PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Risk of myocardial infarction among people living with HIV: an updated systematic review and meta-analysis
AUTHORS	Eyawo, Oghenowede; Brockman, Gwenyth; Goldsmith, Charles; Hull, Mark; Lear, Scott; Bennett, Matthew; Guillemi, Silvia; Franco-Villalobos, Conrado; Adam, Ahmed; Mills, Edward; Montaner, Julio; Hogg, Robert

VERSION 1 – REVIEW

REVIEWER	Alinda Vos University Medical Center Utrecht, The Netherlands
REVIEW RETURNED	16-Oct-2018

GENERAL COMMENTS	This review and meta-analysis seek to answer the question if HIV and ART is associated with an increased risk of myocardial infarction. This is an important issue as the HIV infected population is aging. The authors conducted a comprehensive search using a clear strategy including all evidence up to July 2018. There are, however, some concerns from my side: 1) the critical appraisal is not clear to me: based on what was decided to score a study as plus or minus in the critical appraisal table (appendix 3)? 2) I miss the link between the critical appraisal and the interpretation of the results. All studies get the same weight in the results section, regardless of the outcome of the critical appraisal. 3) Some firm statements are made, while they rely on only a few studies, For example plasma VL and low CD4 count. VL >100,000 cp/mL is addressed in only 2 studies, CD4 count >200cells/mm3 in 3 studies. I would recommend to integrate the critical appraisal in the results section by giving reliable studies more weight and studies with apparent methodological flaws less weight, or to perform a sensitivity analysis including only studies that were deemed to be methodological sound in the critical appraisal. This review and meta-analysis seek to answer the question if HIV and ART is associated with an increased risk of myocardial infarction. This is an important issue as the HIV infected population is aging. The authors conducted a comprehensive search using a clear strategy including all evidence up to July 2018. There are, however, some concerns from my side: 1) the critical appraisal is not clear to me: based on what was decided to score a study as plus or minus in the critical appraisal table (appendix 3)? 2) I miss the link between the critical appraisal and the interpretation of the results. All studies get the same weight in the results section, regardless of the outcome of the critical appraisal. 3) Some firm statements are made, while they rely on only a few studies, For example plasma VL and low CD4 count. VL >100,000 cp/mL is

	addressed in only 2 studies, CD4 count >200cells/mm3 in 3 studies. I would recommend to integrate the critical appraisal in the results section by giving reliable studies more weight and studies with apparent methodological flaws less weight, or to perform a sensitivity analysis including only studies that were deemed to be methodological sound in the critical appraisal. Comments per section Abstract: the conclusion refers to CD4 and VL, while these outcomes were not addressed in the results section Methods: Why did you include stroke and cerebro-vascular disease in the search while they outcome of interest was myocardial infarction? Once sentence to clarify this would be helpful. Page 7: Why were abstracts screened up to 2016 and full text articles up to 2018? A clarification would help. Data analysis Lines 24-26: to minimize…unadjusted estimates. This seems plausible to me. However, I cannot see how you dealt with adjusted versus unadjusted estimates. Which studies presented adjusted, which unadjusted estimates? How were these results analysed? There is nothing about this in table 2 nor in the forest plots. Lines 36-38: 'we assessed heterogeneity… individual studies'. What was the outcome? How did you deal with heterogeneity in the interpretation of the results? Flowchart 1: '36 with unusable data (e.g. uadjusted estimates) were excluded. I do not understand this. You state in lines 24-26 of the data analysis section that unadjusted estimates will be dealt with separately. If so, should not be an exclusion criteria? Results General: incorporate the outcome of the critical appraisal in the interpretation of results What definitions did you use to categorize a study as '+' or '-' in appendix 3? A table with the criteria would be helpful. Page 13, line 26-30: I'm struggling to understand the cumulative treatment exposure. Is this a risk compared to the non-HIV infected population? And did the original studies account for the effects of age? It may be the case as well, as you point out in the discussion, that the effect reflects duration of HIV infection. In general: lots of meta-analysis include only 2 or 3 articles. (figures 2-6). Do you think that results are reliable as the conclusion relies on only 2 or 3 studies? (and I, as reader, do not have an idea about the quality of the studies when I look at the plots) . Discussion I like the paragraph 'Previous meta-analysis … ART per se.' That's well written! Lines 3-8: 'Third, we have …. (D:A:D) study.' I do not understand this argument. You did not use risk estimates, but real risk of myocardial infarction. Can you please clarify this? Minor: figure 6 (page 39) does not have a title

REVIEWER	Matthew Levy George Washington University Milken Institute School of Public Health, United States
REVIEW RETURNED	24-Oct-2018

GENERAL COMMENTS	Thank you for the opportunity to review this manuscript, titled "Risk of myocardial infarction among people living with HIV: an updated systematic review and meta-analysis." I commend the authors for conducting a very comprehensive and well-designed meta-analysis. Specific comments are listed below: 1. Regarding the analysis that quantified the overall relative risk of myocardial infarction associated with HIV infection: while the literature search, data abstraction, and quantitative aspects of this meta-analysis were well designed and well executed, the authors missed an opportunity to more comprehensively and critically examine the epidemiologic study designs of the various research studies that contributed data to this meta-analysis. Given that the authors already took the time to carefully review aspects of each of the study designs (Appendix Table 3), a more critical examination of the literature in relation to the relative risk quantified would have added great value in comparison to what has been done in similar prior reviews and meta-analyses. As the authors recognized, there is heterogeneity across studies. Heterogeneity might include differences in study populations, comparison groups, adjustment for confounding factors, and the measures used to defined myocardial infarction (e.g., diagnosis code, adjudication protocol). The authors could have provided a critical review of, for example, the appropriateness of the HIV-uninfected comparison groups and the extent to which confounding factors were accounted for in analyses. For context, please see this article: Althoff KN, Gange SJ. A critical epidemiological review of cardiovascular disease risk in HIV-infected adults: the importance of the HIV-uninfected comparison group, confounding, and competing risks. HIV Med. 2013;14(3):191-2. Although the authors evaluated the risk of bias in the studies included in the meta-analysis (Appendix Table 3), it was not an area of focus in the manuscript text. What criteria were used to determine whether there was bias in 'case/group representation' and comparability among study groups? The manuscript does not mention to what extent prior publications were excluded from the analysis due to poor quality. Which epidemiologic studies included in the meta-analysis most likely resulted in the most valid results? Was a sub-analysis restricted to the most valid study designs conducted? This sort of discussion might provide more insight that would supplement the relative risk calculated in the meta-analysis. 2. A major strength of this meta-analysis is that the authors not only considered the relative risk of MI associated with HIV, but also considered the associations for various ARV regimens (defined using current, lifetime, and cumulative definitions), CD4 count, and HIV viral load. It might be nice to highlight this novel aspect of the study a bit more in the title, abstract, and/or

	introduction. For instance, the rationale for this analysis in the abstract is that the evidence surrounding specific components of CVD risk remains inconclusive. My understanding was that it is generally well accepted that HIV confers an increased CVD risk. 3. On the other hand, a large amount of results are presented, which at times make the results a little difficult to follow in the text of the results section. Sub-headings would make it easier to follow. The first paragraph of the discussion section is very helpful for highlighting the key findings in a straightforward manner. The figures are also very clear. 4. In the second paragraph of the "Meta-Analysis of the risk of MI" section, consider re-wording the sentences to make it more clear that the RR of 1.80 represents a comparison of HIV+ to HIV- among patients on ARV therapy, and that the RR of 1.25 was among patients not on ARV therapy (and not comparing ARV-treated to ARV-untreated, as it seemed to me, until I looked at the forest plot figure). 5. Regarding that prior point (#4), do the authors take that finding to provide evidence that HIV is not associated with MI among treated patients? Might it be due to differences in characteristics between patients who are treated and not treated in those two study samples, which might not have been able to be accounted for in the analysis (i.e., unmeasured confounding)? 6. Consider making the distinctions between recent treatment exposure, any treatment exposure, and cumulative treatment exposure more explicit upfront in the methods section. Perhaps a better term for any treatment exposure might be lifetime exposure. In the results text, "recent" is compared to "not recent" ARV exposure, which might be confusing because it reads as though recent exposure was being compared to past exposure of the respective regimen, although I believe that recent exposure was compared to a lack of recent exposure (which might have included past exposure or never having been exposed). 7. To what extent do the authors think that the differences in findings for different ARV regimens are due to real differences in the associations between different ARV treatments and MI risk, as opposed to differences in study designs and study samples? 8. In order to support the rationale that it is more appropriate to assess the risk of HIV infection with MI as an outcome separately from other CVD outcomes (as has been done in other meta-analyses), more information on how the pathophysiologic mechanisms differ would be supportive. I noticed that the registered systematic review protocol included in the appendix stated that the primary outcome would be stroke, MI, or cardiac death as a composite outcome. Why was this pre-specified protocol not followed? 9. One limitation that could be helpful to explicitly state in the discussion section is that many of the sub-analyses of this meta-analysis were based on only two or a handful of studies – not all studies included in the meta-analysis overall.

	10. Please confirm that the citations 26-31 provide support that the odds ratio can be used in this context for estimation of relative risk. Thank you. 11. Did the authors assess possible publication bias using a method such as a funnel plot?

REVIEWER	Audrey Rankin Queen's University Belfast
REVIEW RETURNED	27-Nov-2018

GENERAL COMMENTS	This paper reports a meta-analysis which estimates the risk of MI among HIV positive individuals. The statistical analysis conducted utilises a meta-analysis approach which is an appropriate method. The only comment I have is in relation the quality assessment conducted according to the Newcastle-Ottawa (NOS) criteria. The authors should comment on the results of this assessment and implications for the meta-analyses in terms of the certainty of the evidence.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1: Alinda Vos
(University Medical Center Utrecht, The Netherlands)
This review and meta-analysis seek to answer the question if HIV and ART is associated with an increased risk of myocardial infarction. This is an important issue as the HIV infected population is aging. The authors conducted a comprehensive search using a clear strategy including all evidence up to July 2018. There are, however, some concerns from my side:

Comment 1: the critical appraisal is not clear to me: based on what was decided to score a study as plus or minus in the critical appraisal table (appendix 3)?
Response: Thank you for the comment. We have updated the Methods section to include additional details on the critical appraisal including a description of how it was decided to score a study as 'plus' or 'minus' on a given study design feature. The Methods section describing the critical appraisal has been revised to include the following additional text: '…Following guidelines in the Newcastle-Ottawa Scale (NOS) for assessing the quality of observational studies in metaanalyses29 and with slight modification of the scoring system to simplify reporting, the risk of bias assessment was performed based on the adequacy of three key domains of the study design features namely: the group/participant selection; comparability of groups; and the exposure and outcome assessments in the individual studies. For each of these key features, we assigned a "+" (plus) sign when this was clearly and adequately described in the study, and a "–" (minus) sign when it was not clearly described or was missing. A detailed description of the results of the quality assessment is available in the appendix'.
• 29. Wells GA, Shea B, O'Connell D, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses, 2018: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp, Accessed January 17, 2019.

Comment 2 & 3: I miss the link between the critical appraisal and the interpretation of the results. All studies get the same weight in the results section, regardless of the outcome of the critical appraisal. 3) Some firm statements are made, while they rely on only a few studies, For example plasma VL and low CD4 count. VL >100,000 cp/mL is addressed in only 2 studies, CD4 count >200cells/mm3 in 3 studies.

I would recommend to integrate the critical appraisal in the results section by giving reliable studies more weight and studies with apparent methodological flaws less weight, or to perform a sensitivity analysis including only studies that were deemed to be methodological sound in the critical appraisal.
Response: We agree with the reviewer that the link between the critical appraisal and the interpretation of the results could have been clearer. In the revised manuscript, we have included the following additional text to allow readers better contextualize the study findings in light of the critical appraisal where we acknowledge in the Discussion section that '…In terms of the critical appraisal and its impact on the interpretation of the results, variability in the quality of the included studies may have influenced the results of the meta-analyses and thus the conclusions drawn.'
We also thank the reviewer for their suggestions on how to further integrate the critical appraisal into the interpretation of the results. Of note, in meta-analysis, the choice of weight assignment is not driven by the results of the critical appraisal, rather eligible studies are usually weighted by the precision of the estimated effect sizes from the individual studies, and is based on the assumptions surrounding the modelling strategy selected for the meta-analysis, – which is a random-effects model in this manuscript. Therefore, we are unable to arbitrarily assigned weights or "give reliable studies more weight" as suggested by the reviewer. However, we implemented the reviewer's second suggestion and performed a sensitivity analysis where we excluded studies deemed to be of lower quality. This did not lead to an important change in our findings. For example, in the meta-analysis of MI risk associated with HIV infection, excluding two studies (Durand et al 2011, Triant et al 2009) that were deemed to be of lower quality resulted in a pooled relative risk of 1.58 (95% CI: 1.36, 1.83) compared to 1.67 (95%CI: 1.45, 1.94) in our original analysis that used data from all eligible studies.
Regarding the point made by the reviewer that certain comparisons relied only on a few studies, we now acknowledge this as a limitation in the Discussion section where we state: '…Also, some of the comparisons in our study were based on a small number of studies which is a limitation.'

Reviewer 2: Matthew Levy
(George Washington University Milken Institute School of Public Health, United States) Thank you for the opportunity to review this manuscript, titled "Risk of myocardial infarction among people living with HIV: an updated systematic review and meta-analysis." I commend the authors for conducting a very comprehensive and well-designed meta-analysis. Specific comments are listed below:

Comment 1. Regarding the analysis that quantified the overall relative risk of myocardial infarction associated with HIV infection: while the literature search, data abstraction, and quantitative aspects of this meta-analysis were well designed and well executed, the authors missed an opportunity to more comprehensively and critically examine the epidemiologic study designs of the various research studies that contributed data to this meta-analysis. Given that the authors already took the time to carefully review aspects of each of the study designs (Appendix Table 3), a more critical examination of the literature in relation to the relative risk quantified would have added great value in comparison to what has been done in similar prior reviews and meta-analyses. As the authors recognized, there is heterogeneity across studies. Heterogeneity might include differences in study populations, comparison groups, adjustment for confounding factors, and the measures used to defined myocardial infarction (e.g., diagnosis code, adjudication protocol). Response: We thank the reviewer for the supportive feedback and have updated several sections of the manuscript based on his suggestions in the comments below.

Comment: The authors could have provided a critical review of, for example, the appropriateness of the HIV-uninfected comparison groups and the extent to which confounding factors were accounted for in analyses. For context, please see this article: Althoff KN, Gange SJ. A critical epidemiological review of cardiovascular disease risk in HIV-infected adults: the importance of

the HIV-uninfected comparison group, confounding, and competing risks. HIV Med. 2013;14(3):191-2.
Response: In light of the reviewer's comments, we re-ran the primary meta-analysis quantifying the overall relative risk (RR) of MI associated with HIV infection and excluded one study involving a 'general population' comparison group,42 as including this could be considered a weakness in the meta-analysis. Consequently, throughout the manuscript we have now revised our pooled estimate of the overall RR of MI associated with HIV infection to be 1.67 (95%CI: 1.45, 1.94) and not 1.60 (95%CI: 1.38, 1.85) as in the previous version. The excluded study was re-added in a sensitivity analysis to highlight the effect of using an appropriate comparison group.
In terms of providing a critical review on the appropriateness of the HIV-uninfected comparison groups as suggested by the reviewer, we have also updated sections of the manuscript to accommodate discussions on this issue. For example, in the Methods section, we now state that: '…For the analysis that quantified the overall RR of MI associated with HIV infection, we examined the appropriateness of the comparison group by repeating the meta-analysis and including one additional study that involved a general population comparison group,42 as opposed to an HIV-uninfected comparison group.'
In the Results section where we describe our findings on the risk of MI associated with HIV infection, we now further state that: '…In sensitivity analysis (Appendix Figure S1) where we repeated the meta-analysis and included one additional study that involved a general population comparison group,42 the overall pooled RR was 1.60; 95%CI: 1.38, 1.85.'
In the Discussion section where we contextualized the overall RR of MI, we have added the following additional text: '…Regarding studies that quantified the risk of MI associated with HIV infection, the appropriateness of the HIV-uninfected group used for comparison purposes is critical; an issue that has been extensively reviewed elsewhere.73 In sensitivity analysis, the overall RR of MI associated with HIV infection was reduced when we included one additional study involving a 'general population' comparison group, therefore highlighting the importance of using an appropriate control group.'

- 42. Drozd DR, Kitahata MM, Althoff KN, et al. Increased Risk of Myocardial Infarction in HIV-Infected Individuals in North America Compared With the General Population. J Acquir Immune Defic Syndr 2017;75(5):568-76. doi: 10.1097/QAI.0000000000001450
- 73. Althoff KN, Gange SJ. A critical epidemiological review of cardiovascular disease risk in HIV-infected adults: the importance of the HIV-uninfected comparison group, confounding, and competing risks. HIV Med 2013;14(3):191-2.

Regarding the extent to which confounding factors were accounted for, we have added this point to the Discussion section where we now state: '…There is also the potential for residual, unmeasured confounding given the observational nature of the included studies. For example, we noted that the included studies did not consistently control for the exact same set of confounders which may have undermine their internal validity.'

Comment: Although the authors evaluated the risk of bias in the studies included in the metaanalysis (Appendix Table 3), it was not an area of focus in the manuscript text. What criteria were used to determine whether there was bias in 'case/group representation' and comparability among study groups? The manuscript does not mention to what extent prior publications were excluded from the analysis due to poor quality.
Response: We have provided additional information on the risk of bias assessment. Please see tracked changes under the 'Data extraction and quality assessment' sub-section of the Methods.

Comment: Which epidemiologic studies included in the meta-analysis most likely resulted in the most valid results? Was a sub-analysis restricted to the most valid study designs conducted?
Response: This systematic review included various meta-analyses involving data from multiple studies. We are unable to identify a single study that most likely resulted in the pooled RR estimate. Through the synthesis of multiple studies, our meta-analysis has helped to increase the power and precision of the risk estimates of MI and thus resulted in the most valid results on the topic.

Yes, we conducted several sensitivity analyses to test the robustness of our estimates including excluding studies that were deemed to be of lower quality. This did not lead to an important change in our findings. For example, in the meta-analysis of MI risk associated with HIV infection, excluding two studies (Durand et al 2011, Triant et al 2009) that were deemed to be of lower quality resulted in a pooled relative risk of 1.58 (95% CI: 1.36, 1.83) compared to 1.67 (95%CI: 1.45, 1.94) in our original analysis that used data from all eligible studies.

Comment: This sort of discussion might provide more insight that would supplement the relative risk calculated in the meta-analysis.
Response: We agree with the reviewer and have made the necessary revisions throughout.

Comment 2. A major strength of this meta-analysis is that the authors not only considered the relative risk of MI associated with HIV, but also considered the associations for various ARV regimens (defined using current, lifetime, and cumulative definitions), CD4 count, and HIV viral load. It might be nice to highlight this novel aspect of the study a bit more in the title, abstract, and/or introduction. For instance, the rationale for this analysis in the abstract is that the evidence surrounding specific components of CVD risk remains inconclusive. My understanding was that it is generally well accepted that HIV confers an increased CVD risk.
Response: Thank you for the constructive feedback. We have updated the abstract, so this novel aspect of our study is more evident.

Comment 3. On the other hand, a large amount of results are presented, which at times make the results a little difficult to follow in the text of the results section. Sub-headings would make it easier to follow. The first paragraph of the discussion section is very helpful for highlighting the key findings in a straightforward manner. The figures are also very clear.
Response: Thank you. As suggested, we have added sub-headings to the Results section.

Comment 4. In the second paragraph of the “Meta-Analysis of the risk of MI” section, consider re-wording the sentences to make it more clear that the RR of 1.80 represents a comparison of HIV+ to HIV- among patients on ARV therapy, and that the RR of 1.25 was among patients not on ARV therapy (and not comparing ARV-treated to ARV-untreated, as it seemed to me, until I looked at the forest plot figure).
Response: Thank you. We have revised it so this is clearer.

Comment 5. Regarding that prior point (#4), do the authors take that finding to provide evidence that HIV is not associated with MI among treated patients? Might it be due to differences in characteristics between patients who are treated and not treated in those two study samples, which might not have been able to be accounted for in the analysis (i.e., unmeasured confounding)?
Response: We do not think that HIV is not associated with MI among treated patients. In the Discussion section (end of 2nd paragraph), we state that: ‘…We suspect that the higher MI risk among ART-treated HIV+ individuals may not necessarily be attributable to ART alone but rather to the combined effect from a host of factors including HIV itself, ART, and other comorbid risk factors which have been individually shown to contribute to MI risk.’

Comment 6. Consider making the distinctions between recent treatment exposure, any treatment exposure, and cumulative treatment exposure more explicit upfront in the methods section. Perhaps a better term for any treatment exposure might be lifetime exposure. In the results text, “recent” is compared to “not recent” ARV exposure, which might be confusing because it reads as though recent exposure was being compared to past exposure of the respective regimen, although I believe that recent exposure was compared to a lack of recent exposure (which might have included past exposure or never having been exposed).

Response: Thank you. We have now made this distinction in the Methods section. Please see tracked changes under the 'Data extraction and quality assessment' sub-section of the Methods.
Regarding the reviewer's comment "recent" is compared to "not recent" ARV exposure, which might be confusing because it reads as though recent exposure was being compared to past exposure of the respective regimen,": the way it reads is actually correct and is in alignment with the original studies. Recent exposure was compared to exposure that was not recent. Now that we've made the distinction between the various treatment exposures more explicit in the Methods as per your suggestion, it will hopefully help to clear any confusion.

Comment 7. To what extent do the authors think that the differences in findings for different ARV regimens are due to real differences in the associations between different ARV treatments and MI risk, as opposed to differences in study designs and study samples?
Response: The body of evidence supporting associations between some ARV treatments and MI risk is strong, especially for protease inhibitor class of medications. Our meta-analyses, which combined estimates from multiple studies provides additional evidence that these differences are more likely to be real than not. Specific to abacavir and MI risk where there is conflicting evidence between observational studies and RCTS, additional rigorously conducted studies in real-world populations are needed to definitively substantiate our findings and strengthen the existing evidence on this topic.

Comment 8. In order to support the rationale that it is more appropriate to assess the risk of HIV infection with MI as an outcome separately from other CVD outcomes (as has been done in other meta-analyses), more information on how the pathophysiologic mechanisms differ would be supportive. I noticed that the registered systematic review protocol included in the appendix stated that the primary outcome would be stroke, MI, or cardiac death as a composite outcome. Why was this pre-specified protocol not followed?
Response: Thank you for the comment. We elected to focus this meta-analysis on the risk of MI for several reasons. Generally, CVD outcomes have included the presence of ischemic heart disease (which includes MI), cerebrovascular disease (including stroke and TIA), sudden cardiac death or as in many studies, any one of these or angina. Given the variability in the definition of what constitutes CVD,22-25 choosing MI as the outcome in our meta-analysis increases the precision and homogeneity of the included group and ensures a measure of similar risk/outcome. Our decision was further driven by the strong body of literature related to HIV and MI and the ongoing debate around potential MI risk associated with use of specific ART medications such as abacavir. By examining MI separately, we have attempted to eliminate the bias introduced by the inclusion of a varying percentage of patients with MI vs non-MI ischemic heart disease and thus included patients of similar risk. We have revised the text describing our rationale for focusing on MI and provided several references regarding differences in etiology, clinical picture or definitions of CVD events.22-26 Please see tracked changes in the last paragraph of the Introduction section. Future meta-analyses are planned to examine stroke and cardiac death, respectively.

- 22. Iloeje UH, Yuan Y, L'Italien G, et al. Protease inhibitor exposure and increased risk of cardiovascular disease in HIV-infected patients. HIV Med 2005;6(1):37-44. doi: 10.1111/j.1468-1293.2005.00265.x
- 23. Lichtenstein KA, Armon C, Buchacz K, et al. Low CD4+ T cell count is a risk factor for cardiovascular disease events in the HIV outpatient study. Clin Infect Dis 2010;51(4):435-47. doi: 10.1086/655144
- 24. Choi AI, Vittinghoff E, Deeks SG, et al. Cardiovascular risks associated with abacavir and tenofovir exposure in HIV-infected persons. AIDS 2011;25(10):1289-98. doi: 10.1097/QAD.0b013e328347fa16
- 25. Klein MB, Xiao Y, Abrahamowicz M, et al. Re-assessing the cardiovascular risk of abacavir in the Swiss HIV Cohort Study (SHCS) using a flexible marginal structural model [ICPE

Abstract 396]. In: Abstracts of the 29th International Conference on Pharmacoepidemiology & Therapeutic Risk Management. Pharmacoepidemiol Drug Saf 2013;22(S1):193-94.

- 26. Widimsky P, Coram R, Abou-Chebl A. Reperfusion therapy of acute ischaemic stroke and acute myocardial infarction: similarities and differences. Eur Heart J 2014;35(3):14755. doi: 10.1093/eurheartj/eht409

Comment 9. One limitation that could be helpful to explicitly state in the discussion section is that many of the sub-analyses of this meta-analysis were based on only two or a handful of studies – not all studies included in the meta-analysis overall.
Response: We agree with the reviewer. We now mention this as a limitation in the Discussion section where we state: '…Also, some of the comparisons in our study were based on a small number of studies which is a limitation.'

Comment 10. Please confirm that the citations 26-31 provide support that the odds ratio can be used in this context for estimation of relative risk. Thank you.
Response: Yes, we confirm.

Comment 11. Did the authors assess possible publication bias using a method such as a funnel plot?
Response: We assessed publication bias by visually inspecting and testing for funnel plot asymmetry but did not report or interpret it as this was inappropriate given the small number of studies in most of the meta-analysis. We have included some discussion on this under the 'Data analysis' sub-section of the Methods where we now state that: '…Although we assessed publication bias by visually inspecting and testing for funnel plot asymmetry,46 its interpretation was limited by a lack of sufficient number of studies per meta-analysis.47 48'

- 46. Egger M, Davey Smith G, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315(7109):629-34.
- 47. Ioannidis JP, Trikalinos TA. The appropriateness of asymmetry tests for publication bias in meta-analyses: a large survey. CMAJ 2007;176(8):1091-6. doi: 10.1503/cmaj.060410
- 48. Lau J, Ioannidis JP, Terrin N, et al. The case of the misleading funnel plot. BMJ 2006;333(7568):597-600. doi: 10.1136/bmj.333.7568.597

Reviewer:3: Audrey Rankin
(Queen's University Belfast)
Comment: This paper reports a meta-analysis which estimates the risk of MI among HIV positive individuals. The statistical analysis conducted utilises a meta-analysis approach which is an appropriate method. The only comment I have is in relation the quality assessment conducted according to the Newcastle-Ottawa (NOS) criteria. The authors should comment on the results of this assessment and implications for the meta-analyses in terms of the certainty of the evidence.
Response: We thank the reviewer for the supportive feedback. Regarding the result of the quality assessment and implications for the meta-analyses in terms of the certainty of the evidence, we have added the following text to the Discussion section: '…In terms of the critical appraisal and its impact on the interpretation of the results, variability in the quality of the included studies may have influenced the results of the meta-analyses and thus the conclusions drawn.'

Additional reviewer comments from PDF file entitled "BMJ systematic review and metaanalysis risk of MI in HIV.pdf"
Comment: This review and meta-analysis seek to answer the question if HIV and ART is associated with an increased risk of myocardial infarction. This is an important issue as the HIV infected population is aging. The authors conducted a comprehensive search using a clear strategy including all evidence up to July 2018. There are, however, some concerns from my side: 1) the critical appraisal is not clear to me: based on what was decided to score a study as plus or minus in the

critical appraisal table (appendix 3)? 2) I miss the link between the critical appraisal and the interpretation of the results. All studies get the same weight in the results section, regardless of the outcome of the critical appraisal. 3) Some firm statements are made, while they rely on only a few studies, For example plasma VL and low CD4 count. VL >100,000 cp/mL is addressed in only 2 studies, CD4 count >200cells/mm3 in 3 studies.
I would recommend to integrate the critical appraisal in the results section by giving reliable studies more weight and studies with apparent methodological flaws less weight, or to perform a sensitivity analysis including only studies that were deemed to be methodological sound in the critical appraisal.
Response: These are the exact same comments as those from Dr. Alinda Vos (reviewer #1) and have been previously addressed above.

Comments per section
Abstract:
Comment: the conclusion refers to CD4 and VL, while these outcomes were not addressed in the results section
Response: Thank you for pointing this out. We have now added the results of CD4 and VL in the abstract.

Methods:
Comment: Why did you include stroke and cerebro-vascular disease in the search while they outcome of interest was myocardial infarction? Once sentence to clarify this would be helpful.
Response: Thank you for the comment. We originally planned to examine cardiovascular disease outcomes in general, hence the broad search that included stroke and cerebrovascular disease. However, given the variability in the definition of what constitutes CVD,22-25 the strong body of literature related to HIV and MI and the ongoing debate around potential MI risk associated with use of specific ART medications such as abacavir, we elected to limit our study to MI only. In the Introduction section, we have revised the text describing our rationale for focusing on MI and provided several references regarding differences in etiology, clinical picture or definitions of CVD events.22-26 Please see tracked changes in the last paragraph of the Introduction section.
Future meta-analyses are planned to examine stroke/cerebrovascular disease and cardiac death, respectively.

- 22. Iloeje UH, Yuan Y, L'Italien G, et al. Protease inhibitor exposure and increased risk of cardiovascular disease in HIV-infected patients. HIV Med 2005;6(1):37-44. doi: 10.1111/j.1468-1293.2005.00265.x
- 23. Lichtenstein KA, Armon C, Buchacz K, et al. Low CD4+ T cell count is a risk factor for cardiovascular disease events in the HIV outpatient study. Clin Infect Dis 2010;51(4):435-47. doi: 10.1086/655144
- 24. Choi AI, Vittinghoff E, Deeks SG, et al. Cardiovascular risks associated with abacavir and tenofovir exposure in HIV-infected persons. AIDS 2011;25(10):1289-98. doi: 10.1097/QAD.0b013e328347fa16
- 25. Klein MB, Xiao Y, Abrahamowicz M, et al. Re-assessing the cardiovascular risk of abacavir in the Swiss HIV Cohort Study (SHCS) using a flexible marginal structural model [ICPE Abstract 396]. In: Abstracts of the 29th International Conference on Pharmacoepidemiology & Therapeutic Risk Management. Pharmacoepidemiol Drug Saf 2013;22(S1):193-94.
- 26. Widimsky P, Coram R, Abou-Chebl A. Reperfusion therapy of acute ischaemic stroke and acute myocardial infarction: similarities and differences. Eur Heart J 2014;35(3):14755. doi: 10.1093/eurheartj/eht409

Comment: Page 7: Why were abstracts screened up to 2016 and full text articles up to 2018? A clarification would help.

Response: Thank you for the opportunity to clarify. We did not continue with the manual screening of conference abstracts past 2016 as we observed that relevant conference abstracts were also being captured in our primary database searches.

Data analysis
Comment: Lines 24-26: to minimize…unadjusted estimates. This seems plausible to me. However, I cannot see how you dealt with adjusted versus unadjusted estimates. Which studies presented adjusted, which unadjusted estimates? How were these results analysed? There is nothing about this in table 2 nor in the forest plots.
Response: Thank you for the opportunity to clarify. Given their observational nature and the lack of adjustment for confounding, studies that reported only unadjusted estimates were not used in the meta-analysis to minimize bias and were therefore excluded. We have now made this exclusion criteria more explicit in the Methods section. Please see tracked changes under the 'Search strategy and selection criteria' of the Methods.

Comment: Lines 36-38: 'we assessed heterogeneity… individual studies'. What was the outcome? How did you deal with heterogeneity in the interpretation of the results?
Response: Thank you for the comment. We included this point in the Discussion section where we state that: '…Therefore, heterogeneity arising from differences in study design or other features may have influenced the results and thus the overall conclusions drawn. Although we observed heterogeneity across results of studies included in some of the meta-analyses, this is a common limitation in meta-analysis especially those involving observational studies.43 Our a priori choice of employing the random-effects modeling strategy was driven in part by this expected variability among studies.83'

- 43. Mills EJ, Jansen JP, Kanters S. Heterogeneity in meta-analysis of FDG-PET studies to diagnose lung cancer. JAMA 2015;313(4):419. doi: 10.1001/jama.2014.16482
- 83. Hedges LV, Vevea JL. Fixed- and random-effects models in meta-analysis Psychological Methods 1998;3(4):486-504.

Flowchart 1: '36 with unusable data (e.g. uadjusted estimates) were excluded. I do not understand this.
Response: Thank you for the opportunity to clarify. Although relevant to CVD, these set of studies reported data in formats or variable categories that were distinct and could not be combined in our meta-analysis. We have updated the flowchart (Figure 1) to include additional examples of reporting that made the data unusable alongside other measures in the eligible studies.

Comment: You state in lines 24-26 of the data analysis section that unadjusted estimates will be dealt with separately. If so, should not be an exclusion criteria?
Response: Thank you. We have now made this exclusion criteria more explicit in the Methods section. Please see tracked changes under the 'Search strategy and selection criteria' of the Methods.

Results
Comment: General: incorporate the outcome of the critical appraisal in the interpretation of results
Response: Thank you. We have now added the following text to the Discussion section: '…In terms of the critical appraisal and its impact on the interpretation of the results, variability in the quality of the included studies may have influenced the results of the meta-analyses and thus the conclusions drawn.'

Comment: What definitions did you use to categorize a study as '+' or '-' in appendix 3? A table with the criteria would be helpful.

Response: Thank you. We have updated the Methods section to include additional details on the critical appraisal including a description of how it was decided to score a study as 'plus' or 'minus' on a given study design feature. We now state: '…Following guidelines in the Newcastle-Ottawa Scale (NOS) for assessing the quality of observational studies in meta-analyses29 and with slight modification of the scoring system to simplify reporting, the risk of bias assessment was performed based on the adequacy of three key domains of the study design features namely: the group/participant selection; comparability of groups; and the exposure and outcome assessments in the individual studies. For each of these key features, we assigned a "+" (plus) sign when this was clearly and adequately described in the study, and a "–" (minus) sign when it was not clearly described or was missing. A detailed description of the results of the quality assessment is available in the appendix'.

• 29. Wells GA, Shea B, O'Connell D, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses, 2018: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp, Accessed January 17, 2019.

Comment: Page 13, line 26-30: I'm struggling to understand the cumulative treatment exposure. Is this a risk compared to the non-HIV infected population? And did the original studies account for the effects of age? It may be the case as well, as you point out in the discussion, that the effect reflects duration of HIV infection.

Response: Thank you for the comment. Cumulative treatment exposure refers to the total amount of treatment, in this context, antiretroviral therapy, that a participant is exposed to over time. In the Methods section, we have now made the distinction between the various types of exposure to ART as this will hopefully help to clear any confusion. Please see tracked changes under the 'Data extraction and quality assessment' of the Methods.

We also confirm that age was one of the key variables adjusted for in the original studies.

Comment: In general: lots of meta-analysis include only 2 or 3 articles. (figures 2-6). Do you think that results are reliable as the conclusion relies on only 2 or 3 studies? (and I, as reader, do not have an idea about the quality of the studies when I look at the plots) .

Response: We now mention this as a limitation in the Discussion section where we state: '…Also, some of the comparisons in our study were based on a small number of studies which is a limitation.'

Discussion

Comment: I like the paragraph 'Previous meta-analysis … ART per se.' That's well written!

Response: Thank you for the supportive feedback.

Comment: Lines 3-8: 'Third, we have …. (D:A:D) study.' I do not understand this argument. You did not use risk estimates, but real risk of myocardial infarction. Can you please clarify this?

Response: Thank you for the opportunity to clarify. Epidemiologic analyses, including those of studies such as the Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) study, are based on probabilities, or risks, and these probabilities are almost always estimated using data from samples. All studies included in the review are based on samples, not the entire population under study. In each individual study, regression models were then used to estimate the risk of MI in the sampled individuals, adjusted for other factors. Because these are numerical quantities generated from a statistical model, which rely on underlying distributional assumptions, there is a variance associated with each estimate; this variance is captured in the 95% confidence intervals. The precision of the estimates has been considered in the meta-analytic procedures and the final pooled estimate was generated with its own 95% confidence interval. To provide a single point-estimate (and describe it as the "real risk"), without a measure of variability due to the estimation process, would be incorrect.

Comment: Minor: figure 6 (page 39) does not have a title
Response: Thank you. We have now included the title (Figure 6. Forest plot of the meta-analysis of the risk of MI associated with recent exposure to drugs of the protease inhibitor class), which is also reflected under “Figure Titles and Legends” in the manuscript.

VERSION 2 – REVIEW

REVIEWER	Matthew Levy The George Washington University, USA
REVIEW RETURNED	18-Mar-2019

GENERAL COMMENTS	I thank the authors for their responses to and revisions based on the reviewers' comments. Based on my read, what remains weak is that the manuscript does not adequately discuss the differences in the designs and quality of the different studies contributing data to this meta analysis. The authors very nicely present an overview of the different studies and conduct a quantitatively rigorous meta analysis, yet the attempt to conduct and present a quality review of the studies included seems superficial, like an afterthought. For instance, in response to one of the previous comments, the authors repeated the meta analysis with one study excluded because it used a population comparison group, as opposed to an HIV-uninfected comparison group. And presumably all other studies with HIV-uninfected comparison groups were assumed to have adequate comparison groups; however, this is rather superficial. How similar are the HIV+ and HIV- groups across studies? Do they all come from the same source population (like in the Veterans Aging Cohort Study)? The authors still don't mention anything about how the MI or other CVD outcomes were measured across studies, while it has been shown that an adjudication protocol is important for ensuring valid CVD outcome measures in HIV cohorts (see: Crane et al., "Lessons Learned From the Design and Implementation of Myocardial Infarction Adjudication Tailored for HIV Clinical Cohorts", AJE, 2014). There also is no discussion about the extent to which confounding factors were adjusted for in analyses. For instance, in some studies of HIV-associated CVD risk, data on smoking were not available, so smoking was not adjusted for, which is a major limitation. This could also explain in part some of the differences in hazard and risk ratios obtained across studies. More information is included about the quality assessment, but rating a "+" or "-" in a supplementary table without sufficient discussion in the manuscript text does not do the quality assessment justice. In my opinion, a rigorous, well discussed quality assessment is what this manuscript could contribute to the literature above and beyond prior similar meta analyses, as the studies cited have already been used in multiple meta-analyses of the HIV-associated MI risk. For one of the most recent, well-designed ones, see Shah et al., Circulation, 2018, "Global Burden of Atherosclerotic Cardiovascular Disease in People Living With HIV." A rating scale beyond "+" and "-" would have been valuable. Consistent with some of the other reviewers' comments, I don't think we can take much from the different sub-analyses of different ARV regimens, CD4 counts, and HIV viral load, because many are based on only 2-3 studies. It is unclear to the reader whether differences in RRs across different ARV regimens or other CD4/VL values are due to the different study designs and populations, or due to real differences in the variables of interest. More discussion about the interpretation of those results seems especially important. It could

	be considered misleading to present the results for MI risk associated with CD4 counts or different ARV regimens, without sufficient discussion or careful presentation of results, so that different RRs for different ARV/CD4/VL values aren't taken at face value alone. This limitation was added as one sentence, as opposed to embedded within a discussion of each of the different key results.

REVIEWER	Audrey Rankin Queens University Belfast
REVIEW RETURNED	12-Mar-2019

GENERAL COMMENTS	Thank you for the opportunity to review the revised manuscript. It is clear that the authors have made a substantial effort in revising this paper. Regarding my comments, I am happy with the changes and/or responses given.

VERSION 2 – AUTHOR RESPONSE

Response:

We thank the reviewer for his detailed comments and agree that additional discussion about key considerations regarding the quality aspects of the included studies will improve the interpretation of the findings from the meta-analysis. We have made the necessary revisions throughout and updated the Discussion section to include the following text below:

"…. There are several important considerations that should be taken into account in the interpretation of the results of this study. Accurate characterization of the risk of MI and CVD outcomes in general may be confounded by a number of factors that may have affected our conclusions. The first concern has to do with the differences in the risk factors, drug exposure, HIV-related variables, or population considered in the included studies. Indeed, no two studies of HIV+ individuals from different underlying populations can have participants with the same exact demographic, clinical and drug exposure profile – all of which play a role in overall health outcomes. Given that studies typically included in a global meta-analysis such as ours do not come from the same underlying source population, we acknowledge that there may be some differences in the population distribution in the included studies (e.g., in the distribution by age, sex, disease stage, medication profile/history) that we were unable to account for. A second concern relates to the variability in the quality and design features of the included studies, which may have influenced the results of the meta-analyses and thus the conclusions drawn. Although the majority of included studies were cohort-based (90%), almost one half (47%) were retrospective in nature and did not adequately report how the exposure or outcome was ascertained including whether an adjudication protocol was applied in the ascertainment of MIs. It has been shown that the application of an adjudication protocol in the study of MI and other CVD events is important to ensuring accurate identification of events as relying only on administrative diagnostic codes could result in misclassification.82 While some studies retrospectively assessed MI and relied on International Classification of Diseases (ICD) codes alone – something that is quite common in large epidemiological studies of MI,76 others followed participants over time and prospectively assessed and validated the MI events. It is unclear how differences in MI definition across studies may have affected our results although in two studies from the same underlying population (Veterans Aging Cohort Study (VACS)) that used similar but not the same definitions for MI,3 83 the RR differed slightly: 1.48 (95%CI: 1.27, 1.72)3 vs. 1.76 (95%CI: 1.49, 2.07).83 Regarding studies that quantified the risk of MI associated with HIV infection, the available evidence based on the included studies all point in the same direction suggesting an increase in MI risk. However, we noted some variability in the design and quality of the studies, something that may have contributed in

part to the observed heterogeneity. For example, three studies did not provide sufficient information on the exposure or outcome ascertainment in the studies.52 56 69 Furthermore, the appropriateness of the HIV-uninfected group used for comparison purposes is critical in the assessment of MI risk associated with HIV infection; an issue that has been extensively reviewed elsewhere.84 While some studies made this comparison using an HIV-uninfected group, other studies used the general population group for comparison. In sensitivity analysis, the overall RR of MI associated with HIV infection was reduced when we included in the meta-analysis two additional studies involving a 'general population' comparison group,42 43 therefore highlighting the importance of using an appropriate control group.

Another potential concern relates to differences in the extent to which key confounding factors were adjusted for in the individual analysis contributing to the meta-analysis. For example, some studies lacked data on smoking – an important risk factor for CVD in general, and therefore did not account for it in the analyses.52 60 65 In this regard, we noted that the included studies did not consistently control for the exact same set of confounders which may have undermine their internal validity and explained some of the differences in the effect measures from the individual studies. There is also the potential for residual, unmeasured confounding given the observational nature of the included studies. Therefore, heterogeneity arising from differences in study design or other quality features may have influenced the results and thus the overall conclusions drawn. Although we observed heterogeneity across results of studies included in some of the meta-analyses, this is a common limitation in meta-analysis especially those involving observational studies.44 Our a priori choice of employing the random-effects modeling strategy was driven in part by this expected variability among studies.85 Furthermore, our study combined results presented using several different relative effect measures with the assumption that these represent approximately the same numerical value.31-36 In sensitivity analyses, we did not find any evidence of bias in our pooled estimates, as these did not differ importantly from the pooled estimates we obtained when we combined studies reporting results using the same effect measure. Moreover, we reached comparable conclusions with previous meta-analyses that combined,19 or did not combine HR estimates with OR, and RR.16

Also, some of the meta-analyses in our study such as those examining the risk of MI in relation to CD4, pVL, or use of specific ARV regimens were based on a small number of studies (only 2-3 studies), which is a serious limitation. It is important to also consider this point in the interpretation of these specific findings. We acknowledge that the results from such meta-analyses could have been strengthened with the inclusion of additional eligible studies. Nevertheless, in the absence of sufficient number of studies examining these relationships, our results could be viewed as the best available evidence summarizing the risk of MI associated with CD4, pVL, or use of specific ARV regimens among people living with HIV.

Given the foregoing discussion in relation to the design and quality aspects of the included studies as well as issues of sufficiency of available studies examining several potential associations with MI risk, additional rigorously conducted studies with extensive confounding factor stratification/adjustment are needed to further confirm our findings. Furthermore, considering that the majority of the studies on this topic are carried out in North America and Europe, our study highlights the need for more research to be conducted in resource limited settings where most people living with HIV reside.