Association of autoantibodies anti-OxLDL and markers of inflammation with stage of HIV infection

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HIV infected individuals show a wide spectrum of changes caused by systemic immunosuppression homing from the virus itself or therapies used to improve this state. Uncontrolled replication of the virus causes changes in lipid profile, such as increased levels of triglycerides, low density lipoprotein and decreased high density lipoprotein, to promote a dyslipidemia condition [1].

Oxidative stress is also elevated in HIV+ patients due to excessive production of free radicals that play a major role in oxidative modification in LDL particle [2]. HIV+ patients with low counts of T CD4 cells show reduced plasma levels of anti-LDLm (LDL modified), and these low levels of antibodies are also associated with the lipodystrophy syndrome [3]. Serum levels of OxLDL are higher in HIV+ patients on antiretroviral therapy especially in patients with lipodystrophy syndrome compared with soronegative patients [4]. There are also other types of disorders that are frequent in HIV infection, such as endothelial dysfunction and metabolic disorders including insulin resistance [5]. All these factors increase the risk of HIV+ patients to accelerate the progression of the atherosclerosis.

In this cross-section study, we included consecutive 69 adult patients of both gender with sorological positive to HIV infection. In HIV-infected individous were evaluated immune markers, HIV infection parameters and autoimmune response to oxidized low density lipoprotein. Ethical approval was obtained from the local institutional ethics committee and written consent was obtained from all patients.

We evaluated several patient characteristics related to HIV infection, including lypodistrophy, antiretroviral therapy, AIDS, time of treatment with HAART, and AIDS-related diseases (e.g. hepatitis, tuberculosis, Kaposi sarcomas, pneumonia, candidiase, and leucoplasia pilosa). Anthropometric variables, including body mass index (BMI), gender, age, smoking status and treatment with hypolipidaemic, antiretroviral drugs and nadir CD4 T-cell count documented were recorded. The presence of hypertension or diabetes was defined according to standard international criteria [6]. Lipodystrophy was defined as the presence of body fat changes that could be clearly recognized by the patient and confirmed by the doctor. Plasma viral load was measured with the Cobass TaqMan HIV-1 assay (Roche, Basel, Switzerland) and CD4 T-cell count was determined by flow cytometry (Beckman–Coulter). A sample of fasting venous blood was obtained during the clinical examination.

Serum total cholesterol and triglyceride concentrations were measured by standard methods (Beckman–Coulter, Fullerton, CA, USA). HDL cholesterol was analyzed using a homogeneous method (Beckman–Coulter). LDL concentrations were calculated using the Friedewald formula [7].

The cytokines pro-inflammatory and anti-inflammatory were evaluated by ELISA, according to the information provided by the manufacturer (R&D Systems, Minneapolis, MS). To determine the Abs to copper-oxidized LDL, we used an established assay as previously described [8]. Briefly, an aliquot of the plasma sample (3.5 L/ml) was added onto microliter plates coated with 1 μg/ml human oxLDL (20 mM Cu2+, 24 h), blocked with a 1% solution of gelatin and incubated overnight with mouse plasma samples (50 μL; diluted 1:400). A peroxidase-conjugated secondary goat anti-mouse IgG antibody

Table 1
Demographic, clinical and biological characteristics of the subjects infected with HIV virus.

Patients (n)	69
Gender (males/females)	49/20
Age (years)	46 ± 7.6
Cardiovascular disease risk factors	
Diabetes N (%)	4 (6)
Hypertension N (%)	13 (18)
Dyslipidaemia N(%)	6 (9)
Smoking N (%)	13 (18)
Body mass index (kg/m^2), *mean values ± SD*	24.4 ± 3.2
Lipid profile	
Total cholesterol (mg/dl), *mean values ± SD*	181 ± 46
LDL-C (mg/dl), *mean values ± SD*	110 ± 29
HDL-C(mg/dl), *mean values ± SD*	44 ± 12
Triglycerides (mg/dl), *mean values ± SD*	171 ± 112
TC/HDL-C, *mean values ± SD*	4.1 ± 2.9
Risk factors for HIV infection	
Time of treatment with HAART (years)	9.6 ± 3.5
AIDS-related disease (%)	15 (21)
Hepatitis C virus coinfection (%)	9 (13)
Lipodistrophy (%)	11 (16)
Antiretroviral therapy	
NRTIs N(%)	64 (92)
Protease inhibitors N (%)	39 (56)
NNRTIs N (%)	25 (35)
Current CD+4 cell count (cells/μL)	520 ± 266
Nadir CD4+ cell count (cells/μL)	209 ± 157
Viral load<40 copies/ml (%)	65 (94)
Inflammation and oxidation markers	
IL-1β (pg/ml), *median (IQR)*	2.88 (2.48–4.53)
IL-6 (pg/ml), *median (IQR)*	5.36 (3.57–9.05)
IL-10 (pg/ml), *median (IQR)*	7.36 (6.81–10.81)
TNF-α (pg/ml), *median (IQR)*	2.46 (2.39–4.37)
Abs anti-OxLDL (IR), *median (IQR)*	0.25 (0.20–0.35)

Abbreviations: TC/HDL: total cholesterol/high density lipoprotein; HAART: highly active antiretroviral therapy; NRTIs: nucleoside reverse transcriptase inhibitors; NNRTIs: nonnucleoside reverse transcriptase inhibitors; Abs anti-OxLDL: autoantibodies anti-OxLDL; RI: reactivity index, IQR: interquartile range.

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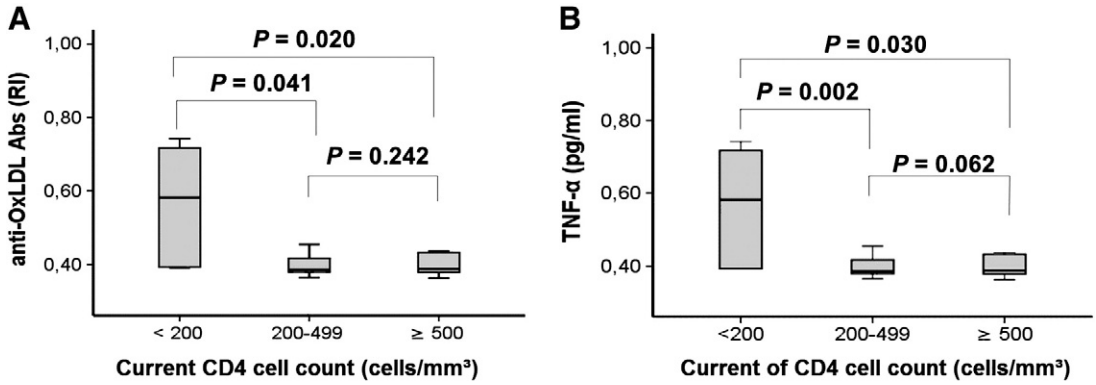

Fig. 1. (A) Titers of the autoantibodies anti-OxLDL in HIV-infected patients according to the stages of infection by CD4 cell counts. (B) Serum concentration TNF-alfa of HIV-infected patients in relationship to CD4 cell counts. RI: reactivity index. Comparisons between groups were made by using the ANOVA test with Tukey pos-test. TNF-alfa presented in log transformed.

(50 μL; diluted 1:400) was used to detect anti-OxLDL. The optical density was measured at 450 nm (Titertek Multiskan MCC/340P, model 2.20; Labsystems). The procedure for the copper-oxidized LDL (7.5 μg/ml) followed smaller modifications as described by Ketelhuth et al. [9].

The present study was approved by institutional ethics committee of Dental School of University of Sao Paulo, and informed consent was obtained from each patient.

Results are expressed as means ± SD or median values and inter-quartile range. Categorical variables were expressed as number of subjects and percent values. Variables with non-Gaussian distribution were log-transformed for comparisons. Analyses of variance with Tukey's post-test were used to examine comparisons between groups of CD4 T-cell counts or in the case of the non-Gaussian distribution, the Mann–Whitney *U*-test was used. Correlations between variables were tested by *Pearson* test. Factors independently associated with anti-OxLDL Abs were identified with multivariable linear regression. A *P* value of <0.05 was considered significant. Statistical analyses were performed with SPSS 17.0.

Demographic characteristics of patients are presented in Table 1. In the present study we have shown that hypertension and smoking are major risk factors of cardiovascular disease. The time of therapy with HAART is elevated to 9.6 years revealing great exposition of HIV infection. The medications very prevalent in use were nucleoside reverse transcriptase inhibitors with 92% and protease inhibitors with 56%. The viral load show nine patients presented values undetectable and with mean of CD4 presented above to 500 cells count. Only four patientes show viral load >40 copies/ml (Table 1).

The patients were separated by CD4 cell counts; <200 cells/μL (10 patients); 201–499 cells/μL (30 patients); ≥500 cells/μL (29 patients), in accordance with the definitions of CDC [10].

However patients who had CD4 counts less than 200 (cells/μL), in AIDS, have higher concentrations of anti-OxLDL Abs, differently according to the stages of the disease (Fig. 1A). These same patients also showed a rise to AIDS elevated concentrations of TNF-α, revealing an inflammatory component associated with lower amounts of CD4 cells (Fig. 1B).

No differences in other cytokines between groups by CD4 cell counts were observed.

We found that current CD4 cells and anti-OxLDL Abs exhibit an inverse correlation ($r=-0.26$, $P=0.02$) as observed in Fig. 2A. This correlation was maintained in relation to the levels of nadir CD4 cells hit by study subjects ($r=-0.34$, $P=0.004$; Fig. 2B).

Multiple linear regression including autoantibodies anti-OxLDL as dependent variable showed that nadir reached CD4+ and TNF-α remained independently associated with anti-OxLDL (Table 2). When patients had low nadir of CD4+ cells and had high TNF-α concentration, titers of anti-OxLDL Abs increased.

In conclusion, our results show that anti-oxLDL IgG Abs are elevated in HIV-infected patients with CD4 cell count less. This phenomenon is associated with increases levels of TNF-alfa and nadir of CD4 cell count. The oxidation process in HIV infection promote modification in LDL particle in which new antigens are generated. This promotes lifting in autoimmune response to epitopes of the oxLDL. One possible cause this response may be the modulation in survival and memory of antigen

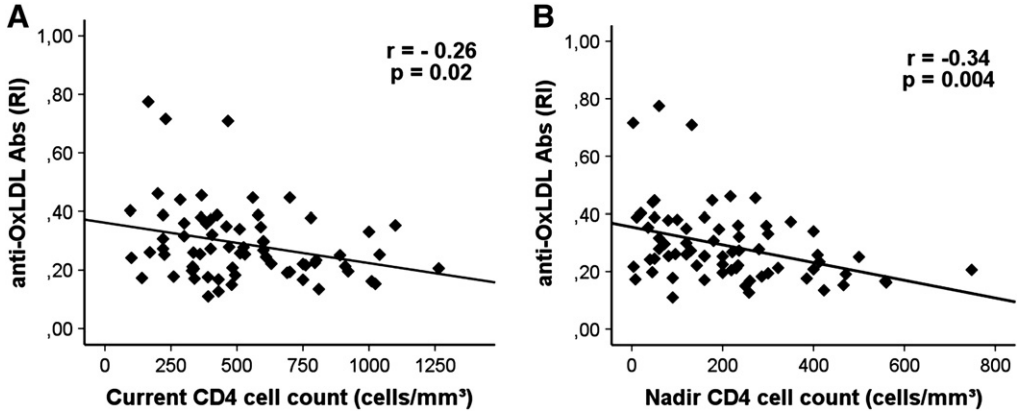

Fig. 2. (A) Scatter plots showing inverse correlation ($r=-0.26$; $P=0.02$) between titers of autoantibodies (Abs) anti-OxLDL and CD4 cell counts in baseline study. (B) Scatter plots showing inverse correlation ($r=-0.36$; $P=0{,}004$) were observed among titers of Abs anti-OxLDL and nadir CD4 cell counts. RI: reactivity index; all correlations were made by *Pearson* correlation test.

Table 2
Multiple linear regression showing significant associations between Abs anti-OxLDL antibodies, biomarkers for HIV infection and inflammation.

	Coefficients		t	P	95% confidence interval	
	B	SE			Lower	Upper
Constant	0.364	0.158	2.298	0.025	0.133	0.666
Age, per years	0.001	0.002	0.285	0.777	−0.003	0.004
Body mass index, kg/m^2	−0.003	0.005	−0.702	0.485	−0.013	0.006
Time of medication, per years	−0.005	0.004	−0.125	0.270	−0.013	0.004
Nadir CD4+, per cells/μL	−0.066	0.031	−2.117	**0.038**	−0.125	−0.004
TNF-α[a] (pg/ml)	0.356	0.103	3.463	<**0.001**	0.150	0.561

In bold statistically significant association (P<0.05).
[a] Variables log transformed.

presenting cells by HIV virus, these cells are responsible for antibodies production.

The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology.

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The effect of remote ischemic preconditioning on exercise-induced plasma troponin I appearance in healthy volunteers

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Following prolonged endurance exercise (e.g. marathon running), circulating levels of cardiac troponin are increased in more than 50% of individuals [1]. Also after controlled laboratory-based exercise of shorter duration plasma troponin concentration increases, but these data are sparse [2,3]. Up to now, the underlying mechanism of troponin appearance after strenuous exercise in humans is unknown. Many theories have been posed, including exercise-induced increase in cardiomyocyte membrane permeability or cardiomyocyte injury due to ischemia reperfusion (IR) [1]. In this study we aimed to investigate whether IR-injury contributes to the rise in circulating troponin after strenuous exercise in healthy volunteers. Remote ischemic preconditioning (RIPC) is a powerful strategy to limit IR-injury [4]. Therefore, in a randomized controlled cross-over study in healthy volunteers, we studied the effect of RIPC on exercise-induced troponin I release. If IR-injury contributes to the plasma appearance of troponin in this model, then exercise-induced troponin release can be used as a model of myocardial IR-injury in healthy volunteers.

The study was approved by the local ethics committee. After informed consent, twenty healthy volunteers (age 22 ± 4 years, 10 males) participated. Before randomization, all volunteers underwent a maximal cycling test to determine maximum heart rate. Subsequently, in a crossover design, all volunteers performed a submaximal exercise test preceded by RIPC or a control intervention. The tests were separated by two weeks and the order was randomized. After 30 min of supine rest, subjects performed bicycle exercise at 80% of their maximum heart rate or heart rate reserve (whatever resulted in the highest target heart rate) for 70 min, immediately followed by cycling at 95% of maximal heart rate reserve until exhaustion or for a maximum of 15 min. The RIPC stimulus was applied during the 30 min immediately before the exercise test. RIPC consisted of three 5-min cycles of bilateral forearm ischemia, induced

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