

## BACKGROUND and OBJECTIVES

Increased cardiovascular risk (CVR) in HIV-patients is multifactorial. HIV infection is related with lipid disturbances and chronic inflammation. The relation between them and HIV is not well known.

The objectives of the study were:

- To compare the changes in LDL particles phenotype and inflammatory biomarker levels in naive HIV-infected patients that started or not combined-ART (c-ART) over a 2-year follow-up
- To investigate associations between LDL particle phenotype, inflammatory biomarkers and HIV factors.
- To investigate correlations between LDL particle phenotype, inflammatory biomarkers and HIV factors with subclinical atherosclerosis assessed by carotid

## METHODOLOGY

**Design:** Prospective, multicenter, comparative study carried out in a Hospital from Barcelona (Hospital Universitari de Bellvitge) and Hospital from Australia (Baker Heart and Diabetes Institute). Two groups of naive HIV-patients (Group A:CD4>500 cel/μL, not starting c-ART at baseline; group B:CD4<500 cel/μL, starting c-ART at baseline) were compared with healthy controls(HC), matched by age and sex. In group A patients experiencing a CD4 decrease to <500 cel/μL were recommended to start c-ART and were not included in the follow-up analyses.

**Inclusion criteria** HIV-infected patients older than 18 years, who had never received ART. **Exclusion criteria:** diabetes mellitus, previous CV disease, secondary dyslipidemia, malignant disease or any active infection or inflammatory disease, body mass index (BMI) >30 kg/m<sup>2</sup> and pregnancy. The control group consisted of healthy, HIV-negative, age- and sex-matched healthcare workers

**At baseline, month 12 and 24 the following variables were analyzed:**

- Clinical assessment: tobacco, ART use, lipid-lowering, antihypertensive drugs, Framingham score, blood pressure, height, weight, waist and hip circumference.
- Laboratory: HIV viral load, CD4 cell count, creatinine, insulin and lipid profile. Plasma biomarkers: sCD163, sCD14, Interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), asymmetric dimethylarginine (ADMA), by ELISA; high-sensitivity C-reactive protein (hs-CRP) by immunocolorimetry; lipoprotein-phospholipase A2 (Lp-PLA2) by 2-thio-PAF.

-Carotid ultrasound: measurement of carotid intima-media thickness (c-IMT) of far wall of left and right common carotid using a semi-automatic software and presence of plaque (focal structure into the arterial lumen of at least 0.5 mm or 50% of the surrounding IMT value or c-IMT ≥1.5 mm) in common, bulb and internal carotid. Subclinical atherosclerosis was defined as the presence of a plaque or common c-IMT > 75<sup>th</sup> percentile of a reference population (Rev Esp Cardiol 2012;65:1086).

-**Statistics:** The Student *t*-test or Mann-Whitney *U*-test was used to compare continuous variables between groups at each study time point. Qualitative variables were compared using the chi-square or Fisher exact test. Comparisons between the baseline and follow-up measures in each group were carried out with the paired *t*-test or Wilcoxon signed rank test. The Pearson correlation coefficient was calculated to estimate the strength of the association between variables

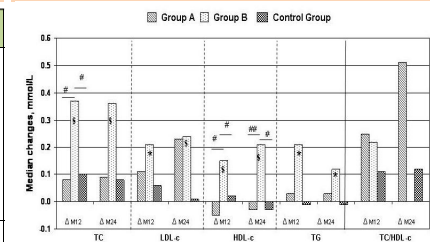
## RESULTS

### 1. Demographical, cardiovascular risk and laboratory characteristics of participants

Variables	Control group n=22	HIV-infected n=62	P- value*	Group A n=31	Group B n=31	P- value*	P- value*	P- value*
Age, y	37 (31-47)	36.5 (30-43)	ns	35 (29-45)	40 (31-42)	ns	ns	ns
Male	15 (68.2)	52 (83.9)	ns	24 (77.4)	26 (83.9)	ns	ns	ns
HIV viral load, log	4.3 (37-49)	3.9 (3.9-4.6)	0.04	4.7 (3.8-5.1)	4.7 (3.8-5.1)	0.04		
CD4 count, cells/μL	510 (355-704)	742 (692-816)	<0.001	380 (259-510)				
BMI, Kg/m <sup>2</sup>	23.9 (20.7-26.1)	25.7 (21.9-26.9)	ns	25.1 (21.9-26.9)	24.1 (22-26.5)	ns	ns	ns
Waist-to-hip ratio	0.88 (0.84-0.97)	0.9 (0.88-0.98)	ns	0.91 (0.88-0.97)	0.96 (0.91-1.1)	0.02	ns	0.028
SBP, mmHg	115.5 (108-133)	125 (119-133)	0.062	123 (115-128)	130 (119-140)	0.022	ns	0.036
DBP, mmHg	77.5 (62-82)	78 (72-84)	ns	78 (69-82)	77 (73-86)	ns	ns	ns
CV risk factors, n (%)								
- Current smoker	6 (31.6)	22 (36.1)	ns	12 (40)	10 (32)	ns	ns	ns
- Hypertension	2 (10.5)	1 (1.6)	ns	0	1 (3.2)	ns	ns	ns
- Family history of premature CHD	5 (26.3)	10 (16.4)	ns	5 (16.7)	5 (16.1)	ns	ns	ns
Framingham risk, %	2.5 (1.2-5.9)	3.9 (2.3-7.9)	ns	3.9 (1.9-5.6)	5.1 (2.4-7.9)	ns	ns	0.086
Carotid ultrasound								
- c-IMT, mm	0.55 (0.51-0.61)	0.53 (0.46-0.64)	ns	0.54 (0.48-0.65)	0.52 (0.44-0.63)	0.082	ns	ns
- c-IMT >75 <sup>th</sup>	4 (22.2)	8 (14.3)	ns	6 (21.4)	2 (7.1)	ns	ns	ns
- Carotid plaque	1 (4.8)	3 (5.4)	ns	1 (3.2)	3 (10.3)	ns	ns	ns

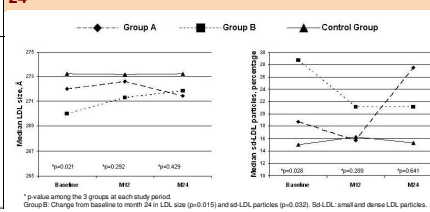
ADMA: asymmetric dimethylarginine; Apo: apolipoprotein; BMI: body mass index; CHD: coronary heart disease; c-IMT: carotid intima-media thickness; CV: cardiovascular; DBP: diastolic blood pressure; HDL-c: high density lipoprotein cholesterol; hs-CRP: high-sensitivity C-reactive protein; IL-6: interleukin-6; LDL-c: low density lipoprotein cholesterol; MCP-1: monocyte chemoattractant protein-1; sLDL: small dense LDL particles; SBP: systolic blood pressure; TG: triglycerides; HDL-c: high density lipoprotein cholesterol.  
Continuous variable: mean (standard deviation), except biomarkers: median (Interquartile range); qualitative variables: n (percentage).  
\* For between HIV-infected patients (Group A and B) vs healthy control group. \* For between A and B group. \* For between A and healthy control group. \*\* For between B and healthy control group.

### 2. Change in lipids at month 12 and 24



Within-group significant change from baseline to month 12 (ΔM12) and month 24 (ΔM24): \*p<0.05, #p<0.01. Between-groups significant change: # p<0.05, ## p<0.01. Abbreviations: Δ, change from baseline; HDL-c: high density lipoprotein cholesterol; LDL-c: low density lipoprotein cholesterol; TC: total cholesterol; TG: triglycerides.

### 3. Change in LDL particles phenotype at month 12 and 24



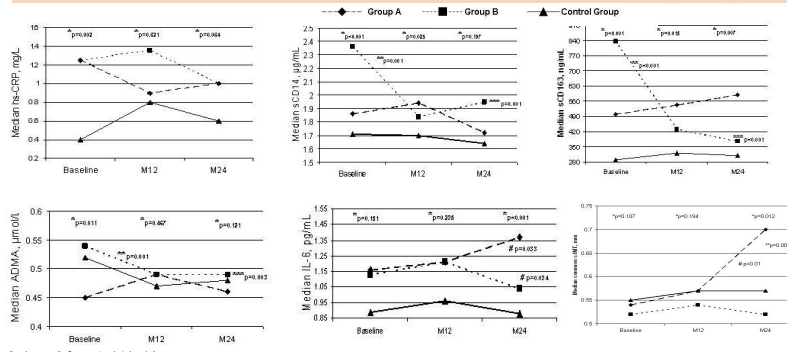
\* p-value among the 3 groups at each study period. Group B: Change from baseline to month 24 in LDL size (p=0.015) and sLDL particles (p=0.032). sLDL: small and dense LDL particles.

### 3. Correlation analyses at baseline and the change at month 24

Variables	LDL Size	Total Lp-PLA2	IL-6	MCP-1	sCD14	sCD163	hs-CRP
HIV viral load							
Baseline						0.214 (0.037)	
Month 24			0.273 (0.097)		0.326 (0.046)		
CD4 count							
Baseline							
Month 24			0.31 (0.022)		-0.241 (0.079)		
TC							
Baseline							
Month 24			0.276 (0.027)		-0.308 (0.006)		
HDL-c							
Baseline							
Month 24	0.425 (<0.001)	-0.337 (0.002)			-0.304 (0.006)		
LDL-c							
Baseline							
Month 24			0.246 (0.028)		-0.311 (0.005)		
LDL-c			0.345 (0.005)		-0.497 (<0.001)		
LDL-c							
Baseline							
Month 24							
CT HDL-c							
Baseline							
Month 24	-0.601 (<0.001)	0.433 (<0.001)			0.226 (0.072)		
Triglycerides							
Baseline							
Month 24	-0.761 (<0.001)	0.374 (0.002)			0.227 (0.043)		
LDL size							
Baseline							
Month 24			-0.192 (0.087)		-0.385 (0.002)		
Total Lp-PLA2							
Baseline							
Month 24							
IL-6							
Baseline							
Month 24							
MCP-1							
Baseline							
Month 24							
sCD14							
Baseline							
Month 24							
sCD163							
Baseline							
Month 24							
hs-CRP							
Baseline							
Month 24							

HDL-c: high density lipoprotein cholesterol; hs-CRP: high-sensitivity C-reactive protein; IL-6: interleukin-6; LDL-c: low density lipoprotein cholesterol; MCP-1: monocyte chemoattractant protein-1; TG: triglycerides; HDL-c: high density lipoprotein cholesterol.

### 4. Change in plasma biomarkers and c-IMT at month 12 and 24



\* p-value among the 3 groups at each study period. Within-group significant change from baseline to month 12 (ΔM12) and month 24 (ΔM24): # p<0.05, ## p<0.01. Between-groups significant differences: Month 12: # p<0.05, ## p<0.01. sCD163: A vs B p=0.019 and A vs control p=0.005. Month 24: hs-CRP: B vs control p=0.037; sCD163: A vs control p=0.004; ADMA: A vs control p=0.046; IL-6: A vs B p=0.001 and A vs control p=0.002.

## CONCLUSIONS

In HIV-infected naive patients, ART was associated with improvements in LDL particles phenotype and inflammatory/immune biomarkers, reaching values similar to those of the controls, except in hs-CRP. ART may be effective in preventing accelerated atherosclerosis. Biomarkers, mainly those associated with macrophage activation, were associated with lipid disturbances.