Journal of Infection (2008) **57**, 47—54

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Association of non-HDL cholesterol with subclinical atherosclerosis in HIV-positive patients☆

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Accepted 17 May 2008
Available online 12 June 2008

KEYWORDS
Apolipoprotein B;
Atherosclerosis;
HIV infection;
Intima-media thickness;
Metabolic syndrome;
Non-HDL cholesterol;
TG/HDL ratio

Summary *Objectives:* To assess the relationship between non-classical cardiovascular (CV) risk factors including non-HDL cholesterol (non-HDL-C), apolipoprotein B, triglycerides to HDL ratio, LDL size, inflammation or oxidative stress parameters and carotid intima-media thickness (CIMT), in order to better identify prevention or therapeutic targets. In addition, we studied the relationship between metabolic syndrome (MS) and CIMT.
Methods: Cross-sectional study including 232 HIV-positive (HIV+) adults (80% treated by combined antiretroviral therapy) extracted from the ANRS CO3 Aquitaine Cohort.
Results: There was a significant association of higher non-HDL-C ($p < 0.01$), apolipoprotein B ($p < 0.01$) levels or TG/HDL ratio ($p < 0.05$) with higher CIMT when compared the first vs fourth quartile, while there is no association between CIMT and LDL-C ($p = 0.09$) or LDL size ($p = 0.55$). In multivariate analysis, only the TG/HDL molar ratio > 1.5 tend toward significance ($p = 0.08$). MS was observed in only 7.3% of patients with the NCEP-ATP III definition and 11.2% with the IDF criteria. Whatever the used definition, there was a significant association between MS presence and increased CIMT ($p < 0.05$) in univariate and multivariate model.
Conclusions: Non-HDL-C, TG/HDL ratio and apolipoprotein B levels, which are closely linked to lipid disorders associated to the MS, appear as stronger predictive markers than LDL-C for

☆ Financial support: ''*The Agence Nationale de Recherches sur le Sida et les hepatites virales*'' (ANRS, Paris, France) supports the ANRS CO3 Aquitaine Cohort. The SUPRA study was sponsored by the French Charity ''*Sidaction.*''

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doi:10.1016/j.jinf.2008.05.007

screening subclinical atherosclerosis in HIV+ populations. Achieving non-HDL-C target defined by the NCEP-ATP III guidelines appears of great importance to reduce CV complications in HIV+ patients.

Introduction

The introduction of combined antiretroviral therapy (c-ART) has significantly increased the survival rates of HIV-positive (HIV+) patients and has improved their quality of life.[1] However, emergence of cardiovascular (CV) complications related to HIV infection *per se* and/or to c-ART has raised clinical and public health concerns[2–5] given the increasing duration of exposure to c-ART. Several classical CV risk factors including age, gender, body mass index (BMI), blood pressure, increased total cholesterol (TC) levels or tobacco consumption have been previously recognized in HIV+ patients.[6] Surprisingly, there are contradictory reports on the association between low density lipoprotein cholesterol (LDL-C), the most recognized lipid risk factor in non-HIV populations,[7] and CIMT in HIV+ populations.[8–11] Because classical risk factors do not account for all atherosclerosis risks in HIV+ patients, a search for non-classical could be of interest. Some of them could be directly related to HIV infection or its treatment such as duration of c-ART, time from HIV diagnosis, HIV plasma viral load or lipodystrophy.[12–15] Other ones including non-high density lipoprotein (HDL) cholesterol, triglyceride (TG) to HDL ratio, apolipoprotein B (apoB), LDL size, inflammation or oxidative stress could be related to metabolic disorders such as metabolic syndrome (MS) and lipid triad (LT). LT defined as low HDL-C, high TG and small dense LDL presence[16] represents the characteristic dyslipidemia of MS. In order to evaluate non-classical CV risk factors, we studied firstly the relationship between CIMT and biological parameters (lipids, inflammation and oxidative stress markers); then the relationship between CIMT and metabolic cluster (MS, LT) presence.

Patients and methods

Study design

This ancillary study was performed on 232 HIV+ patients from the SUPRA (Surveillance of the Progression of Atherosclerosis) study sample extracted from the ANRS CO3 (Agence Nationale de Recherches sur le SIDA et les hépatites virales) Aquitaine cohort. In brief, 423 HIV+ patients were included consecutively between September and December 1999 in order to investigate the extent of atherosclerosis by measuring the CIMT and studying CV risk factors.[8] Blood samples were collected the day of CIMT measurement after an overnight fasting. A subgroup of 232 patients was selected on the basis of available aliquots frozen at −80 °C to perform complementary analysis including inflammation, oxidative stress parameters and LDL size determination. There was no significant difference between the characteristics of patients of this subgroup of 232 patients compared to the entire cohort of 423 patients.

Initial study procedures

CIMT measurement: the ultrasonographic scanning of carotid arteries was performed while the subjects were lying in the decubitus position. IMT of the left common carotid artery was measured by high resolution B-mode ultrasonography (ACUSON, Paris, France) on the far wall carotid arterial with a mechanical sector transducer of 7.5 MHz frequency and a pulsed Doppler, as previously described.[8]

Lipodystrophy was categorized as peripheral fat accumulation, lipoatrophy and mixed syndrome on the basis of clinical examination and standardized questionnaire.[17]

Immuno-virological parameters: $CD4^+$ cell count (flow cytometry) and plasma HIV-1 viral load (Chiron Quantiplex RNA HIV-1, Emeryville, CA, USA) were determined.

Biological parameters: glucose and insulin (enzyme linked immunosorbent assay) were determined to calculate HOMA index with the formula[18]: insulin (μUI/l) × glucose (mmol/l)/22.5. TG, TC and HDL-C concentrations were measured by enzymatic method. LDL-C was calculated with Friedwald's formula for TG < 4.5 mmol/l; non-HDL-C was defined as (TC − HDL-C). ApoA1 and apoB were measured by immunonephelometric method.

Complementary study procedures

LDL size: the size of the predominant LDL subfraction was determined by plasma electrophoretic migration in non-denaturant polyacrylamide gradient gels (Spiragel 1.5–25%, Lara-Spiral, France) as previously described.[19]

Inflammation parameters: high sensitive C-Reactive Protein (hs-CRP), albumin and prealbumin levels were determined using immuno-turbidimetric assay on an Olympus 2700 analyzer (Olympus, Rungis, France).[20]

Oxidative stress parameters:

(i) Total antioxidant capacity of the plasma (TAC) was measured using the Trolox equivalent antioxidant capacity with a colorimetric technique (Randox, Mauguio, France) adapted on an Olympus 2700 analyzer (Olympus, Rungis, France);
(ii) Plasma concentration of advanced oxidation protein products (AOPP), considered as an index of protein oxidation, was measured by spectrophotometry (340 nm) using chloramine T and expressed as micromoles per liter of chloramine T equivalents[21];
(iii) Plasma malonyldialdehyde (MDA) levels, as a marker of lipid peroxidation were determined by spectrofluorometry[22];
(iv) Plasma concentration of advanced glycation end products (AGEs) was determined on diluted (water) plasma sample by fluorescence at 460 nm after excitation at