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ORIGINAL RESEARCH

Relationship between plasma bilirubin level and oxidative stress markers in HIV-infected patients on atazanavir- *vs.* efavirenz-based antiretroviral therapy

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Objectives

Chronic oxidative stress (OS) may play a role in cardiovascular disease in HIV-infected patients, and increased bilirubin levels may have a beneficial role in counteracting OS. Atazanavir (ATV) inhibits UDP-glucuronosyl-transferase 1A1 (UGT1A1), thus increasing unconjugated bilirubin levels. We aimed to compare changes in OS markers in patients on ATV/ritonavir (ATV/r)- *vs.* efavirenz (EFV)-based first-line antiretroviral therapy (ART).

Methods

A multicentre, prospective cohort study of HIV-infected patients who started first-line ART with either ATV/r or EFV was conducted. Lipoprotein-associated phospholipase A2 (Lp-PLA2), myeloperoxidase (MPO) and oxidized low-density lipoprotein (oxLDL) were measured for 145 patients in samples obtained at baseline and after at least 9 months of ART during which the initial regimen was maintained and the patient was virologically suppressed. The change in OS markers was modelled using multiple linear regressions adjusting for baseline values and confounders.

Results

After adjustment for baseline variables, patients on ATV/r had a significantly greater decrease in Lp-PLA2 [estimated difference −16.3; 95% confidence interval (CI) −31.4, −1.25; $P = 0.03$] and a significantly smaller increase in OxLDL (estimated difference −21.8; 95% CI −38.0, −5.6; $P < 0.01$) relative to those on EFV, whereas changes in MPO were not significantly different (estimated difference 1.2; 95% CI −14.3, 16.7; $P = 0.88$). Adjusted changes in bilirubin were significantly greater for the ATV/r group than for the EFV group (estimated difference 1.33 mg/dL; 95% CI 1.03, 1.52 mg/dL; $P < 0.01$). Changes in bilirubin and changes in OS markers were significantly correlated.

Conclusions

When compared with EFV, ATV/r-based therapy was associated with lower levels of oxidative stress biomarkers, which was in part attributable to increased bilirubin levels.

Keywords: atazanavir, bilirubin, efavirenz, oxidative stress, prospective studies

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Spanish AIDS Research Network (CoRIS) are present in Appendix 1.

Introduction

Oxidative stress (OS) may be the result of an imbalance between the production of reactive oxygen species (ROS) and the ability to detoxify oxygen reactive intermediates or repair the resulting damage. A substantial body of evidence now implicates OS in the pathogenesis of

atherosclerosis [1]. ROS generated during cellular metabolic pathways modifies polyunsaturated fatty acids and lipoproteins, resulting in proinflammatory and atherogenic particles such as oxidized low-density lipoprotein (OxLDL) [2]. In addition, high levels of ROS may contribute to endothelial dysfunction through nitric oxide inactivation [3].

Cardiovascular disease (CVD) is one of the main causes of death in the general population and represents a significant cause of morbidity and mortality in HIV-infected patients [4]. CVD occurs in this population at earlier ages than expected, probably reflecting a state of premature aging (PA). The factors involved in PA have not been adequately addressed, but a critical factor involved in the cellular and molecular pathogenesis of accelerated aging is chronic low-grade inflammation [5]. Chronic inflammation eventually induces mitochondrial dysfunction which subsequently results in increased OS [6].

There is increasing evidence suggesting that bilirubin, the metabolic end product of haem catabolism, may have anti-inflammatory properties as well as a potent beneficial antioxidant effect through inhibition of the oxidation of LDL, scavenging of oxygen radicals, and counteracting of OS [7,8]. Epidemiological studies have shown an inverse relationship between serum bilirubin and CVD [9]. Gilbert syndrome (GS), a genetic disorder that affects the enzyme responsible for bilirubin metabolism, UDP-glucuronosyl-transferase 1A1 (UGT1A1), is associated with a low prevalence of ischaemic heart disease [10]. A population study performed as part of the Framingham Heart Study showed that homozygote UGT1A1*28 allele carriers have increased serum bilirubin concentrations and a lower risk of CVD [11]. Genome-wide studies confirmed that genotype (TA)7/(TA)7 carriers of UGT1A*1 had significantly higher bilirubin levels and approximately one-third the risk of CVD compared with carriers of the wild-type (TA)6 allele [12].

Atazanavir (ATV) is a potent HIV protease inhibitor (PI) recommended in all HIV treatment guidelines as part of preferred initial therapy for HIV infection. Its efficacy, safety and tolerability have been demonstrated in both treatment-naïve and treatment-experienced patients [13]. Hyperbilirubinaemia is a common adverse event because ATV is an inhibitor of UGT1A1. There is a significant correlation between plasma concentrations of ATV and bilirubin levels [14]. Thus, bilirubin levels are higher in patients treated with ATV/ritonavir (ATV/r) regardless of UGT1A1 genotype.

We hypothesized that HIV-infected patients on ATV-based therapy would show reduced OS associated with increased plasma bilirubin levels. Therefore, we measured OS biomarkers in blood samples from a cohort of patients starting ATV-based therapy, and compared them with those from a similar population of patients starting non-ATV-based, standard antiretroviral therapy (ART) [efavirenz (EFV)-based ART].

Methods

Study design and population

The study was conducted with patients from the cohort of the Spanish AIDS Research Network (CoRIS) (see Appendix). This is an open, multicentre, prospective cohort of HIV-infected patients over 13 years of age with confirmed HIV infection who were naïve to ART at entry and seen for the first time from 1 January 2004 to 31 May 2012 in any of the 32 participating centres. Samples from patients were kindly provided by the HIV BioBank integrated in the AIDS Research Network, where baseline and follow-up blood samples from CoRIS participants are stored. Ethics approval was obtained from all hospitals ethics committees and every patient provided written informed consent. Detailed descriptions of the cohort and biobank have been previously published [15]. The information is subject to internal quality controls; once every 2 years, information on 10% of the cohort is audited by an externally contracted agency. The biobank possesses a certification of quality under Regulation UNE-EN-ISO 9001:2008 Systems of Quality Management Requirements. UNE-EN-ISO 9001:2008 is a regulation for implementing the Systems of Quality Management that has permitted the standardization and documentation of all the procedures and tasks carried out in the biobank.

We selected previously ART-naïve patients from CoRIS who started first-line ART with either an ATV/r- or EFV-based regimen plus a combination of nucleos(t)ide reverse transcriptase inhibitors (NRTIs), and who had a baseline biobank sample taken within 6 months before ART initiation and a follow-up sample taken after at least 9 months of ART while maintaining the initial regimen and being virologically suppressed. Positive serologies for either hepatitis C or B virus were exclusion criteria. Sample size was calculated for a difference of 50% between groups, 80% power and a 1:2 ratio, resulting in 48 patients for the ATV group and 96 for the EFV group.

Variables

Basic clinical and epidemiological information was obtained from CoRIS, and an ad hoc data collection form was filled in by the clinicians to retrieve additional data. The following markers of OS were measured in paired samples at baseline and follow-up: lipoprotein-associated

phospholipase A2 (Lp-PLA2), myeloperoxidase (MPO) and oxidized LDL (OxLDL).

Measurement of plasma levels of Lp-PLA2, MPO and OxLDL

Commercial enzyme-linked immunosorbent assay (ELISA) kits were used for measurement of the levels of Lp-PLA2 (R&D Systems, Abingdon, UK), MPO (Mercodia, Uppsala, Sweden) and OxLDL (Mercodia) according to the manufacturers' guidelines. For the Lp-PLA2 ELISA, intra-assay variations were < 7% and inter-assay variations were < 10%. The detection range of the assay was 0.781–50 ng/mL. For the MPO ELISA, intra-assay variations were < 5% and inter-assay variations were < 10%. The detection range of the assay was 3.5–200 μL. For the OxLDL assay, intra-assay and inter-assay variations were < 7.5% and < 6.5%, respectively, and the detection range was 1.4–22.5 mU/L.

Statistical analyses

Categorical and continuous variables were described using proportions and medians [with interquartile ranges (IQRs)] and means [with standard deviations (SD)], respectively. The χ^2 test, the nonparametric Mann–Whitney *U*-test or Student's *t*-test was used as appropriate to compare variables across groups. Correlations between the annual change in total bilirubin and annual changes in Lp-PLA2, MPO and OxLDL were explored using Pearson's correlation tests.

Total follow-up time was defined as the time that elapsed between the date of ART initiation and the date on which the follow-up sample was taken. Lp-PLA2, MPO and OxLDL values at 1 year of follow-up were interpolated from available data and used as an endpoint. A similar procedure was used for total bilirubin levels, which were obtained from clinical records. Annual change from baseline in these parameters and the difference in annual change between groups were calculated.

The effect of treatment with ATZ/r *vs*. EFV on annual change in Lp-PLA2, MPO, OxLDL and total bilirubin was estimated using multiple linear regression analysis adjusting by baseline level, to minimize regression to the mean. Models were adjusted by potential baseline confounders, including: sex (male or female), age at ART initiation (< 30, 30 to < 35, 35 to < 45 or ≥ 45 years), transmission category [men who have sex with men (MSM), heterosexual or other/unknown], education level (maximum of lower secondary, or upper secondary or higher), geographical origin (Spanish or other), baseline CD4 T-cell count (< 100, 100 to < 350 or ≥ 350 cells/μL), baseline plasma HIV RNA (< 4, 4 to < 5 or ≥ 5 log HIV-1 RNA copies/mL), AIDS diagnosis ever (no or yes), baseline body mass index (BMI) (< 20, 20 to < 25 or ≥ 25), use of tobacco during the study (no or yes), hypertension (no or yes), diabetes (no or yes) and baseline LDL cholesterol level (< 130, 130 to < 160, 160 to < 190 or ≥ 190 mg/dL). For OS markers, total baseline bilirubin was also considered a potential confounder, and was categorized in quartiles (< 0.39, 0.39 to < 0.48, 0.48 to < 0.57 and ≥ 0.57 mg/dL). Confounding was evaluated using a threshold of 10% change in the regression coefficient.

Multiple imputations using chained equations (MICE) were used to deal with missing values. A logistic model was used for education level and tobacco use, a multinomial model for BMI and LDL cholesterol, and linear regression for total bilirubin at baseline and follow-up, with all models being adjusted by all baseline confounders, including OS markers. Fifteen replicas were created, and Rubin's rules were applied for the estimation of 95% confidence intervals (CIs) and calculation of *P*-values. Wald tests were used to derive *P*-values. All analyses were conducted using STATA software (V.12.0MP; Stata Corporation, College Station, TX, USA).

Results

The study included 145 subjects, 48 on ATV/r-based and 97 on EFV-based therapy. The NRTI backbone was in all cases tenofovir 300 mg and emtricitabine 200 mg, coformulated as a single tablet (Truvada) or in Atripla (Gilead Sciences, Inc Foster City, California, USA) in patients on EFV. The main clinical characteristics are shown in Table 1. The two groups were well balanced for baseline values, apart from higher total bilirubin levels in the EFV group and higher MPO levels in the ATV group. The time from treatment initiation to the follow-up sample being taken was close to 6 weeks longer in the ATV group. We imputed baseline total, high-density lipoprotein (HDL) and LDL cholesterol for four, six and seven patients, respectively, who had missing values, all from the EFV group. For bilirubin, we imputed baseline values for 17 patients in the EFV group and four patients in the ATZ group, and follow-up values for 14 patients in the EFV group and nine patients in the ATV group. The distributions of the variables remained virtually unchanged for both groups (data not shown).

OS and bilirubin changes after 1 year of follow-up were interpolated in the imputed data sets and are shown in Table 2. A significant increase in bilirubin levels was observed only in the ATV/r group, as expected [1.25 mg/dL (0.96, 1.54); $P < 0.01$], while a small decrease was found for patients on EFV [−0.13 mg/dL (−0.19, −0.07);

Table 1 Baseline characteristics according to treatment group

	Efavirenz (n = 97)		Atazanavir (n = 48)		P
Age (years) [median (IQR)]	35.9 (29.9–41.6)		37.0 (29.4–42.9)		0.98
Gender, male [n (%)]	83	(85.5)	37	(77.1)	0.20
Transmission category [n (%)]					
MSM	64	(65.9)	27	(56.2)	0.48
Heterosexual	28	(28.8)	17	(35.4)	
Other/unknown	5	(5.1)	4	(8.3)	
AIDS diagnosis [n (%)]	18	(18.5)	6	(12.5)	0.36
CD4 count (cells/mL) [median (IQR)]	263	(160–355)	289.5	(189.5–399)	0.31
HIV-1 VL (log copies/mL) [median (IQR)]	4.68	(3.9–5.2)	4.69	(3.9–5.2)	0.73
BMI (kg/m^2) [median (IQR)]*	23.4	(21.8–25.2)	23.3	(21.2–25.5)	0.88
Smoking [n (%)]	38	(39.1)	24	(50.0)	0.46
Hypertension [n (%)]	8	(8.2)	7	(14.5)	0.24
Type 2 diabetes [n (%)]	6	(6.1)	2	(4.1)	0.62
Cholesterol (mg/dL) [median (IQR)]†					
Total	163	(133–192)	157.5	(137.5–183]	0.48
HDL	41	(31–45)	39.5	(31–44.5)	0.62
LDL	101.5	(80–127)	96	(72.5–109.5)	0.09
Lipid-lowering therapy [n (%)]	4	(4.1)	3	(6.2)	0.57
Baseline bilirubin (total; mg/dL) [mean (SD)]	0.53	(0.2)	0.45	(0.1)	0.03
Lp-PLA2 (ng/mL) [mean (SD)]	142.2	(72.8)	150.1	(92.8)	0.93
MPO (μg/L) [mean (SD)]	74.3	(48.2)	93.9	(64.3)	0.02
OxLDL (U/L) [mean (SD)]	76.3	(52.3)	82.2	(54.4)	0.40
Follow-up (days) [median (IQR)]	390	(357–495)	429.5	(355.5–669)	< 0.01

BMI, body mass index; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; Lp-PLA2, lipoprotein-associated phospholipase A2; MPO, myeloperoxidase; MSM, men who have sex with men; OxLDL, oxidized low-density lipoprotein; SD, standard deviation; VL, viral load.

Table 2 Change at 1 year in oxidative stress markers and linear regression analysis for the treatment effect of ritonavir-boosted atazanavir (ATV/r) compared with efavirenz (EFV)

	Lp-PLA2 (ng/mL)		MPO (μg/L)		OxLDL (U/L)	
	M/Coef (95% CI)	P	M/Coef (95% CI)	P	M/Coef (95% CI)	P
One-year change*						
EFV	−3.7 (−16.0, 8.6)	0.55	−0.8 (−9.5, 7.9)	0.85	26.1 (14.9, 37.3)	< 0.01
ATZ	−22.6 (−40.4, −4.7)	0.01	−5.4 (−19.4, 8.6)	0.44	3.5 (−6.3, 13.4)	0.48
Crude difference	−18.9 (−40.2, 2.4)	0.08	−4.6 (−20.2, 11.1)	0.57	−22.6 (−39.8, −5.3)	0.01
Adjusted for baseline values	−14.6 (−29.7, 0.4)	0.06	3.7 (−10.0, 17.5)	0.59	−20.6 (−36.8, −4.4)	0.01
Adjusted for confounders†	−16.3 (−31.4, −1.25)	0.03	1.2 (−14.3, 16.7)	0.88	−21.8 (−38.0, −5.6)	< 0.01
Adjusted for all baseline variables‡	−14.9 (−31.7,2.0)	0.08	1.2 (−14.3,16.7)	0.88	−24.6 (−42.8, −6.3)	< 0.01

M, mean (for 1-year change); Coef, regression coefficient (for all other estimates); CI, confidence interval; Lp-PLA2, lipoprotein-associated phospholipase A2; MPO, myeloperoxidase; OxLDL, oxidized low-density lipoprotein.
*P-values for the null hypothesis of mean change equal to zero (Student's t-test).
†Lp-PLA2: adjusted for baseline CD4 count, transmission category and baseline total cholesterol; MPO: adjusted for baseline CD4 count, transmission category, gender, place of birth, education level, baseline VL, AIDS diagnosis, Arterial Hypertension (HTA), type 2 diabetes, baseline total cholesterol and baseline total and conjugated bilirubin; OxLDL: adjusted for baseline CD4 count and smoking.
‡Gender, education level, age, transmission category, place of birth, HTA, type 2 diabetes, smoking, AIDS, CD4 count, viral load, body mass index, total cholesterol, and total and conjugated bilirubin.

$P < 0.01$]. Lp-PLA2 and MPO levels decreased in both treatment arms, although the decrease was only of substantial magnitude and statistical significance for the reduction of Lp-PLA2 in the ATV group [change at 1 year −22.6 ng/mL (−40.4, −4.7); $P = 0.01$]. OxLDL levels increased after the initiation of ART in both the EFV and ATV/r arms, with the increase being greater and of statistical significance in the EFV group [change at 1 year 26.1 U/L (14.9, 37.3); $P < 0.01$] (Fig. 1).

After adjustment for baseline variables and confounders, patients on ATV/r had a significantly greater decrease in Lp-PLA2 [estimated difference −16.3 (95% CI −31.4, −1.25); $P = 0.03$] and a significantly smaller increase in OxLDL [estimated difference −21.8 (95% CI −38.0, −5.6); $P < 0.01$] relative to those on EFV, whereas changes in MPO did not differ [estimated difference 1.2 (−14.3, 16.7); $P = 0.88$]. Adjusted changes in bilirubin were significantly greater for the ATV/r group than for

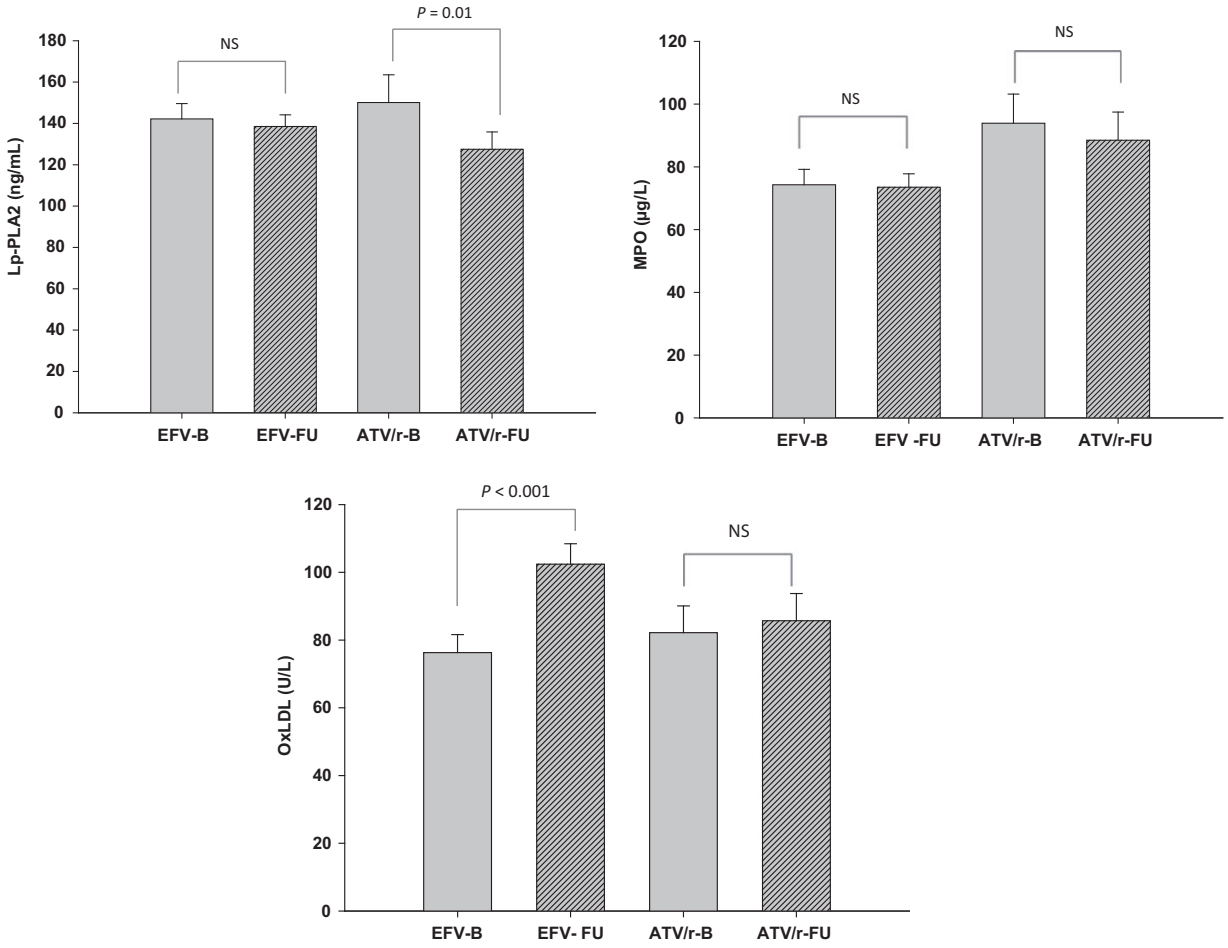

Fig. 1 Oxidative stress biomarker variations during the study period. Values shown are mean plus standard error. EFV, efavirenz; ATV/r, atazanavir/ritonavir; B, baseline; FU, follow-up; NS, nonsignificant; Lp-PLA2, lipoprotein-associated phospholipase A2; MPO, myeloperoxidase; OxLDL, oxidized low-density lipoprotein.

the EFV group [estimated difference 1.33 (1.03, 1.52); $P < 0.01$]. Additional adjustment for the remaining baseline variables that did not fulfill criteria for confounding did not substantially change point estimates, although statistical precision was reduced.

The annual increase in total bilirubin was weakly but significantly correlated with decreases in Lp-PLA2 ($r = -0.22$; $P = 0.01$) and OxLDL ($r = -0.18$; $P = 0.03$) but not with changes in MPO ($r = -0.03$; $P = 0.71$).

Discussion

The present study revealed two main findings. First, the OS biomarkers Lp-PLA2 and Ox-LDL were significantly reduced after ATV/r initiation. Secondly, there was a significant correlation between bilirubin level and changes in OS biomarkers. These data confirm our initial hypothesis that HIV-infected patients on ATV-based therapy would present with reduced OS biomarkers associated with increased plasma bilirubin levels.

The effect of contemporary antiretroviral drugs on OS has not been studied extensively. HIV infection by itself causes OS, which may increase in patients receiving ART [16], in particular those treated with regimens with high mitochondrial toxicity, such as thymidine analogue NRTIs (t-NRTIs). OS and mitochondrial toxicity can partly explain signs of cellular senescence and adipose tissue toxicity induced by t-NRTIs [17]. *In vitro* studies have shown that EFV may cause mitochondrial toxicity and OS of doubtful clinical significance [18]. Similarly, the use of PIs is associated with increased OS biomarkers. It has been proposed that PIs may increase ROS production which induces cell death, impaired mitochondrial function and ubiquitin-proteasome system (UPS) dysregula-

tion [19]. In contrast, Hileman *et al.* [20] reported that neither ATV/r nor higher total bilirubin levels were associated with significant variations of OS and inflammation biomarkers in patients on stable ART. Meanwhile, Dekker *et al.* [21] observed in a double-blind and randomized crossover study that subjects with type 2 diabetes mellitus receiving a 3-day ATV treatment showed a significant improvement in plasma antioxidant capacity.

According to our findings, ART is not associated *per se* with an increase in OS markers. Our data show that in patients on ATV/r-based therapy there was a significant decrease in OS biomarkers (OxLDL and Lp-PLA2) from baseline, suggesting that the primary factor responsible for OS is HIV infection itself.

The different biomarkers used when studying OS may in part explain the different results obtained in previously published studies [22]. We analysed OxLDL, Lp-PLA2 and MPO as well-known biomarkers reflecting oxidative damage. MPO is an extensively studied biomarker of inflammation and oxidation, which also has potential diagnostic and prognostic utility in CVD [23]. OxLDL is a recognized marker of OS, and increased plasma levels are associated with coronary artery disease [24]. HIV-infected patients on ART show increased OxLDL levels, and associations have been found between OxLDL and lipodystrophy [25], increased CVD risk and subclinical atherosclerosis [26]. Considering the role of OxLDL in the development of atherosclerosis, it has been suggested that increased plasma bilirubin levels could reduce this atherogenic risk. Lp-PLA2 is an accepted CVD risk biomarker, and increased values have been described in HIV-infected patients [27], in particular in those on PI-based therapy and with high coronary artery calcium scores [28].

To our knowledge, this is the first study to explore prospectively OS biomarkers in HIV-infected patients after ART initiation. The reduction in OS biomarkers observed with viral suppression supports the concept of a cardioprotective effect of ART. A previously unreported finding is the reduction of Lp-PLA2 and OxLDL levels in patients on ATV/r-based therapy, which correlated with increased levels of bilirubin. These results suggest that bilirubin might exert some protective effect on OS. These findings have been described in the general population in patients with Gilbert syndrome. In this condition, there is also a significant relationship between OS, serum concentration of bilirubin and endothelial function [29]. There is a growing body of evidence suggesting a beneficial role of increased bilirubin as a protective factor for CVD. Some studies found a strong inverse and independent relationship between conjugated bilirubin and coronary artery calcium score [30]. Also, an inverse association has been found between bilirubin levels and the incidence of metabolic syndrome in a healthy population [31].

The interpretation of the potential clinical relevance of these findings is difficult. In large epidemiological studies, the use of ATV has not been associated with an increased CVD risk [32]. CVD is the final result of many pathogenic pathways, including traditional risk factors, such as smoking, hyperlipidaemia, hypertension and diabetes. Chronic low-grade inflammation and OS might have a relevant pathogenic role in individual patients, but this is still a matter of debate.

The study has limitations. The allocation of patients to ART was not randomized. Nevertheless, although cardiovascular risk factors may have contributed to the decision to prescribe an ATV/r-based regimen, the main risk factors (smoking, hypertension and diabetes) were well balanced between the two arms, including those that are not modifiable, i.e. age, sex and race. In clinical practice, patients on PI therapy often have some differential features compared with those receiving EFV. Those patients who are expected to have poorer compliance with ART often receive PIs, and this might have influenced the results of the study. However, the differences found between study arms at baseline were limited to higher MPO levels in patients receiving ATV/r. These differences would go against the hypothesis of the study, so we consider that the final results are unlikely to be explained by these differences.

In contrast, the strengths of this study are the large number of participants, providing adequate power to address the research question; the use of sera collected, processed and banked under rigorous standardized conditions; and, finally, the prospective design which reflects the "real world" situation, as patient care is completely entrusted to the physician.

In summary, this study demonstrated that there was a significant reduction in plasma levels of OS biomarkers in patients after the initiation of ART and that this decline was correlated to plasma levels of bilirubin. These results merit further investigation in randomized clinical trials.

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Conflicts of interest: VE has received honoraria, speakers' fees and/or funds for research from Abbvie, Gilead, Merck Sharp & Dohme, Bristol-Myers Squibb, ViiV Healthcare and Janssen. MM has received honoraria, speakers' fees and/or funds for research from Janssen, Merck Sharp & Dohme, Bristol-Myers Squibb and ViiV Healthcare. JB has received honoraria, speakers' fees and/or funds for research from Abbvie, Bristol-Myers Squibb, Gilead, Janssen, Merck Sharp & Dohme and ViiV Healthcare. JP has received honoraria, speakers' fees and/or funds for research from Janssen, Merck Sharp & Dohme, Gilead, Bristol-Myers Squibb and ViiV Healthcare. JB has received honoraria, speakers' fees and/or funds for research from Abbvie, Bristol-Myers Squibb, Gilead, Janssen, Merck Sharp & Dohme and ViiV Healthcare. EM has received honoraria, speakers' fees and/or funds for research from Abbott, Abbvie, Boehringer-Ingelheim, Bristol-Myers Squibb, Gilead, GlaxoSmithKline, Merck Sharp & Dohme, Theratechnologies, Tibotec, and ViiV Healthcare. JRB has received honoraria, speakers' fees and/or funds for research from Abbvie, Bristol-Myers Squibb, Gilead, Janssen, Merck Sharp & Dohme and ViiV Healthcare. SM, DG-G, PS and CV have no potential conflicts of interest to declare.

Appendix 1: Centres and investigators involved in CoRIS

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References

1 Hulsmans M, Van Dooren E, Holvoet P. Mitochondrial reactive oxygen species and risk of atherosclerosis. *Curr Atheroscler Rep* 2012; **14**: 264–276.

2 Mitra S, Deshmukh A, Sachdeva R *et al*. Oxidized low-density lipoprotein and atherosclerosis implications in antioxidant therapy. *Am J Med Sci* 2011; **342**: 135–142.

3 Heitzer T, Schlinzig T, Krohn K *et al.* Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation* 2001; **104**: 2673–2678.

4 Smith CJ, Ryom L, Weber R *et al.* Trends in underlying causes of death in people with HIV from 1999 to 2011 (D:A:D): a multicohort collaboration. *Lancet* 2014; **384**: 241–248.

5 Pawelec G, Goldeck D, Derhovanessian E. Inflammation, ageing and chronic disease. *Curr Opin Immunol* 2014; **29**: 23–28.

6 Hajjar DP, Gotto AM Jr. Biological relevance of inflammation and oxidative stress in the pathogenesis of arterial diseases. *Am J Pathol* 2013; **182**: 1474–1481.

7 Zelenka J, Muchova L, Zelenkova M *et al.* Intracellular accumulation of bilirubin as a defense mechanism against increased oxidative stress. *Biochimie* 2012; **94**: 1821–1827.

8 Sedlak TW, Saleh M, Higginson DS *et al.* Bilirubin and glutathione have complementary antioxidant and cytoprotective roles. *Proc Natl Acad Sci USA* 2009; **106**: 5171–5176.

9 Novotny L, Vitek L. Inverse relationship between serum bilirubin and atherosclerosis in men: a meta-analysis of published studies. *Exp Biol Med* (Maywood, NJ). 2003;**228**:568–571.

10 Vitek L, Jirsa M, Brodanova M *et al.* Gilbert syndrome and ischemic heart disease: a protective effect of elevated bilirubin levels. *Atherosclerosis* 2002; **160**: 449–456.

11 Lin JP, O'Donnell CJ, Schwaiger JP *et al.* Association between the UGT1A1*28 allele, bilirubin levels, and coronary heart disease in the Framingham Heart Study. *Circulation* 2006; **114**: 1476–1481.

12 Lin JP, Schwaiger JP, Cupples LA *et al.* Conditional linkage and genome-wide association studies identify UGT1A1 as a major gene for anti-atherogenic serum bilirubin levels–the Framingham Heart Study. *Atherosclerosis* 2009; **206**: 228–233.

13 Swainston Harrison T, Scott LJ. Atazanavir: a review of its use in the management of HIV infection. *Drugs* 2005;**65**:2309–2336.

14 Lozano R, Domeque N, Apesteguia AF. Atazanavir-bilirubin interaction: a pharmacokinetic-pharmacodynamic model. *Clin Pharmacol* 2013; **5**: 153–159.

15 Sobrino-Vegas P, Gutierrez F, Berenguer J *et al.* The Cohort of the Spanish HIV Research Network (CoRIS) and its associated biobank; organizational issues, main findings and losses to follow-up. *Enferm Infecc Microbiol Clin* 2011; **29**: 645–653.

16 Sharma B. Oxidative stress in HIV patients receiving antiretroviral therapy. *Curr HIV Res* 2014; **12**: 13–21.

17 Caron M, Auclairt M, Vissian A *et al.* Contribution of mitochondrial dysfunction and oxidative stress to cellular premature senescence induced by antiretroviral thymidine analogues. *Antivir Ther* 2008; **13**: 27–38.

18 Blas-Garcia A, Polo M, Alegre F *et al.* Lack of mitochondrial toxicity of darunavir, raltegravir and rilpivirine in neurons and hepatocytes: a comparison with efavirenz. *J Antimicrob Chemother* 2014; **69**: 2995–3000.

19 Reyskens KM, Essop MF. HIV protease inhibitors and onset of cardiovascular diseases: a central role for oxidative stress and dysregulation of the ubiquitin-proteasome system. *Biochim Biophys Acta* 2014; **1842**: 256–268.

20 Hileman C, Longenecker C, Carman T *et al.* Relationship between total bilirubin and endothelial function, inflammation and oxidative stress in HIV-infected adults on stable antiretroviral therapy. *HIV Med* 2012; **13**: 609–616.

21 Dekker D, Dorresteijn MJ, Pijnenburg M *et al.* The bilirubin-increasing drug atazanavir improves endothelial function in patients with type 2 diabetes mellitus. *Arterioscler Thromb Vasc Biol* 2011; **31**: 458–463.

22 Dalle-Donne I, Rossi R, Colombo R *et al.* Biomarkers of oxidative damage in human disease. *Clin Chem* 2006; **52**: 601–623.

23 Anatoliotakis N, Deftereos S, Bouras G *et al.* Myeloperoxidase: expressing inflammation and oxidative stress in cardiovascular disease. *Curr Top Med Chem* 2013; **13**: 115–138.

24 Indermuehle A, Crake T, Meier P. Oxidized low-density lipoprotein cholesterol and coronary artery disease. *Cardiology* 2011; **119**: 106–107.

25 Duong M, Petit JM, Martha B *et al.* Concentration of circulating oxidized LDL in HIV-infected patients treated with antiretroviral agents: relation to HIV-related lipodystrophy. *HIV Clin Trials* 2006; **7**: 41–47.

26 Parra S, Coll B, Aragones G *et al.* Nonconcordance between subclinical atherosclerosis and the calculated Framingham risk score in HIV-infected patients: relationships with serum markers of oxidation and inflammation. *HIV Med* 2010; **11**: 225–231.

27 Ross Eckard A, Longenecker C, Jiang Y *et al.* Lipoprotein-associated phospholipase A2 and cardiovascular disease risk in HIV infection. *HIV Med* 2014;**15**:537–546.

28 Mangili A, Ahmad R, Wolfert RL *et al.* Lipoprotein-associated phospholipase A2, a novel cardiovascular inflammatory marker, in HIV-infected patients. *Clin Infect Dis* 2014; **58**: 893–900.

29 Maruhashi T, Soga J, Fujimura N *et al.* Hyperbilirubinemia, augmentation of endothelial function, and decrease in oxidative stress in Gilbert syndrome. *Circulation* 2012; **126**: 598–603.

30 Sung KC, Shin J, Lim YH *et al*. Relation of conjugated bilirubin concentrations to the presence of coronary artery calcium. *Am J Cardiol* 2013; **112**: 1873–1879.
31 Lee MJ, Jung CH, Kang YM *et al*. Serum bilirubin as a predictor of incident metabolic syndrome: a 4-year retrospective longitudinal study of 6205 initially healthy Korean men. *Diabetes Metab* 2014; **40**: 305–309.
32 Monforte A, Reiss P, Ryom L *et al*. Atazanavir is not associated with an increased risk of cardio- or cerebrovascular disease events. *AIDS* 2013; **27**: 407–415.

