantifying asymptomatic shedding in the absence of other indications for therapy. We acknowledge in the paragraph on limitations that this strategy could theoretically have lowered our observed HSV shedding rates, although this possibility is unlikely because similar, small numbers of HAART-naïve and HAART-treated participants had data

censored (3/44 HAART-naïve and 1/41 HAART-treated participants). To formally address the reviewer's concern, we have now conducted a sensitivity analysis in which these three HAART-naïve participants were assumed to exhibit maximal HSV shedding, while the single HAART-treated participant was assumed to exhibit minimal HSV shedding during the censored days, and the results of the analysis did not qualitatively impact our conclusions; information about this analysis has been added to the Methods, Results and Discussion sections, respectively.

3. Compare the results to previous reports eg. Mayaud et al. STI 2008.
Response: We agree with the reviewer that this report is relevant to our study, and have added discussion of its findings and limitations in the second paragraph of the Discussion section.

4. In the limitations section, discuss the relatively high HSV detection threshold of the PCR technique and the power of the study to show a significant difference because of the lower than expected HSV shedding rate.
Response: A statement about the relatively high HSV detection threshold of the PCR technique has now been added to the paragraph discussing limitations of the paper, in the second last paragraph of the Discussion section.

5. Consider revising the study title.
Response: On the advice of the reviewer, we have revised the title to the following: "Antiretroviral therapy is not associated with reduced herpes simplex virus shedding in HIV co-infected adults: An observational cohort study"