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The role of C-reactive protein as a marker for cardiovascular risk associated with antiretroviral therapy in HIV-infected patients

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Abstract

C-reactive protein (CRP) has been associated with prognosis of HIV-infection, but its relationship with cardiovascular disease remains unknown. We aimed to evaluate whether CRP may be a marker of cardiovascular risk in HIV-infected patients, and to determine the influence of antiretroviral therapy (ART) on CRP levels. We conducted a cross-sectional study on 245 consecutive HIV-infected patients during a 2-month period. An extensive workup for cardiovascular risk was performed, including determination of CRP levels measured by an ultrasensitive immunoturbidimetric assay (detection limit, 0.003 mg/dl). Ninety-nine (40.4%) patients had serum CRP concentrations above 0.3 mg/dl, considered to represent individuals at high risk for developing cardiovascular complications. In univariate analysis, CRP levels correlated positively with total cholesterol ($p = 0.01$), LDL cholesterol ($p = 0.001$), triglycerides ($p = 0.04$) and Framingham risk score ($p = 0.006$), and negatively with HDL cholesterol ($p = 0.004$). Concentrations of CRP were higher in males ($p = 0.05$) and smokers ($p = 0.002$). No correlation was found between CRP levels and HIV-viral load or CD4 cell counts. In multivariate analysis, independent factors associated with the highest quartile of serum CRP concentrations (0.49 mg/dl) were LDL-cholesterol ($p < 0.001$), HDL-cholesterol ($p = 0.001$), cigarette smoking ($p = 0.019$) and current ART ($p = 0.021$). Our results show that C-reactive protein is associated with traditional cardiovascular risk factors, and may then be a marker for cardiovascular risk linked to HIV infection and ART.

Keywords: C-reactive protein; HIV; Cardiovascular risk; Cardiovascular disease; Antiretroviral therapy

1. Introduction

There is an increasing awareness that cardiovascular disease (CVD) is augmented in HIV-infected patients [1,2]. Factors associated with HIV infection and antiretroviral therapy (ART) have been implicated in the premature development of atherosclerosis and coronary heart disease [1–3]. Antiretroviral therapy may increase lipids, impair glucose metabolism and alter body fat distribution [4,5]. However, hyperlipidemia and other conventional risk factors do not fully account for the association between ART and increased risk of CVD [6], suggesting that additional mechanisms may be implicated in the excess of risk.

Inflammation plays a central role in the pathogenesis of atherosclerosis and CVD [7] in general population, but its contribution in the context of HIV infection is poorly known. Among currently available inflammatory biomarkers, C-reactive protein (CRP) is the best characterized, and constitutes at present the only marker that adds prognostic information to traditional cardiovascular risk assessment [8]. Whether CRP is also a marker of cardiovascular risk in HIV-infected patients remains unknown. Current data evaluating CRP in that setting are very limited, and results are

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contradictory [9–12]. HIV infection is associated with a chronic immune activation and proinflammatory states [13] that might increase CRP concentration, adding complexity on its clinical meaning in HIV-infected patients. Recently published data from HIV-infected males enrolled in the Multicenter AIDS Cohort Study (MACS) found that CRP is a marker for HIV-disease progression [14]. This study, however, was carried out during the pre-highly active antiretroviral therapy (HAART) era, and information on the possible relationship of CRP with cardiovascular risk was not obtained.

In order to deepen in the potential implications of CRP during the HAART era, we evaluated CRP levels in a clinic-based cohort of HIV-infected patients. We aimed to investigate whether any link exists between CRP levels and cardiovascular risk in patients under current antiretroviral regimens. We analyzed the relationship of CRP with traditional cardiovascular risk factors, and with factors linked to HIV infection and ART.

2. Materials and methods

2.1. Study population

A cross-sectional study was conducted at the HIV clinic of the university hospital of Elche, Spain. All consecutive HIV-infected patients attended during a 2-month period were included. Patients were ≥18 years of age, with no other inclusion–exclusion criteria. The study was approved by the local Ethics Committee. All subjects were informed about the study and gave their written consent before participation.

2.2. Clinical and laboratory evaluations

Clinical data including demographic characteristics, cardiovascular risk factors, anthropometric parameters (weight, height, waist and hip circumferences) and current antiretroviral regimen were obtained at the visit. Laboratory workup included CRP levels, fasting lipid levels, CD4 T-lymphocyte counts, plasma HIV RNA, and routine safety blood tests.

To determine CRP levels, serum samples were collected and separated from the red cells on the same day, and stored immediately at −70 °C until analyzed. C reactive protein (CRP) was measured by an immunoturbidimetric assay on a Hitachi 717 automated analyzer (Tina-quant CRP detection method, Roche Diagnostics, detection limit 0.003 mg/dl, between-assay coefficient of variance 3.62%, intra-assay variation 1.09%). Based on population samples, CRP levels of less than 0.1 mg/l, 0.1–0.3 mg/l, and more than 0.3 mg/l have been used to represent individuals at low, moderate, and high risk for developing future cardiovascular complications [15]. Total and high-density lipoprotein (HDL) cholesterol were measured by spectrophotometry (assays kits OSR 6216 and OSR 6187, respectively; Olympus Diagnostic Ireland, Palex Medical, Barcelona, Spain). Low-density lipoprotein (LDL) cholesterol was estimated from quantitative measurements of total and HDL-cholesterol and plasma triglycerides. No calculation of LDL was performed in patients with triglyceride levels above 400 mg/dl. Plasma HIV RNA was performed using the Roche Amplicor Version 1.5 (Roche Diagnostics, Madrid, Spain; lower limit of detection 50 copies/ml plasma). The rest of the laboratory evaluations were measured using standard techniques.

2.3. Statistical analysis

Descriptive statistics were computed by standard methods. To evaluate factors associated with CRP concentrations, demographic, clinical and laboratory variables, and treatment status were analyzed. To investigate the association of CRP levels with current ART, only patients receiving the same ART regimen during the last 3 months were included. To be considered treatment interruption, the patient had to have discontinued all antiretroviral drugs during at least 3 months. In those patients with a shorter interruption, ART related variables were not included in the analysis. We used the Spearman's correlation coefficient to determine the association between levels of CPR and continuous variables. To compare median levels of CPR levels according to categorical variables we applied the Mann–Whitney test. In a second phase of analysis, we categorized all continuous variables under study as follows: CD4 cell counts (<200, 200–499 and ≥500 cells/mm^3), HIV viral load (<200 and ≥200 copies/ml); LDL cholesterol, HDL cholesterol, age and Framingham risk score were categorized in thirds. Multivariable analyses were carried out using logistic regression models to obtain an adjusted measure of the effect of cardiovascular related variables and ART treatment on the risk of high level of CPR, controlling for more than one confounder simultaneously. We used likelihood ratio tests to assess the global significance of each covariate in the final model, and Wald tests to assess the significance of each category of these covariates. Finally we checked for interactions, particularly whether the effect of ART was modified by any of the potential effect modifiers by including in the model interaction terms between ART and the effect modifier under study. Likelihood ratio tests were used to derive p-value of the interaction tests. A p-value of <0.05 was considered to be statistically significant. Statistical analyses were performed using SPSS, Version 12.0 (Chicago, Illinois).

3. Results

3.1. Patient characteristics

Demographic and clinical characteristics of the 245 included patients are shown in Table 1. Patients were in a stable condition, with no concurrent opportunistic infections, as reflected by their median CD4 cell count of 500.5 cells/mm^3. One hundred eighty one (73.9%) were on ART, 105

Table 1
Demographic and clinical characteristics of the 245 HIV-infected patients

Characteristic	Value
Age (years)	40.2 (35.4, 46.2)
Female sex, no. (%)	51 (20.8)
CD4 cell count (cells/mm^3)	500.5 (325.5, 696.5)
Plasma HIV viral load (copies/ml)	<50 (<50, 4750)
HIV viral load < 50 copies/ml, no. (%)	132 (53.9)
Time of exposure to ART (years)	4.0 (2, 6.7)
Current antiretroviral therapy, no. (%)	
None	61 (24.9)
Naïve	30 (12.2)
NNRTI-based regimen	95 (38.8)
Efavirenz	64 (26.1)
Nevirapine	31 (12.7)
Protease inhibitor-based regimen	52 (21.2)
Lopinavir/ritonavir	41 (16.7)
Other	11 (4.5)
NRTI-based regimen	24 (9.8)
NNRTI + protease inhibitor-based regimen	10 (4.1)
Missing	3 (1.2)
Current smoker, no. (%)	157 (64.1)
High blood pressure, no. (%)[a]	21 (8.6)
Diabetes mellitus, no. (%)	15 (6.1)
Total serum cholesterol level >200 mg/dl, no. (%)	56 (22.9)
Triglyceride serum levels >200 mg/dl, no. (%)	54 (22)
Waist-to-hip index	0.93 (0.77, 1.11)
Framingham risk score	3 (1, 10)
C-reactive protein (mg/dl)	0.24 (0.08, 0.49)

All numeric variables are presented as median and interquartile range (IQR).
ART: antiretroviral therapy; NNRTI: non-nucleoside reverse transcriptase inhibitors; NRTI: nucleoside reverse transcriptase inhibitors.
[a] Blood pressure ≥140/90 or use of hypertensive medication.

(42.9%) were receiving non-nucleoside reverse transcriptase inhibitors (NNRTI), 62 (25.3%) protease inhibitors (PI)-based regimens, and 10 (4.1%) patients were receiving both NNRTI and PI. Most (79%) of the patients on PI received lopinavir/ritonavir. The most commonly prescribed nucleoside reverse transcriptase inhibitors (NRTI) were lamivudine in 142 (58%) cases and zidovudine in 85 (34.7%).

3.2. C-reactive protein concentrations

Ninety-nine (40.4%) patients had serum CRP levels above 0.3 mg/dl, considered to represent individuals at high risk for developing future cardiovascular complications [15]. CRP correlated positively with total serum cholesterol level (Spearman's ρ [r_s] = 0.16, $p = 0.01$, $N = 235$), LDL cholesterol level ($r_s = 0.23$, $p = 0.001$, $N = 210$), serum triglyceride level ($r_s = 0.13$, $p = 0.04$, $N = 234$) and Framingham risk score ($r_s = 0.19$, $p = 0.006$, $N = 209$), and negatively with HDL cholesterol level ($r_s = -0.19$, $p = 0.004$, $N = 227$) (Table 2). CRP concentration was higher in males (median, interquartile range [IQR], 0.25 [0.10–0.50] mg/dl versus 0.19 [0.07–0.39] mg/dl; $p = 0.05$) and smokers (median [IQR], 0.27 [0.11–0.58] mg/dl versus 0.14 [0.06–0.31] mg/dl; $p = 0.002$). No correlation was found between CRP levels and HIV-viral load or CD4 cell counts (Table 2). To explore factors associated with the highest CRP concentrations, the variable was dichotomized, and subjects above and below the 75th percentile (0.49 mg/dl) were compared. Patients with CRP concentrations above 0.49 mg/dl had also higher median

Table 2
Univariate analysis of variables associated with C-reactive protein (CRP) concentrations

Variable	CRP concentration (mg/dl), median [interquartile range]	Spearman's ρ	p
Total serum cholesterol level		0.16	0.01
Serum LDL cholesterol level		0.23	0.001
Serum HDL cholesterol level		−0.19	0.004
Serum triglyceride level		0.13	0.04
Framingham risk score		0.19	0.006
CD4 cell count		−0.3	0.6
HIV-viral load		−0.03	0.9
Age		−0.007	0.9
Sex			0.05
Female	0.19 [0.07, 0.39]		
Male	0.25 [0.10, 0.50]		
Current smoker			0.002
Yes	0.27 [0.11, 0.58]		
No	0.14 [0.06, 0.31]		
Current treatment with antiretrovirals			0.7
Yes	0.24 [0.09, 0.50]		
No	0.25 [0.00, 0.40]		
Naïve	0.20 [0.05, 0.43]		0.4
Current treatment with NRTI	0.23 [0.14, 0.63]		0.48
Current treatment with NNRTI	0.23 [0.08, 0.41]		0.5
Current treatment with PI	0.25 [0.10, 0.50]		0.3
Current treatment with NNRTI+PI	0.60 [0.14, 0.89]		0.14

NRTI: nucleoside reverse-transcriptase inhibitors; NNRTI: non-nucleoside reverse-transcriptase inhibitors; PI: protease inhibitors.

Table 3
Logistic regression models for the effect of antiretroviral therapy on the highest levels of C-reactive protein

Model	OR (95% CI)	p^*
Model 1: crude	1.18 (0.59–2.33)	0.64
Model 2: model 1 + HDL cholesterol	1.80 (0.83–3.90)	0.14
Model 3: model 2 + LDL cholesterol	2.54 (1.07–6.04)	0.035
Model 4: model 3 + smoking status	3.09 (1.19–8.02)	0.021

Missing cases are excluded (188 cases included).
* p-Value based on the Wald test.

LDL-cholesterol levels ($p = 0.01$), lower HDL-cholesterol levels ($p = 0.02$), and were more frequently current smokers ($p = 0.01$).

In the multivariate analysis, the top quartile of CPR (0.49 mg/dl) was associated with HDL and LDL cholesterol levels, smoking and ART treatment. Age, sex, history of high blood pressure or diabetes, Framingham risk score, HIV viral load, and CD4 cell counts were not associated with the top quartile of CPR in multivariate analysis. The same results were obtained when the co-variables were analyzed as continuous or categorical. Although CRP was available in 243 of 245 patients, the final model included only 188 observations, due to the sum of missing data of all variables included in multivariate analysis. There were not differences between the 188 included patients and those with any missing data with respect to demographic, clinical and laboratory parameters (data not shown). In the final model, the odds ratio (OR) for the highest quartile of CPR concentrations was 0.16 (95% confidence interval [CI], 0.06–0.46; $p = 0.001$) in a comparison of patients in the top third of HDL cholesterol levels with those in the bottom third. The OR for the comparison between top third and bottom third of LDL cholesterol levels was 6.35 (95% CI, 2.30–17.51; $p < 0.001$). Smoking was also associated with high CPR levels (OR, 3.03; 95% CI, 1.20–776; $p = 0.019$). Finally, although CPR levels were not different according to ART status in the univariate analysis (Table 2), once adjusted for smoking, HDL and LDL cholesterol levels, ART was associated with high CPR level (OR, 3.09; 95% CI, 1.19–8.02; $p = 0.021$), as shown in Table 3. This table shows how the association between ART and the highest CRP levels, not significant in univariate analysis ("crude" model), changes to become significant when other cardiovascular risk factors associated both with ART and CRP are sequentially incorporated in the logistic regression model. A multivariate model that included also the missing values as categorical variables (243 cases) showed similar results. However, in this model the association between ART and high levels of CPR did not reach statistical significance.

4. Discussion

In this study, we have found an association between CRP levels and traditional cardiovascular risk factors in HIV-infected patients. Cigarette smoking, serum LDL-cholesterol, and inversely HDL-cholesterol, were predictors of high CRP concentrations. In addition, antiretroviral therapy was also independently associated with high CRP levels.

The association of high levels of CRP with several established CVD risk factors is consistent with the hypothesis that CRP may be a marker for preclinical cardiovascular disease in HIV-infected patients. Interrelations between CRP concentrations and other risk factors for cardiovascular disease, such as cigarette smoking and cholesterol fractions, mainly HDL-cholesterol, had been reported in general population [16–18], but not in the context of HIV disease. Although this association may account for a substantial component of the relationship of CRP with prevalent CVD, a residual independent effect of CRP has also been demonstrated [19,20]. Indeed, epidemiologic studies have shown that CRP is an independent predictor of risk of myocardial infarction, stroke, peripheral arterial disease, and sudden cardiac death, even in apparently healthy individuals [21].

In contrast to the findings of the MACS cohort [14], where an association between HIV-disease progression and CRP levels was found, patients under ART showed higher levels of CRP than non treated patients in our study, suggesting an enhanced cardiovascular risk under current antiretroviral regimens. Several studies have reported an increased incidence of coronary events in patients receiving ART, thought to be due mainly to the development of dyslipidemia [2,22]. A recent analysis of data from DAD study, however, found that dyslipidemia alone does not fully account for the association between ART and increased risk of myocardial infarction [6]. As stated before, our results support a direct influence of ART on CRP levels that is independent of the lipid abnormalities induced by antiretroviral drugs. This finding suggests that some of the relationships between ART and cardiovascular risk not explained by conventional risk factors could be explained by the increase in CRP levels. Of note, although significant in multivariate analysis, median CRP values were not different among patients receiving and those not receiving ART. Smoking was associated positively with CPR levels but it was inversely related to ART treatment. HDL cholesterol was higher in those patients receiving ART, but was inversely associated with CPR levels. These divergent associations confounded towards the null value the association between ART and CPR levels. These results indicate that any insight into the relationships between ART and CPR should consider the intricate relations among the potential confounding variables.

Several study limitations should be taken into consideration. First, some of the patients who were not receiving ART at the study time-point had been on ART before. Unfortunately, several variables concerning ART in those patients, including previous antiretroviral regimens given and their duration were not recorded. The effect that previous ART could have had on CRP levels is unknown. Second, the cross-sectional design limits the conclusions about the effect of the ART on CRP levels, since potential confounders such as time of exposure to the different antiretroviral regimens

were not controlled. Third, the small number of patients did not allow us to evaluate separately the effect of the different combinations of antiretrovirals, and thus the influence of individual medications or triple-drug regimens on CRP levels remains undetermined. Among antiretroviral drugs, PIs have predominantly been implicated in the development of hyperlipidemia and cardiovascular risk [2,23,24]. In our study, patients treated with PI-based regimens and those receiving both PI and NNRTI showed the highest CRP levels but the differences were not statistically significant.

In summary, serum CRP levels are associated with classic cardiovascular risk factors and may predict a higher risk of future coronary events in HIV-infected patients receiving ART. CRP might be a valuable adjunct to traditional risk factors in estimating overall cardiovascular risk in HIV-infected patients on ART. Future studies are needed to corroborate our results, and assess the clinical applicability of this biomarker.

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