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Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2012-001155
Article Type:	Research
Date Submitted by the Author:	19-Mar-2012
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Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Cardiovascular medicine
Keywords:	HIV & AIDS < INFECTIOUS DISEASES, Myocardial infarction < CARDIOLOGY, Coronary heart disease < CARDIOLOGY

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CD4+ cell count ≥200 cells/mm^3 and Moderate Alcohol Consumption: Evidence of Protection against Coronary and other Arterial Disease Events in an 11-year Cohort of HIV-infected Patients on Antiretroviral Therapy

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Abstract word count: 276 words

Text word count: 3264 words

Funding sources: The ANRS CO8 (APROCO/COPILOTE) cohort is funded by ANRS (Agence Nationale de Recherches sur le Sida et les hépatites virales) and CMIT (Collège des Universitaires de Maladies Infectieuses et Tropicales), which received research grants from Abbott, Boehringer-Ingelheim, Bristol-Myers Squibb, GlaxoSmithKline, Gilead Sciences, Pfizer and Roche.

The funders were responsible for initiating, managing and financing the cohort, but did not interfere with data analysis and interpretation and dissemination of the scientific results.

Competing interests: All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf <http://www.icmje.org/coi_disclosure.pdf> (available on request from the corresponding author) and declare that (1) they have no support from any company for the submitted work; (2) they have no relationships with any company that might have an interest in the submitted work in the previous 3 years; (3) their spouses, partners, or children have no financial relationships that may be relevant to the submitted work; and (4) they have no non-financial interests that may be relevant to the submitted work.

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Data sharing: There is no additional data available.

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Summary

Article Focus

- Major coronary or other arterial disease events (CADE) are frequent in HIV-infected individuals receiving antiretroviral therapy.
- While some antiretroviral classes can increase the risk of CADE, it is still controversial to what extent immune reconstitution and other factors like moderate alcohol consumption can reduce the risk of CADE in this population.

Key Messages

- Moderate alcohol consumption and CD4+ cell count ≥200 cells/mm^3 can be beneficial to reduce the risk of CADE in HIV-infected patients.
- Reduced immunological response to ART over a long follow-up may have a more important impact on CADE than exposure to a specific antiretroviral drug.

Strengths and Limitations

- This paper suggests the importance of combined risk reduction interventions including adherence counselling for assuring long term immunological response and decrease the risk of CADE.

Short title

CD4+, Moderate Alcohol Consumption and Coronary Events in HIV-infected patients

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Abstract

Objective: To investigate the relationship between response to antiretroviral treatment (ART), alcohol use and occurrence of a major coronary or other arterial disease event (CADE) in HIV-infected individuals.

Design: Cohort study. A Cox model was used to identify correlates of a first occurrence of a major CADE.

Setting: The French ANRS CO8 APROCO-COPILOTE cohort was set up in 1997 to study clinical progression and patient-reported outcomes (PRO) after initiating protease inhibitor-containing ART. Clinical data were retrieved from medical records. Self-administered questionnaires collected data on PRO and behaviours, including alcohol use.

Participants: Metabolic data were only available for a subgroup (n=675) of the study group (n=1154).

Main outcomes measures: Major coronary or other arterial disease first event.

Results: Over the 11-year follow-up, 49 major CADE were observed, with an incidence rate [95%CI]=0.75[0.57 to 0.99] per 100 person-years. CD4+ cell count ≥200 cells/mm^3 was associated with a reduced risk of CADE (adjusted hazard ratio AHR[95% CI]=0.40[0.18 to 0.86]) after adjustment for female gender (0.25[0.08 to 0.83]), age (1.07[1.04 to 1.10]) and smoking>20 cigarettes/day (4.19[2.17 to 8.11]). Moreover, individuals with moderate alcohol consumption (≤3 AU/day) had a lower risk of CADE (0.38[0.20 to 0.71]) than alcohol abstainers, while the risk for those drinking>3 AU/day was not significantly different from this latter group. These associations remained valid after adjustment for metabolic disorders. No significant association with exposure to any specific antiretroviral was detected.

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Conclusions: In the long term, CD4+ cell count ≥200 cells/mm^3 and moderate alcohol consumption remain the principal factors associated with a lower risk of CADE. Combined interventions to reduce CADE risk-related behaviours and sustain ART adherence in HIV-infected individuals are now a clinical and public health priority.

Key words: Coronary and other arterial disease events, Alcohol, CD4+ cell count, HIV, ARV.

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Introduction

Cardiovascular disease risk factors (including for example hypertension and tobacco use), as well as immunological status[1] are major predictors of atherosclerosis in HIV-infected women and men. Although antiretroviral (ARV) therapy agents[2-5] have consistently been found to be associated with coronary and other arterial disease events (CADE) in these patients, more recently Butt and colleagues put into evidence the negative effect of HIV viral replication on heart failure[6]. In one study, patients having experienced myocardial infarction (MI) presented lower baseline and proximal CD4+ cell counts than those who did not, while no association was found with specific antiretroviral agents[7]. Moreover, known factors associated with the risk of CADE[8] such as hyperlipidemia and insulin resistance[9-11] are becoming an increasingly major clinical concern in ART-treated individuals.

Though excessive alcohol use may be frequent in HIV-infected individuals, its impact on the occurrence of CADE in this population has been less investigated. Excessive alcohol consumption also plays a major role in HIV risk-taking behaviours, particularly in men who have sex with men and drug users[12, 13]. In HIV-infected individuals, excessive alcohol use is known to compromise patients' response to antiretroviral therapy (ART) through reduced adherence[14-17] and to predict HIV progression and increased mortality[18-21], mostly from liver diseases[22-25]. Some recent cross-sectional analyses in HIV-infected and uninfected men have found that heavy alcohol consumption is associated with a higher risk of CADE[26, 27]. On the other hand, in uninfected adults, moderate alcohol consumption has been found to be associated with a reduced risk[28], improved lipid profiles, increased insulin sensitivity and reduced risk of MI[29]. However, this association has not yet been studied in longitudinal studies involving HIV-infected populations including women. Moreover, there are as yet no consistent results in the literature regarding the role that alcohol use plays in exacerbating the risk of CADE in ART-treated patients and to what extent it can confound or

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boost the effect of immuno-virological response to ART, ART-associated dyslipidemia and insulin resistance on the risk of CADE.

We used data from the 11-year follow-up of the APROCO-COPILOTE cohort of patients receiving ART since 1997 to investigate the relationship between undetectable viral load, CD4+ cell count, alcohol consumption and the first occurrence of a CADE, after adjustment for known risk factors including metabolic disorders.

Methods

The French APROCO-COPILOTE cohort (ANRS CO8) was designed to study the clinical, immunological, virological, and socio-behavioural course of HIV disease in HIV-1 positive individuals who started the first generation of potent ART (treatment regimens including protease inhibitors (PI)). The study was approved by the Ethics Committee of Cochin Hospital (Paris) and informed consent was obtained from all participants.

Setting

Patients were enrolled in the cohort at their first PI-based ART prescription between May 1997 and June 1999 (n=1281) in 47 French centres, and clinically followed-up every 4 months thereafter, up to month 132 (M132).

Patients

In the present study were included all patients who had either two alcohol assessment or one alcohol assessment just preceding the CADE over the whole follow-up (n=1154).

Medical questionnaire

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The medical questionnaire at enrolment (M0) collected retrospective data about patient's HIV history including HIV transmission category, time since HIV diagnosis, previous exposure to antiretroviral treatment before enrolment, co-infection with hepatitis C virus (HCV). This information was complemented by another medical questionnaire, completed by the HIV physician at each follow-up visit, which collected clinical and laboratory data (plasma HIV RNA level, CD4+ cell count, HIV clinical stage) as well as data on the antiretroviral regimen prescribed. A third form, based on updated medical records, detailed all clinical severe events, including CADE, occurring during the study period. Information on metabolic disorders was available at M12 for a subset of cohorts' patients, who participated in a parallel cross-sectional survey, designed to study clinical and laboratory metabolic complications[30]. These "metabolic" data included height, weight, hypertriglyceridemia, hypercholesterolemia, personal history of coronary heart disease (CHD) and hypertension, and finally any family history of CHD.

At each follow-up visit, CD4+ cell count was measured by standardized flow cytometry, and plasma HIV RNA levels were measured using the assay routinely available in each participating centre. HIV clinical stage was defined using the 1993 Centres for Disease Control and Prevention (CDC) clinical classification system[31].

Serum triglyceride and total cholesterol levels were measured after a 12-hour overnight fast. Hypertriglyceridemia was defined as a triglyceride level ≥2.2 mmol/l, while hypercholesterolemia was defined as a total cholesterol level ≥5.5 mmol/l.

Outcome

The clinical severe events occurring during follow-up, including CADE, were initially recovered from medical records and validated by a validation committee[30]. The classification of clinical severe events was based on the 10th Revision of the International

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Statistical Classification of Diseases and Related Health Problems (ICD-10)[i]. The major CADE selected for this study were MI, stroke, coronary heart disease, peripheral arterial disease and cardiovascular surgery for coronary disease.

Self-administered questionnaire

A self-administered questionnaire collected - at M0, M4, M12 and every 8 months thereafter during the first 5 years of follow-up, then yearly thereafter – data on socio-demographic characteristics including age, gender and educational level. Among other psycho-social and behavioural information, it also collected details on depressive symptoms, tobacco use, alcohol consumption, self-reported ART-related symptoms and adherence to ART.

Depression was measured using the validated French version of the Centre for Epidemiologic Studies Depression Scale (CES-D), which is a 20-item scale commonly used in studies involving HIV-infected patients[32].

Tobacco and alcohol consumption were evaluated during the previous 4 weeks. Alcohol consumption was assessed using two questions about frequency of consumption and quantity consumed daily, if applicable.

A 13-item scale comprising the French version of the symptom index validated by Justice *et al*[33] and described elsewhere[34] collected information about self-reported symptoms. Five other questions gathered information about adherence to ART, according to the methodology established by the AIDS Clinical Trial Group[35].

Statistical analysis

As the results of the analysis on the whole dataset (n=1154) could be affected by the lack of important predictors of CADE like hypertriglyceridemia or hypercholesterolemia, we

[i] http://www.who.int/classifications/icd/en/

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conducted a secondary analysis on a subset of patients who had additional metabolic disorders data. Precisely, two analyses on two different study populations were performed to study predictors of major CADE:

1) First analysis: the study population included all patients who had either two alcohol assessment or one alcohol assessment just preceding the event over the whole follow-up (n=1154). The follow-up period was M0-M132.

2) Second analysis: the study population was restricted to the subset of patients with available data on metabolic disorders, measured at M12 (n=675). Consequently, only events occurring after M12 were considered in this second analysis.

The WHO body mass index (BMI) categories were used in the analysis: underweight and overweight/obese patients were defined respectively as having a BMI lower than 18.5 and greater than 25.

A cumulative measure of time exposure to specific ARV medications (abacavir, efavirenz, nevirapine, lopinavir and protease inhibitor) was computed from cohort enrolment to the date of each follow-up visit.

Tobacco consumption was dichotomized in two categories: more than 20 cigarettes/day *vs* other[36]. The average number of alcohol units (AU) consumed per day[37] was computed using the two questions on alcohol consumption.

Patients were classified as non-adherent if they reported taking less than 100% of prescribed medications during the previous 4 weeks, using a validated algorithm[38]. A patient with a CES-D score value above 17 (men) or 23 (women) was considered as presenting depressive symptoms[39].

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Incidence rate of major CADE was computed as the number of cases divided by the number of person-years of follow-up. Follow-up duration was calculated as the difference in days from enrolment to the date of the CADE, death or last visit, whichever occurred first. Follow-up of patients experiencing major CADE was censored after the date of the first CADE. We used a Cox proportional hazards model to identify characteristics associated with the first occurrence of a major CADE. Psychosocial characteristics - depressive symptoms, tobacco and alcohol consumption, self-reported symptoms, CD4+ cell count and viral load - were evaluated at each visit and used as time-varying covariates in the statistical analysis. All the other covariates were used as fixed variables (measured at M0, M1 or M12). For time-varying factors, the last known value was carried forward in the case of missing data at a scheduled visit. In the case of an event occurring between two consecutive follow-up visits, the values for the time-varying variables measured at the visit preceding the event were used. Covariates with a p-value<0.25 in univariate Cox analyses were considered eligible for the multivariate Cox model, which was built using a backward selection procedure based on the Wald test ($p<0.05$). The proportional-hazards assumption was verified globally for the multivariate models and separately with respect to each covariate, using both Kaplan-Meier estimates and tests based on Schoenfeld residuals[40]. A residual analysis for outliers' detection was performed and the sensitivity of the model to influential outliers was tested. All analyses were performed using Stata Intercooled software, version 10.1.

Results

A total of 1154 patients were included in the present study, accounting for 9401 person-visits. When comparing the study patients (n=1154) with those included in the cohort, but excluded from the study due to missing alcohol data (n=127), no significant difference was found for

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gender, age at enrolment, antiretroviral naivety, CDC clinical stage, baseline CD4+ cell counts and HIV viral load at M0 (data not shown).

Median [interquartile range (IQR)] duration of follow-up of the selected patients, from their first visit after the ART prescription (M1) until M132, was 5.9 [1.8-9.4] years.

Over the 11-year follow-up of the cohort, a total of 85 CADE were observed. These included 49 major CADE as follows: myocardial infarction (n=30), coronary heart disease (n=4), stroke (n=7), peripheral arterial disease (n=7) and cardiovascular surgery (n=1). The distribution of the other thirty six CADE was as follows: phlebitis/pulmonary embolism (n=22), cardiomyopathy/congestive heart failure (n=5), cardiac dysrhythmia (n=6), cerebral haemorrhage (n=2) and aortic aneurysm (n=1).

The incidence rate [95% CI] of all CADE was 1.3 [1.0 to 1.6] per 100 person-years, while for major CADE the incidence rate was 0.75 [0.57 to 0.99] per 100 person-years. Of the 49 major CADE, 40 occurred after M12. Furthermore, the major CADE incidence over the period M12-M132 was 0.89 [0.66 to 1.22] per 100 person-years.

The distribution of factors associated with major CADE, as well as the results of univariate and multivariate Cox models, are reported in Table 1 for the entire study population (n=1154). Table 2 focuses instead on the subset of patients with available data on metabolic disorders (n=675).

In the subsample of patients with available metabolic data (Table 2), the majority (73%) had a normal BMI, 17% were classified in the overweight category and a minority was classified in each of the two more extreme categories (6% underweight and 3% obese). About 8% of the patients had hypertriglyceridemia and 6% had hypercholesterolemia at M12. One year after the enrolment in the cohort, 6% of the patients reported a personal history of hypertension. Only 1% and 28% respectively had a personal and family history of CHD.

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In the entire study group (n=1154), the following factors were found to be independent predictors of major CADE: older age at baseline (Adjusted Hazard Ratio (AHR)[95% CI]=1.07[1.04 to 1.10]), tobacco consumption>20 cigarettes/day (4.19[2.17 to 8.11]) and CD4+ cell count ≥200 cells/mm^3 (0.40[0.18 to 0.86]) (see Table 1). In addition, a negative association was found between female gender and major CADE (0.25[0.08 to 0.83]). After accounting for the effect of age, gender, tobacco consumption and CD4+ cell count ≥200 cells/mm^3 in the multivariate Cox model, individuals with moderate alcohol consumption (≤3 AU/day) were at lower risk of major CADE (0.38[0.20 to 0.71]), than alcohol abstainers, while those drinking more than 3 AU/day were not significantly different from abstainers (p=0.229).

These results remained valid even when the analysis was restricted to the subgroup of patients with available metabolic data and the follow-up period M12-M132. After adjusting for age, tobacco consumption, hypertriglyceridemia and a family history of CHD, we consistently found a negative association between moderate alcohol use (0.23[0.11-0.49]), CD4+ cell count ≥200 cells/mm^3 (0.25[0.09-0.69]) and major CADE one year after enrolment (Table 2).

No significant association was found between time of exposure to different ARV drugs and major CADE, the only exceptions being efavirenz and nevirapine, for which a slight negative association was found in univariate analysis only. No significant interaction was found between smoking habits and hypertriglyceridemia or personal history of CHD. Furthermore, detectable viral load was not associated with major CADE in univariate or multivariate analyses.

For the Cox models fitted in this study, proportional-hazards assumption remained valid, either globally and with respect to each covariate. The residual analysis did not alter the results of the multivariate model.

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Discussion

This longitudinal study clearly confirms that in ART-treated individuals, proximal CD4+ cell count higher than 200 cells/mm^3 remains a major risk factor negatively associated with major coronary and other arterial disease events. This result remains valid even after adjustment for metabolic disorders such as hypertriglyceridemia and family history of CHD, the strength of the association remaining unchanged.

The relationship between CADE and CD4+ cell count, in addition to the lack of association between CADE and specific ARV classes, confirms previous results[7] where the association between exposure to certain ARV and CADE was no longer evident after controlling for traditional risk factors and HIV disease markers. Indeed, the association between specific ARV drugs and major CADE, like myocardial infarctus, has been shown in very powerful studies[5], where adjustment for nadir CD4+ lymphocyte count or peak HIV-1 RNA level did not modify the results. However, in the study cited, no adjustment for proximal CD4+ cell count was performed, and the association with the class of ARV became weaker after adjustment for serum lipid levels[5]. Indeed, although ART may modify lipid levels and increase the risk of CADE, it may also reduce this risk because of CD4+ reconstitution following long-term suppression of HIV replication or HIV-associated inflammation. The lack of association with exposure to specific ARV classes in the present study, something usually associated with an increased risk of CADE[41], suggests that reduced immunological response to ART over a long follow-up, as well as known CADE risk factors may have a more important impact than exposure to a specific antiretroviral drug.

It is also possible that patients with altered lipid profiles were switched to other classes of antiretroviral drugs during the follow-up. As a consequence, in the long term, CD4+ cell

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count ≥200 cells/mm^3 remain the main correlate of a decreased risk of CADE, whatever the antiretroviral received.

In our study, individuals reporting elevated alcohol consumption exhibited the same risk of CADE as alcohol abstainers. In contrast, those reporting moderate alcohol consumptions had a lower risk. This result was confirmed after adjustment for additional risk factors including tobacco use, age, gender and CD4+ cell count. This relationship between alcohol consumption and CADE remained significant and J-shaped, even after adjustment for data on metabolic risk factors- such as having a history of CHD and hypertriglyceridemia -which was available for a subset of patients. As highlighted in previous observations of the general population[28, 42-45] and certain populations affected by other diseases[46], these results confirmed that the increased risk of CADE in HIV-infected patients receiving ART is not attributable to elevated alcohol use. Furthermore, we highlighted the apparent protective effect of moderate alcohol consumption in ART treated HIV-infected patients.

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 an increase in non-HIV based mortality[47], cardiovascular diseases becoming increasingly important. This increase can be explained firstly by a reduction in the competing role of HIV-related mortality, which in the long term was much more detrimental to health than other causes of deaths. Secondly, the high prevalence in this population of other risk factors like tobacco use, together with ART-related morbidity including altered lipid profiles and triglycerides, may contribute to an increased risk of CADE.

The association found between moderate alcohol use and reduced CADE risk is not consistent with all previous research in HIV-infected patients. Freiberg *et al*[26] pointed out that among HIV-infected men in the US, hazardous drinking and both alcohol abuse and dependence

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were associated with a higher prevalence of CADE compared with infrequent and moderate drinking, even after adjusting for traditional risk factors, antiretroviral therapy and CD4+ cell count. This difference in the results may be due to the different design of the two studies (longitudinal for ours *vs* cross-sectional for Freidberg's), but probably also to the different types of alcohol mainly consumed in the two populations, red wine consumption being more widespread in France than in the US. This difference in the pattern of alcohol use has already been highlighted in a comparative study on CADE in non HIV-infected Irish and French males, showing an increased protective effect of drinking red wine in the latter population[48]. Increased wine consumption probably brings about an increase in high-density lipoprotein cholesterol levels, which helps protect against CADE. On the other hand, beer and spirits consumption are linked to increased triglyceride levels[49]. We do not know to what extent moderate alcohol users are representative of a population with better health status as alcohol use in France is extremely frequent in the general population and this fact may increase the strength of the association found.

While one hypothesis is that excessive alcohol consumption can increase inflammatory markers' levels, which in turn probably leads to a higher risk of CADE and premature aging in HIV patients, this hypothesis was not demonstrated in a study conducted in older adults without cardiovascular disease, where alcohol intake was found to be associated with lower levels of inflammatory markers[50]. In the meantime it is important to remember that individuals with immune suppression are at a greater risk of cancer, a disease whose pattern of risk factors also include alcohol use, even when moderate[51].

Tobacco smoking has consistently been found to be a major risk factor for CADE[52]. Smoking prevalence and dependence in HIV-infected patients is higher than in the general population[53]. Effective interventions for reducing and quitting smoking[54], especially for patients with several risk factors, are strongly recommended especially considering the

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increased CADE risk which exists in HIV-infected smokers receiving ART[55]. However, results from studies reporting on the effectiveness of interventions for quitting smoking in HIV-infected individuals are inconsistent[56].

Some limitations of this study need to be acknowledged. First, our definition of CADE includes different types of events which might not share the same pattern of risk factors. However, an additional analysis performed on a restricted dataset and which adjusted for other potential risk factors (such as hypertriglyceridemia) confirmed the pattern found in the main analysis. Second, information on alcohol use was mainly based on self-reports and these may tend to underestimate alcohol consumption. However, it has been reported that HIV-HCV co-infected patients tend to underreport alcohol use more to their hepathologist than to other physicians[57]. As our patients were all followed-up by HIV physicians, it is likely that the degree of underreporting did not greatly affect hazard ratios' estimates.

This cohort is representative of the first generation of patients receiving potent ART. As cardiovascular disease is a rising issue in all HIV-infected populations receiving ART, these results can give important information about the pattern of risk and protective factors in all treated HIV-infected populations.

In conclusion, in the long term, CD4+ cell count ≥200 cells/mm^3 and moderate alcohol consumption remain the principal factors negatively associated with the risk of major CADE. Combined interventions to reduce CADE-risk-related behaviours and assure sustained adherence and response to ART in HIV-infected individuals are now a clinical and public health priority.

Acknowledgments

The authors would like to thank all participating patients, nurses and physicians in clinical sites.

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We would also like to thank Jude Sweeney for the English revision and editing of the manuscript.

Authors' contribution:

Maria Patrizia Carrieri planned the data analyses and wrote the manuscript; Camelia Protopopescu analyzed the data and wrote the manuscript; Vincent Le Moing contributed to patients' recruitment and investigation and revised the manuscript; Philippe Reboud contributed to patients' evaluation; François Raffi was responsible for cohort initiation, contributed to patients' recruitment and investigation and revised the manuscript; Sophie Mahy contributed to patients' recruitment and investigation; Perrine Roux contributed to patients' evaluation and revised the manuscript; Lise Cuzin contributed to patients' recruitment and investigation; Bruno Spire contributed to data analyses and revised the manuscript; Catherine Leport was responsible for cohort initiation, contributed to patients' recruitment and investigation and revised the manuscript. All authors approved the final version of the manuscript.

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Promotion: Agence Nationale de Recherches sur le Sida et les hépatites virales (ANRS, Action Coordonnée n°7).

Other support: Collège des Universitaires de Maladies Infectieuses et Tropicales (CMIT ex APPIT), Sidaction Ensemble contre le Sida and associated pharmaceutical companies: Abbott, Boehringer-Ingelheim, Bristol-Myers Squibb, GlaxoSmithKline, Gilead Sciences, Pfizer and Roche.

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Table 1. Factors associated with the first occurrence of a major CADE among HIV-infected patients receiving ART (ANRS CO8 APROCO-COPILOTE cohort - univariate and multivariate Cox proportional hazard models, all patients - n=1154, follow-up period M0-M132)

	% of patients or mean (SD)[@]	% of miss. data	Univariate analyses HR [95% CI]	p-value	Multivariate analysis AHR [95% CI]	p-value
Socio-demographic and psychosocial characteristics						
Female gender	22.0	0	0.26 [0.08-0.84]	0.025	0.25 [0.08-0.83]	0.024
Age at M0° - *years*	37.7 (9.5)	0	1.06 [1.03-1.09]	$<10^{-3}$	1.07 [1.04-1.10]	$<10^{-3}$
Secondary-school certificate at M0°	31.3	7.3	0.51 [0.26-1.01]	0.054		
Alcohol consumption at M0°		0				
- abstainers (ref)	18.8		1			
- <=3 AU/day	75.3		0.47 [0.24-0.89]	0.021		
- >3 AU/day	5.9		0.75 [0.21-2.63]	0.648		
Alcohol consumption*		0				
- abstainers (ref)	18.8		1		1	
- <=3 AU/day	75.3		0.44 [0.24-0.81]	0.009	0.38 [0.20-0.71]	0.002
- >3 AU/day	5.9		1.04 [0.30-3.58]	0.953	0.46 [0.13-1.63]	0.229
Tobacco consumption >20 cig./day*	16.4	0.9	3.17 [1.73-5.83]	$<10^{-3}$	4.19 [2.17-8.11]	$<10^{-3}$
Depressive symptoms[#]*	37.9	8.5	1.22 [0.67-2.21]	0.512		
Number of self-reported symptoms (excluding lipodystrophy)*	3.8 (2.8) [@@]	0	1.01 [0.95-1.08]	0.694		
Number of self-reported lipodystrophy symptoms*	1.1 (2.2) [@@]	0.6	1.07 [0.96-1.18]	0.223		
ART adherence*	57.7 [@@]	0.1	2.42 [1.17-5.02]	0.017		
Clinical characteristics						
HIV transmission category°		0				

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- homosexual	40.6		0.99 [0.42-2.34]	0.988		
- injecting drug use	17.8		0.92 [0.50-1.70]	0.793		
- other (ref)	41.6		1			
CDC clinical stage A at M0°	51.2	0	0.44 [0.23-0.85]	0.014		
HCV infection at M0°	22.4	4.3	1.16 [0.59-2.29]	0.655		
Time since HIV diagnosis at M0- *years*°	4.7 (4.2)	0.9	1.01 [0.94-1.08]	0.822		
Duration of exposure to efavirenz - *years**§	1.0 (2.2) §	0	0.86 [0.71-1.03]	0.102		
Duration of exposure to nevirapine - *years**§	0.9 (2.2) §	0	0.91 [0.78-1.07]	0.279		
Duration of exposure to abacavir - *years**§	1.0 (2.1) §	0	0.98 [0.84-1.13]	0.748		
Duration of exposure to lopinavir - *years**§	0.4 (1.5) §	0	1.01 [0.85-1.20]	0.929		
Duration of exposure to PI-based regimen - *years**§	3.2 (3.1) §	0	1.03 [0.92-1.15]	0.615		
Antiretroviral naivety at M0°	44.4	0	1.14 [0.64-2.02]	0.654		
CD4+ cell count ≥ 200 *cells/mm³**	65.4	0.1	0.40 [0.19-0.87]	0.020	0.40 [0.18-0.86]	0.020
Detectable viral load*	91.9	0.3	0.99 [0.53-1.84]	0.980		

CADE = coronary or other arterial disease event; ART = antiretroviral therapy; CHD = coronary heart disease

SD = standard deviation; (A)HR = (adjusted) hazard ratio; CI = confidence interval

° Fixed variable (measured at M0 or M1); * Time-varying variable (the last available value before each visit)

@ Percentages and averages were computed at the first date of follow-up for time-varying variables

@@ At M12; § At the end of the follow-up

Depressive symptoms: the Centre for Epidemiologic Studies Depression Scale (CES-D) scores is >17 for men and >23 for women

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Table 2. Factors associated with the first occurrence of a major CADE among HIV-infected patients receiving ART (ANRS CO8 APROCO-COPILOTE cohort - univariate and multivariate Cox proportional hazard models, patients with available metabolic data - n=675, follow-up period M12-M132)

	% of patients or mean (SD)[@]	% of miss. data	Univariate analyses HR [95% CI]	p-value	Multivariate analysis AHR [95% CI]	p-value
Socio-demographic and psychosocial characteristics						
Female gender	19.0	0	0.33 [0.08-1.40]	0.134		
Age at M0 – *years*°	38.4 (9.5)	0	1.04 [1.01-1.07]	0.013	1.05 [1.02-1.09]	0.003
Secondary-school certificate at M0°	35.4	6.7	0.34 [0.14-0.84]	0.019		
Alcohol consumption at M0°		0				
- abstainers (ref)	15.1		1			
- <=3 AU/day	78.4		0.44 [0.18-1.03]	0.059		
- >3 AU/day	6.5		1.04 [0.26-4.05]	0.959		
Alcohol consumption*		0				
- abstainers (ref)	15.1		1		1	
- <=3 AU/day	78.4		0.27 [0.13-0.58]	0.001	0.23 [0.11-0.49]	$<10^{-3}$
- >3 AU/day	6.5		1.19 [0.33-4.23]	0.792	0.55 [0.14-2.14]	0.390
Tobacco consumption >20 cig./day*	14.8	1.2	3.58 [1.70-7.54]	0.001	5.59 [2.43-12.8]	$<10^{-3}$
Depressive symptoms[#]*	36.6	8.0	1.83 [0.90-3.73]	0.094		
Number of self-reported symptoms (excluding lipodystrophy)*[@@]	4.0 (2.9)	0	1.03 [0.95-1.12]	0.463		
Number of self-reported lipodystrophy symptoms*[@@]	1.4 (2.4)	0.2	1.10 [0.98-1.24]	0.111		
ARV adherence*[@@]	56.6	0.2	1.63 [0.70-3.81]	0.260		
Clinical characteristics						

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HIV transmission category°		0				
- homosexual (ref)	44.0		1			
- injecting drug use	15.0		2.08 [0.75-5.73]	0.156		
- other	41.0		1.32 [0.59-2.93]	0.499		
CDC clinical stage A at M0°	50.8	0	0.43 [0.19-0.97]	0.041		
HCV infection at M0°	19.8	3.8	2.05 [0.94-4.47]	0.071		
Duration since HIV diagnosis at M0- *years*°	4.7 (4.2)	1.2	1.04 [0.96-1.13]	0.311		
Duration of exposure to efavirenz - *years**§	1.4 (2.5)	0	0.89 [0.73-1.08]	0.236		
Duration of exposure to nevirapine - *years**§	1.0 (2.3)	0	0.83 [0.66-1.06]	0.147		
Duration of exposure to abacavir – *years**§	1.2 (2.3)	0	0.99 [0.85-1.17]	0.971		
Duration of exposure to lopinavir - *years**§	0.5 (1.5)	0	1.07 [0.88-1.30]	0.505		
Duration of exposure to PI-based regimen - *years**§	3.9 (3.1)	0	1.03 [0.89-1.18]	0.714		
Antiretroviral naivety at M0°	41.5	0	0.86 [0.41-1.79]	0.683		
CD4+ cell count ≥ 200 *cells/mm*^{3}*	67.7	0	0.39 [0.15-1.01]	0.052	0.25 [0.09-0.69]	0.007
Detectable viral load*	92.7	0.3	1.24 [0.59-2.59]	0.574		
Metabolic characteristics						
BMI categories°@@		1.6				
- underweight: <18.5 (ref)	5.8		1			
- normal weight: 18.5–24.9	72.8		0.65 [0.15-2.76]	0.556		
- overweight or obese: >25	19.8		0.57 [0.11-2.87]	0.498		
Hypertriglyceridemia°@@	8.4	0	2.06 [0.79-5.37]	0.141	2.98 [1.11-8.01]	0.030
Hypercholesterolemia°@@	6.5	0.1	0.95 [0.22-3.97]	0.939		
Personal history of CHD°@@	1.2	0	9.39 [2.22-39.8]	0.002		
Family history of CHD°@@	28.0	0	1.82 [0.89-3.71]	0.102	2.25 [1.08-4.71]	0.031

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Personal history of hypertension°@@	6.1	0	2.73 [1.05-7.13]	0.040

CADE = coronary or other arterial disease event; ART = antiretroviral therapy; CHD = coronary heart disease

SD = standard deviation; (A)HR = (adjusted) hazard ratio; CI = confidence interval

° Fixed variable (measured at M0, M1 or M12); * Time-varying variable (the last available value before each visit)

@ Percentages and averages are computed at the first date of follow-up for time-varying variables

@@ At M12; § At the end of the follow-up

Depressive symptoms: the Centre for Epidemiologic Studies Depression Scale (CES-D) scores is >17 for men and >23 for women

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STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract "Cohort study."
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found **"Objective**: To investigate the relationship between response to antiretroviral treatment (ART), alcohol use and occurrence of a major coronary or other arterial disease event (CADE) in HIV-infected individuals. **Design**: Cohort study. A Cox model was used to identify correlates of a first occurrence of a major CADE. **Setting**: The French ANRS CO8 APROCO-COPILOTE cohort was set up in 1997 to study clinical progression and patient-reported outcomes (PRO) after initiating protease inhibitor-containing ART. Clinical data were retrieved from medical records. Self-administered questionnaires collected data on PRO and behaviours, including alcohol use. **Participants**: Metabolic data were only available for a subgroup (n=675) of the study group (n=1154). Main outcomes measures: Major coronary or other arterial disease first event. **Results**: Over the 11-year follow-up, 49 major CADE were observed, with an incidence rate [95%CI]=0.75[0.57 to 0.99] per 100 person-years. CD4+ cell count ≥200 cells/mm3 was associated with a reduced risk of CADE (adjusted hazard ratio AHR[95% CI]=0.40[0.18 to 0.86]) after adjustment for female gender (0.25[0.08 to 0.83]), age (1.07[1.04 to 1.10]) and smoking>20 cigarettes/day (4.19[2.17 to 8.11]). Moreover, individuals with moderate alcohol consumption (≤3 AU/day) had a lower risk of CADE (0.38[0.20 to 0.71]) than alcohol abstainers, while the risk for those drinking>3 AU/day was not significantly different from this latter group. These associations remained valid after adjustment for metabolic disorders. No significant association with exposure to any specific antiretroviral was detected. **Conclusions**: In the long term, CD4+ cell count ≥200 cells/mm3 and moderate alcohol consumption remain the principal factors associated with a lower risk of CADE. Combined interventions to reduce CADE risk-related behaviours and sustain ART adherence in HIV-infected individuals are now a clinical and public health priority."
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported "Cardiovascular disease risk factors (including for example hypertension and tobacco use), as well as immunological status are major predictors of atherosclerosis in HIV-infected women and men. Although antiretroviral (ARV) therapy agents have consistently been found to be associated with coronary and other arterial disease events (CADE) in these patients, more recently Butt and colleagues put into evidence the negative effect of HIV viral replication on heart failure. In one study, patients having experienced myocardial infarction (MI) presented lower baseline and proximal CD4+ cell counts than those who did not, while no association was found with specific antiretroviral agents. Moreover, known factors associated with the risk of CADE such as hyperlipidemia and insulin resistance are becoming an increasingly

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		major clinical concern in ART-treated individuals. Though excessive alcohol use may be frequent in HIV-infected individuals, its impact on the occurrence of CADE in this population has been less investigated. Excessive alcohol consumption also plays a major role in HIV risk-taking behaviors, particularly in men who have sex with men and drug users. In HIV-infected individuals, excessive alcohol use is known to compromise patients' response to antiretroviral therapy (ART) through reduced adherence and to predict HIV progression and increased mortality, mostly from liver diseases. Some recent cross-sectional analyses in HIV-infected and uninfected men have found that heavy alcohol consumption is associated with a higher risk of CADE. On the other hand, in uninfected adults, moderate alcohol consumption has been found to be associated with a reduced risk, improved lipid profiles, increased insulin sensitivity and reduced risk of MI. However, this association has not yet been studied in longitudinal studies involving HIV-infected populations including women. Moreover, there are as yet no consistent results in the literature regarding the role that alcohol use plays in exacerbating the risk of CADE in ART-treated patients and to what extent it can confound or boost the effect of immuno-virological response to ART, ART-associated dyslipidemia and insulin resistance on the risk of CADE."
Objectives	3	State specific objectives, including any prespecified hypotheses "We used data from the 11-year follow-up of the APROCO-COPILOTE cohort of patients receiving ART since 1997 to investigate the relationship between undetectable viral load, CD4+ cell count, alcohol consumption and the first occurrence of a CADE, after adjustment for known risk factors including metabolic disorders."
Methods		
Study design	4	Present key elements of study design early in the paper "The French APROCO-COPILOTE cohort (ANRS CO8) was designed to study the clinical, immunological, virological, and socio-behavioural course of HIV disease in HIV-1 positive individuals who started the first generation of potent ART (treatment regimens including protease inhibitors (PI)). The study was approved by the Ethics Committee of Cochin Hospital (Paris) and informed consent was obtained from all participants."
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection "Patients were enrolled in the cohort at their first PI-based ART prescription between May 1997 and June 1999 (n=1281) in 47 French centres, and clinically followed-up every 4 months thereafter, up to month 132 (M132)."
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up "In the present study were included all patients who had either two alcohol assessment or one alcohol assessment just preceding the CADE over the whole follow-up (n=1154)."
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed [Not applicable.]
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect

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		modifiers. Give diagnostic criteria, if applicable *"Medical questionnaire* The medical questionnaire at enrolment (M0) collected retrospective data about patient's HIV history including HIV transmission category, time since HIV diagnosis, previous exposure to antiretroviral treatment before enrolment, co-infection with hepatitis C virus (HCV). This information was complemented by another medical questionnaire, completed by the HIV physician at each follow-up visit, which collected clinical and laboratory data (plasma HIV RNA level, CD4+ cell count, HIV clinical stage) as well as data on the antiretroviral regimen prescribed. A third form, based on updated medical records, detailed all clinical severe events, including CADE, occurring during the study period. Information on metabolic disorders was available at M12 for a subset of cohorts' patients, who participated in a parallel cross-sectional survey, designed to study clinical and laboratory metabolic complications. These "metabolic" data included height, weight, hypertriglyceridemia, hypercholesterolemia, personal history of coronary heart disease (CHD) and hypertension, and finally any family history of CHD. *Outcome* The clinical severe events occurring during follow-up, including CADE, were initially recovered from medical records and validated by a validation committee. The classification of clinical severe events was based on the 10th Revision of the International Statistical Classification of Diseases and Related Health Problems (ICD-10). The major CADE selected for this study were MI, stroke, coronary heart disease, peripheral arterial disease and cardiovascular surgery for coronary disease. *Self-administered questionnaire* A self-administered questionnaire collected - at M0, M4, M12 and every 8 months thereafter during the first 5 years of follow-up, then yearly thereafter – data on socio-demographic characteristics including age, gender and educational level. Among other psycho-social and behavioural information, it also collected details on depressive symptoms, tobacco use, alcohol consumption, self-reported ART-related symptoms and adherence to ART."
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group "At each follow-up visit, CD4+ cell count was measured by standardized flow cytometry, and plasma HIV RNA levels were measured using the assay routinely available in each participating centre. HIV clinical stage was defined using the 1993 Centres for Disease Control and Prevention (CDC) clinical classification system. Serum triglyceride and total cholesterol levels were measured after a 12-hour overnight fast. Hypertriglyceridemia was defined as a triglyceride level ≥2.2 mmol/l, while hypercholesterolemia was defined as a total cholesterol level ≥5.5 mmol/l. Depression was measured using the validated French version of the Centre for Epidemiologic Studies Depression Scale (CES-D), which is a 20-item scale commonly used in studies involving HIV-infected patients. Tobacco and alcohol consumption were evaluated during the previous 4 weeks. Alcohol consumption was assessed using two questions about frequency of consumption and quantity consumed daily, if applicable. A 13-item scale comprising the French version of the symptom index validated by Justice et al and described elsewhere collected information about self-reported

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		symptoms. Five other questions gathered information about adherence to ART, according to the methodology established by the AIDS Clinical Trial Group."
Bias	9	Describe any efforts to address potential sources of bias "As the results of the analysis on the whole dataset (n=1154) could be affected by the lack of important predictors of CADE like hypertriglyceridemia or hypercholesterolemia, we conducted a secondary analysis on a subset of patients who had additional metabolic disorders data." [This secondary analysis confirmed the same pattern of predictors of CADE events after adjustment for metabolic disorders.]
Study size	10	Explain how the study size was arrived at "Precisely, two analyses on two different study populations were performed to study predictors of major CADE: 1) First analysis: the study population included all patients who had either two alcohol assessment or one alcohol assessment just preceding the event over the whole follow-up (n=1154). The follow-up period was M0-M132. 2) Second analysis: the study population was restricted to the subset of patients with available data on metabolic disorders, measured at M12 (n=675). Consequently, only events occurring after M12 were considered in this second analysis."
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why "The WHO body mass index (BMI) categories were used in the analysis: underweight and overweight/obese patients were defined respectively as having a BMI lower than 18.5 and greater than 25. A cumulative measure of time exposure to specific ARV medications (abacavir, efavirenz, nevirapine, lopinavir and protease inhibitor) was computed from cohort enrolment to the date of each follow-up visit. Tobacco consumption was dichotomized in two categories: more than 20 cigarettes/day *vs* other. The average number of alcohol units (AU) consumed per day was computed using the two questions on alcohol consumption. Patients were classified as non-adherent if they reported taking less than 100% of prescribed medications during the previous 4 weeks, using a validated algorithm. A patient with a CES-D score value above 17 (men) or 23 (women) was considered as presenting depressive symptoms." [See references in the paper for the reasons of choosing of these categories].
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding "Incidence rate of major CADE was computed as the number of cases divided by the number of person-years of follow-up. Follow-up duration was calculated as the difference in days from enrolment to the date of the CADE, death or last visit, whichever occurred first. Follow-up of patients experiencing major CADE was censored after the date of the first CADE. We used a Cox proportional hazards model to identify characteristics associated with the first occurrence of a major CADE. Psychosocial characteristics - depressive symptoms, tobacco and alcohol consumption, self-reported symptoms, CD4+ cell count and viral load - were evaluated at each visit and used as time-varying covariates in the statistical analysis. All the other covariates were used as fixed variables (measured at M0, M1 or M12).

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		For time-varying factors, the last known value was carried forward in the case of missing data at a scheduled visit. In the case of an event occurring between two consecutive follow-up visits, the values for the time-varying variables measured at the visit preceding the event were used. Covariates with a p-value<0.25 in univariate Cox analyses were considered eligible for the multivariate Cox model, which was built using a backward selection procedure based on the Wald test (p<0.05). Interaction effects between the factors of the multivariate final model were tested. The proportional-hazards assumption was verified globally for the multivariate models and separately with respect to each covariate, using both Kaplan-Meier estimates and tests based on Schoenfeld residuals. A residual analysis for outliers' detection was performed and the sensitivity of the model to influential outliers was tested. All analyses were performed using Stata Intercooled software, version 10.1."
		(*b*) Describe any methods used to examine subgroups and interactions "Interaction effects between the factors of the multivariate final Cox model were tested."
		(*c*) Explain how missing data were addressed "For time-varying factors, the last known value was carried forward in the case of missing data at a scheduled visit."
		(*d*) If applicable, explain how loss to follow-up was addressed "Follow-up duration was calculated as the difference in days from enrolment to the date of the CADE, death or last visit, whichever occurred first. Follow-up of patients experiencing major CADE was censored after the date of the first CADE." [Patients lost to follow-up were dealt as usual in the Cox model (right-censoring).]
		(*e*) Describe any sensitivity analyses "A residual analysis for outliers' detection was performed and the sensitivity of the models to influential outliers was tested."
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed "A total of 1154 patients were included in the present study, accounting for 9401 person-visits."
		(b) Give reasons for non-participation at each stage "When comparing the study patients (n=1154) with those included in the cohort, but excluded from the study due to missing alcohol data (n=127), no significant difference was found for gender, age at enrolment, antiretroviral naivety, CDC clinical stage, baseline CD4+ cell counts and HIV viral load at M0 (data not shown)."
		(c) Consider use of a flow diagram [Not done because the graph is very simple.]
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders

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		"The distribution of factors associated with major CADE [...] are reported in Table 1 for the entire study population (n=1154). Table 2 focuses instead on the subset of patients with available data on metabolic disorders (n=675). In the subsample of patients with available metabolic data (Table 2), the majority (73%) had a normal BMI, 17% were classified in the overweight category and a minority was classified in each of the two more extreme categories (6% underweight and 3% obese). About 8% of the patients had hypertriglyceridemia and 6% had hypercholesterolemia at M12. One year after the enrolment in the cohort, 6% of the patients reported a personal history of hypertension. Only 1% and 28% respectively had a personal and family history of CHD."
		(b) Indicate number of participants with missing data for each variable of interest [See Tables 1 and 2, second column.]
		(c) Summarise follow-up time (eg, average and total amount) "Median [interquartile range (IQR)] duration of follow-up of the selected patients, from their first visit after the ART prescription (M1) until M132, was 5.9 [1.8-9.4] years."
Outcome data	15*	Report numbers of outcome events or summary measures over time "Over the 11-year follow-up of the cohort, a total of 85 CADE were observed. These included 49 major CADE as follows: myocardial infarction (n=30), coronary heart disease (n=4), stroke (n=7), peripheral arterial disease (n=7) and cardiovascular surgery (n=1). The distribution of the other thirty six CADE was as follows: phlebitis/pulmonary embolism (n=22), cardiomyopathy/congestive heart failure (n=5), cardiac dysrhythmia (n=6), cerebral haemorrhage (n=2) and aortic aneurysm (n=1). The incidence rate [95% CI] of all CADE was 1.3 [1.0 to 1.6] per 100 person-years, while for major CADE the incidence rate was 0.75 [0.57 to 0.99] per 100 person-years. Of the 49 major CADE, 40 occurred after M12. Furthermore, the major CADE incidence over the period M12-M132 was 0.89 [0.66 to 1.22] per 100 person-years."
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included "[...] the results of univariate and multivariate Cox models, are reported in Table 1 for the entire study population (n=1154). Table 2 focuses instead on the subset of patients with available data on metabolic disorders (n=675). [...] In the entire study group (n=1154), the following factors were found to be independent predictors of major CADE: older age at baseline (Adjusted Hazard Ratio (AHR)[95% CI]=1.07[1.04 to 1.10]), tobacco consumption>20 cigarettes/day (4.19[2.17 to 8.11]) and CD4+ cell count ≥200 cells/mm^3 (0.40[0.18 to 0.86]) (see Table 1). In addition, a negative association was found between female gender and major CADE (0.25[0.08 to 0.83]). After accounting for the effect of age, gender, tobacco consumption and CD4+ cell count ≥200 cells/mm^3 in the multivariate Cox model, individuals with moderate alcohol consumption (≤3 AU/day) were at lower risk of major CADE (0.38[0.20 to 0.71]), than alcohol abstainers, while those drinking more than 3 AU/day were not significantly different from abstainers (p=0.229). These results remained valid even when the analysis was restricted to the subgroup of patients with available metabolic data and the follow-up period M12-M132. After

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		adjusting for age, tobacco consumption, hypertriglyceridemia and a family history of CHD, we consistently found a negative association between moderate alcohol use (0.23[0.11-0.49]), CD4+ cell count ≥200 cells/mm^3 (0.25[0.09-0.69]) and major CADE one year after enrolment (Table 2)."
		(*b*) Report category boundaries when continuous variables were categorized [See Tables 1 and 2 for details.]
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period "The incidence rate [95% CI] of all CADE was 1.3 [1.0 to 1.6] per 100 person-years, while for major CADE the incidence rate was 0.75 [0.57 to 0.99] per 100 person-years. Of the 49 major CADE, 40 occurred after M12. Furthermore, the major CADE incidence over the period M12-M132 was 0.89 [0.66 to 1.22] per 100 person-years."
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses "No significant association was found between time of exposure to different ARV drugs and major CADE, the only exceptions being efavirenz and nevirapine, for which a slight negative association was found in univariate analysis only. No significant interaction was found between smoking habits and hypertriglyceridemia or personal history of CHD. Furthermore, detectable viral load was not associated with major CADE in univariate or multivariate analyses. For the Cox models fitted in this study, proportional-hazards assumption remained valid, either globally and with respect to each covariate. The residual analysis did not alter the results of the multivariate model."
Discussion		
Key results	18	Summarise key results with reference to study objectives "This longitudinal study clearly confirms that in ART-treated individuals, proximal CD4+ cell count higher than 200 cells/mm^3 remains a major risk factor negatively associated with major coronary and other arterial disease events. This result remains valid even after adjustment for metabolic disorders such as hypertriglyceridemia and family history of CHD, the strength of the association remaining unchanged."
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias "Some limitations of this study need to be acknowledged. First, our definition of CADE includes different types of events which might not share the same pattern of risk factors. However, an additional analysis performed on a restricted dataset and which adjusted for other potential risk factors (such as hypertriglyceridemia) confirmed the pattern found in the main analysis. Second, information on alcohol use was mainly based on self-reports and these may tend to underestimate alcohol consumption. However, it has been reported that HIV-HCV co-infected patients tend to underreport alcohol use more to their hepathologist than to other physicians. As our patients were all followed-up by HIV physicians, it is likely that the degree of underreporting did not greatly affect hazard ratios' estimates."
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations,

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multiplicity of analyses, results from similar studies, and other relevant evidence

"The relationship between CADE and CD4+ cell count, in addition to the lack of association between CADE and specific ARV classes, confirms previous results where the association between exposure to certain ARV and CADE was no longer evident after controlling for traditional risk factors and HIV disease markers. Indeed, the association between specific ARV drugs and major CADE, like myocardial infarctus, has been shown in very powerful studies, where adjustment for nadir CD4+ lymphocyte count or peak HIV-1 RNA level did not modify the results. However, in the study cited, no adjustment for proximal CD4+ cell count was performed, and the association with the class of ARV became weaker after adjustment for serum lipid levels. Indeed, although ART may modify lipid levels and increase the risk of CADE, it may also reduce this risk because of CD4+ reconstitution following long-term suppression of HIV replication or HIV-associated inflammation. The lack of association with exposure to specific ARV classes in the present study, something usually associated with an increased risk of CADE, suggests that reduced immunological response to ART over a long follow-up, as well as known CADE risk factors may have a more important impact than exposure to a specific antiretroviral drug.

It is also possible that patients with altered lipid profiles were switched to other classes of antiretroviral drugs during the follow-up. As a consequence, in the long term, CD4+ cell count $\geq$200 cells/mm^3 remain the main correlate of a decreased risk of CADE, whatever the antiretroviral received.

In our study, individuals reporting elevated alcohol consumption exhibited the same risk of CADE as alcohol abstainers. In contrast, those reporting moderate alcohol consumptions had a lower risk. This result was confirmed after adjustment for additional risk factors including tobacco use, age, gender and CD4+ cell count. This relationship between alcohol consumption and CADE remained significant and J-shaped, even after adjustment for data on metabolic risk factors- such as having a history of CHD and hypertriglyceridemia -which was available for a subset of patients. As highlighted in previous observations of the general population and certain populations affected by other diseases, these results confirmed that the increased risk of CADE in HIV-infected patients receiving ART is not attributable to elevated alcohol use. Furthermore, we highlighted the apparent protective effect of moderate alcohol consumption in ART treated HIV-infected patients.

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 an increase in non-HIV based mortality, cardiovascular diseases becoming increasingly important. This increase can be explained firstly by a reduction in the competing role of HIV-related mortality, which in the long term was much more detrimental to health than other causes of deaths. Secondly, the high prevalence in this population of other risk factors like tobacco use, together with ART-related morbidity including altered lipid profiles and triglycerides, may contribute to an increased risk of CADE.

The association found between moderate alcohol use and reduced CADE risk is not consistent with all previous research in HIV-infected patients. Freiberg et al pointed out that among HIV-infected men in the US, hazardous drinking and both alcohol abuse and dependence were associated with a higher prevalence of CADE compared with infrequent and moderate drinking, even after adjusting for traditional risk factors, antiretroviral therapy and CD4+ cell count. This difference in the results may be due to the different design of the two studies (longitudinal for ours vs cross-sectional for

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		Freidberg's), but probably also to the different types of alcohol mainly consumed in the two populations, red wine consumption being more widespread in France than in the US. This difference in the pattern of alcohol use has already been highlighted in a comparative study on CADE in non HIV-infected Irish and French males, showing an increased protective effect of drinking red wine in the latter population. Increased wine consumption probably brings about an increase in high-density lipoprotein cholesterol levels, which helps protect against CADE. On the other hand, beer and spirits consumption are linked to increased triglyceride levels. We do not know to what extent moderate alcohol users are representative of a population with better health status as alcohol use in France is extremely frequent in the general population and this fact may increase the strength of the association found. While one hypothesis is that excessive alcohol consumption can increase inflammatory markers' levels, which in turn probably leads to a higher risk of CADE and premature aging in HIV patients, this hypothesis was not demonstrated in a study conducted in older adults without cardiovascular disease, where alcohol intake was found to be associated with lower levels of inflammatory markers. In the meantime it is important to remember that individuals with immune suppression are at a greater risk of cancer, a disease whose pattern of risk factors also include alcohol use, even when moderate. Tobacco smoking has consistently been found to be a major risk factor for CADE. Smoking prevalence and dependence in HIV-infected patients is higher than in the general population. Effective interventions for reducing and quitting smoking, especially for patients with several risk factors, are strongly recommended especially considering the increased CADE risk which exists in HIV-infected smokers receiving ART. However, results from studies reporting on the effectiveness of interventions for quitting smoking in HIV-infected individuals are inconsistent."
Generalisability	21	Discuss the generalisability (external validity) of the study results "This cohort is representative of the first generation of patients receiving potent ART. As cardiovascular disease is a rising issue in all HIV-infected populations receiving ART, these results can give important information about the pattern of risk and protective factors in all treated HIV-infected populations. In conclusion, in the long term, CD4+ cell count ≥200 cells/mm3 and moderate alcohol consumption remain the principal factors negatively associated with the risk of major CADE. Combined interventions to reduce CADE-risk-related behaviours and assure sustained adherence and response to ART in HIV-infected individuals are now a clinical and public health priority. "
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based "Funding sources: The ANRS CO8 (APROCO/COPILOTE) cohort is funded by ANRS (Agence Nationale de Recherches sur le Sida et les hépatites virales) and CMIT (Collège des Universitaires de Maladies Infectieuses et Tropicales), which received research grants from Abbott, Boehringer-Ingelheim, Bristol-Myers Squibb, GlaxoSmithKline, Gilead Sciences, Pfizer and Roche. The funders were responsible for initiating, managing and financing the cohort, but did not interfere with data analysis and interpretation and dissemination of the scientific results."

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*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2012-001155.R1
Article Type:	Research
Date Submitted by the Author:	29-Jun-2012
Complete List of Authors:	Carrieri, Maria Patrizia Protopopescu, Camelia; INSERM, U912 (SESSTIM), Aix Marseille Univ, IRD, UMR-S912, ORS PACA, Observatoire Régional de la Santé Provence Alpes Côte d'Azur Le Moing, Vincent Reboud, Philippe Raffi, François Mahy, Sophie Roux, Perrine Cuzin, Lise Spire, Bruno Leport, Catherine
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Cardiovascular medicine
Keywords:	HIV & AIDS < INFECTIOUS DISEASES, Myocardial infarction < CARDIOLOGY, Coronary heart disease < CARDIOLOGY

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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

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Abstract word count: 285 words

Text word count: 4326 words

Funding sources: The ANRS CO8 (APROCO/COPILOTE) cohort is funded by ANRS (Agence Nationale de Recherches sur le Sida et les hépatites virales) and CMIT (Collège des Universitaires de Maladies Infectieuses et Tropicales), which received research grants from Abbott, Boehringer-Ingelheim, Bristol-Myers Squibb, GlaxoSmithKline, Gilead Sciences, Pfizer and Roche.

The funders were responsible for initiating, managing and financing the cohort, but did not interfere with data analysis and interpretation and dissemination of the scientific results.

Competing interests: All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf <http://www.icmje.org/coi_disclosure.pdf> (available on request from the corresponding author) and declare that (1) they have no support from any company for the submitted work; (2) they have no relationships with any company that might have an interest in the submitted work in the previous 3 years; (3) their spouses, partners, or children have no financial relationships that may be relevant to the submitted work; and (4) they have no non-financial interests that may be relevant to the submitted work.

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Data sharing: There is no additional data available.

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Summary

Article Focus

- Major coronary or other arterial disease events (CADE) are frequent in HIV-infected individuals receiving antiretroviral therapy.
- While some antiretroviral classes can increase the risk of CADE, it is still controversial to what extent immune reconstitution and other factors like moderate alcohol consumption can reduce the risk of CADE in this population.

Key Messages

- Moderate alcohol consumption and absence of immunodepression can be beneficial to reduce the risk of CADE in HIV-infected patients.
- Reduced immunological response to antiretroviral treatment (ART) over a long follow-up may have a more important impact on CADE than exposure to a specific antiretroviral drug.

Strengths and Limitations

- This paper suggests the importance of combined risk reduction interventions including adherence counselling for assuring long term immunological response and decrease the risk of CADE.

Short title

CD4+, Moderate Alcohol Consumption and Coronary Events in HIV-infected patients

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Abstract

Objective: To investigate the relationship between response to antiretroviral treatment (ART), alcohol use and occurrence of a major coronary or other arterial disease event (CADE) in HIV-infected individuals.

Design: Cohort study. A Cox model was used to identify correlates of a first occurrence of a major CADE.

Setting: The French ANRS CO8 APROCO-COPILOTE cohort was set up in 1997 to study clinical progression and patient-reported outcomes (PRO) after initiating protease inhibitor-containing ART. Clinical data were retrieved from medical records. Self-administered questionnaires collected data on PRO and behaviours, including alcohol use.

Participants: Metabolic data were only available for a subgroup (n=675) of the study group (n=1154).

Main outcomes measures: Major coronary or other arterial disease first event.

Results: Over the 11-year follow-up, 49 major CADE were observed, with an incidence rate [95%CI]=0.75[0.57 to 0.99] per 100 person-years. Immunodepression (CD4+ cell count <200 cells/mm^3) was associated with an increased risk of CADE (Adjusted Hazard Ratio AHR[95% CI]= 2.52[1.15 to 5.48]) after adjustment for female gender (0.25[0.08 to 0.83]), age (1.07[1.04 to 1.10]) and smoking>20 cigarettes/day (4.19[2.17 to 8.11]). Moreover, individuals with moderate alcohol consumption (≤4(3) AU/day for men(women)) had a lower risk of CADE (0.38[0.20 to 0.71]) than alcohol abstainers, while the risk for those drinking>4(3) AU/day for men(women) was not significantly different from this latter group. These associations remained valid after adjustment for metabolic disorders. No significant association with exposure to any specific antiretroviral was detected.

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Conclusions: In the long term, absence of immunodepression and moderate alcohol consumption remain associated with a lower risk of major CADE. Combined interventions to reduce CADE risk-related behaviours including adherence counselling for assuring long term immunological response to ART in HIV-infected individuals are now a clinical and public health priority.

Key words: Coronary and other arterial disease events, Alcohol, CD4+ cell count, HIV, ARV.

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Introduction

Cardiovascular disease risk factors (including for example hypertension and tobacco use), as well as immunological status[1] are major predictors of atherosclerosis in HIV-infected women and men. 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, more recently Butt and colleagues put into evidence the negative effect of HIV viral replication on heart failure[6]. In one study, patients having experienced a MI presented lower baseline and proximal CD4+ cell counts than those who did not, while no association was found between cardiovascular disease events and specific antiretroviral agents[7]. Moreover, known factors associated with cardiovascular risk[8] such as hyperlipidemia and insulin resistance[9-11] are becoming an increasingly major clinical concern in ARV-treated individuals.

Though excessive alcohol use may be frequent in HIV-infected individuals, its impact on the occurrence of cardiovascular disease in this population has been less investigated. Excessive alcohol consumption also plays a major role in HIV risk-taking behaviours, particularly in men who have sex with men and drug users[12, 13]. In HIV-infected individuals, excessive alcohol use is known to compromise patients' response to antiretroviral therapy (ART) through reduced adherence[14-17] and to predict HIV progression and increased mortality[18-21], mostly from liver diseases[22-25]. Some recent cross-sectional analyses in HIV-infected and uninfected men have found that heavy alcohol consumption is associated with a higher risk of cardiovascular disease[26, 27]. On the other hand, in uninfected adults, moderate alcohol consumption has been found to be associated with a reduced cardiovascular mortality[28], improved lipid profiles, increased insulin sensitivity and reduced risk of MI[29]. However, this association has not yet been studied in longitudinal studies involving HIV-infected populations including women. Moreover, there are as yet no consistent results

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in the literature regarding the role that alcohol use plays in exacerbating cardiovascular risk in ARV-treated patients and to what extent it can confound or boost the effect of immuno-virological response to ART, ART-associated dyslipidemia and insulin resistance on the risk of cardiovascular disease events.

We used data from the 11-year follow-up of the APROCO-COPILOTE cohort of patients receiving ART since 1997 to investigate the relationship between viral load, CD4+ cell count, alcohol consumption and the first occurrence of a major coronary or other arterial disease event (CADE), after adjustment for known risk factors including metabolic disorders.

Methods

The French APROCO-COPILOTE cohort (ANRS CO8) was designed to study the clinical, immunological, virological, and socio-behavioural course of HIV disease in HIV-1 positive individuals who started the first generation of potent ART (treatment regimens including protease inhibitors (PI)). The study was approved by the Ethics Committee of Cochin Hospital (Paris) and informed consent was obtained from all participants.

Setting

Patients were enrolled in the cohort at their first PI-based ART prescription between May 1997 and June 1999 (n=1281) in 47 French centres, and clinically followed-up every 4 months thereafter, up to month 132 (M132).

Patients

In the present study were included all patients who had either two alcohol assessment or one alcohol assessment just preceding the CADE over the whole follow-up (n=1154).

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Medical questionnaire

The medical questionnaire at enrolment (M0) collected retrospective data about patient's HIV history including HIV transmission category, time since HIV diagnosis, previous exposure to antiretroviral treatment before enrolment, and co-infection with hepatitis C virus (HCV) defined as having positive HCV RNA and/or positive HCV antibodies. This information was complemented by medical questionnaires, completed by the HIV physicians at each follow-up visit, which collected clinical and laboratory data (plasma HIV RNA level, CD4+ cell count, HIV clinical stage, aspartate transaminase (AST) and alanine transaminase (ALT) levels), as well as data on the antiretroviral regimen prescribed. A third form, based on updated medical records, detailed all clinical severe events, including cardiovascular events, occurring during the study period. Information on metabolic disorders was available at M12 for a subset of cohorts' patients, who participated in a parallel cross-sectional survey, designed to study clinical and laboratory metabolic complications[30]. These "metabolic" data included height, weight, hypertriglyceridemia, hypercholesterolemia, personal history of coronary heart disease (CHD) and hypertension, and finally any family history of CHD.

At each follow-up visit, CD4+ cell count was measured by standardized flow cytometry, and plasma HIV RNA levels were measured using the assay routinely available in each participating centre. Immunodepression was defined by CD4+ cell count <200 cells/mm^3; viral load was classified as detectable if it was greater than the threshold value specific to each centre where the assay was performed. HIV clinical stage was defined using the 1993 Centres for Disease Control and Prevention (CDC) clinical classification system[31].

Serum triglyceride and cholesterol levels were measured after a 12-hour overnight fast. Hypertriglyceridemia was defined as a triglyceride level ≥2.2 mmol/l, while hypercholesterolemia was defined as a total cholesterol level ≥5.5 mmol/l.

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Outcome

The details of all clinical severe 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 clinical severe 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.

Self-administered questionnaire

A self-administered questionnaire collected - at M0, M4, M12 and every 8 months thereafter during the first 5 years of follow-up, then yearly thereafter – data on socio-demographic characteristics including age, gender and educational level. Among other psycho-social and behavioural information, it also collected details on depressive symptoms, tobacco use, alcohol consumption, self-reported ART-related symptoms and adherence to ART.

Depression was measured using the validated French version of the Centre for Epidemiologic Studies Depression Scale (CES-D), which is a 20-item scale commonly used in studies involving HIV-infected patients[32].

[i] http://www.who.int/classifications/icd/en/

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Tobacco and alcohol consumption were evaluated during the previous 4 weeks. Alcohol consumption was assessed using two questions about frequency of consumption and quantity consumed daily, if applicable.

A 13-item scale comprising the French version of the symptom index validated by Justice *et al*[33] and described elsewhere[34] collected information about self-reported symptoms. Five other questions gathered information about adherence to ART, according to the methodology established by the AIDS Clinical Trial Group[35].

Statistical analysis

As the results of the analysis on the whole dataset (n=1154) could be affected by the lack of important predictors of CADE like hypertriglyceridemia or hypercholesterolemia, we conducted a secondary analysis on a subset of patients who had additional metabolic disorders data. More specifically, two analyses on the following study populations were performed to study predictors of major CADE:

1) First analysis: the study population included all patients who had either two alcohol assessment or one alcohol assessment just preceding the event over the whole follow-up (n=1154). The follow-up period was M0-M132.

2) Second analysis: the study population was restricted to the subset of patients with available data on metabolic disorders, measured at M12 (n=675). Consequently, only events occurring after M12 were considered in this second analysis.

The WHO body mass index (BMI) categories were used in the analysis: underweight and overweight/obese patients were defined respectively as having a BMI lower than 18.5 and greater than 25.

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

Tobacco consumption was dichotomized in two categories: more than 20 cigarettes/day *vs* other[36]. 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 ≤4(3) AU/day for men(women); >4(3) AU/day for men(women)), in order to test a gradient effect. 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.

Patients were classified as non-adherent if they reported taking less than 100% of prescribed medications during the previous 4 weeks, using a validated algorithm[38]. A patient with a CES-D score value above 17 (men) or 23 (women) was considered as presenting depressive symptoms[39].

Incidence rate of major CADE was computed as the number of cases divided by the number of person-years of follow-up. Follow-up duration was calculated as the difference in days

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from enrolment to the date of the CADE, death or last visit, whichever occurred first. Follow-up of patients experiencing major CADE was censored after the date of the first CADE. We used a Cox proportional hazards model to identify characteristics associated with the first occurrence of a major CADE. Psychosocial characteristics - depressive symptoms, tobacco and alcohol consumption, self-reported symptoms, CD4+ cell count and viral load - were evaluated at each visit and used as time-varying covariates in the statistical analysis. All the other covariates were used as fixed variables (measured at M0, M1 or M12). For time-varying factors, the last known value was carried forward in the case of missing data at a scheduled visit. In the case of an event occurring between two consecutive follow-up visits, the values for the time-varying variables measured at the visit preceding the event were used. Covariates with a p-value<0.25 in univariate Cox analyses were considered eligible for the multivariate Cox model, which was built using a backward stepwise selection procedure based on the Wald test ($p<0.05$). Interaction effects between the factors of the multivariate final model were tested. The proportional-hazards assumption was verified globally for the multivariate models and separately with respect to each covariate, using both Kaplan-Meier estimates and tests based on Schoenfeld residuals[40]. A residual analysis for outliers' detection was performed and the sensitivity of the model to influential outliers was tested.

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. A third analysis was performed separately on the subgroups of patients co-infected with HCV and those not co-infected with HCV.

All analyses were performed using Stata Intercooled software, version 12.1.

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Results

A total of 1154 patients were included in the present study, accounting for 9401 person-visits. When comparing the study patients (n=1154) with those included in the cohort, but excluded from the study due to missing alcohol data (n=127), no significant difference was found for gender, age at enrolment, antiretroviral naivety, CDC clinical stage, baseline CD4+ cell counts and HIV viral load at M0 (data not shown).

Median [interquartile range (IQR)] duration of follow-up of the selected patients, from their first visit after the ART prescription (M1) until M132, was 5.9 [1.8-9.4] years. During the 11 years of the study, accounting for 6544 person-years, the loss to follow-up rate [95% CI] was 17.6 [16.3 to 18.7] per 100 person-years.

Over the 11-year follow-up of the cohort, a total of 85 severe cardiovascular events were observed. These included 49 major CADE as follows: myocardial infarction (n=30), coronary heart disease (n=4), stroke (n=7), peripheral arterial disease (n=7) and cardiovascular surgery for coronary disease (n=1). The distribution of the other thirty six severe cardiovascular events was as follows: phlebitis/pulmonary embolism (n=22), cardiomyopathy/congestive heart failure (n=5), cardiac dysrhythmia (n=6), cerebral haemorrhage (n=2) and aortic aneurysm (n=1).

The incidence rate [95% CI] of all severe cardiovascular events was 1.3 [1.0 to 1.6] per 100 person-years, while for major CADE the incidence rate was 0.75 [0.57 to 0.99] per 100 person-years. Of the 49 major CADE, 40 occurred after M12. Furthermore, the major CADE incidence over the period M12-M132 was 0.89 [0.66 to 1.22] per 100 person-years.

The distribution of factors associated with major CADE, as well as the results of univariate and multivariate Cox models, are reported in Table 2 for the entire study population (n=1154).

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Table 3 focuses instead on the subset of patients with available data on metabolic disorders (n=675).

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 ≤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.

In the subsample of patients with available metabolic data (Table 3), the majority (73%) had a normal BMI, 17% were classified in the overweight category and a minority was classified in each of the two more extreme categories (6% underweight and 3% obese). About 8% of the patients had hypertriglyceridemia and 6% had hypercholesterolemia at M12. One year after the enrolment in the cohort, 6% of the patients reported a personal history of hypertension. Only 1% and 28% respectively had a personal and family history of CHD.

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In the entire study group (n=1154), the following factors were found to be independent predictors of major CADE: older age at baseline (AHR[95% CI]=1.07[1.04 to 1.10]), tobacco consumption>20 cigarettes/day (4.19[2.17 to 8.11]) and CD4+ cell count <200 cells/mm^3 (2.52[1.15 to 5.48]) (see Table 2). In addition, a negative association was found between female gender and major CADE (0.25[0.08 to 0.83]). After accounting for the effect of age, gender, tobacco consumption and CD4+ cell count <200 cells/mm^3 in the multivariate Cox model, individuals with moderate alcohol consumption (≤4(3) AU/day for men(women)) were at lower risk of major CADE (0.38[0.20 to 0.71]), than alcohol abstainers, while those drinking more than 4(3) AU/day for men(women) were not significantly different from abstainers (p=0.229).

These results remained valid also when the analysis was restricted to the subgroup of patients with available metabolic data and the follow-up period M12-M132. After adjusting for age, tobacco consumption, hypertriglyceridemia and family history of CHD, we consistently found a negative association between moderate alcohol use (0.23[0.11 to 0.49]), and a positive association between CD4+ cell count <200 cells/mm^3 (4.02[1.45 to 11.1]) and major CADE after one year after enrolment (Table 3).

No significant association was found between time of exposure to different ARV drugs and major CADE. No significant interaction was found between smoking habits and hypertriglyceridemia or personal history of CHD. Furthermore, detectable viral load was not associated with major CADE in univariate or multivariate analyses.

For the Cox models fitted in this study, proportional-hazards assumption remained valid, either globally and with respect to each covariate. The residual analysis did not alter the results of the multivariate model.

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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. 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).

Discussion

This longitudinal study clearly confirms that in ARV-treated individuals, proximal CD4+ cell count lower than 200 cells/mm^3 remains a risk factor associated with major coronary and other arterial disease events. This result remains valid even after adjustment for metabolic disorders such as hypertriglyceridemia and family history of CHD, the strength of the association remaining unchanged.

The relationship between CADE and CD4+ cell count, in addition to the lack of association between CADE and specific ARV classes, confirms previous results[7] where the association between exposure to certain ARV and CADE was no longer evident after controlling for traditional risk factors and HIV disease markers. Indeed, the association between specific ARV drugs and major CADE, like myocardial infarctus, has been shown in very powerful studies[5], where adjustment for nadir CD4+ lymphocyte count or peak HIV-1 RNA level did not modify the results. However, in the study cited, no adjustment for proximal CD4+ cell count was performed, and the association with the class of ARV became weaker after adjustment for serum lipid levels[5]. Indeed, although ART may modify lipid levels and increase the risk of CADE, it may also reduce this risk by reducing HIV-associated

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inflammation following long-term suppression of HIV replication[41]. The lack of association with exposure to some specific ARV classes in the present study, factors usually associated with an increased risk of CADE[42, 43], suggests that reduced immunological response to ART over a long follow-up, as well as known CADE risk factors may have a more important impact than exposure to a specific antiretroviral drug.

It is also possible that patients with altered lipid profiles were switched to other classes of antiretroviral drugs during the follow-up. As a consequence, in the long term, CD4+ cell count <200 cells/mm^3 remain a correlate of an increased risk of CADE, whatever the antiretroviral received.

In our study, individuals reporting elevated alcohol consumption exhibited the same risk of CADE as alcohol abstainers. In contrast, those reporting moderate alcohol consumptions had a lower risk. This result was confirmed after adjustment for additional risk factors including tobacco use, age, gender and CD4+ cell count. This relationship between alcohol consumption and CADE remained significant and J-shaped, even after adjustment for data on metabolic risk factors- such as having a history of CHD and hypertriglyceridemia -which was available for a subset of patients. As highlighted in previous observations in the general population[28, 44-47] and certain populations affected by other diseases[48], these results confirmed that the increased risk of CADE in HIV-infected patients receiving ART is not attributable to elevated alcohol use. Furthermore, we highlighted the apparent protective effect of moderate alcohol consumption in ART treated HIV-infected patients.

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 non-HIV based mortality as a part of the total mortality of HIV-infected patients [49], cardiovascular diseases becoming increasingly important. This increase can be explained

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firstly by a reduction in the competing role of HIV-related mortality, which in the long term was much more detrimental to health than other causes of deaths. Secondly, the high prevalence in this population of other risk factors like tobacco use, together with ART-related morbidity including altered lipid profiles and triglycerides, may contribute to an increased risk of CADE.

The association found in our study between moderate alcohol use and reduced CADE risk is not consistent with all previous research in HIV-infected patients. Freiberg *et al*[26] pointed out that among HIV-infected men in the US, hazardous drinking and both alcohol abuse and dependence were associated with a higher prevalence of cardiovascular disease compared with infrequent and moderate drinking, even after adjusting for traditional risk factors, antiretroviral therapy and CD4+ cell count. This difference in the results may be due to the different design of the two studies (longitudinal for ours *vs* cross-sectional for Freiberg's), but probably also to the different types of alcohol mainly consumed in the two populations, red wine consumption being more widespread in France than in the US. This difference in the pattern of alcohol use has already been highlighted in a comparative study on cardiovascular disease in non HIV-infected Irish and French males, showing an increased protective effect of drinking red wine in the latter population[50]. Increased wine consumption probably brings about an increase in high-density lipoprotein cholesterol levels, which helps protect against cardiovascular events. On the other hand, beer and spirits consumption are linked to increased triglyceride levels[51]. We do not know to what extent moderate alcohol users are representative of a population with better health status as alcohol use in France is relatively frequent in the general population and this fact may increase the strength of the association found.

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

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aging in HIV patients. However, this hypothesis was not demonstrated in a study conducted in older adults without cardiovascular disease, where alcohol intake was found to be associated with lower levels of inflammatory markers[53]. In the meantime it is important to remember that individuals with immune suppression are at a greater risk of cancer, a disease whose pattern of risk factors also include alcohol use, even when moderate[54].

Tobacco smoking has consistently been found to be a major risk factor for cardiovascular disease[55]. Smoking prevalence and dependence in HIV-infected patients is higher than in the general population[56]. Effective interventions for reducing and quitting smoking[57], especially for patients with several risk factors, are strongly recommended especially considering the increased cardiovascular risk which exists in HIV-infected smokers receiving ART[58]. However, results from studies reporting on the effectiveness of interventions for quitting smoking in HIV-infected individuals are inconsistent[59].

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.

Some limitations of this study need to be acknowledged. First, our definition of CADE includes different types of events which might not share the same pattern of risk factors. However, an additional analysis performed on a restricted dataset and which adjusted for other potential risk factors (such as hypertriglyceridemia) confirmed the pattern found in the main analysis.

Second, information on alcohol use was mainly based on self-reports and these may tend to underestimate alcohol consumption. However, it has been reported that HIV-HCV co-infected patients tend to underreport alcohol use more to their hepathologist than to other

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physicians[60]. As our patients were all followed-up by HIV physicians, it is likely that the degree of underreporting did not greatly affect hazard ratios' estimates. Moreover, the association found between the excessive alcohol consumption and the biomarkers of alcoholic liver injury indicate a good accuracy of self-reported data on alcohol consumption.

Then, 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.

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.

This cohort is representative of the first generation of patients receiving potent ART. As cardiovascular disease is a rising issue in all HIV-infected populations receiving ART, these

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results can give important information about the pattern of risk and protective factors in all treated HIV-infected populations.

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

Acknowledgments

The authors would like to thank all participating patients, nurses and physicians in clinical sites.

We would also like to thank Jude Sweeney for the English revision and editing of the manuscript.

Authors' contribution:

Maria Patrizia Carrieri planned the data analyses and wrote the manuscript; Camelia Protopopescu analyzed the data and wrote the manuscript; Vincent Le Moing contributed to patients' recruitment and investigation and revised the manuscript; Philippe Reboud contributed to patients' evaluation; François Raffi was responsible for cohort initiation, contributed to patients' recruitment and investigation and revised the manuscript; Sophie Mahy contributed to patients' recruitment and investigation; Perrine Roux contributed to patients' evaluation and revised the manuscript; Lise Cuzin contributed to patients' recruitment and investigation; Bruno Spire contributed to data analyses and revised the manuscript; Catherine Leport was responsible for cohort initiation, contributed to patients' recruitment and investigation and revised the manuscript. All authors approved the final version of the manuscript.

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Other support: Collège des Universitaires de Maladies Infectieuses et Tropicales (CMIT ex APPIT), Sidaction Ensemble contre le Sida and associated pharmaceutical companies:

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Abbott, Boehringer-Ingelheim, Bristol-Myers Squibb, GlaxoSmithKline, Gilead Sciences, Pfizer and Roche.

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Table 1. Association between alcohol consumption and living injury indicators, after adjustment for HCV status, among HIV-infected patients receiving ART (ANRS CO8 APROCO-COPILOTE cohort - random effects logistic models, all patients - n=1154, follow-up period M0-M132)

	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

ART = antiretroviral therapy; ALT= alanine transaminase; AST= aspartate transaminase
AOR = Adjusted Odds-Ratios from a random effects logistic model; CI = confidence interval
* Time-varying variable (the last available value before each visit)

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Table 2. Factors associated with the first occurrence of a major CADE among HIV-infected patients receiving ART (ANRS CO8 APROCO-COPILOTE cohort - univariate and multivariate Cox proportional hazard models, all patients - n=1154, follow-up period M0-M132)

	% of patients or mean (SD)	% of miss. data	Univariate analyses HR [95% CI]	p-value	Multivariate analysis AHR [95% CI]	p-value
Socio-demographic and psychosocial characteristics						
Female gender	22.0	0	0.26 [0.08-0.84]	0.025	0.25 [0.08-0.83]	0.024
Age at M0° - *years*	37.7 (9.5)	0	1.06 [1.03-1.09]	$<10^{-3}$	1.07 [1.04-1.10]	$<10^{-3}$
Secondary-school certificate at M0°	31.3	7.3	0.51 [0.26-1.01]	0.054		
Alcohol consumption at M0°		0				
- abstainers (ref)	18.8		1			
- ≤4(3) AU/day for men(women)	75.3		0.47 [0.24-0.89]	0.021		
- >4(3) AU/day for men(women)	5.9		0.75 [0.21-2.63]	0.648		
Alcohol consumption*		0				
- abstainers (ref)	18.8		1		1	
- ≤4(3) AU/day for men(women)	75.3		0.44 [0.24-0.81]	0.009	0.38 [0.20-0.71]	0.002
- >4(3) AU/day for men(women)	5.9		1.04 [0.30-3.58]	0.953	0.46 [0.13-1.63]	0.229
Tobacco consumption >20 cig./day*	16.4	0.9	3.17 [1.73-5.83]	$<10^{-3}$	4.19 [2.17-8.11]	$<10^{-3}$
Depressive symptoms[#]*	37.9	8.5	1.22 [0.67-2.21]	0.512		
Number of self-reported symptoms (excluding lipodystrophy)*[@]	3.8 (2.8)	0	1.01 [0.95-1.08]	0.694		
Number of self-reported lipodystrophy symptoms*[@]	1.1 (2.2)	0.6	1.07 [0.96-1.18]	0.223		
ART adherence*[@]	57.7	0.1	2.42 [1.17-5.02]	0.017		
Clinical characteristics						
HIV transmission category°		0				
- homosexual	40.6		0.99 [0.42-2.34]	0.988		

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- injecting drug use	17.8		0.92 [0.50-1.70]	0.793		
- other (ref)	41.6		1			
CDC clinical stage A at M0°	51.2	0	0.44 [0.23-0.85]	0.014		
HCV infection at M0°	22.4	4.3	1.16 [0.59-2.29]	0.655		
Time since HIV diagnosis at M0- *years*°	4.7 (4.2)	0.9	1.01 [0.94-1.08]	0.822		
Duration of exposure to efavirenz - *years**§	1.0 (2.2)	0	0.86 [0.71-1.03]	0.102		
Duration of exposure to nevirapine - *years**§	0.9 (2.2)	0	0.91 [0.78-1.07]	0.279		
Duration of exposure to abacavir - *years**§	1.0 (2.1)	0	0.98 [0.84-1.13]	0.748		
Duration of exposure to lopinavir - *years**§	0.4 (1.5)	0	1.01 [0.85-1.20]	0.929		
Duration of exposure to PI-based regimen - *years**§	3.2 (3.1)	0	1.03 [0.92-1.15]	0.615		
Antiretroviral naivety at M0°	44.4	0	1.14 [0.64-2.02]	0.654		
CD4+ cell count < 200 *cells/mm³* at M0°	35.9	0.1	0.99 [0.55-1.80]	0.978		
Detectable viral load at M0°	94.0	0.3	0.81 [0.25-2.61]	0.725		
CD4+ cell count < 200 *cells/mm³**	34.6	0.1	2.48 [1.15-5.33]	0.020	2.52 [1.15-5.48]	0.020
Detectable viral load*	91.9	0.3	0.99 [0.53-1.84]	0.980		

CADE = coronary or other arterial disease event; ART = antiretroviral therapy; CHD = coronary heart disease

SD = standard deviation; (A)HR = (adjusted) hazard ratio; CI = confidence interval

° Fixed variable (measured at M0 or M1); * Time-varying variable (the last available value before each visit); percentages and averages were computed at the first date of follow-up for time-varying variables

@ At M12; § At the end of the follow-up (last available visit for each patient)

Depressive symptoms: the Centre for Epidemiologic Studies Depression Scale (CES-D) scores is >17 for men and >23 for women

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Table 3. Factors associated with the first occurrence of a major CADE among HIV-infected patients receiving ART (ANRS CO8 APROCO-COPILOTE cohort - univariate and multivariate Cox proportional hazard models, patients with available metabolic data - n=675, follow-up period M12-M132)

	% of patients or mean (SD)	% of miss. data	Univariate analyses HR [95% CI]	p-value	Multivariate analysis AHR [95% CI]	p-value
Socio-demographic and psychosocial characteristics						
Female gender	19.0	0	0.33 [0.08-1.40]	0.134		
Age at M0 – *years*°	38.4 (9.5)	0	1.04 [1.01-1.07]	0.013	1.05 [1.02-1.09]	0.003
Secondary-school certificate at M0°	35.4	6.7	0.34 [0.14-0.84]	0.019		
Alcohol consumption at M0°		0				
- abstainers (ref)	15.1		1			
- ≤4(3) AU/day for men(women)	78.4		0.44 [0.18-1.03]	0.059		
- >4(3) AU/day for men(women)	6.5		1.04 [0.26-4.05]	0.959		
Alcohol consumption*		0				
- abstainers (ref)	15.1		1		1	
- ≤4(3) AU/day for men(women)	78.4		0.27 [0.13-0.58]	0.001	0.23 [0.11-0.49]	$<10^{-3}$
- >4(3) AU/day for men(women)	6.5		1.19 [0.33-4.23]	0.792	0.55 [0.14-2.14]	0.390
Tobacco consumption >20 cig./day*	14.8	1.2	3.58 [1.70-7.54]	0.001	5.59 [2.43-12.8]	$<10^{-3}$
Depressive symptoms[#]*	36.6	8.0	1.83 [0.90-3.73]	0.094		
Number of self-reported symptoms (excluding lipodystrophy)*[@]	4.0 (2.9)	0	1.03 [0.95-1.12]	0.463		
Number of self-reported lipodystrophy symptoms*[@]	1.4 (2.4)	0.2	1.10 [0.98-1.24]	0.111		
ARV adherence*[@]	56.6	0.2	1.63 [0.70-3.81]	0.260		
Clinical characteristics						

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HIV transmission category°		0				
- homosexual (ref)	44.0		1			
- injecting drug use	15.0		2.08 [0.75-5.73]	0.156		
- other	41.0		1.32 [0.59-2.93]	0.499		
CDC clinical stage A at M0°	50.8	0	0.43 [0.19-0.97]	0.041		
HCV infection at M0°	19.8	3.8	2.05 [0.94-4.47]	0.071		
Duration since HIV diagnosis at M0- *years*°	4.7 (4.2)	1.2	1.04 [0.96-1.13]	0.311		
Duration of exposure to efavirenz - *years**§	1.4 (2.5)	0	0.89 [0.73-1.08]	0.236		
Duration of exposure to nevirapine - *years**§	1.0 (2.3)	0	0.83 [0.66-1.06]	0.147		
Duration of exposure to abacavir – *years**§	1.2 (2.3)	0	0.99 [0.85-1.17]	0.971		
Duration of exposure to lopinavir - *years**§	0.5 (1.5)	0	1.07 [0.88-1.30]	0.505		
Duration of exposure to PI-based regimen - *years**§	3.9 (3.1)	0	1.03 [0.89-1.18]	0.714		
Antiretroviral naivety at M0°	41.5	0	0.86 [0.41-1.79]	0.683		
CD4+ cell count < 200 *cells/mm*^{3} at M0°	33.9	0	0.88 [0.41-1.88]	0.749		
Detectable viral load at M0°	94.8	0.1	0.70 [0.17-2.94]	0.627		
CD4+ cell count < 200 *cells/mm*^{3}*	32.3	0	2.58 [0.99-6.75]	0.052	4.02 [1.45-11.1]	0.007
Detectable viral load*	92.7	0.3	1.24 [0.59-2.59]	0.574		
Metabolic characteristics						
BMI categories°@		1.6				
- underweight: <18.5 (ref)	5.8		1			
- normal weight: 18.5–24.9	72.8		0.65 [0.15-2.76]	0.556		
- overweight or obese: >25	19.8		0.57 [0.11-2.87]	0.498		
Hypertriglyceridemia°@	8.4	0	2.06 [0.79-5.37]	0.141	2.98 [1.11-8.01]	0.030

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Hypercholesterolemia°@	6.5	0.1	0.95 [0.22-3.97]	0.939		
Personal history of CHD°@	1.2	0	9.39 [2.22-39.8]	0.002		
Family history of CHD°@	28.0	0	1.82 [0.89-3.71]	0.102	2.25 [1.08-4.71]	0.031
Personal history of hypertension°@	6.1	0	2.73 [1.05-7.13]	0.040		

CADE = coronary or other arterial disease event; ART = antiretroviral therapy; CHD = coronary heart disease

SD = standard deviation; (A)HR = (adjusted) hazard ratio; CI = confidence interval

° Fixed variable (measured at M0, M1 or M12); * Time-varying variable (the last available value before each visit); percentages and averages are computed at the first date of follow-up for time-varying variables

@ At M12; § At the end of the follow-up (last available visit for each patient)

Depressive symptoms: the Centre for Epidemiologic Studies Depression Scale (CES-D) scores is >17 for men and >23 for women

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STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract "Cohort study."
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found **"**Objective: To investigate the relationship between response to antiretroviral treatment (ART), alcohol use and occurrence of a major coronary or other arterial disease event (CADE) in HIV-infected individuals. Design: Cohort study. A Cox model was used to identify correlates of a first occurrence of a major CADE. Setting: The French ANRS CO8 APROCO-COPILOTE cohort was set up in 1997 to study clinical progression and patient-reported outcomes (PRO) after initiating protease inhibitor-containing ART. Clinical data were retrieved from medical records. Self-administered questionnaires collected data on PRO and behaviours, including alcohol use. Participants: Metabolic data were only available for a subgroup (n=675) of the study group (n=1154). Main outcomes measures: Major coronary or other arterial disease first event. Results: Over the 11-year follow-up, 49 major CADE were observed, with an incidence rate [95%CI]=0.75[0.57 to 0.99] per 100 person-years. Immunodepression (CD4+ cell count <200 cells/mm^3) was associated with an increased risk of CADE (Adjusted Hazard Ratio AHR[95% CI]= 2.52[1.15 to 5.48]) after adjustment for female gender (0.25[0.08 to 0.83]), age (1.07[1.04 to 1.10]) and smoking>20 cigarettes/day (4.19[2.17 to 8.11]). Moreover, individuals with moderate alcohol consumption (≤4(3) AU/day for men(women)) had a lower risk of CADE (0.38[0.20 to 0.71]) than alcohol abstainers, while the risk for those drinking>4(3) AU/day for men(women) was not significantly different from this latter group. These associations remained valid after adjustment for metabolic disorders. No significant association with exposure to any specific antiretroviral was detected. Conclusions: In the long term, absence of immunodepression and moderate alcohol consumption remain associated with a lower risk of major CADE. Combined interventions to reduce CADE risk-related behaviours including adherence counselling for assuring long term immunological response to ART in HIV-infected individuals are now a clinical and public health priority."
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported "Cardiovascular disease risk factors (including for example hypertension and tobacco use), as well as immunological status[1] are major predictors of atherosclerosis in HIV-infected women and men. 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, more recently Butt and colleagues put into evidence the negative effect of HIV viral replication on heart failure[6]. In one study, patients having experienced a MI presented lower baseline and proximal CD4+ cell counts than those who did not, while no association was found between cardiovascular disease events and specific antiretroviral agents[7]. Moreover, known

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		factors associated with cardiovascular risk[8] such as hyperlipidemia and insulin resistance[9-11] are becoming an increasingly major clinical concern in ARV-treated individuals. Though excessive alcohol use may be frequent in HIV-infected individuals, its impact on the occurrence of cardiovascular disease in this population has been less investigated. Excessive alcohol consumption also plays a major role in HIV risk-taking behaviours, particularly in men who have sex with men and drug users[12, 13]. In HIV-infected individuals, excessive alcohol use is known to compromise patients' response to antiretroviral therapy (ART) through reduced adherence[14-17] and to predict HIV progression and increased mortality[18-21], mostly from liver diseases[22-25]. Some recent cross-sectional analyses in HIV-infected and uninfected men have found that heavy alcohol consumption is associated with a higher risk of cardiovascular disease[26, 27]. On the other hand, in uninfected adults, moderate alcohol consumption has been found to be associated with a reduced cardiovascular mortality[28], improved lipid profiles, increased insulin sensitivity and reduced risk of MI[29]. However, this association has not yet been studied in longitudinal studies involving HIV-infected populations including women. Moreover, there are as yet no consistent results in the literature regarding the role that alcohol use plays in exacerbating cardiovascular risk in ARV-treated patients and to what extent it can confound or boost the effect of immuno-virological response to ART, ART-associated dyslipidemia and insulin resistance on the risk of cardiovascular disease events."
Objectives	3	State specific objectives, including any prespecified hypotheses "We used data from the 11-year follow-up of the APROCO-COPILOTE cohort of patients receiving ART since 1997 to investigate the relationship between viral load, CD4+ cell count, alcohol consumption and the first occurrence of a major coronary or other arterial disease event (CADE), after adjustment for known risk factors including metabolic disorders."
Methods		
Study design	4	Present key elements of study design early in the paper "The French APROCO-COPILOTE cohort (ANRS CO8) was designed to study the clinical, immunological, virological, and socio-behavioural course of HIV disease in HIV-1 positive individuals who started the first generation of potent ART (treatment regimens including protease inhibitors (PI)). The study was approved by the Ethics Committee of Cochin Hospital (Paris) and informed consent was obtained from all participants."
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection "Patients were enrolled in the cohort at their first PI-based ART prescription between May 1997 and June 1999 (n=1281) in 47 French centres, and clinically followed-up every 4 months thereafter, up to month 132 (M132)."
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up "In the present study were included all patients who had either two alcohol assessment or one alcohol assessment just preceding the CADE over the whole follow-up (n=1154)."

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		(*b*) For matched studies, give matching criteria and number of exposed and unexposed [Not applicable.]
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable *"Medical questionnaire* The medical questionnaire at enrolment (M0) collected retrospective data about patient's HIV history including HIV transmission category, time since HIV diagnosis, previous exposure to antiretroviral treatment before enrolment, and co-infection with hepatitis C virus (HCV) defined as having positive HCV RNA and/or positive HCV antibodies. This information was complemented by medical questionnaires, completed by the HIV physicians at each follow-up visit, which collected clinical and laboratory data (plasma HIV RNA level, CD4+ cell count, HIV clinical stage, aspartate transaminase (AST) and alanine transaminase (ALT) levels), as well as data on the antiretroviral regimen prescribed. A third form, based on updated medical records, detailed all clinical severe events, including cardiovascular events, occurring during the study period. Information on metabolic disorders was available at M12 for a subset of cohorts' patients, who participated in a parallel cross-sectional survey, designed to study clinical and laboratory metabolic complications[30]. These "metabolic" data included height, weight, hypertriglyceridemia, hypercholesterolemia, personal history of coronary heart disease (CHD) and hypertension, and finally any family history of CHD. *Outcome* The details of all clinical severe 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 clinical severe events was based on the 10th Revision of the International Statistical Classification of Diseases and Related Health Problems (ICD-10)[1]. 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. *Self-administered questionnaire* A self-administered questionnaire collected - at M0, M4, M12 and every 8 months thereafter during the first 5 years of follow-up, then yearly thereafter – data on socio-demographic characteristics including age, gender and educational level. Among other psycho-social and behavioural information, it also collected details on depressive symptoms, tobacco use, alcohol consumption, self-reported ART-related symptoms and adherence to ART."
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is

[1] http://www.who.int/classifications/icd/en/

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		more than one group "At each follow-up visit, CD4+ cell count was measured by standardized flow cytometry, and plasma HIV RNA levels were measured using the assay routinely available in each participating centre. Immunodepression was defined by CD4+ cell count <200 cells/mm^3; viral load was classified as detectable if it was greater than the threshold value specific to each centre where the assay was performed. HIV clinical stage was defined using the 1993 Centres for Disease Control and Prevention (CDC) clinical classification system[31]. Serum triglyceride and cholesterol levels were measured after a 12-hour overnight fast. Hypertriglyceridemia was defined as a triglyceride level ≥2.2 mmol/l, while hypercholesterolemia was defined as a total cholesterol level ≥5.5 mmol/l. Depression was measured using the validated French version of the Centre for Epidemiologic Studies Depression Scale (CES-D), which is a 20-item scale commonly used in studies involving HIV-infected patients[32]. Tobacco and alcohol consumption were evaluated during the previous 4 weeks. Alcohol consumption was assessed using two questions about frequency of consumption and quantity consumed daily, if applicable. A 13-item scale comprising the French version of the symptom index validated by Justice *et al*[33] and described elsewhere[34] collected information about self-reported symptoms. Five other questions gathered information about adherence to ART, according to the methodology established by the AIDS Clinical Trial Group[35]."
Bias	9	Describe any efforts to address potential sources of bias "As the results of the analysis on the whole dataset (n=1154) could be affected by the lack of important predictors of CADE like hypertriglyceridemia or hypercholesterolemia, we conducted a secondary analysis on a subset of patients who had additional metabolic disorders data." [This secondary analysis confirmed the same pattern of predictors of CADE events after adjustment for metabolic disorders.]
Study size	10	Explain how the study size was arrived at "More specifically, two analyses on the following study populations were performed to study predictors of major CADE: 1) First analysis: the study population included all patients who had either two alcohol assessment or one alcohol assessment just preceding the event over the whole follow-up (n=1154). The follow-up period was M0-M132. 2) Second analysis: the study population was restricted to the subset of patients with available data on metabolic disorders, measured at M12 (n=675). Consequently, only events occurring after M12 were considered in this second analysis."
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why "The WHO body mass index (BMI) categories were used in the analysis: underweight and overweight/obese patients were defined respectively as having a BMI lower than 18.5 and greater than 25. A time-varying cumulative measure of time exposure to specific ARV medications (abacavir, efavirenz, nevirapine, lopinavir and protease inhibitors) was computed during which ART included a specific drug from this list, from cohort enrolment to the date of each follow-up visit.

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		Tobacco consumption was dichotomized in two categories: more than 20 cigarettes/day *vs* other[36]. 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 ≤4(3) AU/day for men(women); >4(3) AU/day for men(women)), in order to test a gradient effect. 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. Patients were classified as non-adherent if they reported taking less than 100% of prescribed medications during the previous 4 weeks, using a validated algorithm[38]. A patient with a CES-D score value above 17 (men) or 23 (women) was considered as presenting depressive symptoms[39]. " [See references in the paper for the reasons of choosing of these categories].
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding "Incidence rate of major CADE was computed as the number of cases divided by the number of person-years of follow-up. Follow-up duration was calculated as the difference in days from enrolment to the date of the CADE, death or last visit, whichever occurred first. Follow-up of patients experiencing major CADE was censored after the date of the first CADE. We used a Cox proportional hazards model to identify characteristics associated with the first occurrence of a major CADE. Psychosocial characteristics - depressive symptoms, tobacco and alcohol consumption, self-reported symptoms, CD4+ cell count and viral load - were evaluated at each visit and used as time-varying covariates in the statistical analysis. All the other covariates were used as fixed variables (measured at M0, M1 or M12). For time-varying factors, the last known value was carried forward in the case of missing data at a scheduled visit. In the case of an event occurring between two consecutive follow-up visits, the values for the time-varying variables measured at the visit preceding the event were used. Covariates with a p-value<0.25 in univariate Cox analyses were considered eligible for the multivariate Cox model, which was built using a backward stepwise selection procedure based on the Wald test (p<0.05). Interaction effects between the factors of the multivariate final model were tested. The proportional-hazards assumption was verified globally for the multivariate models and separately with respect to each covariate, using both Kaplan-Meier estimates and tests based on Schoenfeld residuals[40]. A residual analysis for outliers' detection was performed and the sensitivity of the model to influential outliers was tested. 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. A third analysis was performed separately on the subgroups of patients co-infected with HCV and those not co-infected with HCV.

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		All analyses were performed using Stata Intercooled software, version 12.1."
		(*b*) Describe any methods used to examine subgroups and interactions "Interaction effects between the factors of the multivariate final Cox model were tested."
		(*c*) Explain how missing data were addressed "For time-varying factors, the last known value was carried forward in the case of missing data at a scheduled visit."
		(*d*) If applicable, explain how loss to follow-up was addressed "Follow-up duration was calculated as the difference in days from enrolment to the date of the CADE, death or last visit, whichever occurred first. Follow-up of patients experiencing major CADE was censored after the date of the first CADE." [Patients lost to follow-up were dealt as usual in the Cox model (right-censoring).]
		(*e*) Describe any sensitivity analyses "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. A third analysis was performed separately on the subgroups of patients co-infected with HCV and those not co-infected with HCV."
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed "A total of 1154 patients were included in the present study, accounting for 9401 person-visits."
		(b) Give reasons for non-participation at each stage "When comparing the study patients (n=1154) with those included in the cohort, but excluded from the study due to missing alcohol data (n=127), no significant difference was found for gender, age at enrolment, antiretroviral naivety, CDC clinical stage, baseline CD4+ cell counts and HIV viral load at M0 (data not shown)."
		(c) Consider use of a flow diagram [Not done because the graph is very simple.]
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders "The distribution of factors associated with major CADE [...] are reported in Table 2 for the entire study population (n=1154). Table 3 focuses instead on the subset of patients with available data on metabolic disorders (n=675). 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

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		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 ≤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. In the subsample of patients with available metabolic data (Table 3), the majority (73%) had a normal BMI, 17% were classified in the overweight category and a minority was classified in each of the two more extreme categories (6% underweight and 3% obese). About 8% of the patients had hypertriglyceridemia and 6% had hypercholesterolemia at M12. One year after the enrolment in the cohort, 6% of the patients reported a personal history of hypertension. Only 1% and 28% respectively had a personal and family history of CHD."
		(b) Indicate number of participants with missing data for each variable of interest [See Tables 2 and 3, second column.]
		(c) Summarise follow-up time (eg, average and total amount) "Median [interquartile range (IQR)] duration of follow-up of the selected patients, from their first visit after the ART prescription (M1) until M132, was 5.9 [1.8-9.4] years. During the 11 years of the study, accounting for 6544 person-years, the loss to follow-up rate [95% CI] was 17.6 [16.3 to 18.7] per 100 person-years."
Outcome data	15*	Report numbers of outcome events or summary measures over time "Over the 11-year follow-up of the cohort, a total of 85 severe cardiovascular events were observed. These included 49 major CADE as follows: myocardial infarction (n=30), coronary heart disease (n=4), stroke (n=7), peripheral arterial disease (n=7) and cardiovascular surgery for coronary disease (n=1). The distribution of the other thirty six severe cardiovascular events was as follows: phlebitis/pulmonary embolism (n=22), cardiomyopathy/congestive heart failure (n=5), cardiac dysrhythmia (n=6), cerebral haemorrhage (n=2) and aortic aneurysm (n=1). The incidence rate [95% CI] of all severe cardiovascular events was 1.3 [1.0 to 1.6] per 100 person-years, while for major CADE the incidence rate was 0.75 [0.57 to 0.99] per 100 person-years. Of the 49 major CADE, 40 occurred after M12. Furthermore, the major CADE incidence over the period M12-M132 was 0.89 [0.66 to 1.22] per 100 person-years."
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included

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		"[...] the results of univariate and multivariate Cox models, are reported in Table 2 for the entire study population (n=1154). Table 3 focuses instead on the subset of patients with available data on metabolic disorders (n=675). [...] In the entire study group (n=1154), the following factors were found to be independent predictors of major CADE: older age at baseline (AHR[95% CI]=1.07[1.04 to 1.10]), tobacco consumption>20 cigarettes/day (4.19[2.17 to 8.11]) and CD4+ cell count <200 cells/mm^3 (2.52[1.15 to 5.48]) (see Table 2). In addition, a negative association was found between female gender and major CADE (0.25[0.08 to 0.83]). After accounting for the effect of age, gender, tobacco consumption and CD4+ cell count <200 cells/mm^3 in the multivariate Cox model, individuals with moderate alcohol consumption (≤4(3) AU/day for men(women)) were at lower risk of major CADE (0.38[0.20 to 0.71]), than alcohol abstainers, while those drinking more than 4(3) AU/day for men(women) were not significantly different from abstainers (p=0.229). These results remained valid also when the analysis was restricted to the subgroup of patients with available metabolic data and the follow-up period M12-M132. After adjusting for age, tobacco consumption, hypertriglyceridemia and family history of CHD, we consistently found a negative association between moderate alcohol use (0.23[0.11 to 0.49]), and a positive association between CD4+ cell count <200 cells/mm^3 (4.02[1.45 to 11.1]) and major CADE after one year after enrolment (Table 3)."
		(*b*) Report category boundaries when continuous variables were categorized [See Tables 2 and 3 for details.]
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period "The incidence rate [95% CI] of all severe cardiovascular events was 1.3 [1.0 to 1.6] per 100 person-years, while for major CADE the incidence rate was 0.75 [0.57 to 0.99] per 100 person-years. Of the 49 major CADE, 40 occurred after M12. Furthermore, the major CADE incidence over the period M12-M132 was 0.89 [0.66 to 1.22] per 100 person-years."
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses "No significant association was found between time of exposure to different ARV drugs and major CADE. No significant interaction was found between smoking habits and hypertriglyceridemia or personal history of CHD. Furthermore, detectable viral load was not associated with major CADE in univariate or multivariate analyses. For the Cox models fitted in this study, proportional-hazards assumption remained valid, either globally and with respect to each covariate. The residual analysis did not alter the results of the multivariate model. 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. 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)."

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Discussion		
Key results	18	Summarise key results with reference to study objectives "This longitudinal study clearly confirms that in ARV-treated individuals, proximal CD4+ cell count lower than 200 cells/mm^3 remains a risk factor associated with major coronary and other arterial disease events. This result remains valid even after adjustment for metabolic disorders such as hypertriglyceridemia and family history of CHD, the strength of the association remaining unchanged."
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias "Some limitations of this study need to be acknowledged. First, our definition of CADE includes different types of events which might not share the same pattern of risk factors. However, an additional analysis performed on a restricted dataset and which adjusted for other potential risk factors (such as hypertriglyceridemia) confirmed the pattern found in the main analysis. Second, information on alcohol use was mainly based on self-reports and these may tend to underestimate alcohol consumption. However, it has been reported that HIV-HCV co-infected patients tend to underreport alcohol use more to their hepathologist than to other physicians[60]. As our patients were all followed-up by HIV physicians, it is likely that the degree of underreporting did not greatly affect hazard ratios' estimates. Moreover, the association found between the excessive alcohol consumption and the biomarkers of alcoholic liver injury indicate a good accuracy of self-reported data on alcohol consumption. Then, 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. 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."
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence "The relationship between CADE and CD4+ cell count, in addition to the lack of association between CADE and specific ARV classes, confirms previous results[7]

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where the association between exposure to certain ARV and CADE was no longer evident after controlling for traditional risk factors and HIV disease markers. Indeed, the association between specific ARV drugs and major CADE, like myocardial infarctus, has been shown in very powerful studies[5], where adjustment for nadir CD4+ lymphocyte count or peak HIV-1 RNA level did not modify the results. However, in the study cited, no adjustment for proximal CD4+ cell count was performed, and the association with the class of ARV became weaker after adjustment for serum lipid levels[5]. 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[41]. The lack of association with exposure to some specific ARV classes in the present study, factors usually associated with an increased risk of CADE[42, 43], suggests that reduced immunological response to ART over a long follow-up, as well as known CADE risk factors may have a more important impact than exposure to a specific antiretroviral drug.

It is also possible that patients with altered lipid profiles were switched to other classes of antiretroviral drugs during the follow-up. As a consequence, in the long term, CD4+ cell count <200 cells/mm^3 remain a correlate of an increased risk of CADE, whatever the antiretroviral received.

In our study, individuals reporting elevated alcohol consumption exhibited the same risk of CADE as alcohol abstainers. In contrast, those reporting moderate alcohol consumptions had a lower risk. This result was confirmed after adjustment for additional risk factors including tobacco use, age, gender and CD4+ cell count. This relationship between alcohol consumption and CADE remained significant and J-shaped, even after adjustment for data on metabolic risk factors- such as having a history of CHD and hypertriglyceridemia -which was available for a subset of patients. As highlighted in previous observations in the general population[28, 44-47] and certain populations affected by other diseases[48], these results confirmed that the increased risk of CADE in HIV-infected patients receiving ART is not attributable to elevated alcohol use. Furthermore, we highlighted the apparent protective effect of moderate alcohol consumption in ART treated HIV-infected patients.

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 non-HIV based mortality as a part of the total mortality of HIV-infected patients [49], cardiovascular diseases becoming increasingly important. This increase can be explained firstly by a reduction in the competing role of HIV-related mortality, which in the long term was much more detrimental to health than other causes of deaths. Secondly, the high prevalence in this population of other risk factors like tobacco use, together with ART-related morbidity including altered lipid profiles and triglycerides, may contribute to an increased risk of CADE.

The association found in our study between moderate alcohol use and reduced CADE risk is not consistent with all previous research in HIV-infected patients. Freiberg *et al*[26] pointed out that among HIV-infected men in the US, hazardous drinking and both alcohol abuse and dependence were associated with a higher prevalence of cardiovascular disease compared with infrequent and moderate drinking, even after adjusting for traditional risk factors, antiretroviral therapy and CD4+ cell count. This difference in the results may be due to the different design of the two studies (longitudinal for ours *vs* cross-sectional for Freiberg's), but probably also to the different types of alcohol mainly consumed in the two populations, red wine

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		consumption being more widespread in France than in the US. This difference in the pattern of alcohol use has already been highlighted in a comparative study on cardiovascular disease in non HIV-infected Irish and French males, showing an increased protective effect of drinking red wine in the latter population[50]. Increased wine consumption probably brings about an increase in high-density lipoprotein cholesterol levels, which helps protect against cardiovascular events. On the other hand, beer and spirits consumption are linked to increased triglyceride levels[51]. We do not know to what extent moderate alcohol users are representative of a population with better health status as alcohol use in France is relatively frequent in the general population and this fact may increase the strength of the association found. 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. However, this hypothesis was not demonstrated in a study conducted in older adults without cardiovascular disease, where alcohol intake was found to be associated with lower levels of inflammatory markers[53]. In the meantime it is important to remember that individuals with immune suppression are at a greater risk of cancer, a disease whose pattern of risk factors also include alcohol use, even when moderate[54]. Tobacco smoking has consistently been found to be a major risk factor for cardiovascular disease[55]. Smoking prevalence and dependence in HIV-infected patients is higher than in the general population[56]. Effective interventions for reducing and quitting smoking[57], especially for patients with several risk factors, are strongly recommended especially considering the increased cardiovascular risk which exists in HIV-infected smokers receiving ART[58]. However, results from studies reporting on the effectiveness of interventions for quitting smoking in HIV-infected individuals are inconsistent[59]. 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."
Generalisability	21	Discuss the generalisability (external validity) of the study results "This cohort is representative of the first generation of patients receiving potent ART. As cardiovascular disease is a rising issue in all HIV-infected populations receiving ART, these results can give important information about the pattern of risk and protective factors in all treated HIV-infected populations. In conclusion, in the long term, absence of immunodepression and moderate alcohol consumption remain associated with a lower risk of major CADE. 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."
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based "Funding sources: The ANRS CO8 (APROCO/COPILOTE) cohort is funded by ANRS (Agence Nationale de Recherches sur le Sida et les hépatites virales) and CMIT (Collège des Universitaires de Maladies Infectieuses et Tropicales), which

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	received research grants from Abbott, Boehringer-Ingelheim, Bristol-Myers Squibb, GlaxoSmithKline, Gilead Sciences, Pfizer and Roche. The funders were responsible for initiating, managing and financing the cohort, but did not interfere with data analysis and interpretation and dissemination of the scientific results."

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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27-Apr-2012

Dear Dr. Protopopescu:

Manuscript ID bmjopen-2012-001155 entitled "CD4+ cell count ≥200 cells/mm3 and Moderate Alcohol Consumption: Evidence of Protection against Coronary and other Arterial Disease Events in an 11-year Cohort of HIV-infected Patients on Antiretroviral Therapy" which you submitted to BMJ Open, has been reviewed. The comments of the reviewer(s) are included at the bottom of this letter.

Articles don't usually receive four reviews but this paper seemed popular with reviewers!

The reviewer(s) have recommended revisions to your manuscript. Therefore, I invite you to respond to the reviewer(s)' comments and revise your manuscript. Please remember that the reviewers' comments and the previous drafts of your manuscript will be published as supplementary information alongside the final version.

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Sincerely,
Mr. Richard Sands
Managing Editor, BMJ Open
rsands@bmjgroup.com

>From the managing editor:

Please reword the title to address the research question rather than the results.

We have changed the title as follows:

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

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.
he 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:

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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 ≤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 ≤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 HR [95% CI]	***p-value***	***Multivariate analysis AHR [95% CI]***	***p-value***
Socio-demographic and psychosocial characteristics				
*Alcohol consumption**				
- abstainers (ref)	*1*		*1*	
- ≤1 AU/day	*0.40 [0.21-0.78]*	*0.007*	*0.40 [0.21-0.78]*	*0.007*
- >1 and ≤ 4(3) AU/day for men(women)	*0.58 [0.25-1.35]*	*0.207*	*0.33 [0.14-0.79]*	*0.013*
- >4(3) AU/day for men(women)	*1.04 [0.30-3.58]*	*0.951*	*0.45 [0.13-1.62]*	*0.223*

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

	Univariate analyses HR [95% CI]	***p-value***	***Multivariate analysis AHR [95% CI]***	***p-value***
Socio-demographic and psychosocial characteristics				
*Alcohol consumption**				
- abstainers (ref)	*1*		*1*	
- ≤1 AU/day	*0.35 [0.17-0.71]*	*0.004*	*0.24 [0.11-0.55]*	*0.001*
- >1 and ≤ 4(3) AU/day for men(women)	*0.36 [0.13-1.01]*	*0.053*	*0.19 [0.06-0.62]*	*0.006*
- >4(3) AU/day for men(women)	*1.09 [0.31-3.81]*	*0.889*	*0.54 [0.14-2.11]*	*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 ≤4(3) AU/day for men(women); >4(3) AU/day for men(women)), in order to test for a gradient effect.

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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 ≤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/mm^3 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

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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 producted 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.

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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*	

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-≤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.

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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

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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.
Unversity Clinic of Infectious Diseases
Bern University Hospital and University of Bern
Switzerland

No competing interest.

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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

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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.

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

Immunodepression was defined by CD4+ cell count <200 cells/mm^3; 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 virables and not as continuous ones,. And if binary, why CD4 of 200 as strata limit.

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A cut-off of 200 cells/mm^3 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/mm^3 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 HV-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 cempeting interest in raltion to this paper

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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

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et al. CliniInfect 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/mm^3 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 ≤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.

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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

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2012-001155.R2
Article Type:	Research
Date Submitted by the Author:	07-Sep-2012
Complete List of Authors:	Carrieri, Maria Patrizia Protopopescu, Camelia; INSERM, U912 (SESSTIM), Aix Marseille Univ, IRD, UMR-S912, ORS PACA, Observatoire Régional de la Santé Provence Alpes Côte d'Azur Le Moing, Vincent Reboud, Philippe Raffi, François Mahy, Sophie Roux, Perrine Cuzin, Lise Spire, Bruno Leport, Catherine
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Cardiovascular medicine, HIV/AIDS, Nutrition and metabolism
Keywords:	HIV & AIDS < INFECTIOUS DISEASES, Myocardial infarction < CARDIOLOGY, Coronary heart disease < CARDIOLOGY

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STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract "Cohort study."
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found **"**Objective: To investigate the relationship between response to antiretroviral treatment (ART), alcohol use and occurrence of a major coronary or other arterial disease event (CADE) in HIV-infected individuals. Design: Cohort study. A Cox model was used to identify correlates of a first occurrence of a major CADE. Setting: The French ANRS CO8 APROCO-COPILOTE cohort was set up in 1997 to study clinical progression and patient-reported outcomes (PRO) after initiating protease inhibitor-containing ART. Clinical data were retrieved from medical records. Self-administered questionnaires collected data on PRO and behaviours, including alcohol use. Participants: Metabolic data were only available for a subgroup (n=675) of the study group (n=1154). Main outcomes measures: Major coronary or other arterial disease first event. Results: Over the 11-year follow-up, 49 major CADE were observed, with an incidence rate [95%CI]=0.75[0.57 to 0.99] per 100 person-years. Immunodepression (CD4+ cell count <200 cells/mm^3) was associated with an increased risk of CADE (Adjusted Hazard Ratio AHR[95% CI]= 2.52[1.15 to 5.48]) after adjustment for female gender (0.25[0.08 to 0.83]), age (1.07[1.04 to 1.10]) and smoking>20 cigarettes/day (4.19[2.17 to 8.11]). Moreover, individuals with moderate alcohol consumption (≤4(3) AU/day for men(women)) had a lower risk of CADE (0.38[0.20 to 0.71]) than alcohol abstainers, while the risk for those drinking>4(3) AU/day for men(women) was not significantly different from this latter group. These associations remained valid after adjustment for metabolic disorders. No significant association with exposure to any specific antiretroviral was detected. Conclusions: In the long term, absence of immunodepression and moderate alcohol consumption remain associated with a lower risk of major CADE. Combined interventions to reduce CADE risk-related behaviours including adherence counselling for assuring long term immunological response to ART in HIV-infected individuals are now a clinical and public health priority."
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported "Cardiovascular disease risk factors (including for example hypertension and tobacco use), as well as immunological status[1] are major predictors of atherosclerosis in HIV-infected women and men. 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, more recently Butt and colleagues put into evidence the negative effect of HIV viral replication on heart failure[6]. In one study, patients having experienced a MI presented lower baseline and proximal CD4+ cell counts than those who did not, while no association was found between cardiovascular disease events and specific antiretroviral agents[7]. Moreover, known

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		factors associated with cardiovascular risk[8] such as hyperlipidemia and insulin resistance[9-11] are becoming an increasingly major clinical concern in ARV-treated individuals. Though excessive alcohol use may be frequent in HIV-infected individuals, its impact on the occurrence of cardiovascular disease in this population has been less investigated. Excessive alcohol consumption also plays a major role in HIV risk-taking behaviours, particularly in men who have sex with men and drug users[12, 13]. In HIV-infected individuals, excessive alcohol use is known to compromise patients' response to antiretroviral therapy (ART) through reduced adherence[14-17] and to predict HIV progression and increased mortality[18-21], mostly from liver diseases[22-25]. Some recent cross-sectional analyses in HIV-infected and uninfected men have found that heavy alcohol consumption is associated with a higher risk of cardiovascular disease[26, 27]. On the other hand, in uninfected adults, moderate alcohol consumption has been found to be associated with a reduced cardiovascular mortality[28], improved lipid profiles, increased insulin sensitivity and reduced risk of MI[29]. However, this association has not yet been studied in longitudinal studies involving HIV-infected populations including women. Moreover, there are as yet no consistent results in the literature regarding the role that alcohol use plays in exacerbating cardiovascular risk in ARV-treated patients and to what extent it can confound or boost the effect of immuno-virological response to ART, ART-associated dyslipidemia and insulin resistance on the risk of cardiovascular disease events."
Objectives	3	State specific objectives, including any prespecified hypotheses "We used data from the 11-year follow-up of the APROCO-COPILOTE cohort of patients receiving ART since 1997 to investigate the relationship between viral load, CD4+ cell count, alcohol consumption and the first occurrence of a major coronary or other arterial disease event (CADE), after adjustment for known risk factors including metabolic disorders."
Methods		
Study design	4	Present key elements of study design early in the paper "The French APROCO-COPILOTE cohort (ANRS CO8) was designed to study the clinical, immunological, virological, and socio-behavioural course of HIV disease in HIV-1 positive individuals who started the first generation of potent ART (treatment regimens including protease inhibitors (PI)). The study was approved by the Ethics Committee of Cochin Hospital (Paris) and informed consent was obtained from all participants."
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection "Patients were enrolled in the cohort at their first PI-based ART prescription between May 1997 and June 1999 (n=1281) in 47 French centres, and clinically followed-up every 4 months thereafter, up to month 132 (M132)."
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up "In the present study were included all patients who had either two alcohol assessment or one alcohol assessment just preceding the CADE over the whole follow-up (n=1154)."

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		(*b*) For matched studies, give matching criteria and number of exposed and unexposed [Not applicable.]
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable *"Medical questionnaire* The medical questionnaire at enrolment (M0) collected retrospective data about patient's HIV history including HIV transmission category, time since HIV diagnosis, previous exposure to antiretroviral treatment before enrolment, and co-infection with hepatitis C virus (HCV) defined as having positive HCV RNA and/or positive HCV antibodies. This information was complemented by medical questionnaires, completed by the HIV physicians at each follow-up visit, which collected clinical and laboratory data (plasma HIV RNA level, CD4+ cell count, HIV clinical stage, aspartate transaminase (AST) and alanine transaminase (ALT) levels), as well as data on the antiretroviral regimen prescribed. A third form, based on updated medical records, detailed all clinical severe events, including cardiovascular events, occurring during the study period. Information on metabolic disorders was available at M12 for a subset of cohorts' patients, who participated in a parallel cross-sectional survey, designed to study clinical and laboratory metabolic complications[30]. These "metabolic" data included height, weight, hypertriglyceridemia, hypercholesterolemia, personal history of coronary heart disease (CHD) and hypertension, and finally any family history of CHD. *Outcome* The details of all clinical severe 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 clinical severe events was based on the 10th Revision of the International Statistical Classification of Diseases and Related Health Problems (ICD-10)[1]. 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. *Self-administered questionnaire* A self-administered questionnaire collected - at M0, M4, M12 and every 8 months thereafter during the first 5 years of follow-up, then yearly thereafter – data on socio-demographic characteristics including age, gender and educational level. Among other psycho-social and behavioural information, it also collected details on depressive symptoms, tobacco use, alcohol consumption, self-reported ART-related symptoms and adherence to ART."
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is

[1] http://www.who.int/classifications/icd/en/

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		more than one group "At each follow-up visit, CD4+ cell count was measured by standardized flow cytometry, and plasma HIV RNA levels were measured using the assay routinely available in each participating centre. Immunodepression was defined by CD4+ cell count <200 cells/mm^3; viral load was classified as detectable if it was greater than the threshold value specific to each centre where the assay was performed. HIV clinical stage was defined using the 1993 Centres for Disease Control and Prevention (CDC) clinical classification system[31]. Serum triglyceride and cholesterol levels were measured after a 12-hour overnight fast. Hypertriglyceridemia was defined as a triglyceride level ≥2.2 mmol/l, while hypercholesterolemia was defined as a total cholesterol level ≥5.5 mmol/l. Depression was measured using the validated French version of the Centre for Epidemiologic Studies Depression Scale (CES-D), which is a 20-item scale commonly used in studies involving HIV-infected patients[32]. Tobacco and alcohol consumption were evaluated during the previous 4 weeks. Alcohol consumption was assessed using two questions about frequency of consumption and quantity consumed daily, if applicable. A 13-item scale comprising the French version of the symptom index validated by Justice *et al*[33] and described elsewhere[34] collected information about self-reported symptoms. Five other questions gathered information about adherence to ART, according to the methodology established by the AIDS Clinical Trial Group[35]."
Bias	9	Describe any efforts to address potential sources of bias "As the results of the analysis on the whole dataset (n=1154) could be affected by the lack of important predictors of CADE like hypertriglyceridemia or hypercholesterolemia, we conducted a secondary analysis on a subset of patients who had additional metabolic disorders data." [This secondary analysis confirmed the same pattern of predictors of CADE events after adjustment for metabolic disorders.]
Study size	10	Explain how the study size was arrived at "More specifically, two analyses on the following study populations were performed to study predictors of major CADE: 1) First analysis: the study population included all patients who had either two alcohol assessment or one alcohol assessment just preceding the event over the whole follow-up (n=1154). The follow-up period was M0-M132. 2) Second analysis: the study population was restricted to the subset of patients with available data on metabolic disorders, measured at M12 (n=675). Consequently, only events occurring after M12 were considered in this second analysis."
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why "The WHO body mass index (BMI) categories were used in the analysis: underweight and overweight/obese patients were defined respectively as having a BMI lower than 18.5 and greater than 25. A time-varying cumulative measure of time exposure to specific ARV medications (abacavir, efavirenz, nevirapine, lopinavir and protease inhibitors) was computed during which ART included a specific drug from this list, from cohort enrolment to the date of each follow-up visit.

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		Tobacco consumption was dichotomized in two categories: more than 20 cigarettes/day *vs* other[36]. 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 ≤4(3) AU/day for men(women); >4(3) AU/day for men(women)), in order to test a gradient effect. 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. Patients were classified as non-adherent if they reported taking less than 100% of prescribed medications during the previous 4 weeks, using a validated algorithm[38]. A patient with a CES-D score value above 17 (men) or 23 (women) was considered as presenting depressive symptoms[39]. " [See references in the paper for the reasons of choosing of these categories].
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding "Incidence rate of major CADE was computed as the number of cases divided by the number of person-years of follow-up. Follow-up duration was calculated as the difference in days from enrolment to the date of the CADE, death or last visit, whichever occurred first. Follow-up of patients experiencing major CADE was censored after the date of the first CADE. We used a Cox proportional hazards model to identify characteristics associated with the first occurrence of a major CADE. Psychosocial characteristics - depressive symptoms, tobacco and alcohol consumption, self-reported symptoms, CD4+ cell count and viral load - were evaluated at each visit and used as time-varying covariates in the statistical analysis. All the other covariates were used as fixed variables (measured at M0, M1 or M12). For time-varying factors, the last known value was carried forward in the case of missing data at a scheduled visit. In the case of an event occurring between two consecutive follow-up visits, the values for the time-varying variables measured at the visit preceding the event were used. Covariates with a p-value<0.25 in univariate Cox analyses were considered eligible for the multivariate Cox model, which was built using a backward stepwise selection procedure based on the Wald test (p<0.05). Interaction effects between the factors of the multivariate final model were tested. The proportional-hazards assumption was verified globally for the multivariate models and separately with respect to each covariate, using both Kaplan-Meier estimates and tests based on Schoenfeld residuals[40]. A residual analysis for outliers' detection was performed and the sensitivity of the model to influential outliers was tested. 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. A third analysis was performed separately on the subgroups of patients co-infected with HCV and those not co-infected with HCV.

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		All analyses were performed using Stata Intercooled software, version 12.1."
		(*b*) Describe any methods used to examine subgroups and interactions "Interaction effects between the factors of the multivariate final Cox model were tested."
		(*c*) Explain how missing data were addressed "For time-varying factors, the last known value was carried forward in the case of missing data at a scheduled visit."
		(*d*) If applicable, explain how loss to follow-up was addressed "Follow-up duration was calculated as the difference in days from enrolment to the date of the CADE, death or last visit, whichever occurred first. Follow-up of patients experiencing major CADE was censored after the date of the first CADE." [Patients lost to follow-up were dealt as usual in the Cox model (right-censoring).]
		(*e*) Describe any sensitivity analyses "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. A third analysis was performed separately on the subgroups of patients co-infected with HCV and those not co-infected with HCV."
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed "A total of 1154 patients were included in the present study, accounting for 9401 person-visits."
		(b) Give reasons for non-participation at each stage "When comparing the study patients (n=1154) with those included in the cohort, but excluded from the study due to missing alcohol data (n=127), no significant difference was found for gender, age at enrolment, antiretroviral naivety, CDC clinical stage, baseline CD4+ cell counts and HIV viral load at M0 (data not shown)."
		(c) Consider use of a flow diagram [Not done because the graph is very simple.]
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders "The distribution of factors associated with major CADE [...] are reported in Table 2 for the entire study population (n=1154). Table 3 focuses instead on the subset of patients with available data on metabolic disorders (n=675). 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

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		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 ≤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. In the subsample of patients with available metabolic data (Table 3), the majority (73%) had a normal BMI, 17% were classified in the overweight category and a minority was classified in each of the two more extreme categories (6% underweight and 3% obese). About 8% of the patients had hypertriglyceridemia and 6% had hypercholesterolemia at M12. One year after the enrolment in the cohort, 6% of the patients reported a personal history of hypertension. Only 1% and 28% respectively had a personal and family history of CHD."
		(b) Indicate number of participants with missing data for each variable of interest [See Tables 2 and 3, second column.]
		(c) Summarise follow-up time (eg, average and total amount) "Median [interquartile range (IQR)] duration of follow-up of the selected patients, from their first visit after the ART prescription (M1) until M132, was 5.9 [1.8-9.4] years. During the 11 years of the study, accounting for 6544 person-years, the loss to follow-up rate [95% CI] was 17.6 [16.3 to 18.7] per 100 person-years."
Outcome data	15*	Report numbers of outcome events or summary measures over time "Over the 11-year follow-up of the cohort, a total of 85 severe cardiovascular events were observed. These included 49 major CADE as follows: myocardial infarction (n=30), coronary heart disease (n=4), stroke (n=7), peripheral arterial disease (n=7) and cardiovascular surgery for coronary disease (n=1). The distribution of the other thirty six severe cardiovascular events was as follows: phlebitis/pulmonary embolism (n=22), cardiomyopathy/congestive heart failure (n=5), cardiac dysrhythmia (n=6), cerebral haemorrhage (n=2) and aortic aneurysm (n=1). The incidence rate [95% CI] of all severe cardiovascular events was 1.3 [1.0 to 1.6] per 100 person-years, while for major CADE the incidence rate was 0.75 [0.57 to 0.99] per 100 person-years. Of the 49 major CADE, 40 occurred after M12. Furthermore, the major CADE incidence over the period M12-M132 was 0.89 [0.66 to 1.22] per 100 person-years."
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included

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		"[...] the results of univariate and multivariate Cox models, are reported in Table 2 for the entire study population (n=1154). Table 3 focuses instead on the subset of patients with available data on metabolic disorders (n=675). [...] In the entire study group (n=1154), the following factors were found to be independent predictors of major CADE: older age at baseline (AHR[95% CI]=1.07[1.04 to 1.10]), tobacco consumption>20 cigarettes/day (4.19[2.17 to 8.11]) and CD4+ cell count <200 cells/mm^3 (2.52[1.15 to 5.48]) (see Table 2). In addition, a negative association was found between female gender and major CADE (0.25[0.08 to 0.83]). After accounting for the effect of age, gender, tobacco consumption and CD4+ cell count <200 cells/mm^3 in the multivariate Cox model, individuals with moderate alcohol consumption (≤4(3) AU/day for men(women)) were at lower risk of major CADE (0.38[0.20 to 0.71]), than alcohol abstainers, while those drinking more than 4(3) AU/day for men(women) were not significantly different from abstainers (p=0.229). These results remained valid also when the analysis was restricted to the subgroup of patients with available metabolic data and the follow-up period M12-M132. After adjusting for age, tobacco consumption, hypertriglyceridemia and family history of CHD, we consistently found a negative association between moderate alcohol use (0.23[0.11 to 0.49]), and a positive association between CD4+ cell count <200 cells/mm^3 (4.02[1.45 to 11.1]) and major CADE after one year after enrolment (Table 3)."
		(*b*) Report category boundaries when continuous variables were categorized [See Tables 2 and 3 for details.]
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period "The incidence rate [95% CI] of all severe cardiovascular events was 1.3 [1.0 to 1.6] per 100 person-years, while for major CADE the incidence rate was 0.75 [0.57 to 0.99] per 100 person-years. Of the 49 major CADE, 40 occurred after M12. Furthermore, the major CADE incidence over the period M12-M132 was 0.89 [0.66 to 1.22] per 100 person-years."
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses "No significant association was found between time of exposure to different ARV drugs and major CADE. No significant interaction was found between smoking habits and hypertriglyceridemia or personal history of CHD. Furthermore, detectable viral load was not associated with major CADE in univariate or multivariate analyses. For the Cox models fitted in this study, proportional-hazards assumption remained valid, either globally and with respect to each covariate. The residual analysis did not alter the results of the multivariate model. 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. 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)."

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Discussion		
Key results	18	Summarise key results with reference to study objectives "This longitudinal study clearly confirms that in ARV-treated individuals, proximal CD4+ cell count lower than 200 cells/mm^3 remains a risk factor associated with major coronary and other arterial disease events. This result remains valid even after adjustment for metabolic disorders such as hypertriglyceridemia and family history of CHD, the strength of the association remaining unchanged."
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias "Some limitations of this study need to be acknowledged. First, our definition of CADE includes different types of events which might not share the same pattern of risk factors. However, an additional analysis performed on a restricted dataset and which adjusted for other potential risk factors (such as hypertriglyceridemia) confirmed the pattern found in the main analysis. Second, information on alcohol use was mainly based on self-reports and these may tend to underestimate alcohol consumption. However, it has been reported that HIV-HCV co-infected patients tend to underreport alcohol use more to their hepathologist than to other physicians[60]. As our patients were all followed-up by HIV physicians, it is likely that the degree of underreporting did not greatly affect hazard ratios' estimates. Moreover, the association found between the excessive alcohol consumption and the biomarkers of alcoholic liver injury indicate a good accuracy of self-reported data on alcohol consumption. Then, 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. 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."
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence "The relationship between CADE and CD4+ cell count, in addition to the lack of association between CADE and specific ARV classes, confirms previous results[7]

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where the association between exposure to certain ARV and CADE was no longer evident after controlling for traditional risk factors and HIV disease markers. Indeed, the association between specific ARV drugs and major CADE, like myocardial infarctus, has been shown in very powerful studies[5], where adjustment for nadir CD4+ lymphocyte count or peak HIV-1 RNA level did not modify the results. However, in the study cited, no adjustment for proximal CD4+ cell count was performed, and the association with the class of ARV became weaker after adjustment for serum lipid levels[5]. 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[41]. The lack of association with exposure to some specific ARV classes in the present study, factors usually associated with an increased risk of CADE[42, 43], suggests that reduced immunological response to ART over a long follow-up, as well as known CADE risk factors may have a more important impact than exposure to a specific antiretroviral drug.

It is also possible that patients with altered lipid profiles were switched to other classes of antiretroviral drugs during the follow-up. As a consequence, in the long term, CD4+ cell count <200 cells/mm^3 remain a correlate of an increased risk of CADE, whatever the antiretroviral received.

In our study, individuals reporting elevated alcohol consumption exhibited the same risk of CADE as alcohol abstainers. In contrast, those reporting moderate alcohol consumptions had a lower risk. This result was confirmed after adjustment for additional risk factors including tobacco use, age, gender and CD4+ cell count. This relationship between alcohol consumption and CADE remained significant and J-shaped, even after adjustment for data on metabolic risk factors- such as having a history of CHD and hypertriglyceridemia -which was available for a subset of patients. As highlighted in previous observations in the general population[28, 44-47] and certain populations affected by other diseases[48], these results confirmed that the increased risk of CADE in HIV-infected patients receiving ART is not attributable to elevated alcohol use. Furthermore, we highlighted the apparent protective effect of moderate alcohol consumption in ART treated HIV-infected patients.

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 non-HIV based mortality as a part of the total mortality of HIV-infected patients [49], cardiovascular diseases becoming increasingly important. This increase can be explained firstly by a reduction in the competing role of HIV-related mortality, which in the long term was much more detrimental to health than other causes of deaths. Secondly, the high prevalence in this population of other risk factors like tobacco use, together with ART-related morbidity including altered lipid profiles and triglycerides, may contribute to an increased risk of CADE.

The association found in our study between moderate alcohol use and reduced CADE risk is not consistent with all previous research in HIV-infected patients. Freiberg *et al*[26] pointed out that among HIV-infected men in the US, hazardous drinking and both alcohol abuse and dependence were associated with a higher prevalence of cardiovascular disease compared with infrequent and moderate drinking, even after adjusting for traditional risk factors, antiretroviral therapy and CD4+ cell count. This difference in the results may be due to the different design of the two studies (longitudinal for ours *vs* cross-sectional for Freiberg's), but probably also to the different types of alcohol mainly consumed in the two populations, red wine

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		consumption being more widespread in France than in the US. This difference in the pattern of alcohol use has already been highlighted in a comparative study on cardiovascular disease in non HIV-infected Irish and French males, showing an increased protective effect of drinking red wine in the latter population[50]. Increased wine consumption probably brings about an increase in high-density lipoprotein cholesterol levels, which helps protect against cardiovascular events. On the other hand, beer and spirits consumption are linked to increased triglyceride levels[51]. We do not know to what extent moderate alcohol users are representative of a population with better health status as alcohol use in France is relatively frequent in the general population and this fact may increase the strength of the association found. 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. However, this hypothesis was not demonstrated in a study conducted in older adults without cardiovascular disease, where alcohol intake was found to be associated with lower levels of inflammatory markers[53]. In the meantime it is important to remember that individuals with immune suppression are at a greater risk of cancer, a disease whose pattern of risk factors also include alcohol use, even when moderate[54]. Tobacco smoking has consistently been found to be a major risk factor for cardiovascular disease[55]. Smoking prevalence and dependence in HIV-infected patients is higher than in the general population[56]. Effective interventions for reducing and quitting smoking[57], especially for patients with several risk factors, are strongly recommended especially considering the increased cardiovascular risk which exists in HIV-infected smokers receiving ART[58]. However, results from studies reporting on the effectiveness of interventions for quitting smoking in HIV-infected individuals are inconsistent[59]. 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."
Generalisability	21	Discuss the generalisability (external validity) of the study results "This cohort is representative of the first generation of patients receiving potent ART. As cardiovascular disease is a rising issue in all HIV-infected populations receiving ART, these results can give important information about the pattern of risk and protective factors in all treated HIV-infected populations. In conclusion, in the long term, absence of immunodepression and moderate alcohol consumption remain associated with a lower risk of major CADE. 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."
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based "Funding sources: The ANRS CO8 (APROCO/COPILOTE) cohort is funded by ANRS (Agence Nationale de Recherches sur le Sida et les hépatites virales) and CMIT (Collège des Universitaires de Maladies Infectieuses et Tropicales), which

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	received research grants from Abbott, Boehringer-Ingelheim, Bristol-Myers Squibb, GlaxoSmithKline, Gilead Sciences, Pfizer and Roche. The funders were responsible for initiating, managing and financing the cohort, but did not interfere with data analysis and interpretation and dissemination of the scientific results."

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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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

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Abstract word count: 285 words

Text word count: 4548 words

Funding sources: The ANRS CO8 (APROCO/COPILOTE) cohort is funded by ANRS (Agence Nationale de Recherches sur le Sida et les hépatites virales) and CMIT (Collège des Universitaires de Maladies Infectieuses et Tropicales), which received research grants from Abbott, Boehringer-Ingelheim, Bristol-Myers Squibb, GlaxoSmithKline, Gilead Sciences, Pfizer and Roche.

The funders were responsible for initiating, managing and financing the cohort, but did not interfere with data analysis and interpretation and dissemination of the scientific results.

Competing interests: All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf <http://www.icmje.org/coi_disclosure.pdf> (available on request from the corresponding author) and declare that (1) they have no support from any company for the submitted work; (2) they have no relationships with any company that might have an interest in the submitted work in the previous 3 years; (3) their spouses, partners, or children have no financial relationships that may be relevant to the submitted work; and (4) they have no non-financial interests that may be relevant to the submitted work.

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Data sharing: There is no additional data available.

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Summary

Article Focus

- Major coronary or other arterial disease events (CADE) are frequent in HIV-infected individuals receiving antiretroviral therapy.
- While some antiretroviral classes can increase the risk of CADE, it is still controversial to what extent immune reconstitution and other factors like moderate alcohol consumption can reduce the risk of CADE in this population.

Key Messages

- Moderate alcohol consumption and absence of immunodepression can be beneficial to reduce the risk of CADE in HIV-infected patients.
- Reduced immunological response to antiretroviral treatment (ART) over a long follow-up may have a more important impact on CADE than exposure to a specific antiretroviral drug.

Strengths and Limitations

- This paper suggests the importance of combined risk reduction interventions including adherence counselling for assuring long term immunological response and decrease the risk of CADE.

Short title

CD4+, Moderate Alcohol Consumption and Coronary Events in HIV-infected patients

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Abstract

Objective: To investigate the relationship between response to antiretroviral treatment (ART), alcohol use and occurrence of a major coronary or other arterial disease event (CADE) in HIV-infected individuals.

Design: Cohort study. A Cox model was used to identify correlates of a first occurrence of a major CADE.

Setting: The French ANRS CO8 APROCO-COPILOTE cohort was set up in 1997 to study clinical progression and patient-reported outcomes (PRO) after initiating protease inhibitor-containing ART. Clinical data were retrieved from medical records. Self-administered questionnaires collected data on PRO and behaviours, including alcohol use.

Participants: Metabolic data were only available for a subgroup (n=675) of the study group (n=1154).

Main outcomes measures: Major coronary or other arterial disease first event.

Results: Over the 11-year follow-up, 49 major CADE were observed, with an incidence rate [95%CI]=0.75[0.57 to 0.99] per 100 person-years. Immunodepression (CD4+ cell count <200 cells/mm^3) was associated with an increased risk of CADE (Adjusted Hazard Ratio AHR[95% CI]= 2.52[1.15 to 5.48]) after adjustment for female gender (0.25[0.08 to 0.83]), age (1.07[1.04 to 1.10]) and smoking>20 cigarettes/day (4.19[2.17 to 8.11]). Moreover, individuals with moderate alcohol consumption (≤4(3) AU/day for men(women)) had a lower risk of CADE (0.38[0.20 to 0.71]) than alcohol abstainers, while the risk for those drinking>4(3) AU/day for men(women) was not significantly different from this latter group. These associations remained valid after adjustment for metabolic disorders. No significant association with exposure to any specific antiretroviral was detected.

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Conclusions: In the long term, absence of immunodepression and moderate alcohol consumption remain associated with a lower risk of major CADE. Combined interventions to reduce CADE risk-related behaviours including adherence counselling for assuring long term immunological response to ART in HIV-infected individuals are now a clinical and public health priority.

Key words: Coronary and other arterial disease events, Alcohol, CD4+ cell count, HIV, ARV.

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Introduction

Cardiovascular disease risk factors (including for example hypertension and tobacco use), as well as immunological status[1] are major predictors of atherosclerosis in HIV-infected women and men. 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, more recently Butt and colleagues put into evidence the negative effect of HIV viral replication on heart failure[6]. In one study, patients having experienced a MI presented lower baseline and proximal CD4+ cell counts than those who did not, while no association was found between cardiovascular disease events and specific antiretroviral agents[7]. Moreover, known factors associated with cardiovascular risk[8] such as hyperlipidemia and insulin resistance[9-11] are becoming an increasingly major clinical concern in ARV-treated individuals.

Though excessive alcohol use may be frequent in HIV-infected individuals, its impact on the occurrence of cardiovascular disease in this population has been less investigated. Excessive alcohol consumption also plays a major role in HIV risk-taking behaviours, particularly in men who have sex with men and drug users[12, 13]. In HIV-infected individuals, excessive alcohol use is known to compromise patients' response to antiretroviral therapy (ART) through reduced adherence[14-17] and to predict HIV progression and increased mortality[18-21], mostly from liver diseases[22-25]. Some recent cross-sectional analyses in HIV-infected and uninfected men have found that heavy alcohol consumption is associated with a higher risk of cardiovascular disease[26, 27]. On the other hand, in uninfected adults, moderate alcohol consumption has been found to be associated with a reduced cardiovascular mortality[28], improved lipid profiles, increased insulin sensitivity and reduced risk of MI[29]. However, this association has not yet been studied in longitudinal studies involving HIV-infected populations including women. Moreover, there are as yet no consistent results

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in the literature regarding the role that alcohol use plays in exacerbating cardiovascular risk in ARV-treated patients and to what extent it can confound or boost the effect of immuno-virological response to ART, ART-associated dyslipidemia and insulin resistance on the risk of cardiovascular disease events.

We used data from the 11-year follow-up of the APROCO-COPILOTE cohort of patients receiving ART since 1997 to investigate the relationship between viral load, CD4+ cell count, alcohol consumption and the first occurrence of a major coronary or other arterial disease event (CADE), after adjustment for known risk factors including metabolic disorders.

Methods

The French APROCO-COPILOTE cohort (ANRS CO8) was designed to study the clinical, immunological, virological, and socio-behavioural course of HIV disease in HIV-1 positive individuals who started the first generation of potent ART (treatment regimens including protease inhibitors (PI)). The study was approved by the Ethics Committee of Cochin Hospital (Paris) and informed consent was obtained from all participants.

Setting

Patients were enrolled in the cohort at their first PI-based ART prescription between May 1997 and June 1999 (n=1281) in 47 French centres, and clinically followed-up every 4 months thereafter, up to month 132 (M132).

Patients

In the present study were included all patients who had either two alcohol assessment or one alcohol assessment just preceding the CADE over the whole follow-up (n=1154).

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Medical questionnaire

The medical questionnaire at enrolment (M0) collected retrospective data about patient's HIV history including HIV transmission category, time since HIV diagnosis, previous exposure to antiretroviral treatment before enrolment, and co-infection with hepatitis C virus (HCV) defined as having positive HCV RNA and/or positive HCV antibodies. This information was complemented by medical questionnaires, completed by the HIV physicians at each follow-up visit, which collected clinical and laboratory data (plasma HIV RNA level, CD4+ cell count, HIV clinical stage, aspartate transaminase (AST) and alanine transaminase (ALT) levels), as well as data on the antiretroviral regimen prescribed. A third form, based on updated medical records, detailed all clinical severe events, including cardiovascular events, occurring during the study period. Information on metabolic disorders was available at M12 for a subset of cohorts' patients, who participated in a parallel cross-sectional survey, designed to study clinical and laboratory metabolic complications[30]. These "metabolic" data included height, weight, hypertriglyceridemia, hypercholesterolemia, personal history of coronary heart disease (CHD) and hypertension, and finally any family history of CHD.

At each follow-up visit, CD4+ cell count was measured by standardized flow cytometry, and plasma HIV RNA levels were measured using the assay routinely available in each participating centre. Immunodepression was defined by CD4+ cell count <200 cells/mm^3; 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. HIV clinical stage was defined using the 1993 Centres for Disease Control and Prevention (CDC) clinical classification system[31].

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Serum triglyceride and cholesterol levels were measured after a 12-hour overnight fast. Hypertriglyceridemia was defined as a triglyceride level ≥2.2 mmol/l, while hypercholesterolemia was defined as a total cholesterol level ≥5.5 mmol/l.

Outcome

The details of all clinical severe 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 clinical severe 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.

Self-administered questionnaire

A self-administered questionnaire collected - at M0, M4, M12 and every 8 months thereafter during the first 5 years of follow-up, then yearly thereafter – data on socio-demographic characteristics including age, gender and educational level. Among other psycho-social and behavioural information, it also collected details on depressive symptoms, tobacco use, alcohol consumption, self-reported ART-related symptoms and adherence to ART.

[i] http://www.who.int/classifications/icd/en/

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Depression was measured using the validated French version of the Centre for Epidemiologic Studies Depression Scale (CES-D), which is a 20-item scale commonly used in studies involving HIV-infected patients[32].

Tobacco and alcohol consumption were evaluated during the previous 4 weeks. Alcohol consumption was assessed using two questions about frequency of consumption and quantity consumed daily, if applicable.

A 13-item scale comprising the French version of the symptom index validated by Justice *et al*[33] and described elsewhere[34] collected information about self-reported symptoms. Five other questions gathered information about adherence to ART, according to the methodology established by the AIDS Clinical Trial Group[35].

Statistical analysis

As the results of the analysis on the whole dataset (n=1154) could be affected by the lack of important predictors of CADE like hypertriglyceridemia or hypercholesterolemia, we conducted a secondary analysis on a subset of patients who had additional metabolic disorders data. More specifically, two analyses on the following study populations were performed to study predictors of major CADE:

1) First analysis: the study population included all patients who had either two alcohol assessment or one alcohol assessment just preceding the event over the whole follow-up (n=1154). The follow-up period was M0-M132.

2) Second analysis: the study population was restricted to the subset of patients with available data on metabolic disorders, measured at M12 (n=675). Consequently, only events occurring after M12 were considered in this second analysis.

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The WHO body mass index (BMI) categories were used in the analysis: underweight and overweight/obese patients were defined respectively as having a BMI lower than 18.5 and greater than 25.

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

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[36].

Tobacco consumption was dichotomized in two categories: more than 20 cigarettes/day *vs* other[37]. The average number of alcohol units (AU) consumed per day[38] was computed using the two questions on alcohol consumption and then recoded in four categories (abstainers; ≤1 AU/day; >1 and ≤4(3) AU/day for men(women); >4(3) AU/day for men(women)), in order to test a gradient effect. 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

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HCV status, see Table 1), which would suggest good accuracy of the self-reported data on alcohol consumption.

Patients were classified as non-adherent if they reported taking less than 100% of prescribed medications during the previous 4 weeks, using a validated algorithm[39]. A patient with a CES-D score value above 17 (men) or 23 (women) was considered as presenting depressive symptoms[40].

Incidence rate of major CADE was computed as the number of cases divided by the number of person-years of follow-up. Follow-up duration was calculated as the difference in days from enrolment to the date of the CADE, death or last visit, whichever occurred first. Follow-up of patients experiencing major CADE was censored after the date of the first CADE. We used a Cox proportional hazards model to identify characteristics associated with the first occurrence of a major CADE. Psychosocial characteristics - depressive symptoms, tobacco and alcohol consumption, self-reported symptoms, CD4+ cell count and viral load - were evaluated at each visit and used as time-varying covariates in the statistical analysis. All the other covariates were used as fixed variables (measured at M0, M1 or M12). For time-varying factors, the last known value was carried forward in the case of missing data at a scheduled visit. In the case of an event occurring between two consecutive follow-up visits, the values for the time-varying variables measured at the visit preceding the event were used. 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[36], and finally a selection procedure based on the Schwartz Bayesian information criterion BIC[41]. All three strategies selected the same final multivariate model. Interaction effects between the factors of the multivariate final model were tested. The proportional-hazards assumption was verified

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globally for the multivariate models and separately with respect to each covariate, using both Kaplan-Meier estimates and tests based on Schoenfeld residuals[42]. A residual analysis for outliers' detection was performed and the sensitivity of the model to influential outliers was tested.

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. A third analysis was performed separately on the subgroups of patients co-infected with HCV and those not co-infected with HCV.

All analyses were performed using Stata Intercooled software, version 12.1.

Results

A total of 1154 patients were included in the present study, accounting for 9401 person-visits. When comparing the study patients (n=1154) with those included in the cohort, but excluded from the study due to missing alcohol data (n=127), no significant difference was found for gender, age at enrolment, antiretroviral naivety, CDC clinical stage, baseline CD4+ cell counts and HIV viral load at M0 (data not shown).

Median [interquartile range (IQR)] duration of follow-up of the selected patients, from their first visit after the ART prescription (M1) until M132, was 5.9 [1.8-9.4] years. 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.

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Over the 11-year follow-up of the cohort, a total of 85 severe cardiovascular events were observed. These included 49 major CADE as follows: myocardial infarction (n=30), coronary heart disease (n=4), stroke (n=7), peripheral arterial disease (n=7) and cardiovascular surgery for coronary disease (n=1). The distribution of the other thirty six severe cardiovascular events was as follows: phlebitis/pulmonary embolism (n=22), cardiomyopathy/congestive heart failure (n=5), cardiac dysrhythmia (n=6), cerebral haemorrhage (n=2) and aortic aneurysm (n=1).

The incidence rate [95% CI] of all severe cardiovascular events was 1.3 [1.0 to 1.6] per 100 person-years, while for major CADE the incidence rate was 0.75 [0.57 to 0.99] per 100 person-years. Of the 49 major CADE, 40 occurred after M12. Furthermore, the major CADE incidence over the period M12-M132 was 0.89 [0.66 to 1.22] per 100 person-years.

The distribution of factors associated with major CADE, as well as the results of univariate and multivariate Cox models, are reported in Table 2 for the entire study population (n=1154). Table 3 focuses instead on the subset of patients with available data on metabolic disorders (n=675).

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/mm^3 during the follow-up. During ART, more than half of the patients (63%) were

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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 ≤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.

In the subsample of patients with available metabolic data (Table 3), the majority (73%) had a normal BMI, 17% were classified in the overweight category and a minority was classified in each of the two more extreme categories (6% underweight and 3% obese). About 8% of the patients had hypertriglyceridemia and 6% had hypercholesterolemia at M12. One year after the enrolment in the cohort, 6% of the patients reported a personal history of hypertension. Only 1% and 28% respectively had a personal and family history of CHD.

In the entire study group (n=1154), the following factors were found to be independent predictors of major CADE: older age at baseline (AHR[95% CI]=1.07[1.04 to 1.10]), tobacco consumption>20 cigarettes/day (4.19[2.17 to 8.11]) and CD4+ cell count <200 cells/mm^3 (2.52[1.15 to 5.48]) (see Table 2). In addition, a negative association was found between female gender and major CADE (0.25[0.08 to 0.83]). After accounting for the effect of age, gender, tobacco consumption and CD4+ cell count <200 cells/mm^3 in the multivariate Cox model, individuals with moderate alcohol consumption (≤4(3) AU/day for men(women)) were at lower risk of major CADE (0.38[0.20 to 0.71]), than alcohol abstainers, while those

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drinking more than 4(3) AU/day for men(women) were not significantly different from abstainers (p=0.229).

These results remained valid also when the analysis was restricted to the subgroup of patients with available metabolic data and the follow-up period M12-M132. After adjusting for age, tobacco consumption, hypertriglyceridemia and family history of CHD, we consistently found a negative association between moderate alcohol use (0.23[0.11 to 0.49]), and a positive association between CD4+ cell count <200 cells/mm^3 (4.02[1.45 to 11.1]) and major CADE after one year after enrolment (Table 3).

No significant association was found between time of exposure to different ARV drugs and major CADE. No significant interaction was found between smoking habits and hypertriglyceridemia or personal history of CHD. Furthermore, detectable viral load was not associated with major CADE in univariate or multivariate analyses.

For the Cox models fitted in this study, proportional-hazards assumption remained valid, either globally and with respect to each covariate. The residual analysis did not alter the results of the multivariate model.

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. 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).

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Discussion

This longitudinal study clearly confirms that in ARV-treated individuals, proximal CD4+ cell count lower than 200 cells/mm^3 remains a risk factor associated with major coronary and other arterial disease events. This result remains valid even after adjustment for metabolic disorders such as hypertriglyceridemia and family history of CHD, the strength of the association remaining unchanged.

The relationship between CADE and CD4+ cell count, in addition to the lack of association between CADE and specific ARV classes, confirms previous results[7] where the association between exposure to certain ARV and CADE was no longer evident after controlling for traditional risk factors and HIV disease markers. Indeed, the association between specific ARV drugs and major CADE, like myocardial infarctus, has been shown in very powerful studies[5], where adjustment for nadir CD4+ lymphocyte count or peak HIV-1 RNA level did not modify the results. However, in the study cited, no adjustment for proximal CD4+ cell count was performed, and the association with the class of ARV became weaker after adjustment for serum lipid levels[5]. 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[43]. The lack of association with exposure to some specific ARV classes in the present study, factors usually associated with an increased risk of CADE[44, 45], suggests that reduced immunological response to ART over a long follow-up, as well as known CADE risk factors may have a more important impact than exposure to a specific antiretroviral drug.

It is also possible that patients with altered lipid profiles were switched to other classes of antiretroviral drugs during the follow-up. As a consequence, in the long term, CD4+ cell

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count <200 cells/mm^3 remain a correlate of an increased risk of CADE, whatever the antiretroviral received.

In our study, individuals reporting elevated alcohol consumption exhibited the same risk of CADE as alcohol abstainers. In contrast, those reporting moderate alcohol consumptions had a lower risk. This result was confirmed after adjustment for additional risk factors including tobacco use, age, gender and CD4+ cell count. This relationship between alcohol consumption and CADE remained significant and J-shaped, even after adjustment for data on metabolic risk factors- such as having a history of CHD and hypertriglyceridemia -which was available for a subset of patients. As highlighted in previous observations in the general population[28, 46-49] and certain populations affected by other diseases[50], these results confirmed that the increased risk of CADE in HIV-infected patients receiving ART is not attributable to elevated alcohol use. Furthermore, we highlighted the apparent protective effect of moderate alcohol consumption in ART treated HIV-infected patients.

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 non-HIV based mortality as a part of the total mortality of HIV-infected patients [51], cardiovascular diseases becoming increasingly important. This increase can be explained firstly by a reduction in the competing role of HIV-related mortality, which in the long term was much more detrimental to health than other causes of deaths. Secondly, the high prevalence in this population of other risk factors like tobacco use, together with ART-related morbidity including altered lipid profiles and triglycerides, may contribute to an increased risk of CADE.

The association found in our study between moderate alcohol use and reduced CADE risk is not consistent with all previous research in HIV-infected patients. Freiberg *et al*[26] pointed

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out that among HIV-infected men in the US, hazardous drinking and both alcohol abuse and dependence were associated with a higher prevalence of cardiovascular disease compared with infrequent and moderate drinking, even after adjusting for traditional risk factors, antiretroviral therapy and CD4+ cell count. This difference in the results may be due to the different design of the two studies (longitudinal for ours *vs* cross-sectional for Freiberg's), but probably also to the different types of alcohol mainly consumed in the two populations, red wine consumption being more widespread in France than in the US. This difference in the pattern of alcohol use has already been highlighted in a comparative study on cardiovascular disease in non HIV-infected Irish and French males, showing an increased protective effect of drinking red wine in the latter population[52]. Increased wine consumption probably brings about an increase in high-density lipoprotein cholesterol levels, which helps protect against cardiovascular events. On the other hand, beer and spirits consumption are linked to increased triglyceride levels[53]. We do not know to what extent moderate alcohol users are representative of a population with better health status as alcohol use in France is relatively frequent in the general population and this fact may increase the strength of the association found.

Another possible hypothesis is that excessive alcohol consumption can increase inflammatory markers' levels[54], which in turn probably leads to a higher risk of CADE and premature aging in HIV patients. However, this hypothesis was not demonstrated in a study conducted in older adults without cardiovascular disease, where alcohol intake was found to be associated with lower levels of inflammatory markers[55]. In the meantime it is important to remember that individuals with immune suppression are at a greater risk of cancer, a disease whose pattern of risk factors also include alcohol use, even when moderate[56].

Tobacco smoking has consistently been found to be a major risk factor for cardiovascular disease[57]. Smoking prevalence and dependence in HIV-infected patients is higher than in

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the general population[58]. Effective interventions for reducing and quitting smoking[59], especially for patients with several risk factors, are strongly recommended especially considering the increased cardiovascular risk which exists in HIV-infected smokers receiving ART[60]. However, results from studies reporting on the effectiveness of interventions for quitting smoking in HIV-infected individuals are inconsistent[61].

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. Some limitations of this study need to be acknowledged. First, our definition of CADE includes different types of events which might not share the same pattern of risk factors. However, an additional analysis performed on a restricted dataset and which adjusted for other potential risk factors (such as hypertriglyceridemia) confirmed the pattern found in the main analysis.

Second, information on alcohol use was mainly based on self-reports and these may tend to underestimate alcohol consumption. However, it has been reported that HIV-HCV co-infected patients tend to underreport alcohol use more to their hepathologist than to other physicians[62]. As our patients were all followed-up by HIV physicians, it is likely that the degree of underreporting did not greatly affect hazard ratios' estimates. Moreover, the association found between the excessive alcohol consumption and the biomarkers of alcoholic liver injury indicate a good accuracy of self-reported data on alcohol consumption.

Then, 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

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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.

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 (HDL) 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.

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.

This cohort is representative of the first generation of patients receiving potent ART. As cardiovascular disease is a rising issue in all HIV-infected populations receiving ART, these

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results can give important information about the pattern of risk and protective factors in all treated HIV-infected populations.

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

Acknowledgments

The authors would like to thank all participating patients, nurses and physicians in clinical sites.

We would also like to thank Jude Sweeney for the English revision and editing of the manuscript.

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Maria Patrizia Carrieri planned the data analyses and wrote the manuscript; Camelia Protopopescu analyzed the data and wrote the manuscript; Vincent Le Moing contributed to patients' recruitment and investigation and revised the manuscript; Philippe Reboud contributed to patients' evaluation; François Raffi was responsible for cohort initiation, contributed to patients' recruitment and investigation and revised the manuscript; Sophie Mahy contributed to patients' recruitment and investigation; Perrine Roux contributed to patients' evaluation and revised the manuscript; Lise Cuzin contributed to patients' recruitment and investigation; Bruno Spire contributed to data analyses and revised the manuscript; Catherine Leport was responsible for cohort initiation, contributed to patients' recruitment and investigation and revised the manuscript. All authors approved the final version of the manuscript.

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Promotion: Agence Nationale de Recherches sur le Sida et les hépatites virales (ANRS, Action Coordonnée n°7).

Other support: Collège des Universitaires de Maladies Infectieuses et Tropicales (CMIT ex APPIT), Sidaction Ensemble contre le Sida and associated pharmaceutical companies:

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Abbott, Boehringer-Ingelheim, Bristol-Myers Squibb, GlaxoSmithKline, Gilead Sciences, Pfizer and Roche.

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Table 1. Association between alcohol consumption and living injury indicators, after adjustment for HCV status, among HIV-infected patients receiving ART (ANRS CO8 APROCO-COPILOTE cohort - random effects logistic models, all patients - n=1154, follow-up period M0-M132)

	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

ART = antiretroviral therapy; ALT= alanine transaminase; AST= aspartate transaminase
AOR = Adjusted Odds-Ratios from a random effects logistic model; CI = confidence interval
* Time-varying variable (the last available value before each visit)

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Table 2. Factors associated with the first occurrence of a major CADE among HIV-infected patients receiving ART (ANRS CO8 APROCO-COPILOTE cohort - univariate and multivariate Cox proportional hazard models, all patients - n=1154, follow-up period M0-M132)

	% of patients or mean (SD)	% of miss. data	Univariate analyses HR [95% CI]	p-value	Multivariate analysis AHR [95% CI]	p-value
Socio-demographic and psychosocial characteristics						
Female gender	22.0	0	0.26 [0.08-0.84]	0.025	0.25 [0.08-0.83]	0.024
Age at M0° - *years*	37.7 (9.5)	0	1.06 [1.03-1.09]	$<10^{-3}$	1.07 [1.04-1.10]	$<10^{-3}$
Secondary-school certificate at M0°	31.3	7.3	0.51 [0.26-1.01]	0.054		
Alcohol consumption at M0°		0				
- abstainers (ref)	18.8		1			
- ≤4(3) AU/day for men(women)	75.3		0.47 [0.24-0.89]	0.021		
- >4(3) AU/day for men(women)	5.9		0.75 [0.21-2.63]	0.648		
Alcohol consumption*		0				
- abstainers (ref)	18.6		1		1	
- ≤4(3) AU/day for men(women)	77.2		0.44 [0.24-0.81]	0.009	0.38 [0.20-0.71]	0.002
- >4(3) AU/day for men(women)	4.1		1.04 [0.30-3.58]	0.953	0.46 [0.13-1.63]	0.229
Tobacco consumption >20 cig./day*	15.8	0.1	3.17 [1.73-5.83]	$<10^{-3}$	4.19 [2.17-8.11]	$<10^{-3}$
Depressive symptoms[#]*	32.0	1.8	1.22 [0.67-2.21]	0.512		
Number of self-reported symptoms (excluding lipodystrophy)*	4.8 (3.8)	0.1	1.01 [0.95-1.08]	0.694		
Number of self-reported lipodystrophy symptoms*	2.5 (2.6)	0.4	1.07 [0.96-1.18]	0.223		
ART adherence*	63.2	0.3	2.42 [1.17-5.02]	0.017		
Clinical characteristics						
HIV transmission category°		0				
- homosexual	40.6		0.99 [0.42-2.34]	0.988		

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- injecting drug use	17.8		0.92 [0.50-1.70]	0.793		
- other (ref)	41.6		1			
CDC clinical stage A at M0°	51.2	0	0.44 [0.23-0.85]	0.014		
HCV infection at M0°	22.4	4.3	1.16 [0.59-2.29]	0.655		
Time since HIV diagnosis at M0- *years*°	4.7 (4.2)	0.9	1.01 [0.94-1.08]	0.822		
Duration of exposure to efavirenz - *years**§	1.0 (2.2)	0	0.86 [0.71-1.03]	0.102		
Duration of exposure to nevirapine - *years**§	0.9 (2.2)	0	0.91 [0.78-1.07]	0.279		
Duration of exposure to abacavir - *years**§	1.0 (2.1)	0	0.98 [0.84-1.13]	0.748		
Duration of exposure to lopinavir - *years**§	0.4 (1.5)	0	1.01 [0.85-1.20]	0.929		
Duration of exposure to PI-based regimen - *years**§	3.2 (3.1)	0	1.03 [0.92-1.15]	0.615		
Antiretroviral naivety at M0°	44.4	0	1.14 [0.64-2.02]	0.654		
CD4+ cell count < 200 *cells/mm*^{3} at M0°	35.9	0.1	0.99 [0.55-1.80]	0.978		
Detectable viral load at M0°	94.0	0.3	0.81 [0.25-2.61]	0.725		
CD4+ cell count < 200 *cells/mm*^{3}*	13.7	0.02	2.48 [1.15-5.33]	0.020	2.52 [1.15-5.48]	0.020
Detectable viral load*	43.4	0.7	0.99 [0.53-1.84]	0.980		

CADE = coronary or other arterial disease event; ART = antiretroviral therapy; CHD = coronary heart disease

SD = standard deviation; (A)HR = (adjusted) hazard ratio; CI = confidence interval

° Fixed variable (measured at M0 or M1); * Time-varying variable (the last available value before each visit); percentages and averages were computed on all follow-up visits for time-varying variables

§ Percentages and averages are computed at the end of the follow-up (last available visit for each patient)

Depressive symptoms: the Centre for Epidemiologic Studies Depression Scale (CES-D) scores is >17 for men and >23 for women

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Table 3. Factors associated with the first occurrence of a major CADE among HIV-infected patients receiving ART (ANRS CO8 APROCO-COPILOTE cohort - univariate and multivariate Cox proportional hazard models, patients with available metabolic data - n=675, follow-up period M12-M132)

	% of patients or mean (SD)	% of miss. data	Univariate analyses HR [95% CI]	p-value	Multivariate analysis AHR [95% CI]	p-value
Socio-demographic and psychosocial characteristics						
Female gender	19.0	0	0.33 [0.08-1.40]	0.134		
Age at M0 – *years*°	38.4 (9.5)	0	1.04 [1.01-1.07]	0.013	1.05 [1.02-1.09]	0.003
Secondary-school certificate at M0°	35.4	6.7	0.34 [0.14-0.84]	0.019		
Alcohol consumption at M0°		0				
- abstainers (ref)	15.1		1			
- ≤4(3) AU/day for men(women)	78.4		0.44 [0.18-1.03]	0.059		
- >4(3) AU/day for men(women)	6.5		1.04 [0.26-4.05]	0.959		
Alcohol consumption*		0				
- abstainers (ref)	16.5		1		1	
- ≤4(3) AU/day for men(women)	79.1		0.27 [0.13-0.58]	0.001	0.23 [0.11-0.49]	$<10^{-3}$
- >4(3) AU/day for men(women)	4.4		1.19 [0.33-4.23]	0.792	0.55 [0.14-2.14]	0.390
Tobacco consumption >20 cig./day*	15.7	0.1	3.58 [1.70-7.54]	0.001	5.59 [2.43-12.8]	$<10^{-3}$
Depressive symptoms[#]*	31.9	1.50	1.83 [0.90-3.73]	0.094		
Number of self-reported symptoms (excluding lipodystrophy)*	4.8 (3.8)	0.02	1.03 [0.95-1.12]	0.463		
Number of self-reported lipodystrophy symptoms*	2.6 (2.7)	0.16	1.10 [0.98-1.24]	0.111		
ARV adherence*	63.6	0.2	1.63 [0.70-3.81]	0.260		
Clinical characteristics						

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HIV transmission category°		0				
- homosexual (ref)	44.0		1			
- injecting drug use	15.0		2.08 [0.75-5.73]	0.156		
- other	41.0		1.32 [0.59-2.93]	0.499		
CDC clinical stage A at M0°	50.8	0	0.43 [0.19-0.97]	0.041		
HCV infection at M0°	19.8	3.8	2.05 [0.94-4.47]	0.071		
Duration since HIV diagnosis at M0- *years*°	4.7 (4.2)	1.2	1.04 [0.96-1.13]	0.311		
Duration of exposure to efavirenz - *years**§	1.4 (2.5)	0	0.89 [0.73-1.08]	0.236		
Duration of exposure to nevirapine - *years**§	1.0 (2.3)	0	0.83 [0.66-1.06]	0.147		
Duration of exposure to abacavir – *years**§	1.2 (2.3)	0	0.99 [0.85-1.17]	0.971		
Duration of exposure to lopinavir - *years**§	0.5 (1.5)	0	1.07 [0.88-1.30]	0.505		
Duration of exposure to PI-based regimen - *years**§	3.9 (3.1)	0	1.03 [0.89-1.18]	0.714		
Antiretroviral naivety at M0°	41.5	0	0.86 [0.41-1.79]	0.683		
CD4+ cell count < 200 *cells/mm*^{3} at M0°	33.9	0	0.88 [0.41-1.88]	0.749		
Detectable viral load at M0°	94.8	0.1	0.70 [0.17-2.94]	0.627		
CD4+ cell count < 200 *cells/mm*^{3}*	11.8	0	2.58 [0.99-6.75]	0.052	4.02 [1.45-11.1]	0.007
Detectable viral load*	40.2	0.03	1.24 [0.59-2.59]	0.574		
Metabolic characteristics						
BMI categories°@		1.6				
- underweight: <18.5 (ref)	5.8		1			
- normal weight: 18.5–24.9	72.8		0.65 [0.15-2.76]	0.556		
- overweight or obese: >25	19.8		0.57 [0.11-2.87]	0.498		
Hypertriglyceridemia°@	8.4	0	2.06 [0.79-5.37]	0.141	2.98 [1.11-8.01]	0.030

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Hypercholesterolemia°@	6.5	0.1	0.95 [0.22-3.97]	0.939		
Personal history of CHD°@	1.2	0	9.39 [2.22-39.8]	0.002		
Family history of CHD°@	28.0	0	1.82 [0.89-3.71]	0.102	2.25 [1.08-4.71]	0.031
Personal history of hypertension°@	6.1	0	2.73 [1.05-7.13]	0.040		

CADE = coronary or other arterial disease event; ART = antiretroviral therapy; CHD = coronary heart disease

SD = standard deviation; (A)HR = (adjusted) hazard ratio; CI = confidence interval

° Fixed variable (measured at M0, M1 or M12); * Time-varying variable (the last available value before each visit); percentages and averages are computed on all follow-up visits for time-varying variables

@ At M12; § Percentages and averages are computed at the end of the follow-up (last available visit for each patient)

Depressive symptoms: the Centre for Epidemiologic Studies Depression Scale (CES-D) scores is >17 for men and >23 for women

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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

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Abstract word count: 285 words

Text word count: 4548 words

Funding sources: The ANRS CO8 (APROCO/COPILOTE) cohort is funded by ANRS (Agence Nationale de Recherches sur le Sida et les hépatites virales) and CMIT (Collège des Universitaires de Maladies Infectieuses et Tropicales), which received research grants from Abbott, Boehringer-Ingelheim, Bristol-Myers Squibb, GlaxoSmithKline, Gilead Sciences, Pfizer and Roche.

The funders were responsible for initiating, managing and financing the cohort, but did not interfere with data analysis and interpretation and dissemination of the scientific results.

Competing interests: All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf <http://www.icmje.org/coi_disclosure.pdf> (available on request from the corresponding author) and declare that (1) they have no support from any company for the submitted work; (2) they have no relationships with any company that might have an interest in the submitted work in the previous 3 years; (3) their spouses, partners, or children have no financial relationships that may be relevant to the submitted work; and (4) they have no non-financial interests that may be relevant to the submitted work.

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Data sharing: There is no additional data available.

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Summary

Article Focus

- Major coronary or other arterial disease events (CADE) are frequent in HIV-infected individuals receiving antiretroviral therapy.
- While some antiretroviral classes can increase the risk of CADE, it is still controversial to what extent immune reconstitution and other factors like moderate alcohol consumption can reduce the risk of CADE in this population.

Key Messages

- Moderate alcohol consumption and absence of immunodepression can be beneficial to reduce the risk of CADE in HIV-infected patients.
- Reduced immunological response to antiretroviral treatment (ART) over a long follow-up may have a more important impact on CADE than exposure to a specific antiretroviral drug.

Strengths and Limitations

- This paper suggests the importance of combined risk reduction interventions including adherence counselling for assuring long term immunological response and decrease the risk of CADE.

Short title

CD4+, Moderate Alcohol Consumption and Coronary Events in HIV-infected patients

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Abstract

Objective: To investigate the relationship between response to antiretroviral treatment (ART), alcohol use and occurrence of a major coronary or other arterial disease event (CADE) in HIV-infected individuals.

Design: Cohort study. A Cox model was used to identify correlates of a first occurrence of a major CADE.

Setting: The French ANRS CO8 APROCO-COPILOTE cohort was set up in 1997 to study clinical progression and patient-reported outcomes (PRO) after initiating protease inhibitor-containing ART. Clinical data were retrieved from medical records. Self-administered questionnaires collected data on PRO and behaviours, including alcohol use.

Participants: Metabolic data were only available for a subgroup (n=675) of the study group (n=1154).

Main outcomes measures: Major coronary or other arterial disease first event.

Results: Over the 11-year follow-up, 49 major CADE were observed, with an incidence rate [95%CI]=0.75[0.57 to 0.99] per 100 person-years. Immunodepression (CD4+ cell count <200 cells/mm^3) was associated with an increased risk of CADE (Adjusted Hazard Ratio AHR[95% CI]= 2.52[1.15 to 5.48]) after adjustment for female gender (0.25[0.08 to 0.83]), age (1.07[1.04 to 1.10]) and smoking>20 cigarettes/day (4.19[2.17 to 8.11]). Moreover, individuals with moderate alcohol consumption (≤4(3) AU/day for men(women)) had a lower risk of CADE (0.38[0.20 to 0.71]) than alcohol abstainers, while the risk for those drinking>4(3) AU/day for men(women) was not significantly different from this latter group. These associations remained valid after adjustment for metabolic disorders. No significant association with exposure to any specific antiretroviral was detected.

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Conclusions: In the long term, absence of immunodepression and moderate alcohol consumption remain associated with a lower risk of major CADE. Combined interventions to reduce CADE risk-related behaviours including adherence counselling for assuring long term immunological response to ART in HIV-infected individuals are now a clinical and public health priority.

Key words: Coronary and other arterial disease events, Alcohol, CD4+ cell count, HIV, ARV.

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Introduction

Cardiovascular disease risk factors (including for example hypertension and tobacco use), as well as immunological status[1] are major predictors of atherosclerosis in HIV-infected women and men. 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, more recently Butt and colleagues put into evidence the negative effect of HIV viral replication on heart failure[6]. In one study, patients having experienced a MI presented lower baseline and proximal CD4+ cell counts than those who did not, while no association was found between cardiovascular disease events and specific antiretroviral agents[7]. Moreover, known factors associated with cardiovascular risk[8] such as hyperlipidemia and insulin resistance[9-11] are becoming an increasingly major clinical concern in ARV-treated individuals.

Though excessive alcohol use may be frequent in HIV-infected individuals, its impact on the occurrence of cardiovascular disease in this population has been less investigated. Excessive alcohol consumption also plays a major role in HIV risk-taking behaviours, particularly in men who have sex with men and drug users[12, 13]. In HIV-infected individuals, excessive alcohol use is known to compromise patients' response to antiretroviral therapy (ART) through reduced adherence[14-17] and to predict HIV progression and increased mortality[18-21], mostly from liver diseases[22-25]. Some recent cross-sectional analyses in HIV-infected and uninfected men have found that heavy alcohol consumption is associated with a higher risk of cardiovascular disease[26, 27]. On the other hand, in uninfected adults, moderate alcohol consumption has been found to be associated with a reduced cardiovascular mortality[28], improved lipid profiles, increased insulin sensitivity and reduced risk of MI[29]. However, this association has not yet been studied in longitudinal studies involving HIV-infected populations including women. Moreover, there are as yet no consistent results

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in the literature regarding the role that alcohol use plays in exacerbating cardiovascular risk in ARV-treated patients and to what extent it can confound or boost the effect of immuno-virological response to ART, ART-associated dyslipidemia and insulin resistance on the risk of cardiovascular disease events.

We used data from the 11-year follow-up of the APROCO-COPILOTE cohort of patients receiving ART since 1997 to investigate the relationship between viral load, CD4+ cell count, alcohol consumption and the first occurrence of a major coronary or other arterial disease event (CADE), after adjustment for known risk factors including metabolic disorders.

Methods

The French APROCO-COPILOTE cohort (ANRS CO8) was designed to study the clinical, immunological, virological, and socio-behavioural course of HIV disease in HIV-1 positive individuals who started the first generation of potent ART (treatment regimens including protease inhibitors (PI)). The study was approved by the Ethics Committee of Cochin Hospital (Paris) and informed consent was obtained from all participants.

Setting

Patients were enrolled in the cohort at their first PI-based ART prescription between May 1997 and June 1999 (n=1281) in 47 French centres, and clinically followed-up every 4 months thereafter, up to month 132 (M132).

Patients

In the present study were included all patients who had either two alcohol assessment or one alcohol assessment just preceding the CADE over the whole follow-up (n=1154).

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Medical questionnaire

The medical questionnaire at enrolment (M0) collected retrospective data about patient's HIV history including HIV transmission category, time since HIV diagnosis, previous exposure to antiretroviral treatment before enrolment, and co-infection with hepatitis C virus (HCV) defined as having positive HCV RNA and/or positive HCV antibodies. This information was complemented by medical questionnaires, completed by the HIV physicians at each follow-up visit, which collected clinical and laboratory data (plasma HIV RNA level, CD4+ cell count, HIV clinical stage, aspartate transaminase (AST) and alanine transaminase (ALT) levels), as well as data on the antiretroviral regimen prescribed. A third form, based on updated medical records, detailed all clinical severe events, including cardiovascular events, occurring during the study period. Information on metabolic disorders was available at M12 for a subset of cohorts' patients, who participated in a parallel cross-sectional survey, designed to study clinical and laboratory metabolic complications[30]. These "metabolic" data included height, weight, hypertriglyceridemia, hypercholesterolemia, personal history of coronary heart disease (CHD) and hypertension, and finally any family history of CHD.

At each follow-up visit, CD4+ cell count was measured by standardized flow cytometry, and plasma HIV RNA levels were measured using the assay routinely available in each participating centre. Immunodepression was defined by CD4+ cell count <200 cells/mm^3; 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. HIV clinical stage was defined using the 1993 Centres for Disease Control and Prevention (CDC) clinical classification system[31].

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Serum triglyceride and cholesterol levels were measured after a 12-hour overnight fast. Hypertriglyceridemia was defined as a triglyceride level ≥2.2 mmol/l, while hypercholesterolemia was defined as a total cholesterol level ≥5.5 mmol/l.

Outcome

The details of all clinical severe 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 clinical severe 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.

Self-administered questionnaire

A self-administered questionnaire collected - at M0, M4, M12 and every 8 months thereafter during the first 5 years of follow-up, then yearly thereafter – data on socio-demographic characteristics including age, gender and educational level. Among other psycho-social and behavioural information, it also collected details on depressive symptoms, tobacco use, alcohol consumption, self-reported ART-related symptoms and adherence to ART.

[i] http://www.who.int/classifications/icd/en/

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Depression was measured using the validated French version of the Centre for Epidemiologic Studies Depression Scale (CES-D), which is a 20-item scale commonly used in studies involving HIV-infected patients[32].

Tobacco and alcohol consumption were evaluated during the previous 4 weeks. Alcohol consumption was assessed using two questions about frequency of consumption and quantity consumed daily, if applicable.

A 13-item scale comprising the French version of the symptom index validated by Justice *et al*[33] and described elsewhere[34] collected information about self-reported symptoms. Five other questions gathered information about adherence to ART, according to the methodology established by the AIDS Clinical Trial Group[35].

Statistical analysis

As the results of the analysis on the whole dataset (n=1154) could be affected by the lack of important predictors of CADE like hypertriglyceridemia or hypercholesterolemia, we conducted a secondary analysis on a subset of patients who had additional metabolic disorders data. More specifically, two analyses on the following study populations were performed to study predictors of major CADE:

1) First analysis: the study population included all patients who had either two alcohol assessment or one alcohol assessment just preceding the event over the whole follow-up (n=1154). The follow-up period was M0-M132.

2) Second analysis: the study population was restricted to the subset of patients with available data on metabolic disorders, measured at M12 (n=675). Consequently, only events occurring after M12 were considered in this second analysis.

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The WHO body mass index (BMI) categories were used in the analysis: underweight and overweight/obese patients were defined respectively as having a BMI lower than 18.5 and greater than 25.

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

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[36].

Tobacco consumption was dichotomized in two categories: more than 20 cigarettes/day *vs* other[37]. The average number of alcohol units (AU) consumed per day[38] was computed using the two questions on alcohol consumption and then recoded in four categories (abstainers; ≤1 AU/day; >1 and ≤4(3) AU/day for men(women); >4(3) AU/day for men(women)), in order to test a gradient effect. 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

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HCV status, see Table 1), which would suggest good accuracy of the self-reported data on alcohol consumption.

Patients were classified as non-adherent if they reported taking less than 100% of prescribed medications during the previous 4 weeks, using a validated algorithm[39]. A patient with a CES-D score value above 17 (men) or 23 (women) was considered as presenting depressive symptoms[40].

Incidence rate of major CADE was computed as the number of cases divided by the number of person-years of follow-up. Follow-up duration was calculated as the difference in days from enrolment to the date of the CADE, death or last visit, whichever occurred first. Follow-up of patients experiencing major CADE was censored after the date of the first CADE. We used a Cox proportional hazards model to identify characteristics associated with the first occurrence of a major CADE. Psychosocial characteristics - depressive symptoms, tobacco and alcohol consumption, self-reported symptoms, CD4+ cell count and viral load - were evaluated at each visit and used as time-varying covariates in the statistical analysis. All the other covariates were used as fixed variables (measured at M0, M1 or M12). For time-varying factors, the last known value was carried forward in the case of missing data at a scheduled visit. In the case of an event occurring between two consecutive follow-up visits, the values for the time-varying variables measured at the visit preceding the event were used. 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[36], and finally a selection procedure based on the Schwartz Bayesian information criterion BIC[41]. All three strategies selected the same final multivariate model. Interaction effects between the factors of the multivariate final model were tested. The proportional-hazards assumption was verified

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globally for the multivariate models and separately with respect to each covariate, using both Kaplan-Meier estimates and tests based on Schoenfeld residuals[42]. A residual analysis for outliers' detection was performed and the sensitivity of the model to influential outliers was tested.

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. A third analysis was performed separately on the subgroups of patients co-infected with HCV and those not co-infected with HCV.

All analyses were performed using Stata Intercooled software, version 12.1.

Results

A total of 1154 patients were included in the present study, accounting for 9401 person-visits. When comparing the study patients (n=1154) with those included in the cohort, but excluded from the study due to missing alcohol data (n=127), no significant difference was found for gender, age at enrolment, antiretroviral naivety, CDC clinical stage, baseline CD4+ cell counts and HIV viral load at M0 (data not shown).

Median [interquartile range (IQR)] duration of follow-up of the selected patients, from their first visit after the ART prescription (M1) until M132, was 5.9 [1.8-9.4] years. 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.

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Over the 11-year follow-up of the cohort, a total of 85 severe cardiovascular events were observed. These included 49 major CADE as follows: myocardial infarction (n=30), coronary heart disease (n=4), stroke (n=7), peripheral arterial disease (n=7) and cardiovascular surgery for coronary disease (n=1). The distribution of the other thirty six severe cardiovascular events was as follows: phlebitis/pulmonary embolism (n=22), cardiomyopathy/congestive heart failure (n=5), cardiac dysrhythmia (n=6), cerebral haemorrhage (n=2) and aortic aneurysm (n=1).

The incidence rate [95% CI] of all severe cardiovascular events was 1.3 [1.0 to 1.6] per 100 person-years, while for major CADE the incidence rate was 0.75 [0.57 to 0.99] per 100 person-years. Of the 49 major CADE, 40 occurred after M12. Furthermore, the major CADE incidence over the period M12-M132 was 0.89 [0.66 to 1.22] per 100 person-years.

The distribution of factors associated with major CADE, as well as the results of univariate and multivariate Cox models, are reported in Table 2 for the entire study population (n=1154). Table 3 focuses instead on the subset of patients with available data on metabolic disorders (n=675).

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/mm^3 during the follow-up. During ART, more than half of the patients (63%) were

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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 ≤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.

In the subsample of patients with available metabolic data (Table 3), the majority (73%) had a normal BMI, 17% were classified in the overweight category and a minority was classified in each of the two more extreme categories (6% underweight and 3% obese). About 8% of the patients had hypertriglyceridemia and 6% had hypercholesterolemia at M12. One year after the enrolment in the cohort, 6% of the patients reported a personal history of hypertension. Only 1% and 28% respectively had a personal and family history of CHD.

In the entire study group (n=1154), the following factors were found to be independent predictors of major CADE: older age at baseline (AHR[95% CI]=1.07[1.04 to 1.10]), tobacco consumption>20 cigarettes/day (4.19[2.17 to 8.11]) and CD4+ cell count <200 cells/mm^3 (2.52[1.15 to 5.48]) (see Table 2). In addition, a negative association was found between female gender and major CADE (0.25[0.08 to 0.83]). After accounting for the effect of age, gender, tobacco consumption and CD4+ cell count <200 cells/mm^3 in the multivariate Cox model, individuals with moderate alcohol consumption (≤4(3) AU/day for men(women)) were at lower risk of major CADE (0.38[0.20 to 0.71]), than alcohol abstainers, while those

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drinking more than 4(3) AU/day for men(women) were not significantly different from abstainers (p=0.229).

These results remained valid also when the analysis was restricted to the subgroup of patients with available metabolic data and the follow-up period M12-M132. After adjusting for age, tobacco consumption, hypertriglyceridemia and family history of CHD, we consistently found a negative association between moderate alcohol use (0.23[0.11 to 0.49]), and a positive association between CD4+ cell count <200 cells/mm^3 (4.02[1.45 to 11.1]) and major CADE after one year after enrolment (Table 3).

No significant association was found between time of exposure to different ARV drugs and major CADE. No significant interaction was found between smoking habits and hypertriglyceridemia or personal history of CHD. Furthermore, detectable viral load was not associated with major CADE in univariate or multivariate analyses.

For the Cox models fitted in this study, proportional-hazards assumption remained valid, either globally and with respect to each covariate. The residual analysis did not alter the results of the multivariate model.

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. 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).

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Discussion

This longitudinal study clearly confirms that in ARV-treated individuals, proximal CD4+ cell count lower than 200 cells/mm^3 remains a risk factor associated with major coronary and other arterial disease events. This result remains valid even after adjustment for metabolic disorders such as hypertriglyceridemia and family history of CHD, the strength of the association remaining unchanged.

The relationship between CADE and CD4+ cell count, in addition to the lack of association between CADE and specific ARV classes, confirms previous results[7] where the association between exposure to certain ARV and CADE was no longer evident after controlling for traditional risk factors and HIV disease markers. Indeed, the association between specific ARV drugs and major CADE, like myocardial infarctus, has been shown in very powerful studies[5], where adjustment for nadir CD4+ lymphocyte count or peak HIV-1 RNA level did not modify the results. However, in the study cited, no adjustment for proximal CD4+ cell count was performed, and the association with the class of ARV became weaker after adjustment for serum lipid levels[5]. 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[43]. The lack of association with exposure to some specific ARV classes in the present study, factors usually associated with an increased risk of CADE[44, 45], suggests that reduced immunological response to ART over a long follow-up, as well as known CADE risk factors may have a more important impact than exposure to a specific antiretroviral drug.

It is also possible that patients with altered lipid profiles were switched to other classes of antiretroviral drugs during the follow-up. As a consequence, in the long term, CD4+ cell

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count <200 cells/mm^3 remain a correlate of an increased risk of CADE, whatever the antiretroviral received.

In our study, individuals reporting elevated alcohol consumption exhibited the same risk of CADE as alcohol abstainers. In contrast, those reporting moderate alcohol consumptions had a lower risk. This result was confirmed after adjustment for additional risk factors including tobacco use, age, gender and CD4+ cell count. This relationship between alcohol consumption and CADE remained significant and J-shaped, even after adjustment for data on metabolic risk factors- such as having a history of CHD and hypertriglyceridemia -which was available for a subset of patients. As highlighted in previous observations in the general population[28, 46-49] and certain populations affected by other diseases[50], these results confirmed that the increased risk of CADE in HIV-infected patients receiving ART is not attributable to elevated alcohol use. Furthermore, we highlighted the apparent protective effect of moderate alcohol consumption in ART treated HIV-infected patients.

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 non-HIV based mortality as a part of the total mortality of HIV-infected patients [51], cardiovascular diseases becoming increasingly important. This increase can be explained firstly by a reduction in the competing role of HIV-related mortality, which in the long term was much more detrimental to health than other causes of deaths. Secondly, the high prevalence in this population of other risk factors like tobacco use, together with ART-related morbidity including altered lipid profiles and triglycerides, may contribute to an increased risk of CADE.

The association found in our study between moderate alcohol use and reduced CADE risk is not consistent with all previous research in HIV-infected patients. Freiberg *et al*[26] pointed

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out that among HIV-infected men in the US, hazardous drinking and both alcohol abuse and dependence were associated with a higher prevalence of cardiovascular disease compared with infrequent and moderate drinking, even after adjusting for traditional risk factors, antiretroviral therapy and CD4+ cell count. This difference in the results may be due to the different design of the two studies (longitudinal for ours *vs* cross-sectional for Freiberg's), but probably also to the different types of alcohol mainly consumed in the two populations, red wine consumption being more widespread in France than in the US. This difference in the pattern of alcohol use has already been highlighted in a comparative study on cardiovascular disease in non HIV-infected Irish and French males, showing an increased protective effect of drinking red wine in the latter population[52]. Increased wine consumption probably brings about an increase in high-density lipoprotein cholesterol levels, which helps protect against cardiovascular events. On the other hand, beer and spirits consumption are linked to increased triglyceride levels[53]. We do not know to what extent moderate alcohol users are representative of a population with better health status as alcohol use in France is relatively frequent in the general population and this fact may increase the strength of the association found.

Another possible hypothesis is that excessive alcohol consumption can increase inflammatory markers' levels[54], which in turn probably leads to a higher risk of CADE and premature aging in HIV patients. However, this hypothesis was not demonstrated in a study conducted in older adults without cardiovascular disease, where alcohol intake was found to be associated with lower levels of inflammatory markers[55]. In the meantime it is important to remember that individuals with immune suppression are at a greater risk of cancer, a disease whose pattern of risk factors also include alcohol use, even when moderate[56].

Tobacco smoking has consistently been found to be a major risk factor for cardiovascular disease[57]. Smoking prevalence and dependence in HIV-infected patients is higher than in

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the general population[58]. Effective interventions for reducing and quitting smoking[59], especially for patients with several risk factors, are strongly recommended especially considering the increased cardiovascular risk which exists in HIV-infected smokers receiving ART[60]. However, results from studies reporting on the effectiveness of interventions for quitting smoking in HIV-infected individuals are inconsistent[61].

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. Some limitations of this study need to be acknowledged. First, our definition of CADE includes different types of events which might not share the same pattern of risk factors. However, an additional analysis performed on a restricted dataset and which adjusted for other potential risk factors (such as hypertriglyceridemia) confirmed the pattern found in the main analysis.

Second, information on alcohol use was mainly based on self-reports and these may tend to underestimate alcohol consumption. However, it has been reported that HIV-HCV co-infected patients tend to underreport alcohol use more to their hepathologist than to other physicians[62]. As our patients were all followed-up by HIV physicians, it is likely that the degree of underreporting did not greatly affect hazard ratios' estimates. Moreover, the association found between the excessive alcohol consumption and the biomarkers of alcoholic liver injury indicate a good accuracy of self-reported data on alcohol consumption.

Then, 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

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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.

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 (HDL) 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.

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.

This cohort is representative of the first generation of patients receiving potent ART. As cardiovascular disease is a rising issue in all HIV-infected populations receiving ART, these

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results can give important information about the pattern of risk and protective factors in all treated HIV-infected populations.

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

Acknowledgments

The authors would like to thank all participating patients, nurses and physicians in clinical sites.

We would also like to thank Jude Sweeney for the English revision and editing of the manuscript.

Authors' contribution:

Maria Patrizia Carrieri planned the data analyses and wrote the manuscript; Camelia Protopopescu analyzed the data and wrote the manuscript; Vincent Le Moing contributed to patients' recruitment and investigation and revised the manuscript; Philippe Reboud contributed to patients' evaluation; François Raffi was responsible for cohort initiation, contributed to patients' recruitment and investigation and revised the manuscript; Sophie Mahy contributed to patients' recruitment and investigation; Perrine Roux contributed to patients' evaluation and revised the manuscript; Lise Cuzin contributed to patients' recruitment and investigation; Bruno Spire contributed to data analyses and revised the manuscript; Catherine Leport was responsible for cohort initiation, contributed to patients' recruitment and investigation and revised the manuscript. All authors approved the final version of the manuscript.

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Promotion: Agence Nationale de Recherches sur le Sida et les hépatites virales (ANRS, Action Coordonnée n°7).

Other support: Collège des Universitaires de Maladies Infectieuses et Tropicales (CMIT ex APPIT), Sidaction Ensemble contre le Sida and associated pharmaceutical companies:

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Abbott, Boehringer-Ingelheim, Bristol-Myers Squibb, GlaxoSmithKline, Gilead Sciences, Pfizer and Roche.

Clinical Centres (investigators): Amiens (Pr JL. Schmit), Angers (Dr JM. Chennebault), Belfort (Dr JP. Faller), Besançon (Pr N. Mgy-Bertrand, Pr B. Hoen, Dr Drobachef), Bobigny (Pr O. Bouchaud), Bordeaux (Pr M. Dupon, Pr Longy-Boursier, Pr P. Morlat, Pr JM. Ragnaud), Bourg-en-Bresse (Dr P. Granier), Brest (Pr M. Garré), Caen (Pr R. Verdon), Compiègne (Dr D. Merrien), Corbeil Essonnes (Dr A. Devidas), Créteil (Pr A. Sobel), Dijon (Pr L. Piroth), Garches (Pr C. Perronne), Lagny (Dr E. Froguel), Libourne (Dr J. Ceccaldi), Lyon (Pr D. Peyramond), Meaux (Dr C. Allard), Montpellier (Pr J. Reynes), Nancy (Pr T. May), Nantes (Pr F. Raffi), Nice (Pr JG Fuzibet, Pr P. Dellamonica), Orléans (Dr P. Arsac), Paris (Pr E. Bouvet, Pr F. Bricaire, Pr P. Bergmann, Pr J. Cabane, Dr J. Monsonego, Pr PM. Girard, Pr L. Guillevin, Pr S. Herson, Pr C.Leport, Pr MC. Meyohas, Pr JM. Molina, Pr G. Pialoux, Pr D. Salmon), Poitiers (Pr P. Roblot), Reims (Pr R. Jaussaud), Rennes (Pr C. Michelet), Saint-Etienne (Pr F. Lucht), Saint-Mandé (Pr T. Debord), Strasbourg (Dr D. Rey), Toulon (Dr JP. De Jaureguiberry), Toulouse (Pr B. Marchou), Tours (Pr L. Bernard).

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Table 1. Association between alcohol consumption and living injury indicators, after adjustment for HCV status, among HIV-infected patients receiving ART (ANRS CO8 APROCO-COPILOTE cohort - random effects logistic models, all patients - n=1154, follow-up period M0-M132)

	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

ART = antiretroviral therapy; ALT= alanine transaminase; AST= aspartate transaminase
AOR = Adjusted Odds-Ratios from a random effects logistic model; CI = confidence interval
* Time-varying variable (the last available value before each visit)

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Table 2. Factors associated with the first occurrence of a major CADE among HIV-infected patients receiving ART (ANRS CO8 APROCO-COPILOTE cohort - univariate and multivariate Cox proportional hazard models, all patients - n=1154, follow-up period M0-M132)

	% of patients or mean (SD)	% of miss. data	Univariate analyses HR [95% CI]	p-value	Multivariate analysis AHR [95% CI]	p-value
Socio-demographic and psychosocial characteristics						
Female gender	22.0	0	0.26 [0.08-0.84]	0.025	0.25 [0.08-0.83]	0.024
Age at M0° - *years*	37.7 (9.5)	0	1.06 [1.03-1.09]	$<10^{-3}$	1.07 [1.04-1.10]	$<10^{-3}$
Secondary-school certificate at M0°	31.3	7.3	0.51 [0.26-1.01]	0.054		
Alcohol consumption at M0°		0				
- abstainers (ref)	18.8		1			
- ≤4(3) AU/day for men(women)	75.3		0.47 [0.24-0.89]	0.021		
- >4(3) AU/day for men(women)	5.9		0.75 [0.21-2.63]	0.648		
Alcohol consumption*		0				
- abstainers (ref)	18.6		1		1	
- ≤4(3) AU/day for men(women)	77.2		0.44 [0.24-0.81]	0.009	0.38 [0.20-0.71]	0.002
- >4(3) AU/day for men(women)	4.1		1.04 [0.30-3.58]	0.953	0.46 [0.13-1.63]	0.229
Tobacco consumption >20 cig./day*	15.8	0.1	3.17 [1.73-5.83]	$<10^{-3}$	4.19 [2.17-8.11]	$<10^{-3}$
Depressive symptoms[#]*	32.0	1.8	1.22 [0.67-2.21]	0.512		
Number of self-reported symptoms (excluding lipodystrophy)*	4.8 (3.8)	0.1	1.01 [0.95-1.08]	0.694		
Number of self-reported lipodystrophy symptoms*	2.5 (2.6)	0.4	1.07 [0.96-1.18]	0.223		
ART adherence*	63.2	0.3	2.42 [1.17-5.02]	0.017		
Clinical characteristics						
HIV transmission category°		0				
- homosexual	40.6		0.99 [0.42-2.34]	0.988		

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- injecting drug use	17.8		0.92 [0.50-1.70]	0.793		
- other (ref)	41.6		1			
CDC clinical stage A at M0°	51.2	0	0.44 [0.23-0.85]	0.014		
HCV infection at M0°	22.4	4.3	1.16 [0.59-2.29]	0.655		
Time since HIV diagnosis at M0- *years*°	4.7 (4.2)	0.9	1.01 [0.94-1.08]	0.822		
Duration of exposure to efavirenz - *years**§	1.0 (2.2)	0	0.86 [0.71-1.03]	0.102		
Duration of exposure to nevirapine - *years**§	0.9 (2.2)	0	0.91 [0.78-1.07]	0.279		
Duration of exposure to abacavir - *years**§	1.0 (2.1)	0	0.98 [0.84-1.13]	0.748		
Duration of exposure to lopinavir - *years**§	0.4 (1.5)	0	1.01 [0.85-1.20]	0.929		
Duration of exposure to PI-based regimen - *years**§	3.2 (3.1)	0	1.03 [0.92-1.15]	0.615		
Antiretroviral naivety at M0°	44.4	0	1.14 [0.64-2.02]	0.654		
CD4+ cell count < 200 *cells/mm*^{3} at M0°	35.9	0.1	0.99 [0.55-1.80]	0.978		
Detectable viral load at M0°	94.0	0.3	0.81 [0.25-2.61]	0.725		
CD4+ cell count < 200 *cells/mm*^{3}*	13.7	0.02	2.48 [1.15-5.33]	0.020	2.52 [1.15-5.48]	0.020
Detectable viral load*	43.4	0.7	0.99 [0.53-1.84]	0.980		

CADE = coronary or other arterial disease event; ART = antiretroviral therapy; CHD = coronary heart disease

SD = standard deviation; (A)HR = (adjusted) hazard ratio; CI = confidence interval

° Fixed variable (measured at M0 or M1); * Time-varying variable (the last available value before each visit); percentages and averages were computed on all follow-up visits for time-varying variables

§ Percentages and averages are computed at the end of the follow-up (last available visit for each patient)

Depressive symptoms: the Centre for Epidemiologic Studies Depression Scale (CES-D) scores is >17 for men and >23 for women

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Table 3. Factors associated with the first occurrence of a major CADE among HIV-infected patients receiving ART (ANRS CO8 APROCO-COPILOTE cohort - univariate and multivariate Cox proportional hazard models, patients with available metabolic data - n=675, follow-up period M12-M132)

	% of patients or mean (SD)	% of miss. data	Univariate analyses HR [95% CI]	p-value	Multivariate analysis AHR [95% CI]	p-value
Socio-demographic and psychosocial characteristics						
Female gender	19.0	0	0.33 [0.08-1.40]	0.134		
Age at M0 – *years*°	38.4 (9.5)	0	1.04 [1.01-1.07]	0.013	1.05 [1.02-1.09]	0.003
Secondary-school certificate at M0°	35.4	6.7	0.34 [0.14-0.84]	0.019		
Alcohol consumption at M0°		0				
- abstainers (ref)	15.1		1			
- ≤4(3) AU/day for men(women)	78.4		0.44 [0.18-1.03]	0.059		
- >4(3) AU/day for men(women)	6.5		1.04 [0.26-4.05]	0.959		
Alcohol consumption*		0				
- abstainers (ref)	16.5		1		1	
- ≤4(3) AU/day for men(women)	79.1		0.27 [0.13-0.58]	0.001	0.23 [0.11-0.49]	$<10^{-3}$
- >4(3) AU/day for men(women)	4.4		1.19 [0.33-4.23]	0.792	0.55 [0.14-2.14]	0.390
Tobacco consumption >20 cig./day*	15.7	0.1	3.58 [1.70-7.54]	0.001	5.59 [2.43-12.8]	$<10^{-3}$
Depressive symptoms[#]*	31.9	1.50	1.83 [0.90-3.73]	0.094		
Number of self-reported symptoms (excluding lipodystrophy)*	4.8 (3.8)	0.02	1.03 [0.95-1.12]	0.463		
Number of self-reported lipodystrophy symptoms*	2.6 (2.7)	0.16	1.10 [0.98-1.24]	0.111		
ARV adherence*	63.6	0.2	1.63 [0.70-3.81]	0.260		
Clinical characteristics						

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HIV transmission category°		0				
- homosexual (ref)	44.0		1			
- injecting drug use	15.0		2.08 [0.75-5.73]	0.156		
- other	41.0		1.32 [0.59-2.93]	0.499		
CDC clinical stage A at M0°	50.8	0	0.43 [0.19-0.97]	0.041		
HCV infection at M0°	19.8	3.8	2.05 [0.94-4.47]	0.071		
Duration since HIV diagnosis at M0- *years*°	4.7 (4.2)	1.2	1.04 [0.96-1.13]	0.311		
Duration of exposure to efavirenz - *years**§	1.4 (2.5)	0	0.89 [0.73-1.08]	0.236		
Duration of exposure to nevirapine - *years**§	1.0 (2.3)	0	0.83 [0.66-1.06]	0.147		
Duration of exposure to abacavir – *years**§	1.2 (2.3)	0	0.99 [0.85-1.17]	0.971		
Duration of exposure to lopinavir - *years**§	0.5 (1.5)	0	1.07 [0.88-1.30]	0.505		
Duration of exposure to PI-based regimen - *years**§	3.9 (3.1)	0	1.03 [0.89-1.18]	0.714		
Antiretroviral naivety at M0°	41.5	0	0.86 [0.41-1.79]	0.683		
CD4+ cell count < 200 *cells/mm*^{3} at M0°	33.9	0	0.88 [0.41-1.88]	0.749		
Detectable viral load at M0°	94.8	0.1	0.70 [0.17-2.94]	0.627		
CD4+ cell count < 200 *cells/mm*^{3}*	11.8	0	2.58 [0.99-6.75]	0.052	4.02 [1.45-11.1]	0.007
Detectable viral load*	40.2	0.03	1.24 [0.59-2.59]	0.574		
Metabolic characteristics						
BMI categories°@		1.6				
- underweight: <18.5 (ref)	5.8		1			
- normal weight: 18.5–24.9	72.8		0.65 [0.15-2.76]	0.556		
- overweight or obese: >25	19.8		0.57 [0.11-2.87]	0.498		
Hypertriglyceridemia°@	8.4	0	2.06 [0.79-5.37]	0.141	2.98 [1.11-8.01]	0.030

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Hypercholesterolemia°@	6.5	0.1	0.95 [0.22-3.97]	0.939		
Personal history of CHD°@	1.2	0	9.39 [2.22-39.8]	0.002		
Family history of CHD°@	28.0	0	1.82 [0.89-3.71]	0.102	2.25 [1.08-4.71]	0.031
Personal history of hypertension°@	6.1	0	2.73 [1.05-7.13]	0.040		

CADE = coronary or other arterial disease event; ART = antiretroviral therapy; CHD = coronary heart disease

SD = standard deviation; (A)HR = (adjusted) hazard ratio; CI = confidence interval

° Fixed variable (measured at M0, M1 or M12); * Time-varying variable (the last available value before each visit); percentages and averages are computed on all follow-up visits for time-varying variables

@ At M12; § Percentages and averages are computed at the end of the follow-up (last available visit for each patient)

Depressive symptoms: the Centre for Epidemiologic Studies Depression Scale (CES-D) scores is >17 for men and >23 for women

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