

PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Impact of Immunodepression and Moderate Alcohol Consumption on Coronary and other Arterial Disease Events in an 11-year Cohort of HIV-infected Patients on Antiretroviral Therapy
AUTHORS	Protopopescu, Camelia; Carrieri, Maria Patrizia; Le Moing, Vincent; Reboud, Philippe; Raffi, François; Mahy, Sophie; Roux, Perrine; Cuzin, Lise; Spire, Bruno; Lepout, Catherine

VERSION 1 - REVIEW

REVIEWER	Lai, Shenghan Johns Hopkins School of Medicine
REVIEW RETURNED	02-Apr-2012

THE STUDY	Item 2. The main outcome was major coronary or other arterial disease first event. However, the participants with CHD history were included. Item 9. The statistical analyses need to be redone. Please see my comments.
RESULTS & CONCLUSIONS	Item 2. The data analysis should be redone to justify the results are credible.
REPORTING & ETHICS	I am not sure that I enjoy compulsory open perr review.
GENERAL COMMENTS	The topic of this study is important. My concerns with study design and data analyses are as follows: 1. This study was designed to investigate risk factors for coronary and other arterial disease events (CADE) in HIV-infected men and women. Thus, those with history of CHD or any other arterial diseases should not be included in the data analysis. 2. Since 75% of participants had ≤ 3 AU/day, it would be interesting to split this category into two categories: 1 AU/day and 2 AU/day. Then, rerun the analysis. 3. Baseline (M0) CD4 count and viral load should be included in the Cox models. 4. Some key risk factors for coronary heart disease, such as blood pressure were missing in the Cox models. I strongly recommend that Framingham risk score be calculated and treated as a covariate in the regression models.

REVIEWER	Robert Kaplan Professor Albert Einstein College of Medicine Bronx NY USA
REVIEW RETURNED	09-Apr-2012

REPORTING & ETHICS	I am unaware of any concerns about these issues such as
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	<p>plagiarism but of course I would have no way of knowing this so I cannot answer yes to your question about whether I know the article to be free of these concerns.</p>
<p>GENERAL COMMENTS</p>	<p>This report from a french cohort of HIV-infected adults examined risk factors for the occurrence of cardiovascular disease events including myocardial infarction, heart failure, and various other arterial and venous events. The authors identified several independent risk factors for incident CVD events, including low CD4 count, age, sex, and a protective effect of moderate alcohol consumption. They conclude that risk behaviors are important determinants of CVD events in HIV-infected adults, while antiretroviral drugs did not have an association with CVD and moreover that any influence of ARVs on cardiovascular disease is likely mediated by immune responses to the medications.</p> <p>General comments The research addresses an important question, and the authors have assembled a well-characterized cohort with incident events -- while the study is relatively small in terms of the number of events, it adds importantly to the literature which contains a small number of such prior cohort studies.</p> <p>Specific comments With alcohol use as a protective cardiovascular risk factor being a major finding of the paper, the authors need to pay more attention to potential risks associated with alcohol consumption:</p> <ol style="list-style-type: none"> 1. They need to draw attention to the fact that liver disease is a major cause of death in HIV-infected patients. They also might address the possibility that competing risks may explain their findings for alcohol use, as liver-related deaths among drinkers may be an important competing risk that produced an artifactual association with lower CVD risks. 2. Were patients queried about past alcohol use, diagnosis of alcoholism, or reasons for quitting alcohol (ie because of health reasons or physicians advice). This is important because their reference group of abstainers may be enriched by a high-CVD-risk group who has quit drinking for health reasons. The potential for this kind of bias must be emphasized, and it is difficult to exclude. 3. Was there information on ALT, AST, or other markers of liver injury or fibrosis? 4. The HCV-coinfected subgroup is important to examine in secondary analyses. Subgroup analysis based on HCV status should be reported. Please clarify whether HCV was defined by active infection, or only by antibodies. <p>Table 2: The lack of association between hypercholesterolemia and risk of CADE is very interesting and important. Because LDL-c and TC are low in HIV-infected patients, providers need to be careful about basing clinical decisions upon measured lipid levels in HIV-infected patients. It is striking that most traditional CVD risk factors (smoking, age, gender, hypertension) predicted CADE in this study, but hypercholesterolemia did not, and this is worthy of comment in the discussison.s</p> <p>The discussion section mentions several potential mechanisms relating to effects of alcohol on lipid metabolism. In this regard, it will be interesting to present the association between alcohol use and levels of HDL-c, triglycerides, etc</p> <p>Page 14, the authors seem to indicate that an association was found</p>

	<p>between efavirez and nevirapine and risk of CADE, but the results presented in the Table do not support this (ie nonsignificant p-values).</p> <p>While they describe the outcome as the first CADE event, it was unclear whether patients with a baseline history of prior CVD events were excluded.</p> <p>What was the methodology and criteria for CADE event adjudication. 1, how were potential cardiovascular events identified (ie, what triggered the collection and review of medical records)? 2, were events classified as either primary (ie reason for admission) or secondary (ie occurred during a hospitalization for some other disease, occurred during surgery, occurred in a septic patient, occurred due to arterial spasm associated with cocaine use)?</p> <p>What was loss-to-followup rate in the cohort?</p> <p>Minor comments Abstract, it is unclear why they call CD4 count and alcohol consumption the "principal factors" associated with CADE, since several other risk factors were identified which seem just as strongly related to CADE.</p> <p>The terminology of "CADE" (coronary or other arterial disease events) to denote the major endpoint is somewhat non-standard, and moreover is not accurate terminology given that deep venous thrombosis was one of the captured outcomes.</p>
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REVIEWER	<p>Prof. Hansjakob Furrer, MD Chief a.i. University Clinic of Infectious Diseases Bern University Hospital and University of Bern Switzerland</p> <p>No competing interest.</p>
REVIEW RETURNED	10-Apr-2012

RESULTS & CONCLUSIONS	<p>The credibility of the results is reduced by the fact, that over 90% of the patients follow-up viral loads are said to be detectable. This should be reassessed (cf. comment 3d). This may be typo in the tables but need explanation.</p> <p>Otherwise, the results are credible.</p>
GENERAL COMMENTS	<p>1. This is an interesting paper assessing factors associated with coronary and other arterial disease events (CADE) in a cohort of French patients with HIV infection who started antiretroviral therapy using a protease inhibitor containing regime. Because they have data on alcohol consumption in the their database they are able to analyse the effect of this behaviour on CADE and find that moderate alcohol consumption is associated with reduced CADE risk in HIV infected persons. They also find CD4 counts below 200 associated with CADE risk, but not detectable viral load or antiretroviral drug classes.</p> <p>2. The statistical approach (Cox regression with baseline and time-updated co-variates) is sound, done by experts in the field, and they pretend that the proportional hazards assumptions are not violated.</p> <p>3. There are some weaker points in the paper a. CADE definitions: CADE also include phlebitis, congestive heart</p>

	<p>failure, cardiac arrhythmia; difficult for me to see these as “coronary and other arterial disease events”. Then a subgroup of major was chosen. Was the definition of a major CADE made a priori?</p> <p>b. Only “severe” CADE are taken into account, but we lack a definition what a “severe” CADE is, especially what is e.g. a severe peripheral artery disease, or a severe coronary disease other than MI or cardiovascular surgery for coronary disease. Also coronary angioplasty should be included in the list.</p> <p>c. The analysis is performed by an experienced team, but I’m surprised that they chose a two step covariate elimination strategy, eliminating first covariables who had a $p < 0.25$, therefore missing masked associations. Secondly they chose a backward elimination procedure in the multivariable model, a very controversial strategy suspicious of “data mining”. I think that in studying diseases such as CADE one should a priori define the covariates, knowing about the risk factors of arteriosclerosis, and not eliminate them if they do not seem to be significant.</p> <p>d. There is a problem with the database or with building up the tables: I can’t believe that in a treated population in France detectable viral loads are found in more than 90% of the measurements (Table 1 and 2). Such a mistake could explain the somewhat unexpected missing association if HIV replication and increased CADE risk. In addition, I miss a definition of undetectable viral load.</p> <p>4. Moderate alcohol consumption is found to be associated with a lower CADE risk in this cohort. This is only one side of the coin: The impact of this finding would be much stronger, if moderate alcohol consumption was associated with a reduced overall mortality or at least not associated with a higher mortality. I propose to include such an analysis in the paper.</p> <p>Minor comments</p> <p>5. Page 7, introduction, 1st para: Antiretroviral drugs have not been consistently found to be associated with CADE, only some of them. Antiretroviral therapy has been associated with lower incidence of CADE (SMART trial)</p> <p>6. Page 9 last para: What does the first sentence mean: Were events only initially validated or were all events validated?</p> <p>7. Page 11 first para: the two study population are not entirely different, but one is a sub-group of the other.</p> <p>8. Page 12: why were CD4 count and viral load taken as binary variables and not as continuous ones,. And if binary, why CD4 of 200 as strata limit.</p> <p>9. Page 14, last sentence 2nd para should read: ... and major CADE after one year after enrolment.</p> <p>10. Page 15 2nd para : “it may also reduce this risk because of CD4+ reconstitution following long-term suppression of HIV replication or HIV-associated inflammation” , is not correctly written, rather: “it may also reduce the risk by reducing HIV-associated inflammation following long-term suppression of HIV-replication (and give some references about reduced inflammation markers on ART).</p> <p>11. Page 16, 3rd para: I don’t think that there are clear data that age adjusted Non-HIV associated mortality has increased in the cART era. In fact, in the cited paper (47) is not age adjusted and gives no statistics in this regards, and the cardiovascular mortality rather decreased in the cited paper.</p> <p>12. Page 17, 2nd para: reference the “one hypothesis”.</p>
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REVIEWER	Enrico Girardi
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	Director, Department of Epidemiology Istituto nazionale per le malattie infettive "L. Spallanzani" Roma, Italy
	I have no competing interest in relation to this paper
REVIEW RETURNED	26-Apr-2012

THE STUDY	<p>As far as I understand, alcohol consumption was included in the multivariable model as a time-varying covariate. However this approach does not take into account the history of alcohol consumption which may be relevant for any detrimental or protective effect of alcohol. For example what about a person who has been reporting alcohol consumption at each visit for 3 years but not at the visit preceding a CHD event?</p> <p>The authors did not find any association between specific ARV drugs and the risk of CHD. The association between ARV and CHD has been demonstrated in several studies, see for example the review by Islam et al (HIV Medicine 2012) which demonstrate that persons with HIV without ART have an increased risk compared with HIV-uninfected people with treatment-naïve persons and that lopinavir/ritonavir and abacavir were associated with the greater risk and the relative risk of MI for PI-based versus non-PI-based ART was 1.41. In the manuscript the analysis of the role of single ARV is not clearly presented. In particular it is not clear what is compared to what. Moreover the number of patients receiving different drugs (or the person-time spent on different drugs) is not reported. This aspect needs further clarification and more in-depth discussion.</p> <p>I wonder whether the use of CD4 cell count and HIV viremia as time-varying covariates is appropriate in this analysis. A commonly accepted theory holds that chronic inflammation, which may be reflected by the time spent with uncontrolled viremia (or "Viremia Copy-Years" as suggested by Mugavero et al. Clin Infect Dis 2012) and probably with low CD4, may contribute to accelerate vascular damage. This phenomenon may not be captured by single transversal measures. This point should also be addressed in the discussion.</p> <p>A table describing the characteristics of individuals included in the analysis may be useful</p>
RESULTS & CONCLUSIONS	<p>Some issue should be discussed in greater detail, see above</p> <p>The conclusions about the role of adherence is not clear to me</p>

VERSION 1 – AUTHOR RESPONSE

Reviewer: Shenghan Lai
Johns Hopkins School of Medicine

The main outcome was major coronary or other arterial disease first event. However, the participants with CHD history were included.
The statistical analyses need to be redone. Please see my comments.

The topic of this study is important. My concerns with study design and data analyses are as follows:

1. This study was designed to investigate risk factors for coronary and other arterial disease events (CADE) in HIV-infected men and women. Thus, those with history of CHD or any other arterial diseases should not be included in the data analysis.

Some HIV-infected patients have a history of CHD but there seems to be no evident reason why they should be excluded. As our objective was to study all major CADE occurring after starting HAART and as a history of CHD concerned the pre-HAART period, we decided to treat this variable as an additional risk factor; interaction effects between history of CHD and the other factors were also tested. The results showed no interaction effect and that adding this factor into the model did not modify the other effects. Moreover, a personal history of CHD was not significant in the final multivariate model (see Table 3).

In addition, to answer the reviewer's request and those of the other reviewers (who did not ask to remove these patients but asked for additional analyses), we performed supplementary "sensitivity analyses" which revealed that the relative impact of the factors was the same once the estimation was performed on patients with no history of CHD (only 7 patients were eliminated from the multivariate analysis of Table 3). Another reason why we decided to keep the patients with a history of CHD in the analysis is that this information was not available for all the patients selected for the study, but only for the subgroup of those with available data on metabolic disorders.

Therefore we added the following text in the Methods section:

Several sensitivity analyses were performed. First, we excluded all patients with a history of CHD and re-ran the model to verify whether the pattern of factors would remain unchanged.

and in the Results section:

The sensitivity analyses performed by first excluding patients with a history of CHD (n=7), and then patients who died due to alcoholic cirrhosis (n=6), confirmed the same pattern of risk factors observed for the whole population.

2. Since 75% of participants had ≤ 3 AU/day, it would be interesting to split this category into two categories: 1 AU/day and 2 AU/day. Then, rerun the analysis.

This is an interesting comment as we initially performed the analyses with alcohol consumption in four categories (abstainers; ≤ 1 AU/day; >1 and $\leq 4(3)$ AU/day for men(women); $>4(3)$ AU/day for men(women)), in order to test for a gradient effect. As the two categories of moderate consumption (≤ 1 AU/day; >1 and $\leq 4(3)$ AU/day for men(women)) had similar AHR and p-values in both multivariate analyses, we decided to aggregate them to keep the model parsimonious.

To illustrate this, we present below the comparison of the results of the impact of time-varying alcohol consumption in the four categories on the first occurrence of a major CADE (univariate and multivariate Cox proportional hazard models) - the multivariate results are adjusted for all the factors in each multivariate model presented in the paper.

For the all patients (n=1154), follow-up period M0-M132 (Table 2):

Univariate analyses	Multivariate analysis	
HR [95% CI]	p-value AHR [95% CI]	p-value
Socio-demographic and psychosocial characteristics		
Alcohol consumption*		
- abstainers (ref)		
- ≤ 1 AU/day		
- >1 and $\leq 4(3)$ AU/day for men(women)		
- $>4(3)$ AU/day for men(women)		
1		
0.40 [0.21-0.78]		
0.58 [0.25-1.35]		

1.04 [0.30-3.58]

0.007

0.207

0.951

1

0.40 [0.21-0.78]

0.33 [0.14-0.79]

0.45 [0.13-1.62]

0.007

0.013

0.223

For the subgroup of patients with available metabolic data (n=675), follow-up period M12-M132 (Table 3):

Univariate analyses Multivariate analysis
HR [95% CI] p-value AHR [95% CI] p-value
Socio-demographic and psychosocial characteristics
Alcohol consumption*

- abstainers (ref)

- ≤1 AU/day

- >1 and ≤ 4(3) AU/day for men(women)

- >4(3) AU/day for men(women)

1

0.35 [0.17-0.71]

0.36 [0.13-1.01]

1.09 [0.31-3.81]

0.004

0.053

0.889

1

0.24 [0.11-0.55]

0.19 [0.06-0.62]

0.54 [0.14-2.11]

0.001

0.006

0.381

We added the following sentence in the Methods section:

The average number of alcohol units (AU) consumed per day[37] was computed using the two questions on alcohol consumption and then recoded in four categories (abstainers; ≤ 1 AU/day; >1 and $\leq 4(3)$ AU/day for men(women); $>4(3)$ AU/day for men(women)), in order to test for a gradient effect.

We also added a description of the four categories in the Results section and argued the aggregation of the two intermediate categories as follows:

Nearly 19% of the patients reported they were alcohol abstainers, three quarters reported moderate alcohol consumption (54% reporting less than 1 AU/day and 21% between 1 and 4(3) AU/day for men(women)), while only a minority of patients (6%) reported elevated alcohol consumption. As the two intermediate categories (≤ 1 AU/day; >1 and $\leq 4(3)$ AU/day for men(women)) had similar estimated Adjusted Hazard Ratios (AHR) and p-values in both multivariate analyses, we decided to aggregate them for model parsimony.

3. Baseline (M0) CD4 count and viral load should be included in the Cox models.

We already tested both of these factors and found that neither was significantly associated with the outcome, nor confounded the effect of the other variables included in the model. In line with strategies for model building, which suggest avoiding unnecessary over adjustment, we decided not to include them in the multivariate model. In addition, the follow-up time was so long that CD4 and VL at baseline have limited clinical value as potential factors having an impact on CADE.

To be more explicit on this subject, we added the univariate results for these factors in Tables 2 and 3 and the following description in the Results section:

At baseline, [...] 94% had a detectable viral load, 36% had a CD4+ cell count <200 cells/mm³ and 22% were co-infected with HCV.

4. Some key risk factors for coronary heart disease, such as blood pressure were missing in the Cox models. I strongly recommend that Framingham risk score be calculated and treated as a covariate in the regression models.

This is a very pertinent comment and it is a limitation of our study as complete data about treated and untreated systolic blood pressure, as well as total and high-density lipoprotein cholesterol were not available during the first part of the cohort follow-up. Because of this the Framingham risk score could not be used as a covariate in our models. Only partial information about these factors was available during all the follow-up period, and only for the subgroup of patients with available data on metabolic disorders. This partial information concerned the variables used in our analysis: hypertriglyceridemia, hypercholesterolemia, and hypertension. Moreover, our results showed a different pattern of factors than those found in the Framingham study (some of the traditional risk factors, such as BMI and hypercholesterolemia, were not significant), so using this score would have been less informative than using all the factors separately.

We added this limitation to the Discussion section as follows:

Finally, it would have been interesting to compute the Framingham risk score[61] and use it as a covariate in the multivariate model. Unfortunately, as our study was not initially designed to thoroughly assess the impact of all possible CHD risk factors, some of the variables used in the construction of

this score (such as treated and untreated systolic blood pressure, as well as total and high-density lipoprotein cholesterol) were not available during the two first years of our study. Moreover, our results showed a different pattern of factors than those found in the Framingham study (some of the traditional risk factors, such as BMI and hypercholesterolemia, were not significant), so using this score would have been less informative than using all the factors separately.

Reviewer: Robert Kaplan
Professor
Albert Einstein College of Medicine
Bronx NY USA

This report from a french cohort of HIV-infected adults examined risk factors for the occurrence of cardiovascular disease events including myocardial infarction, heart failure, and various other arterial and venous events. The authors identified several independent risk factors for incident CVD events, including low CD4 count, age, sex, and a protective effect of moderate alcohol consumption. They conclude that risk behaviors are important determinants of CVD events in HIV-infected adults, while antiretroviral drugs did not have an association with CVD and moreover that any influence of ARVs on cardiovascular disease is likely mediated by immune responses to the medications.

General comments

The research addresses an important question, and the authors have assembled a well-characterized cohort with incident events -- while the study is relatively small in terms of the number of events, it adds importantly to the literature which contains a small number of such prior cohort studies.

Specific comments

With alcohol use as a protective cardiovascular risk factor being a major finding of the paper, the authors need to pay more attention to potential risks associated with alcohol consumption:

1. They need to draw attention to the fact that liver disease is a major cause of death in HIV-infected patients. They also might address the possibility that competing risks may explain their findings for alcohol use, as liver-related deaths among drinkers may be an important competing risk that produced an artifactual association with lower CVD risks.
2. Were patients queried about past alcohol use, diagnosis of alcoholism, or reasons for quitting alcohol (ie because of health reasons or physicians advice). This is important because their reference group of abstainers may be enriched by a high-CVD-risk group who has quit drinking for health reasons. The potential for this kind of bias must be emphasized, and it is difficult to exclude.

We completely agree with the reviewer's points of view, as liver disease has become a leading cause of death among people with HIV and alcohol may have played a role in liver-related mortality of our cohort, especially in HIV-HCV co-infected individuals.

For these reasons we explored deaths due to liver diseases in the cohort. Among the study patients, 6 died because of alcoholic cirrhosis and 11 other deaths were due to non alcoholic liver-related causes (HCV, HCB, hepatocellular carcinoma, cirrhosis). More than half of the observations for the 6 patients with alcoholic cirrhosis were classified under excessive alcohol consumption, and one of the patients experienced a CADE. No excessive consumption of alcohol was reported by the 11 other patients mentioned above. We performed a sensitivity analysis, removing the 6 patients with alcoholic cirrhosis from the study, and found the same pattern of risk factors for CADE. However, the small number of deaths limited the possibility of estimating a competing risk model.

The patients were not asked about alcohol use before inclusion in the cohort, or about diagnosis of

alcoholism or their reasons for quitting alcohol.

To take into account both comments above, we introduced the following sentence about this sensitivity analysis in the Methods section:

Several sensitivity analyses were performed. First, we excluded all patients with a history of CHD and re-ran the model to verify whether the pattern of factors would remain unchanged. Second, the patients who died due to alcoholic cirrhosis were excluded from the study.

and in the Results section:

The sensitivity analyses performed by first excluding patients with a history of CHD (n=7), and then patients who died due to alcoholic cirrhosis (n=6), confirmed the same pattern of risk factors observed for the whole population.

We added also the following limitation in the Discussion section:

[...] the fact that excessive alcohol users exhibit similar HRs to abstainers may be also attributable to the two following reasons. First, some individuals with excessive alcohol consumption may have died due to liver failure before experiencing a cardiovascular event and this could have reduced the impact of excessive alcohol use on CADE. However, when we performed a sensitivity analysis excluding patients who died due to alcoholic cirrhosis, the results remained unchanged. Second, a proportion of abstainers may have been past (heavy) alcohol users and may have had to stop drinking for health reasons. Unfortunately, we do not have information about alcohol use before the beginning of the cohort, or about diagnosis of alcoholism or reasons for quitting alcohol.

3. Was there information on ALT, AST, or other markers of liver injury or fibrosis?

Yes, AST and ALT levels were available at each visit during follow-up, but this was not the case for other markers of liver injury. We used information on liver enzymes as a validation test of self-reported alcohol consumption. This is stated in the following paragraph, added to the Methods section (also see the Table below):

We used information on AST and ALT liver enzymes to test their correlation with alcohol consumption as a validation test of self-reported alcohol consumption. As normal liver enzymes' reference ranges for each laboratory were not available in our data set, we used a universal cut-off of ALT>50 units per litre of serum as an indicator of liver injury, and the AST/ALT ratio (in conjunction with ALT>50 IU/l) as a marker of possible cirrhosis and/or alcoholic liver disease (for AST/ALT>1) and advanced alcoholic liver disease (for AST/ALT>2). We found a positive significant association between excessive alcohol consumption and these two indicators of liver injury (after adjusting for HCV status, see Table 1), which would suggest good accuracy of the self-reported data on alcohol consumption.

ALT>50 IU/l & AST/ALT>1 ALT>50 IU/l & AST/ALT>2

aOR* [95%CI] p-value aOR* [95%CI] p-value

Alcohol consumption

-abstainers (ref.)

1

1

-≤1 AU/day 0.7 [0.5-1.2] 0.228 2.8 [0.4-18.4] 0.292

->1 and ≤4(3) AU/day for men(women)

1.0 [0.6-1.8]
0.847
0.7 [0.0-10.1]
0.772
->4(3) AU/day for men(women)
4.9 [2.4-9.8]
<10-3
29.0 [3.4-250]
0.002
HCV infection at M0 12.9 [7.6-21.8] <10-3 11.2 [2.7-46.4] 0.001

*adjusted Odds-Ratios from a random effects logistic model.

However neither indicator of liver disease was associated with the risk of CADE - something highlighted by univariate analyses - and neither modified the pattern of the other factors in any of the multivariate models. This latter result is not mentioned in the paper.

4. The HCV-coinfected subgroup is important to examine in secondary analyses. Subgroup analysis based on HCV status should be reported. Please clarify whether HCV was defined by active infection, or only by antibodies.

We added the following definition of HCV infection in the Methods section:

The medical questionnaire at enrolment (M0) collected retrospective data about patient's HIV history [...], and co-infection with hepatitis C virus (HCV) defined as having positive HCV RNA and/or positive HCV antibodies.

To answer the question above, despite the lack of statistical power of the group of HIV-HCV infected patients, we performed subgroup analyses based on HCV status. We added the following text in the Methods section:

Several sensitivity analyses were performed. [...] A third analysis was performed separately on the subgroups of patients co-infected with HCV and those not co-infected with HCV.

and in the Results section:

The other sensitivity analyses, performed separately on the subgroups of patients with HCV co-infection and on those not co-infected with HCV, revealed the same effect of alcohol consumption on CADE, although some variables among the other factors were less significant for the co-infected patients (results not shown, available on request).

Table 2: The lack of association between hypercholesterolemia and risk of CADE is very interesting and important. Because LDL-c and TC are low in HIV-infected patients, providers need to be careful about basing clinical decisions upon measured lipid levels in HIV-infected patients. It is striking that most traditional CVD risk factors (smoking, age, gender, hypertension) predicted CADE in this study, but hypercholesterolemia did not, and this is worthy of comment in the discussion.

We thank the reviewer for noting this additional result which may be important in clinical practice. Taking into account his suggestion we added the following sentence in the Discussion section:

It is worth noting the lack of association between hypercholesterolemia and the risk of CADE in our

study. This result suggests that HIV physicians need to be cautious about basing clinical decisions merely upon measured lipid levels in HIV-infected patients. Other risk factors, including behavioural ones, deserve greater consideration for clinical management.

The discussion section mentions several potential mechanisms relating to effects of alcohol on lipid metabolism. In this regard, it will be interesting to present the association between alcohol use and levels of HDL-c, triglycerides, etc

Unfortunately, information on total and high-density lipoprotein (HDL) cholesterol and triglycerides was available in the data set only from the M28 visit onwards, and even then it was not collected systematically. For this reason it was not possible to comment on the association between alcohol use and lipid metabolism over the whole follow-up.

Page 14, the authors seem to indicate that an association was found between efavirez and nevirapine and risk of CADE, but the results presented in the Table do not support this (ie nonsignificant p-values).

We removed the sentence and inserted the following one:

No significant association was found between time of exposure to different ARV drugs and major CADE.

While they describe the outcome as the first CADE event, it was unclear whether patients with a baseline history of prior CVD events were excluded.

As stated in the response to the first reviewer, we decided to keep the patients with a history of CHD in the analysis. As our objective was to study all major CADE occurring after starting HAART and as the history of CHD concerned the pre-HAART period, we decided to treat this variable as an additional risk factor; interaction effects between history of CHD and the other factors were also tested. The results showed no interaction effect and that the addition of this factor to the model did not modify the other effects. Moreover, personal history of CHD is not significant in the final multivariate model (see Table 3). Furthermore, the information on CHD history was not available for all the patients selected for the study, but only for the subgroup of those with available data on metabolic disorders.

In addition, we performed a sensitivity analysis which revealed that the relative impact of the factors was the same when the estimation was performed on patients with no history of CHD. Therefore we added the following text in the Methods section:

Several sensitivity analyses were performed. First, we excluded all patients with a history of CHD and re-ran the model to verify whether the pattern of factors would remain unchanged.

and in the Results section:

The sensitivity analyses performed by first excluding patients with a history of CHD (n=7), and then patients who died due to alcoholic cirrhosis (n=6), confirmed the same pattern of risk factors observed for the whole population.

What was the methodology and criteria for CADE event adjudication. 1, how were potential cardiovascular events identified (ie, what triggered the collection and review of medical records)? 2, were events classified as either primary (ie reason for admission) or secondary (ie occurred during a hospitalization for some other disease, occurred during surgery, occurred in a septic patient, occurred

due to arterial spasm associated with cocaine use?

We have now explained the methodology of severe events recording and validation in our cohort more thoroughly, as well as the selection methodology of major CADE as a part of all cardiovascular events. The rewritten Outcome paragraph in the Methods section is as follows:

The details of all severe clinical events, including cardiovascular events, which occurred during follow-up, were obtained from medical records and validated by the cohort's validation committee[30]. The classification of severe clinical events was based on the 10th Revision of the International Statistical Classification of Diseases and Related Health Problems (ICD-10)i. An event was considered severe when it required medical intervention, hospitalization, when hospitalization was extended due to the event's occurrence, when it led to a life-threatening condition, or when it resulted in death. A group of cardiologists specifically validated the events selected as outcomes for this study. Among cardiovascular events, only major CADE were selected for this analysis as follows (listed in singular form): MI, stroke, coronary heart disease, peripheral arterial disease and cardiovascular surgery for coronary disease. We excluded the following from the definition of the outcome: heart failure, cardiac arrhythmia, phlebitis, pulmonary embolism, and peripheral venous diseases to keep only severe or life-threatening cardiovascular events in the analysis.

What was loss-to-followup rate in the cohort?

We added the loss to follow-up rate in the Results section as follows:

During the 11 years of the study, accounting for 6544 person-years, the loss to follow-up rate was 17.6 [16.3 to 18.7] per 100 person-years.

Minor comments

Abstract, it is unclear why they call CD4 count and alcohol consumption the "principal factors" associated with CADE, since several other risk factors were identified which seem just as strongly related to CADE.

We agree with the reviewer, so we have modified the abstract and the conclusion of the paper, as follows:

In the long term, absence of immunodepression and moderate alcohol consumption remain associated with a lower risk of major CADE.

The terminology of "CADE" (coronary or other arterial disease events) to denote the major endpoint is somewhat non-standard, and moreover is not accurate terminology given that deep venous thrombosis was one of the captured outcomes.

The terminology of "CADE" (coronary or other arterial disease event) was chosen in coordination with a group of cardiologists which validated the selection of the events. It is true that the terminology is somewhat non-standard, but we preferred it instead of the general terminology "cardiovascular events" as the events selected as outcomes included only "MI, stroke, coronary heart disease, peripheral arterial disease and cardiovascular surgery for coronary disease, but not "deep venous thrombosis" (page 7). See also our response above on CADE selection.

Reviewer: Prof. Hansjakob Furrer, MD
Chief a.i.
University Clinic of Infectious Diseases

Bern University Hospital and University of Bern
Switzerland

No competing interest.

The credibility of the results is reduced by the fact, that over 90% of the patients follow-up viral loads are said to be detectable. This should be reassessed (cf. comment 3d). This may be typo in the tables but need explanation.

Otherwise, the results are credible.

Please see response to comment 3d below.

General comments:

1. This is an interesting paper assessing factors associated with coronary and other arterial disease events (CADE) in a cohort of French patients with HIV infection who started antiretroviral therapy using a protease inhibitor containing regime. Because they have data on alcohol consumption in the their database they are able to analyse the effect of this behaviour on CADE and find that moderate alcohol consumption is associated with reduced CADE risk in HIV infected persons. They also find CD4 counts below 200 associated with CADE risk, but not detectable viral load or antiretroviral drug classes.
2. The statistical approach (Cox regression with baseline and time-updated co-variates) is sound, done by experts in the field, and they pretend that the proportional hazards assumptions are not violated.
3. There are some weaker points in the paper
 - a. CADE definitions: CADE also include phlebitis, congestive heart failure, cardiac arrhythmia; difficult for me to see these as "coronary and other arterial disease events". Then a subgroup of major was chosen. Was the definition of a major CADE made a priori?

The terminology of "CADE" (coronary or other arterial disease event) was chosen in coordination with a group of cardiologists who validated the selection of the events. We chose this particular terminology, rather than "cardiovascular events", because our outcome included only "MI, stroke, coronary heart disease, peripheral arterial disease and cardiovascular surgery for coronary disease" as stated at page 7 of the paper. We excluded heart failure, cardiac arrhythmia, phlebitis, pulmonary embolism, and peripheral venous diseases from the selected events to keep only major (severe or life-threatening) cardiovascular events in the analysis.

We explained the selection of events in the "Outcome" paragraph of the Methods section more thoroughly, as follows:

A group of cardiologists specifically validated the events selected as outcomes for this study. Among cardiovascular events, only major CADE were selected for this analysis as follows (listed in singular form): MI, stroke, coronary heart disease, peripheral arterial disease and cardiovascular surgery for coronary disease. We excluded the following from the definition of the outcome: heart failure, cardiac arrhythmia, phlebitis, pulmonary embolism, and peripheral venous diseases to keep only severe or life-threatening cardiovascular events in the analysis.

b. Only "severe" CADE are taken into account, but we lack a definition what a "severe" CADE is, especially what is e.g. a severe peripheral artery disease, or a severe coronary disease other than MI or cardiovascular surgery for coronary disease. Also coronary angioplasty should be included in the list.

We added the definition of a severe event (in general) to the "Outcome" paragraph of the Methods

section:

An event was considered severe when it required medical intervention, hospitalization, when hospitalization was extended due to the event's occurrence, when it led to a life-threatening condition, or when it resulted in death.

Among all severe events recorded in the cohort, we first selected cardiovascular events - according to the ICD-10 classification. Then, among the latter, we selected those corresponding to major CADE as defined for this study. We included coronary angioplasty in the definition of the outcome (one event was recorded in our cohort), which we labelled as "cardiovascular surgery for coronary disease".

c. The analysis is performed by an experienced team, but I'm surprised that they chose a two step covariate elimination strategy, eliminating first covariables who had a $p < 0.25$, therefore missing masked associations. Secondly they chose a backward elimination procedure in the multivariable model, a very controversial strategy suspicious of "data mining". I think that in studying diseases such as CADE one should a priori define the covariates, knowing about the risk factors of arteriosclerosis, and not eliminate them if they do not seem to be significant.

We completely agree with the reviewer about the selection procedure. It is true that the use of a backward elimination procedure among a large set of covariables may be a controversial strategy. But our strategy was to first pre-select covariables before statistical modelling, in accordance with the literature on the risk factors for cardiovascular events. We then used the 0.25 cut-off as a standard threshold point - something suggested in many statistical books - in order to choose variables eligible for the multivariate model. To build this model we compared several strategies, including a backward stepwise elimination procedure, choosing the 0.05 cut-off point as the significance level for the final model. Moreover, in this revised version of the paper we also used the selection strategy based on information criteria, and found identical results. We think that including variables which do not significantly improve the model - even if reported in the literature - may also be controversial, and generally, strategies for model building strongly recommend avoiding unnecessary over adjustment. The issue is that if some variables do not significantly contribute to explain the outcome, it is possible that modest associations are not highlighted due to the lack of power of the study. This may be the case for hypercholesterolemia which is interesting because its impact here is much lower than for other behavioural risk factors and the lack of a strong association may provide a strong note of caution to physicians not to use only this factor for clinical decisions (we added a comment about this in the discussion section).

d. There is a problem with the database or with building up the tables: I can't believe that in a treated population in France detectable viral loads are found in more than 90% of the measurements (Table 1 and 2). Such a mistake could explain the somewhat unexpected missing association if HIV replication and increased CADE risk. In addition, I miss a definition of undetectable viral load.

As stated in the notes at the bottom of these tables, renumbered as Tables 2 and 3, for all time-varying variables, percentages and averages are computed at the first date of follow-up (we have not give a value for the entire duration of follow-up for these time-varying variables). Therefore the descriptive statistics in the two tables correspond to the moment preceding the first PI-based ART prescription (M0), which explains the large percentage of detectable viral load in our population.

We added a definition for undetectable viral load and immunodepression in the Methods section:

Immunodepression was defined by CD4+ cell count <200 cells/mm³; viral load was classified as detectable if it was greater than the threshold value specific to each centre where the assay was performed.

4. Moderate alcohol consumption is found to be associated with a lower CADE risk in this cohort. This is only one side of the coin: The impact of this finding would be much stronger, if moderate alcohol consumption was associated with a reduced overall mortality or at least not associated with a higher mortality. I propose to include such an analysis in the paper.

This is an interesting comment, but a mortality analysis lies beyond the limit of the object of our study. In addition, moderate alcohol consumption is known to prevent cardiovascular events in the general population but not other events leading to death which are frequent in the HIV population. For example, alcohol use may be associated with death due to liver failure, especially in co-infected individuals. As all the reviewers think that the results need to be reinforced, we conducted several sensitivity analyses: the first excluded patients with a history of CHD, the second excluded those who died due to liver-related diseases and in the third we selected HIV-HCV co-infected patients. The same pattern of alcohol consumption as a risk factor was consistently found in all these sensitivity analyses.

Minor comments

5. Page 7, introduction, 1st para: Antiretroviral drugs have not been consistently found to be associated with CADE, only some of them. Antiretroviral therapy has been associated with lower incidence of CADE (SMART trial)

The sentence in the Introduction referred not to antiretroviral therapy in general, but to antiretroviral (ARV) therapy agents. For more clarity, we have modified the sentence as follows:

Although some antiretroviral (ARV) therapy drugs[2-5] have consistently been found to be associated with coronary arterial disease or myocardial infarction (MI) in these patients [...].

6. Page 9 last para: What does the first sentence mean: Were events only initially validated or were all events validated?

All events were validated; we have rewritten the sentence, as follows:

The details of all severe clinical events, including cardiovascular events, which occurred during follow-up, were obtained from medical records and validated by the cohort's validation committee.

7. Page 11 first para: the two study population are not entirely different, but one is a sub-group of the other.

The sentence has been rephrased as follows:

More specifically, two analyses on the following study populations were performed in order to study predictors of major CADE:

8. Page 12: why were CD4 count and viral load taken as binary variables and not as continuous ones,. And if binary, why CD4 of 200 as strata limit.

A cut-off of 200 cells/mm³ was chosen for CD4+ because this threshold was found to be the most

associated with the outcome. Moreover, as well as the detectability threshold for the viral load, CD4+ <200 cells/mm³ is a standard cut-off used to indicate risk of HIV progression.

9. Page 14, last sentence 2nd para should read: ... and major CADE after one year after enrolment.

We have corrected the sentence.

10. Page 15 2nd para : "it may also reduce this risk because of CD4+ reconstitution following long-term suppression of HIV replication or HIV-associated inflammation" , is not correctly written, rather: "it may also reduce the risk by reducing HIV-associated inflammation following long-term suppression of HIV-replication (and give some references about reduced inflammation markers on ART).

We thank the reviewer for noting this and suggesting rephrasing. We have modified the sentence accordingly:

Indeed, although ART may modify lipid levels and increase the risk of CADE, it may also reduce this risk by reducing HIV-associated inflammation following long-term suppression of HIV replication[49].

We also added a reference [49] about reduced inflammation markers during ART.

11. Page 16, 3rd para: I don't think that there are clear data that age adjusted Non-HIV associated mortality has increased in the cART era. In fact, in the cited paper (47) is not age adjusted and gives no statistics in this regards, and the cardiovascular mortality rather decreased in the cited paper.

The cited sentence referred to the relative increase of the mortality due to the non-HIV causes as a part of the total mortality of HIV-infected patients, which has decreased in the cART era. For more clarity, the sentence has been rephrased as follows:

It is important to note that after the introduction of ART in 1996, the sudden decrease observed in HIV-related mortality was nonetheless accompanied by a relative increase of the non-HIV based mortality as a part of the total mortality of HIV-infected patients[49], cardiovascular diseases becoming increasingly important.

12. Page 17, 2nd para: reference the "one hypothesis".

We added the reference number 52 about the impact of excessive alcohol consumption on inflammatory markers' levels, as follows:

Another possible hypothesis is that excessive alcohol consumption can increase inflammatory markers' levels[52], which in turn probably leads to a higher risk of CADE and premature aging in HIV patients.

Reviewer: Enrico Girardi
Director, Department of Epidemiology
Istituto nazionale per le malattie infettive "L. Spallanzani"
Roma, Italy

I have no competing interest in relation to this paper

As far as I understand, alcohol consumption was included in the multivariable model as a time-varying covariate. However this approach does not take into account the history of alcohol consumption which

may be relevant for any detrimental or protective effect of alcohol. For example what about a persons who has been reporting alcohol consumption at each visit for 3 years but not at the visit preceding a CHD event?

To verify the result on the protective effect of alcohol on CADE, we performed another sensitivity analysis based on the individual patient alcohol histories. We addressed the problem in two ways. The first approach was to use a time-lagged alcohol covariate. At the first two visits (M0 and M1), the baseline alcohol status was used. For subsequent visits, the next to last follow-up value was used rather than the last assessment. The second approach used the percentage of the follow-up period during which the patient had been classified in each of the four alcohol consumption categories. Both these sensitivity analyses (not presented in the paper) resulted in similar patterns of risk factors on CADE to that for the principal analysis.

The authors did not find any association between specific ARV drugs and the risk of CHD. The association between ARV and CHD has been demonstrated in several studies, see for example the review by Islam et al (HIV Medicine 2012) which demonstrate that persons with HIV without ART have an increased risk compared with HIV-uninfected people with treatment-naïve persons and that lopinavir/ritonavir and abacavir were associated with the greater risk and the relative risk of MI for PI-based versus non-PI-based ART was 1.41.

We already acknowledged in the Introduction and the Discussion of the paper that some specific antiretroviral (ARV) therapy drugs have consistently been found to be associated with coronary and other arterial disease events (CADE) or myocardial infarction (MI) in HIV-patients; we added the reference suggested by the reviewer.

In the manuscript the analysis of the role of single ARV is not clearly presented. In particular it is not clear what is compared to what.

We tested the effect of a time-varying variable “duration of exposure to specific ARV drugs” on the risk of CADE. We explained the construction of this variable in the Methods section in greater detail, as follows:

A time-varying cumulative measure of time exposure to specific ARV medications (abacavir, efavirenz, nevirapine, lopinavir and protease inhibitors) was computed as the number of years during which ART included a specific drug from this list, from cohort enrolment to the date of each follow-up visit.

Moreover the number of patients receiving different drugs (or the person-time spent on different drugs) is not reported. This aspect needs further clarification and more in-depth discussion.

Descriptive statistics (mean, standard deviation) for duration of exposure to each specific drug are reported at the end of the follow-up (last available visit for each patient), in Tables 2 and 3.

I wonder whether the use of CD4cell count and HIV viremia as time-varying covariates is appropriate in this analysis. A commonly accepted theory holds that chronic inflammation, which may be reflected by the time spent with uncontrolled viremia (or “Viremia Copy-Years” as suggested by Mugavero et al. Clin Infect Dis 2012) and probably with low CD4, may contribute to accelerate vascular damage. This phenomenon may not be captured by single transversal measures. This point should also be addressed in the discussion.

To answer this question we performed another sensitivity analysis (not shown in the paper), using the percentage of the follow-up period that the patient had both a detectable viral load and a CD4+ <200 cells/mm3 instead of the values at each visit for the two time-varying covariables. This analysis confirmed the results of the main analysis.

A table describing the characteristics of individuals include in the analysis may be useful

The characteristics of individuals are described in the first column of the Tables 2 and 3.

Some issue should be discussed in greater detail, see above

We have provided an additional paragraph with the description of the study population's characteristics in the first section of the results section, as follows:

Women represented 22% of the study population (n=1154) (Table 2). Mean (SD) age at baseline was 37.7 (9.5) years and approximately one third of the patients had a secondary school certificate. Individuals HIV-infected through drug use accounted for 18% of the study patients. At baseline, 44% of all the patients were ARV naive, half were in CDC stage A and more than three quarters (80%) had not experienced opportunistic infections. Furthermore, 94% had a detectable viral load, 36% had a CD4+ cell count <200 cells/mm3 and 22% were co-infected with HCV. Depressive symptoms were reported at baseline in 38% of the patients. After one year of ART, more than half of the patients (58%) were highly adherent, the median [IQR] number of self-reported symptoms excluding lipodystrophy was 3 [2 to 6], while this value was 0 [0 to 1] for lipodystrophy symptoms. Only 16% of the patients declared smoking more than 20 cigarettes per day at cohort enrolment. Nearly 19% of the patients reported they were alcohol abstainers, three quarters reported moderate alcohol consumption (54% reporting less than 1 AU/day and 21% between 1 and 4(3) AU/day for men(women)), while only a minority of patients (6%) reported elevated alcohol consumption. As the two intermediate categories (≤ 1 AU/day; > 1 and $\leq 4(3)$ AU/day for men(women)) had similar estimated Adjusted Hazard Ratios (AHR) and p-values in both multivariate analyses, we decided to aggregate them for model parsimony.

The conclusions about the role of adherence is not clear to me

To be clearer, we modified the abstract and the conclusion of the paper as follows:

Combined interventions to reduce CADE-risk-related behaviours, including adherence counselling to assure long term immunological response to ART in HIV-infected individuals, are now a clinical and public health priority.

VERSION 2 – REVIEW

REVIEWER	Kaplan, Robert Yeshiva University, Albert Einstein College of Medicine
REVIEW RETURNED	29-Jun-2012

GENERAL COMMENTS	Solid paper and excellent revision Minor point, loss to followup would typically be a cumulative percent rather than rate per person-year
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REVIEWER	Reviewer: Prof. Hansjakob Furrer, MD
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	<p>Chief a.i. University Clinic of Infectious Diseases Bern University Hospital and University of Bern Switzerland</p> <p>Competing interest: My brother has a small vineyard and produces less than 1000 bottles of red wine.</p>
REVIEW RETURNED	16-Jul-2012

THE STUDY	The content of the manuscript is ok
GENERAL COMMENTS	<p>Major comment</p> <p>A) The authors have adequately answered to most of the reviewers comments. The results are interesting, showing moderate alcohol consumption being protective for CAD in HIV-infected patients (at least in France), and they deserve to be published. I still would like to see that moderate alcohol consumption is not associated with overall mortality in this population, even if the study is not designed and powered for this outcome. Already knowing that there is no signal would be important for daily clinical practice and recommendations to patients.</p> <p>Minor Comments</p> <ol style="list-style-type: none"> 1. page 9 2nd last paragraph: Definition of immunodepression as categorical 0/1 variable with a threshold of 200 CD4 does not seem to be the best model since immunodeficiency is a continuous biological process which does not start at 200 CD4. In addition we have a continuous surrogate marker for it (CD4 count). I don't understand why the authors did not use CD4 as continuous variable in their model. 2. page 9 2nd last paragraph: "the threshold value specific to each centre where the assay was performed": give range of the threshold value (e.g. 20 to 400 copies/mL) 3. Table 2/3 The way time-varying CD4 count and viral load are displayed is not at all informative (too similar to M0 values). Rather display the results of all measured values, e.g. 78% undetectable viral load. This would help to better define the external validity of the results. And as I said above I would rather like CD4 counts describes as a continuous variable. At least by describing the population studied CD4 counts should be given also as medians and IQR or range. 4. In the answers to reviewers comments the authors mention that they "also used the selection strategy based on information criteria", but I can't find this in the manuscript. 5. As they note it is a bit surprising not to find the classical risk factor cholesterolemia associate with CADE. This may be due to the fact that rather LDL Cholesterol or the Total Cholesterol / HDL quotient represent CV risk than Total cholesterol. 6. Nevertheless I stick to my opinion that one sensitivity analysis should include the classical CV risk factors, time varying CD4 count and HIV RNA and the alcohol strata, because the backward elimination procedures do not fully persuade me.

VERSION 2 – AUTHOR RESPONSE

Reviewer: Robert Kaplan
Yeshiva University, Albert Einstein College of Medicine

No conflicts

If you have any further comments for the authors please enter them below.
Solid paper and excellent revision

Minor point, loss to followup would typically be a cumulative percent rather than rate per person-year

Following the reviewer's suggestion, we modified the sentence on the loss to follow-up in the Results section as follows:

The loss to follow-up of the cohort was 0.14% at M12, 0.31% at M36, 0.44% at M60 and 0.76% at M120.

Reviewer: 3

The content of the manuscript is ok

Prof. Hansjakob Furrer, MD
Chief a.i.
University Clinic of Infectious Diseases
Bern University Hospital and University of Bern
Switzerland

Competing interest: My brother has a small vineyard and produces less than 1000 bottles of red wine.

Major comment

A) The authors have adequately answered to most of the reviewers comments. The results are interesting, showing moderate alcohol consumption being protective for CAD in HIV-infected patients (at least in France), and they deserve to be published. I still would like to see that moderate alcohol consumption is not associated with overall mortality in this population, even if the study is not designed and powered for this outcome. Already knowing that there is no signal would be important for daily clinical practice and recommendations to patients.

The analysis of the association between alcohol consumption and overall mortality was not reported in this paper, as it deserves further study and will be the subject of another paper.

In this secondary analysis, not reported in the paper, we studied the association between alcohol consumption and overall mortality measured by all-cause deaths occurring in the cohort (with the exception of accidents, suicides and overdoses). The multivariate Cox model results revealed that moderate alcohol consumption was associated with reduced overall mortality, after adjustment for known risk factors (age, HCV co-infection, CDC HIV stage at M0, CD4+<200 cells/mm³, detectable viral load and depressive symptoms, data not shown). These results reinforce the conclusions of the present paper and will be complemented shortly by additional analyzes on the protective effect of moderate alcohol consumption on different indicators of morbidity and mortality in this population.

Minor Comments

1. page 9 2nd last paragraph: Definition of immunodepression as categorical 0/1 variable with a threshold of 200 CD4 does not seem to be the best model since immunodeficiency is a continuous biological process which does not start at 200 CD4. In addition we have a continuous surrogate marker for it (CD4 count). I don't understand why the authors did not use CD4 as continuous variable in their model.

This is true, but in our case the dichotomous variable was more associated with the outcome than was the continuous one. In addition, by testing different cut-off values, we were able to highlight that the best threshold was at 200 cells/mm³. It is also important to remember that this cut-off has already been used in various previous studies on similar topics, such as Kaplan et al (AIDS 2008, see reference in the paper).

We added the following sentence to the definition of immunodepression in the Methods section of the paper (page 12):

CD4+ cell count was tested either as a continuous variable or recoded in categories. The dichotomous variable using the cut-off of 200 cells/mm³ was found to be the most predictive of the outcome (using Cox models and bias corrected Akaike's information criterion AICc (Sugiura 1978)).

2. page 9 2nd last paragraph: "the threshold value specific to each centre where the assay was performed": give range of the threshold value (e.g. 20 to 400 copies/mL)

We added the following sentence concerning the detectable viral load definition in the Methods section of the paper (page 9):

Immunodepression was defined by CD4+ cell count <200 cells/mm³; viral load was classified as detectable if it was greater than the threshold value specific to each centre where the assay was performed and to each period. The detectability threshold of the viral load ranged from 20 to 500 copies/ml for the M0-M20 period and from 20 to 400 copies/ml for the rest of the follow-up.

3. Table 2/3

The way time-varying CD4 count and viral load are displayed is not at all informative (too similar to M0 values). Rather display the results of all measured values, e.g. 78% undetectable viral load. This would help to better define the external validity of the results. And as I said above I would rather like CD4 counts describes as a continuous variable. At least by describing the population studied CD4 counts should be given also as medians and IQR or range.

As suggested by the reviewer we changed the descriptive results reported in Tables 2 and 3 to display the results of all measured values for time-varying variables rather than at the first date of follow-up.

We also changed the presentation of the characteristics of the population in the Results section (page 15), as follows:

Women represented 22% of the study population (n=1154) (Table 2). Mean (SD) age at baseline was 37.7 (9.5) years and approximately one third of the patients had a secondary school certificate. Individuals HIV-infected through injecting drug use accounted for 18% of the study patients. At baseline, 44% of all the patients were ARV naive, half were in CDC stage A, more than three quarters (80%) had not experienced opportunistic infections and 22% were co-infected with HCV. The selected patients had a detectable viral load at 43% of the follow-up visits, had a CD4+ cell count <200 cells/mm³ at 14% and reported depressive symptoms at 32% of them. The median [IQR] of CD4+ cell

count was 442 [284-633] cells/mm³ during the follow-up. During ART, more than half of the patients (63%) were highly adherent, and after one year of ART the median [IQR] number of self-reported symptoms excluding lipodystrophy was 4 [2 to 7], while this value was 1 [0 to 5] for lipodystrophy symptoms. Only 16% of the patients declared smoking more than 20 cigarettes per day during cohort follow-up. Nearly 19% of the patients reported they were alcohol abstainers, three quarters reported moderate alcohol consumption (59% reporting less than 1 AU/day and 18% between 1 and 4(3) AU/day for men(women)), while only a minority of patients (4%) reported elevated alcohol consumption. As the two intermediate categories (≤ 1 AU/day; >1 and $\leq 4(3)$ AU/day for men(women)) had similar estimated Adjusted Hazard Ratios (AHR) and p-values in both multivariate analyses, we decided to aggregate them for model parsimony.

4. In the answers to reviewers comments the authors mention that they "also used the selection strategy based on information criteria", but I can't find this in the manuscript.

The additional selection method based on information criteria was not detailed in the previous version of the paper, as the results of the final multivariate models were identical.

We have now modified the model selection paragraph in the Methods section as follows (page 13):

Covariates with a p-value <0.25 in univariate Cox analyses were considered eligible for the multivariate Cox model. Three building strategies for the final model were compared: a backward stepwise selection procedure based on the Wald test ($p<0.05$), a selection procedure based on the second-order or bias-corrected Akaike's information criterion AICc (Sugiura 1978), and finally a selection procedure based on the Schwartz Bayesian information criterion BIC (Schwartz 1978). All three strategies selected the same final multivariate model.

5. As they note it is a bit surprising not to find the classical risk factor cholesterolemia associate with CADE. This may be due to the fact that rather LDL Cholesterol or the Total Cholesterol / HDL quotient represent CV risk than Total cholesterol.

The reviewer is correct but unfortunately, information on total, LDL and HDL cholesterol was available in the data set only from the M28 visit onwards, and even then it was not collected systematically.

For this reason we added the following comment in the limitations section of the study (page 21):

It is surprising that the classical risk factor hypercholesterolemia was not found to be associated with CADE risk in this study. This may be due to the fact that rather low-density lipoprotein cholesterol and/or the total/HDL cholesterol quotient may be predictors of CADE risk but not total cholesterol. Unfortunately, the complete data regarding cholesterol levels were not available during the two first years of our study, and therefore these factors could not be assessed in this analysis.

6. Nevertheless I stick to my opinion that one sensitivity analysis should include the classical CV risk factors, time varying CD4 count and HIV RNA and the alcohol strata, because the backward elimination procedures do not fully persuade me.

We used three different model selection approaches and we always obtained the same multivariate model. As already said, in multivariate analysis over adjustment works against the concept of model parsimony, unless non-significant variables in the multivariate analysis are confounding factors (i.e. if entered, they modify the coefficient of the other variables in the model).