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Abstract

Aim. – Ageing HIV-infected patients controlled by antiretroviral therapy (ART) frequently present age-related comorbidities, such as cardiovascular (CV) events, diabetes, dyslipidaemia, hypertension and chronic kidney disease (CKD). The prevalence of these comorbidities was evaluated in a cohort of long-term-monitored ART-controlled HIV-infected patients, then followed by a search into whether oxidative stress, like inflammation, might be associated with metabolic parameters and/or comorbidities.

Methods. – Included were 352 long-term ART patients who started with protease inhibitors (PIs) in 1997–1999. They were evaluated at their final visit, 11 years later, for previous CV events, prevalence of diabetes, LDL-related and atherogenic (high TG/HDL) dyslipidaemias, hypertension and CKD. Also measured were circulating biomarkers to explore oxidative stress (Lp-PLA2, oxLDL, oxLDL/LDL ratio, paraoxonase and arylesterase activities), inflammation/immune activation (hsCRP, hsIL-6, D dimer, soluble CD14, β2 microglobulin, cystatin C), adipokines and insulin resistance. Levels were compared in patients with and without each comorbidity or condition using non-parametric correlation tests and multivariate adjusted analyses.

Results. – At the final visit, 81.5% of patients were male and were aged (median, IQR) 49 years (45–56); BMI was 23.0 kg/m^2 (21.1–25.4), CD4+ lymphocytes were 620 cells/mm^3 (453–790) and 91.5% had undetectable HIV-1 viral loads. The prevalence of diabetes was 11%, and LDL-related dyslipidaemia 28%, atherogenic dyslipidaemia 9%, hypertension 28%, CKD 9% and previous CV events 9%. Diabetes and atherogenic dyslipidaemia were associated with increased oxidative stress and independently with inflammation. LDL-related dyslipidaemia and impaired fasting glucose were associated with increased oxidative stress. No association of these biomarkers was detected with hypertension, CKD and previous CV events.

Conclusion. – In long-term-treated HIV-infected patients with frequent comorbid conditions, oxidative stress could be contributing to diabetes and LDL-related and atherogenic dyslipidaemias independently of inflammation.

Key words: Diabetes; Dyslipidaemia; HIV-infected patients; Oxidative stress; Inflammation

Introduction

Ageing human immunodeficiency virus (HIV)-infected patients present with a high prevalence of age-related comorbidities, including cardiovascular disease (CVD) and those associated with increased CV risk, such as diabetes, hypertension, dyslipidaemia and chronic kidney disease (CKD) [1–3]. This prevalence is higher than that observed in HIV-negative subjects [2], and could result from impaired levels of metabolic parameters commonly observed in such patients as well as with other classic factors, such as smoking, age, and familial histories of diabetes and CVD. Atherogenic dyslipidaemia [low levels of high-density lipoprotein (HDL) and high levels of triglycerides (TG)] can also contribute to CVD risk in these patients, and may be assessed by measuring TG/HDL ratios [4,5].

Impaired levels of metabolic factors could result from HIV infection itself, but also from antiretroviral treatment (ART). In particular, increased risks of diabetes, dyslipidaemia and/or CVD have been reported in patients receiving a protease inhibitor (PI) boosted by ritonavir (r) [6–9]. In addition, increased risks of diabetes, hypertension, hypercholesterolaemia and low HDL have been associated with previous treatment with first-generation non-nucleoside analogues, such as stavudine and zidovudine, and possibly related to long-lasting alterations in adipose tissue distribution [10–13].

Previously, our team evaluated the risk of diabetes in 1046 HIV-infected patients from the APROCO-COPILOTE cohort who started treatment in 1997–1998 with a first-generation PI-containing regimen, which was regularly followed and ultimately evaluated 10 years after inclusion. A high incidence of diabetes was observed, linked to the use of some ART agents, including indinavir, stavudine, zidovudine and didanosine [11]. Moreover, our team also previously reported that 352 patients from the same cohort, upon close evaluation 11 years after inclusion and despite having good immunovirological status, presented with increased levels of inflammation and immune activation compared with healthy controls [14].

The present report of the same group of 352 patients here presents additional findings on a panel of biomarkers evaluating oxidative stress [lipoprotein-associated phospholipase A2 (Lp-PLA2), oxidized low-density lipoprotein (oxLDL), oxLDL/LDL ratios, paraoxonase and arylesterase activities], insulin resistance and adipokines [adiponectin, leptin, homoeostasis model assessment of insulin resistance (HOMA-IR) and plasminogen activator inhibitor 1 (PAI-1)]. Our main objective was to search for any association between these biomarkers and diabetes, LDL-linked and atherogenic dyslipidaemias, hypertension, CKD and previous CV events in addition to inflammation and immune activation.

Patients and methods

Patients

The study population has previously been described elsewhere [14]. Briefly, the 352 patients included here were from the French APROCO-COPILOTE cohort, which enrolled patients between 1997 and 1999 when they initiated PI-containing ART. All received first-generation ART, stavudine and/or zidovudine in combination with indinavir, nelfinavir, saquinavir or full-dose ritonavir.

The ethics committee of Cochin Hospital in Paris, France, approved the study, and informed consent was obtained from all participants. Patients were prospectively followed using standardized case-report forms, and all underwent complete clinical and biological evaluations after a median duration of 11 years of follow-up [interquartile range (IQR): 10–12]. All patients' characteristics (including clinical evaluation of fat distribution), metabolic and adipose-related parameters, biomarkers of oxidative stress, and inflammatory and immune activation were recorded or measured during the same final visit. Thus, the median ART duration was 12.3 years (IQR: 11.4–13.9), CD4 cell count was 620 cells/mm^3 (IQR: 453–790) and CD4/CD8 ratio was 0.81 (IQR: 0.56–1.10), with a viral load (VL) < 50 and < 400 copies/mL for 91.5% and 97.2% of patients, respectively.

Diagnosis of comorbidities

Comorbidities were also assessed at the final visit. Diabetes was diagnosed by fasting glycaemia ≥ 7.0 mmol/L and/or treatment, impaired fasting glucose (IFG) by glycaemia at 6.1–6.9 mmol/L, hypertension by systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg, LDL-linked dyslipidaemia by LDL ≥ 4.1 mmol/L and/or statin treatment, CKD by Modification of Diet in Renal Disease (MDRD) Study equation with an estimated glomerular filtration rate (eGFR) ≤ 60 mL/min, and atherogenic dyslipidaemia by a TG (mg/dL)/HDL (mg/dL) ratio ≥ 3.5 in men and ≥ 2.5 in women [5]. Insulin resistance was also evaluated by HOMA-IR score: fasting glycaemia (mmol/L) × fasting insulinaemia (mU/L)/22.5 ≥ 2.5. The lipid accumulation product (LAP) index was used as a surrogate marker of steatosis [15]: for men, waist circumference (WC) – 65 × TG (mmol/L); and for women, WC – 58 × TG (mmol/L). Also addressed were any previous CV events, defined as a diagnosis of cardiac or vascular disease reported in the patient's medical records during the 11-year follow-up.

Biomarkers

Whole blood was recovered at the final visit with/without ethylenediaminetetraacetic acid (EDTA) or citrate, and the plasma/serum was stored at -80° until assayed. The following markers were also analyzed:

Oxidative stress biomarkers as oxLDL by enzyme-linked immunosorbent assay (ELISA) (Mercodia, Uppsala, Sweden); Lp-PLA2 by immunoturbidimetric assay (Le PLAC Test, Eurobio, Courtaboeuf, France) adapted for an automated chemistry analyzer (AU480, Beckman Coulter, Brea, CA, USA); and antioxidant HDL-linked paraoxonase activities using hydrolysis rates of its two substrates, paraoxon (PON) and phenylacetate (arylesterase, ARE), as previously described [16];

Inflammatory and immune-activation biomarkers, high-sensitivity interleukin 6 (hsIL-6) and soluble CD14 (sCD14) by ELISA (Quantikine®, R&D Systems, Abingdon, OX, UK); high-sensitivity C-reactive protein (hsCRP) by immunonephelometry (IMMAGE® Immunochemistry System, Beckman Coulter); D dimer (plasma) and β2 microglobulin (B2M) by enzyme-linked fluorescent assay (ELFA) (VIDAS®, Biomérieux, Marcy-l'Etoile, France); and cystatin C by immunoturbidimetry (Architect, Abbott Laboratories, Abbott Park, IL, USA); and

Adipose-derived and metabolic biomarkers by ELISA, including leptin (Quantikine, R&D Systems), adiponectin (ALPCO Diagnostics, Salem, NH, USA) and PAI-1 (Diagnostica Stago, Asnières-sur-Seine, France).

Statistics

Continuous variables are presented as medians and 25^{th}–75^{th} percentile values (IQR), and categorical variables as numbers (n) and proportions (%). Prevalences of each comorbidity and multimorbidity were presented with 95% confidence intervals (CI).

Correlations between quantitative parameters were assessed by calculation of item-to-item Spearman correlation coefficients, whereas Kruskal–Wallis, Wilcoxon rank-sum and Fisher's exact tests were used to compare continuous and categorical variables between patients with *vs* without each comorbidity or condition. Given the number of comorbidities evaluated (diabetes, LDL-related and atherogenic dyslipidaemias, hypertension, CKD, previous CV events), differences were considered statistically significant when the two-sided P value was < 0.01. These variables were also compared after the exclusion of patients taking statins.

In addition, levels of metabolic parameters and biomarkers were evaluated according to ongoing treatment with antiretroviral agents (PIs, efavirenz, nevirapine). For multivariate

analyses of levels of oxLDL and oxLDL/LDL ratios, values for age, gender, body mass index (BMI) and inflammation were adjusted for three biomarkers: hsCRP, hsIL-6 and D dimer.

All analyses were performed by a dedicated statistician using either R version 3.4.2 software (R Foundation for Statistical Computing, Vienna, Austria) or SAS version 9.4 software (SAS Institute Inc., Cary, NC, USA).

Results

Patients' main clinical and HIV-related characteristics at the final visit, levels of metabolic and adipose-derived biomarkers, and prevalence of comorbidities and multimorbidity are presented in Table I. Patients were aged (median) 49 years (IQR: 45–56) with a median BMI of 23 kg/m^2 (IQR: 21.1–25.4). In these long-term ART patients, clinical features of lipodystrophy, associated mainly with first-generation ART regimens [17], were often evident: 27% (n = 96) presented with clinical signs of lipoatrophy; 11% (n = 37) showed trunk fat hypertrophy; and 22% (n = 76) had both peripheral lipoatrophy and trunk fat hypertrophy. The median SBP level was 123 mmHg (IQR: 118–137) with a median DBP of 80 mmHg (IQR: 70–80). In addition, 97% had received nucleoside analogues, 52% had taken PIs, 47% had been treated with non-nucleoside analogues (21% efavirenz, 26% nevirapine) and 2% with an integrase inhibitor (raltegravir).

The prevalences of metabolic comorbidities were high and according to age: previous CV events, 9% (32 out of 352, 95% CI: 7.5–10.5%); diabetes, 11% (37 out of 346, 95% CI: 8–14%); hypertension, 28% (90 out of 322, 95% CI: 23–33%); LDL-related dyslipidaemia, 27.5% (90 out of 328, 95% CI: 23–32%); atherogenic dyslipidaemia, 8% (29 out of 352, 95% CI: 5–11%); and CKD, 9% (30 out of 348, 95% CI: 6–11%). The mean HOMA-IR score was 2.0 (IQR: 1.4–3.2), and 36% (122 out of 339) were insulin-resistant.

Regarding the prevalence of multimorbidity, including previous CV events, diabetes, LDL-related and atherogenic dyslipidaemias, hypertension and CKD, 42.6% (95% CI: 40–45.2%) had no comorbidity, 34.9% (95% CI: 32.4–37.4%) had one, 16.5% (95% CI: 14.5–18.5%) had two, 4.3% (95% CI: 3.2–5.4%) had three and 1.7% (95% CI: 1.0–2.4%) had four.

Biomarker levels according to diabetes

Patients with diabetes had, as expected, higher BMIs (25.7 *vs* 22.9 kg/m^2; $P = 0.005$) and waist-to-hip ratios (WHRs; 0.99 *vs* 0.95; $P < 0.001$). Body fat distribution differed, with 61% of diabetes patients presenting with trunk fat accumulation (alone or associated with

peripheral lipoatrophy) *vs* 32% of non-diabetic subjects. Only 9% had peripheral lipoatrophy on its own *vs* 29% of non-diabetic subjects ($P < 0.001$). Diabetes patients also had increased levels of TG, TG/HDL ratios and HOMA-IR scores (5.6 *vs* 1.9; $P < 0.00001$), reduced levels of adiponectin (3.4 *vs* 6.1 mg/L; $P < 0.0001$), but higher oxLDL/LDL ratios (17.8 *vs* 15.6 U/L-mmoL; $P < 0.01$) and CRP levels (3.85 *vs* 1.94 mg/L; $P < 0.005$; Table II). On multivariate analyses adjusted for age, gender, BMI and inflammation, diabetes was associated with increased oxLDL/LDL ratios [adjusted odds ratio (ORa): 1.15, 95% CI: 1.05–1.25]. Thus, diabetes was independently associated with oxidative stress and inflammation (Fig. 1).

Patients with impaired fasting glucose (IFG) were also evaluated and compared with normoglycaemic patients: BMI did not differ and WHR was only slightly increased in IFG subjects (0.96 *vs* 0.94; $P = 0.04$). Interestingly, oxLDL levels were 60.2 U/L in IFG patients *vs* 49.9 U/L in normoglycaemic subjects ($P = 0.01$). HOMA-IR scores were also higher (3.3 *vs* 1.8; $P < 0.0001$), although CRP levels did not differ. In addition, glycaemia did not correlate with biomarkers of inflammation, immune activation or oxidative stress except for cystatin C (R = -0.16; Table III).

Patients with insulin resistance had higher levels of CRP and oxLDL/LDL ratios compared with those without insulin resistance [2.9 *vs* 1.8 mL/L ($P < 0.005$) and 16.4 *vs* 15.4 U/L-mmol/L ($P < 0.01$), respectively]. PAI-1, a marker of insulin resistance and increased CVD risk, correlated positively with oxidative stress (oxLDL and oxLDL/LDL ratio; Table III), whereas adiponectin correlated negatively with CRP and oxidative stress (Lp-PLA2, oxLDL and oxLDL/LDL ratio; Table III).

The median LAP value was 39 (IQR: 20–60). As expected, LAP was associated with metabolic parameters: HOMA-IR 0.421 ($P < 0.0001$); adiponectin -0.279 ($P < 0.0001$); leptin 0.336 ($P < 0.0001$); and PAI-1 0.319 ($P < 0.0001$). LAP was also associated with oxidative stress [oxLDL 0.439 ($P < 0.0001$) and oxLDL/LDL ratio 0.384 ($P < 0.0001$)] and with inflammation [CRP 0.251 ($P < 0.0001$) and IL-6 0.184 ($P = 0.003$)].

Biomarker levels according to LDL-related or atherogenic dyslipidaemia

Patients with *vs* without LDL-related dyslipidaemia had increased values of oxLDL (61.5 *vs* 48.3 U/L; $P < 0.0001$; Table II). On multivariate analyses adjusted for age, gender, BMI and inflammation, LDL-related dyslipidaemia was associated with increased oxLDL (ORa: 1.05, 95% CI: 1.03–1.07) and decreased oxLDL/LDL ratio (ORa: 0.90, 95% CI: 0.82–0.97; Fig. 1). Because statins alter inflammatory/immune-activation profiles, these parameters were also

evaluated in patients not receiving statins. In these patients (n = 291), those with LDL-related dyslipidaemia exhibited higher levels of oxLDL (64.4 *vs* 48.3 U/L; $P < 0.0001$) and ARE (23.8 *vs* 21.6 mmol/L-min; $P < 0.01$), and lower levels of D dimer (201 *vs* 259.5 ng/mL; $P < 0.005$) and oxLDL/LDL ratios (14.5 *vs* 16.2 U/L-mmoL; $P < 0.001$) than those without dyslipidaemia.

Thus, LDL-related dyslipidaemia was not related to treatment with PI and was associated with oxidative stress, but not with inflammation or immune activation. Moreover, LDL levels were related to biomarkers of oxidative stress (Lp-PLA2, oxLDL, oxLDL/LDL ratios, ARE; Table III), but not of inflammation and immune activation [except for B2M (R = -0.14)].

Patients with atherogenic dyslipidaemia had higher levels of oxidative stress biomarkers: oxLDL (65.5 *vs* 49.8 U/L; $P < 0.0001$); oxLDL/LDL ratio (18.7 *vs* 15.5 U/L-mmoL; $P < 0.0001$); and CRP (3.81 *vs* 1.93 mg/L; $P = 0.003$; Table II). On multivariate analyses adjusted for age, gender, BMI and inflammation, atherogenic dyslipidaemia was associated with increased oxLDL (ORa: 1.05, 95% CI: 1.02–1.08) and oxLDL/LDL ratio (ORa: 1.25, 95% CI: 1.11–1.40; Fig. 1), and also with PI use ($P = 0.01$). Thus, atherogenic dyslipidaemia was independently associated with oxidative stress and inflammation.

HDL was negatively correlated with biomarkers assessing oxidative stress (Lp-PLA2, oxLDL, oxLDL/LDL ratios), inflammation (CRP, IL-6) and immune activation (cystatin C, B2M), and positively with PON and ARE (Table III), whereas TG levels were positively associated with most of the biomarkers negatively associated with HDL (oxLDL, oxLDL/LDL ratios, CRP, cystatin C, B2M). TG/HDL ratios correlated positively with anthropometric parameters, WHR (r = 0.293, $P < 0.0001$) and HOMA-IR (R = 0.309, $P < 0.0001$), and negatively with adiponectin (R = -0.307, $P < 0.0001$). In addition, TG/HDL ratios were associated with biomarkers of oxidative stress (Lp-PLA2, oxLDL, oxLDL/LDL ratios) as well as inflammation and immune activation (CRP, IL-6, cystatin C, B2M; Table III).

Biomarker levels according to hypertension, CKD or previous cardiovascular events

Patients with hypertension presented with higher values of HOMA, BMI and WHR (data not shown), but had similar levels of oxidative stress and inflammatory/immune-activation biomarkers (Table II). In multivariate analyses, oxLDL and oxLDL/LDL ratio were not associated with hypertension (ORa: 1.01, 95% CI: 0.99–1.03 and ORa: 1.02, 95% CI: 0.95–1.08, respectively). SBP and DBP correlated (with low Spearman coefficients) with oxLDL (Table III), but not with other biomarkers.

CKD was associated with no biomarkers except for cystatin C and B2M, as expected, as these are markers excreted by the kidney (Table II). On multivariate analyses, oxLDL and oxLDL/LDL ratio were not associated with CKD (ORa: 1.00, 95% CI: 0.97–1.02 and ORa: 1.01, 95% CI: 0.91–1.12, respectively). Creatinine levels correlated with IL-6 and oxLDL (r = 0.15 and r = 0.16, respectively; Table III) in addition to cystatin C and B2M.

Patients with a history of CV events during follow-up were of older age (54 *vs* 49 years; *P* = 0.002), and eGFR was decreased (75.6 *vs* 87.3 mL/min; *P* = 0.003) while, as expected, B2M and cystatin C were increased. However, inflammatory and oxidative stress biomarkers did not differ (Table II). On multivariate analyses adjusted for age, BMI, gender and inflammation, oxLDL and oxLDL/LDL ratio were not associated with previous CV events (ORa: 0.99, 95% CI: 0.96–1.02 and ORa: 1.07, 95% CI: 0.99–1.16, respectively).

Biomarker levels according to antiretroviral molecules

Patients treated with PIs had lower levels of HDL (1.23 *vs* 1.36 mmol/L; *P* = 0.002), and higher levels of TG (1.77 *vs* 1.35 mmol/L; *P* < 0.0001), IL-6 (1.6 *vs* 1.1 pg/mL; *P* < 0.0001), cystatin C (0.98 *vs* 0.87 mg/L; *P* < 0.0001), B2M (2.6 *vs* 2.3 mg/L; *P* < 0.0001) and oxLDL/LDL ratio (16.4 *vs* 14.9 U/L-mmol/L; *P* = 0.004) compared with those following a PI-free regimen.

Regarding non-nucleoside reverse transcriptase inhibitors (NNRTIs), 21% were receiving an efavirenz (EFV)-based and 26% a nevirapine-based regimen of combination ART (cART). Levels of eGFR were higher in those receiving *vs* not receiving EFV (93 *vs* 85 mL/min; *P* = 0.005) whereas levels of D dimer were lower (204 *vs* 266 ng/mL, respectively; *P* = 0.004). Treatment with *vs* without nevirapine was associated with higher HDL (1.45 *vs* 1.21 mmol/L, respectively; *P* = 0.00001), but with lower PAI-1 (26.5 *vs* 34.5 ng/mL, respectively; *P* = 0.006), cystatin C (0.86 *vs* 0.96 mg/L, respectively; *P* = 0.002) and oxLDL/LDL ratio (14.3 *vs* 16.3 U/L-mmol/L, respectively; *P* = 0.0001).

Biomarker levels according to smoking, body fat redistribution and HIV infection severity

No oxidative stress, inflammation or immune-activation biomarkers differed between groups of patients according to fat redistribution (absence or presence of lipoatrophy and/or trunk fat hypertrophy). However, oxLDL and oxLDL/LDL ratios were positively associated with WHR (0.319 and 0.266, respectively; both *P* < 0.0001) and oxLDL was associated with BMI (0.21; *P* < 0.0001). In addition, CRP was associated with WHR and BMI [0.224 (*P* = 0.0003) and 0.200 (*P* = 0.0002), respectively] whereas, as expected, adiponectin was negatively related to

WHR and BMI [-0.200 (P = 0.0002) and -0.324 (P < 0.0001), respectively]. Thus, increased trunk fat was associated with oxidative stress and inflammation as well as with dysmetabolic features.

As regards smoking, increased levels of IL-6 in current *vs* never and past smokers had previously been reported [14]. Moreover, oxLDL/LDL ratios differed across the three groups (P = 0.03) and between past and never smokers (16.76 U/mmol, 95% CI: 13.45–19.49 *vs* 15.14 U/mmol, 95% CI: 12.62–17.62, respectively; P = 0.01).

Levels of biomarkers did not differ in patients with VL < or > 50 copies/mL, CD4/CD8 ratios < or > 0.4, CD4 < or > 350 cells/mm^3, < or > 500 copies/mL at the last follow-up visit, or CD4 < or > 200 cells/mm^3 on entry into the study, as previously reported for inflammatory/immune-activation biomarkers [14].

Discussion

The present study has found that, in long-term HIV-infected and ART-controlled patients from the Agence Nationale de Recherches sur le Sida et les Hépatites Virales (ANRS; French National Agency for Research on AIDS and Viral Hepatitis) CO3 APROCO-COPILOTE cohort, diabetes and atherogenic dyslipidaemia are each independently associated with oxidative stress and inflammation, whereas LDL-related dyslipidaemia and IFG are associated with only oxidative stress.

Our study population is representative of the long-term HIV-infected and treated population with prevalent age-related comorbidities, as observed in our participants and in the total group of 1227 patients [18]. Similar prevalences of comorbidities were reported in 876 patients of the French Chronic Immune Activation and Senescence (CIADIS) study (ANRS CO3 Aquitaine cohort) who were aged 50.5 years [1]. In addition, in a cohort of 524 HIV-infected patients aged 52 years from the AGEhIV study, the prevalence of CVD-linked comorbidities was similar to that observed in our present study, yet was greater overall than that observed in a well-matched control group of 540 HIV-negative subjects [2].

Regarding diabetes, a prevalence of 11% was found in our cohort of adults (median age: 49 years) with normal BMI (median 23 kg/m^2) and receiving long-term ART (median duration: 12.3 years), which is a high proportion compared with the general population. However, this prevalence is comparable to that reported in the ANRS CO3 Aquitaine cohort (12.8%), which also included long-term ART patients (median duration: 13.2 years) [1], albeit higher than that reported in the AGEhIV cohort (5.5%) [2], which had a median ART duration of 10 years. This difference might account for the greater prevalence of diabetes, as those treated

for longer durations had probably received first-generation ART, which was associated with a greater risk of diabetes [11,12]. In fact, some ART agents also clearly involved in ageing, family history of diabetes, ethnic origin, overweight/obesity, lipodystrophy and dysmetabolic profiles [19]. Interestingly, our diabetes patients frequently presented with a lipodystrophic phenotype and trunk fat redistribution, which accords with the deleterious role of trunk fat in metabolic disorders and the insulin resistance that often precede overt diabetes [20]. Indeed, long-lasting alterations in adipose tissue distribution have recently been confirmed in patients with previous exposures to stavudine, zidovudine and didanosine in the Copenhagen Comorbidity in HIV Infection (COCOMO) study [13]. Moreover, a recent report of HIV-infected ART-controlled subjects from sub-Saharan Africa found an increased prevalence of diabetes, with increased LDL levels, which was attributed to ART [21].

LDL-linked dyslipidaemia, also attributed to ART, is frequent in HIV-infected patients [22] and plays an important role. Previous exposures to thymidine analogues and didanosine have been associated with excess risks of hypercholesterolaemia and low HDL levels [13], while PIs have been associated with dyslipidaemia [17]. In addition, our present study has shown that 9% had atherogenic dyslipidaemia, which is predictive of increased CV risk as well as insulin resistance [4,5] and diabetes [23]. Furthermore, in HIV-infected patients, high TG/HDL ratios have been shown to predict risk of new-onset diabetes independently of other traditional risk factors [24], whereas atherogenic dyslipidaemia has recently proved to be more prevalent in HIV-infected *vs* non-infected subjects and associated with low adiponectin [25]. In our present study population, TG/HDL ratio was associated positively with diabetes and insulin resistance (by HOMA-IR and PAI-1), and negatively with adiponectin. In HIV-negative subjects, this ratio has been associated with CRP and oxLDL [26] and, in our present study, was associated with oxidative stress, immune activation and inflammatory markers, which could reflect causal relationships that contribute, together with insulin resistance, to higher CVD risk.

Regarding oxidative stress, it was observed that oxLDL levels were higher in subjects with trunk fat accumulation, as indicated by WHR, which accords with the deleterious role of this fat depot and its involvement in the insulin-resistant phenotype [27]. Also, an association between markers of oxidative stress and insulin resistance has been reported in the general population, suggesting that oxidative stress is an important trigger of insulin resistance [28], an association that was confirmed in our study patients who presented with high HOMA-IR scores according to their BMI. Our study also found that diabetes is associated with oxidative stress independently of inflammation. Interestingly, patients with IFG had increased levels of

oxLDL as well as increased HOMA-IR scores, suggesting a causal relationship. In addition, while increased levels of oxLDL have been positively associated with type 2 diabetes (T2D) in the general population [29], no such significant association was found between oxLDL and incident T2D in our study patients. In fact, our analysis indicating increased levels of oxLDL in IFG patients could be suggesting that oxidative stress plays a greater role in the development of diabetes in HIV-infected patients than in the general population.

Our study also found that a surrogate marker of liver steatosis, LAP, is associated with oxidative stress and inflammation. Increased oxidative stress has been proposed as a critical factor in the pathogenesis of non-alcoholic fatty liver disease [30].

In HIV-infected ART patients, PAI-1, a marker reflecting insulin resistance, was also associated with oxidative stress [31]. Indeed, high levels of PAI-1 were associated with a risk of myocardial infarction independently of CVD risk factors, HIV parameters and ART, suggesting that PAI-1 may be used for risk stratification and prediction of CVD [32].

In our present cohort, Lp-PLA2 was associated with lipid parameters and adiponectin, although its levels did not differ with the presence or absence of the evaluated comorbidities. Increased levels of Lp-PLA2 have been associated with increased risk of coronary heart disease in the general population [33] and in HIV-infected patients [34,35], and Lp-PLA2 has been associated with TG, LDL, HDL and insulin in HIV-infected patients initiated with either darunavir/r or atazanavir/r [36]. However, in a population of ART-controlled patients with normal LDL, Lp-PLA2 was associated with sCD14, but not with lipid parameters or inflammatory biomarkers [34].

OxLDL is thought to play a central role in atherosclerosis development [35] and is clearly associated with atherosclerotic CVD [37]. OxLDL is increased in HIV-infected compared with HIV-negative individuals [38], and is associated with the Framingham coronary heart disease risk score and subclinical atherosclerosis as evaluated by carotid intima–media thickness (cIMT) [39]. These data argue for atherosclerosis being particularly related to an increased oxidative and inflammatory status in HIV patients. In addition, as reported here, oxidative stress is associated with LDL-related and atherogenic dyslipidaemias independently of inflammation. However, when levels of oxidative stress markers were evaluated in patients with a history of CV events during the 11-year follow-up, no increased levels were observed in multivariate analyses, perhaps due to the time lag since the occurrence of such events.

Importantly, statin treatment decreased oxidative stress biomarker levels, and increased antioxidant PON and ARE activities in the general population [40,41]. Also, in HIV-infected patients, statins reduced oxidative stress markers (Lp-PLA2, oxLDL), leading to a reduced

number of coronary plaques independently of LDL levels [35,42]. These data further emphasize the deleterious impact of oxidative stress on the CV complications encountered by HIV-infected patients.

Taken as a whole, our study found that oxLDL and oxLDL/LDL ratios were strongly associated with diabetes and atherogenic dyslipidaemia independently of inflammation. In fact, a causal relationship with insulin resistance is suggested. Further studies are now required to validate their involvement in ageing HIV-infected patients. Moreover, these biomarkers were not related to the severity of the current HIV infection, as evaluated by HIV-1 VL, CD4/CD8 ratios or CD4 numbers. However, increased oxidative stress could be related to persistent effects of stavudine and/or zidovudine, drugs that are responsible for mitochondrial toxicity. Indeed, 80.5% of our study patients had received stavudine and 54% were taking zidovudine.

A previous study had shown that biomarkers of inflammation and immune activation were increased in patients in the APROCO-COPILOTE cohort [14]. In addition, as noted in the present study, they were also associated with diabetes and atherogenic dyslipidaemia, but not with the other evaluated comorbidities.

Several studies have suggested that, in the general population, diabetes is associated with increased inflammation [43]. In HIV-infected patients, inflammatory biomarkers were previously associated with the development or presence of diabetes [11,44–46]. In addition, the following markers have previously been associated with CVD risk in HIV-infected patients: increased levels of IL-6, CRP and/or D dimer with myocardial infarction [32,47,48], and sCD14 with progressive or pathological cIMT and calcified coronary plaques [49,50].

However, no consistent association was detected between hypertension and the tested biomarkers. Likewise, in the AGEhIV cohort, hypertension was not associated with markers of either inflammation or immune activation [51]. Regarding CKD, our study found that, except for cystatin C and B2M as expected, there was no difference in biomarker levels between patients with or without CKD. In the CIADIS study, only 4% of patients presented with CKD, and levels of CRP, IL-6 and sCD14 did not differ between patients with or without CKD, although a soluble weighted score of inflammation [including soluble tumour necrosis factor receptor 1 (sTNFR1) and D dimer] was independently associated with CKD [52]. In the ACTIVIH study, some biomarkers were associated with some comorbidities, but not with others, and different comorbid conditions presented with specific patterns of alteration [53]. Nevertheless, the absence of an association between the tested biomarkers and hypertension or CKD in our present study does not preclude an association with other biomarkers related to

oxidative stress or inflammation.

Some ART molecules can modify patients' metabolic profiles and, thus, could be involved in metabolic comorbidities. Indeed, our study found that PI treatment was, as expected, associated with differences in lipid levels, but also with biomarkers of oxidative stress, inflammation and immune activation that might participate in elevating CVD risk. Our study also confirmed the generally favourable effect of nevirapine on metabolic and inflammatory markers.

Nevertheless, it is necessary to acknowledge the limitations of our study. Regarding diabetes, hypertension, LDL-related and atherogenic dyslipidaemias and CKD, it was decided to consider only the situation at the time of evaluation and, therefore, only previous CV events were evaluated. Also, our study was cross-sectional in design and, as such, cannot address causality of the different biomarkers in the development of the various studied comorbidities.

In conclusion, in our present APROCO-COPILOTE cohort of long-term virologically suppressed patients, a high level of metabolic comorbidities was observed in association, to variable extents, with biomarkers of oxidative stress independently of inflammation. These biomarkers are most probably involved in diabetes and LDL-related and atherogenic dyslipidaemias, and less so with CKD and hypertension. Increased oxidative stress was observed in diabetes patients and those with IFG, and was also associated with insulin resistance. Moreover, there was an association with trunk fat accumulation that might be related to previous ART regimens, but also to HIV present in fat reservoirs and responsible for fat dysfunction and fibrosis [54].

Our findings suggest that oxidative stress could play a particular role in patients presenting with lipodystrophic features and insulin resistance and explain, at least in part, the high frequencies of some comorbidities, especially diabetes and dyslipidaemia, in HIV-infected ART-controlled patients. Thus, therapeutic strategies to control these comorbidities should target oxidative stress in addition to inflammation, and should also consider their differential associations with these biomarkers.

Conflicts of interest and source of funding

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Legend for figure

Fig. 1. Levels of oxidized low-density lipoprotein (oxLDL) and oxLDL/LDL ratios according to presence or absence of LDL-related or atherogenic dyslipidaemias or diabetes in patients from the APROCO-COPILOTE study. Medians and interquartile ranges (IQRs) are shown as boxplots and as individual values. Results were adjusted for age, gender, body mass index and inflammatory markers.

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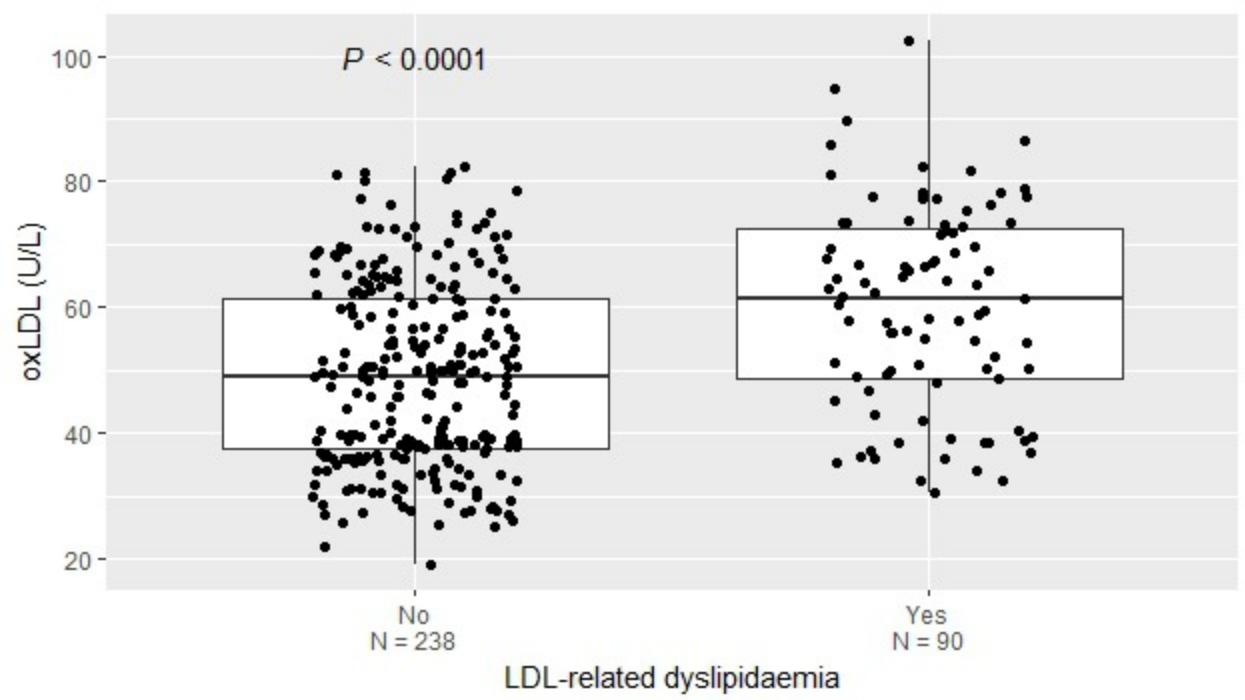
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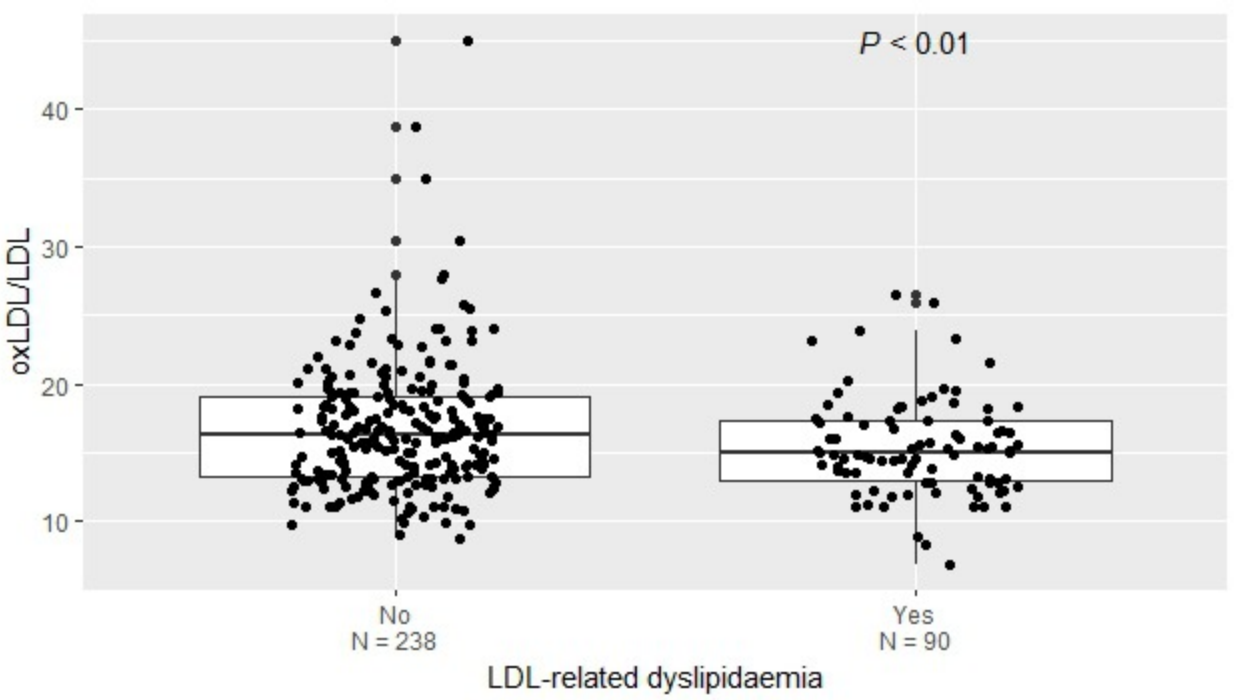
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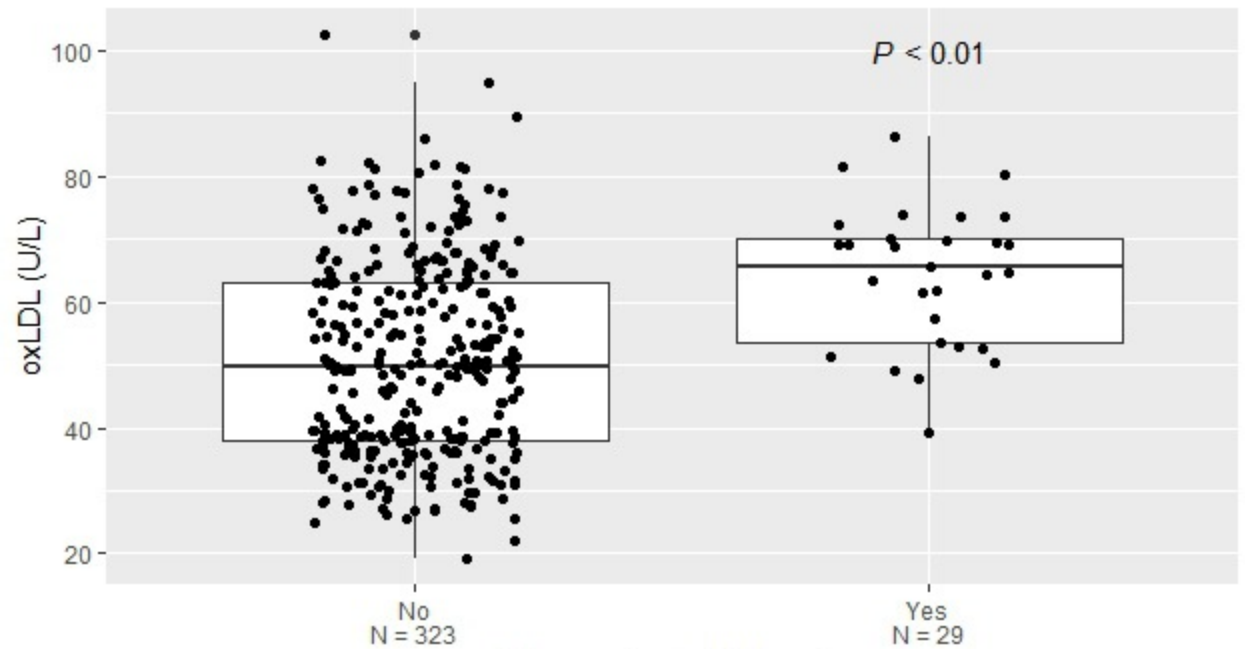
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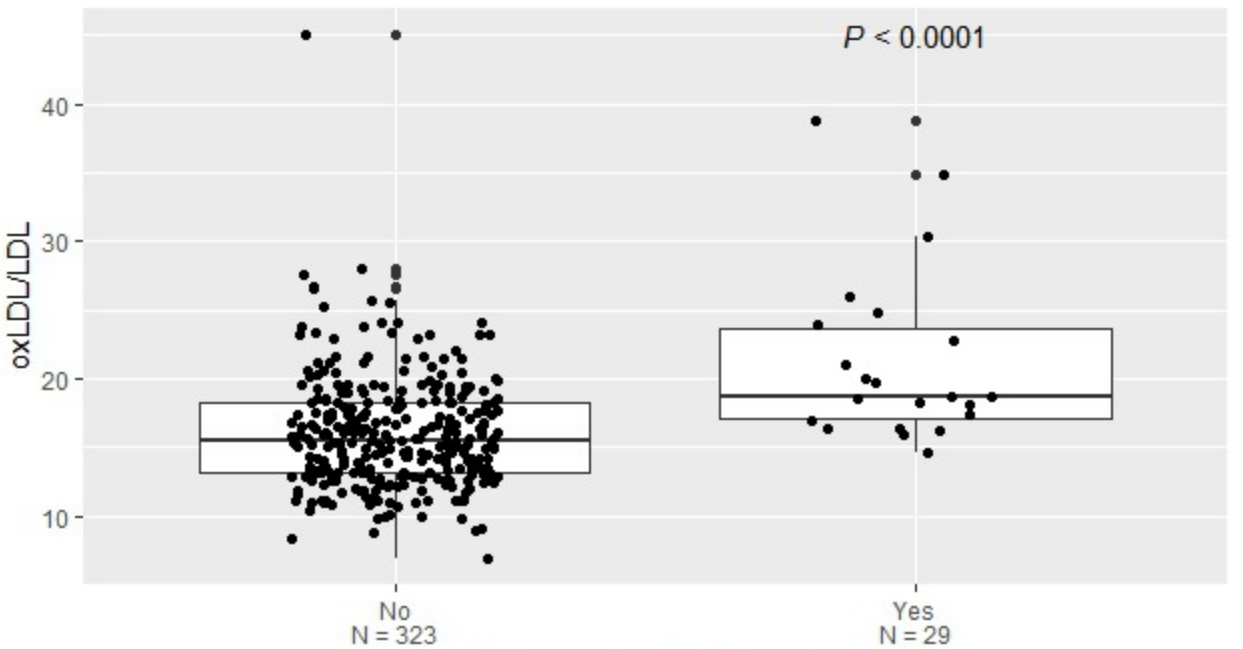
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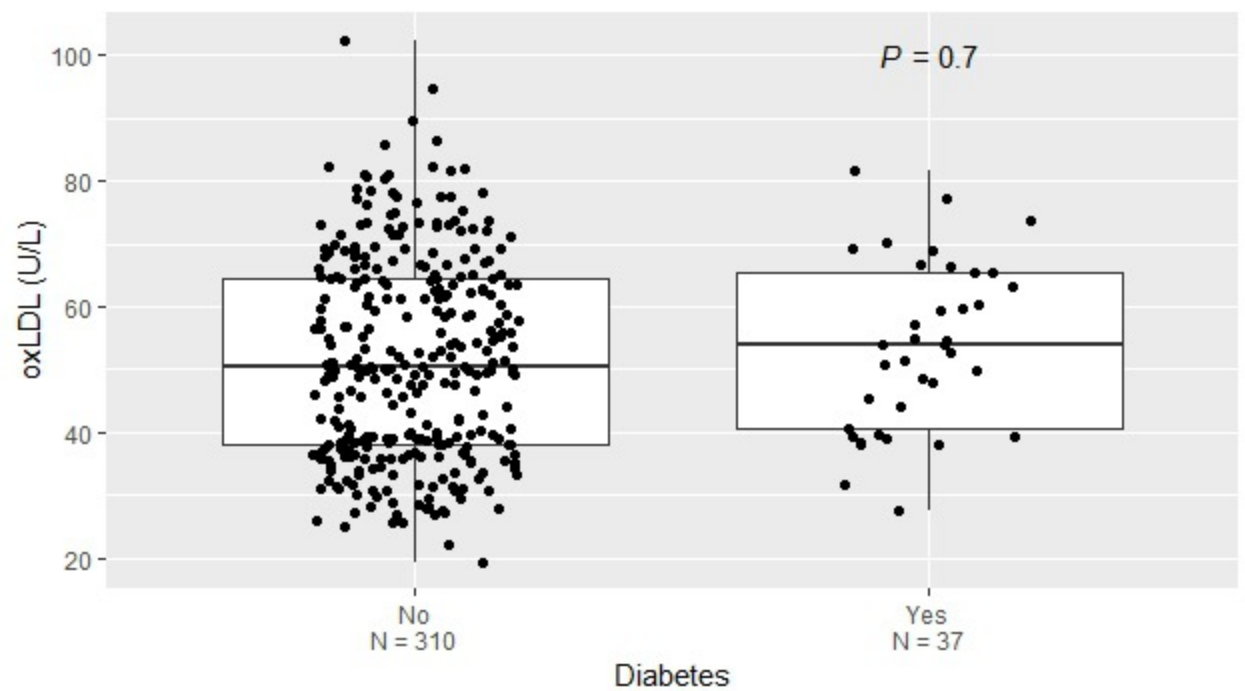
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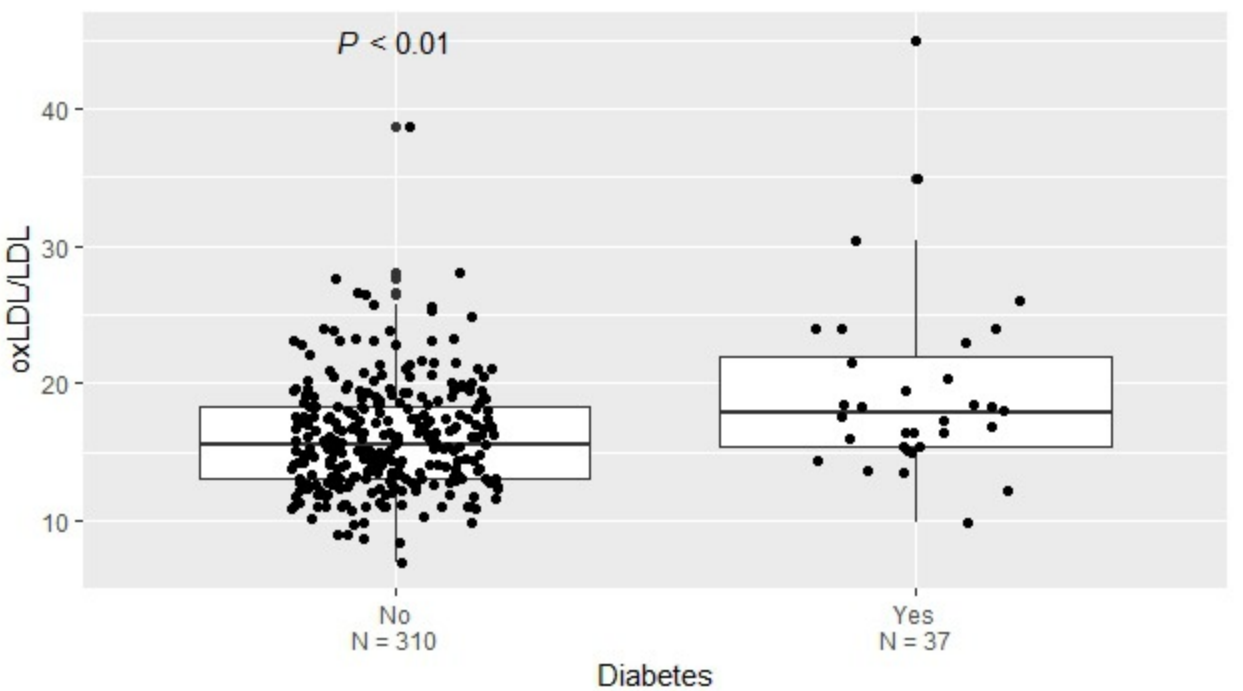
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Diabetes

Table I

Characteristics of patients included from the French APROCO-COPILOTE cohort, including levels of metabolic and adipose-derived parameters and biomarkers at the final follow-up visit

HIV-infected patients (n = 352)	**Median (IQR) or n (%)***
Demographic, blood pressure and anthropometric parameters	
Age (years), current	49 (45–56)
Male gender	287 (81.5%)
Body mass index (kg/m^2)	23.0 (21.1–25.4)
Waist circumference (cm)	**88 (81–95)**
Waist-to-hip ratio	0.95 (0.90–1.00)
No fat redistribution (n = 349)	140 (40%)
Lipoatrophy	96 (27%)
Lipohypertrophy	37 (11%)
Lipoatrophy + lipohypertrophy	76 (22%)
Systolic blood pressure (mmHg; n = 322)	123 (118–137)
Diastolic blood pressure (mmHg; n = 322)	80 (70–80)
Never smokers (n = 342)	112 (32.7%)
Current smokers	138 (40.4%)
Past smokers	92 (26.9%)
Hepatitis C virus co-infection (n = 333)	63 (18.9%)
HIV infection-related characteristics	
Current CD4 cell count (cells/mm^3)	620 (453–790)
Current CD8 cell count (cells/mm^3)	756 (544–1025)
Current CD4/CD8 ratio	0.81 (0.56–1.10)
Current HIV-1 RNA < 50 copies/mL	322 (91.5%)
Total duration of ART (years)	12.3 (11.5–13.8)
Current ART (n = 349)	
Protease inhibitors	183 (52.4%)
Nucleoside analogues	337 (96.6%)
Non-nucleoside analogues	164 (46.7%)
Efavirenz	74 (21.2%)
Nevirapine	90 (25.8%)
Integrase inhibitors	7 (2%)
Metabolic and adipose-related parameters	
Total cholesterol (mmol/L; n = 341)	4.94 (3.96–5.93)
LDL cholesterol (mmol/L; n = 328)	2.91 (2.01–3.74)
HDL cholesterol (mmol/L; n = 334)	1.16 (0.87–1.49)
Triglycerides (mmol/L; n = 341)	1.56 (1.02–2.13)
Glycaemia (mmol/L; n = 347)	5.3 (4.9–5.8)
Insulin (mU/L; n = 344)	8.5 (6.3–12.6)
HOMA-IR (n = 339)	2.02 (1.42–3.16)
PAI-1 (ng/mL; n = 331)	31.9 (19.7–50.9)
Adiponectin (mg/L; n = 347)	5.75 (3.53–9.6)

Leptin (□(pt; n = 342)	4.14 (2.41–8.04)
eGFR (mL/min/1.73 m^2; n = 328)	93 (79–109)
Bilirubin (□mol/L; n = 329)	6.8 (4.6–11.3)
Oxidative stress biomarkers	
Lp-PLA2 (ng/mL; n = 347)	183 (153–213)
oxLDL (U/L; n = 350)	50.6 (38.3–64.5)
oxLDL/LDL (U/mmol; n = 326)	15.9 (13.2–18.5)
Arylesterase (mmol/L-min; n = 350)	21.9 (17.5–27.3)
Paraoxonase (□mol/L-min; n = 350)	133 (67–204)
Inflammatory biomarkers	
hsCRP (mg/L; n = 347)	2.1 (0.9–4.5)
hsIL-6 (pg/mL; n = 347)	1.3 (0.7–2.6)
D dimer (ng/mL; n = 331)	252 (177–374)
Immune activation biomarkers	
sCD14 (ng/mL; n = 347)	1356 (1027–1818)
β2 microglobulin (mg/L; n = 347)	2.4 (2.0–3.1)
Cystatin C (mg/L; n = 347)	0.93 (0.82–1.10)

* Continuous or categorical variables;

HIV: human immunodeficiency virus; IQR: interquartile range; RNA: ribonucleic acid; ART: antiretroviral therapy; LDL/HDL: low-density/high-density lipoprotein; HOMA-IR: homoeostasis model assessment of insulin resistance; PAI-1: plasminogen activator inhibitor 1; eGFR: estimated glomerular filtration rate; Lp-PLA2: lipoprotein-associated phospholipase A2; oxLDL: oxidized LDL; hsCRP/hsIL-6: high-sensitivity C-reactive protein/interleukin 6; sCD14: soluble CD14

Table II

Median levels of oxidative stress, inflammation and immune-activation biomarkers in patients with/without diabetes, low-density lipoprotein (LDL)-related and atherogenic dyslipidaemias (DYSLIP), hypertension and chronic kidney disease (CKD)

	DiabetesYes No n = 37 n = 310		**LDL-related DYSLIP** Yes No n = 90 n = 238		**Atherogenic DYSLIP** Yes No n = 29 n = 323		**Hypertension** Yes No n = 90 n = 232		**CKD** Yes No n = 30 n = 318		**PCE** **Yes No n = 32 n = 320**	
Oxidative stress biomarkers												
Lp-PLA2 (ng/mL)	166	186	181	184	192	183	178	185	181	184	**181**	**184**
oxLDL (U/L)	54.0	50.2	61.5	48.3***	65.5	49.8***	52.5	49.8	49.5	50.6	**49.5**	**50.6**
oxLDL/LDL (U/mmol)	17.8	15.6**	15.0	16.2	18.7	15.5***	16.1	15.3	15.9	15.9	**18.1**	**15.7**
Arylesterase (mmol/L-min)	21.2	22.2	23.6	21.6	22.8	21.7	22.4	21.6	24.8	21.6	**21.0**	**22.2**
Paraoxonase (□mol/L-min)	92	136	119	141	175	132	158	128	73	145	**133**	**133**
Inflammatory biomarkers												
hsCRP (mg/L)	3.85	1.94*	2.25	1.94	3.81	1.93*	2.19	2.11	2.83	1.96	**2.95**	**1.96**
hsIL-6 (pg/mL)	1.43	1.28	1.12	1.34	2.11	1.25	1.22	1.27	1.92	1.28	**1.91**	**1.25**
D dimer (ng/mL)	269	252	237	259	234	252	289	249	287	252	**287**	**251**
Immune activation biomarkers												
sCD14 (ng/mL)	1260	1360	1290	1378	1452	1353	1442	1330	1472	1329	**1343**	**1356**
Cystatin C (mg/L)	0.91	0.93	0.93	0.92	0.95	0.92	0.94	0.92	1.31	0.91***	**1.13**	**0.92*** **
β2 microglobulin(mg/L)	2.40	2.40	2.30	2.40	2.65	2.40	2.35	2.40	3.80	2.40***	**3.25**	**2.40****

* $P < 0.01$, ** $P < 0.001$, *** $P < 0.0001$;

PCE: previous cardiovascular events; Lp-PLA2: lipoprotein-associated phospholipase A2; oxLDL: oxidized low-density lipoprotein; hsCRP/hsIL-6: high-sensitivity C-reactive protein/interleukin 6; sCD14: soluble CD14

Table III
Correlations between metabolic, anthropometric and blood pressure parameters and biomarkers assessing oxidative stress, inflammation and innate immune activation in 352 human immunodeficiency virus (HIV)-infected patients

	Oxidative stress biomarkers					**Inflammatory biomarkers**			**Immune-activation biomarkers**		
	Lp-PLA2	**oxLDL**	**oxLDL /LDL**	**PON**	**ARE**	**hsCRP**	**hsIL-6**	**D dimer**	**sCD14**	**Cystatin C**	**B2M**
Glucose metabolism-related parameters											
Glycaemia	–	–	–	–	–	–	–	–	–	-0.16 $P < 0.05$	–
Insulin	–	–	0.16 $P < 0.005$	–	–	0.17 $P < 0.005$	–	–	–	0.16 $P < 0.005$	–
HOMA	–	–	0.16 $P < 0.005$	–	–	0.14 $P < 0.01$	–	–	–	–	–
PAI-1	–	0.18 $P < 0.001$	0.23 $P < 0.0001$	–	–	–	–	–	–	–	–
Adipo	-0.23 $P < 0.0001$	-0.28 $P < 0.0001$	-0.35 $P < 0.0001$	–		-0.14 $P < 0.01$	–	–	–	–	–
Leptin	–	–	–	0.16 $P < 0.005$	–	–	–	0.16 $P < 0.005$	–	–	–
Lipid parameters											
LDL	0.27 $P < 0.0001$	0.68 $P < 0.0001$	-0.32 $P < 0.0001$	–	0.22 $P < 0.0001$	–	–	–	–	–	-0.14 $P < 0.01$
HDL	-0.37 $P < 0.0001$	-0.29 $P < 0.0001$	-0.47 $P < 0.0001$	0.17 $P < 0.005$	0.17 $P < 0.002$	-0.16 $P < 0.005$	-0.27 $P < 0.0001$	–	–	-0.34 $P < 0.0001$	-0.33 $P < 0.0001$
TG/HDL	0.23 $P < 0.0001$	0.42 $P < 0.0001$	0.44 $P < 0.0001$	–	–	0.20 $P < 0.001$	0.20 $P < 0.001$	–	–	0.30 $P < 0.0001$	0.28 $P < 0.0001$
TG	–	0.41 $P < 0.0001$	0.35 $P < 0.0001$	–	0.15 $P < 0.01$	0.20 $P < 0.001$	–	–	–	0.20 $P < 0.001$	0.19 $P < 0.001$
Anthropometric parameters											
BMI	–	0.21 $P < 0.0001$	–	–	–	0.20 $P < 0.005$	–	–	–	–	–

WHR	–	0.32 *P* < 0.0001	0.27 *P* < 0.0001	–	–	0.22 *P* < 0.001	–	–	–	–	–
Blood pressure											
Diastolic	–	0.18 *P* < 0.005	–	–	–	–	–	–	–	–	–
Systolic	–	0.15 *P* < 0.01	–	–	–	–	–	–	–	–	–
Other biochemical parameters											
Creatinine	–	0.15 *P* < 0.005	–	–	–	–	0.16 *P* < 0.005	–	–	0.41 *P* < 0.0001	0.33 *P* < 0.0001

NB: Only *P* values < 0.01 are presented (Spearman's correlation coefficients);
Lp-PLA2: lipoprotein-associated phospholipase A2; oxLDL: oxidized LDL; PON: paraoxonase activity; ARE: arylesterase activity; hsCRP/hsIL-6: high-sensitivity C-reactive protein/interleukin 6; sCD14: soluble CD14; B2M: β2 microglobulin; HOMA: homoeostasis model assessment; PAI-1: plasminogen activator inhibitor 1; Adipo: adiponectin; LDL/HDL: low-density/high-density lipoprotein; TG: triglycerides; BMI: body mass index; WHR: waist-to-hip ratio