# Both HIV-Infection and Long-Term Antiretroviral Therapy are Associated with Increased Common Carotid Intima-Media Thickness in HIV-Infected Adolescents and Young Adults[§]

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**Abstract:** *Objective*: To evaluate common carotid artery intima-media thickness (CCIMT) and cardiovascular risk factors in HIV-infected adolescents on combination antiretroviral therapy (cART).

*Methods*: 23 HIV-infected adolescents were matched with 19 healthy subjects by gender, age and body mass index (BMI). CCIMT was measured by Echo-Doppler ultrasound. Bootstrapped multiple linear regression was used to identify potential predictors of CCIMT including HIV status, gender, age, BMI, waist circumference, HDL-cholesterol, LDL- cholesterol, triglycerides, folate, homocysteine and insulin resistance as detected by the homeostasis model assessment, mean blood pressure, and CD36 expression.

*Results*: In the pooled sample, age ranged from 17 to 23 years and BMI between 16.0 and 25.6 kg/m$^2$. Mean (SD) CCIMT was higher in HIV-infected than in healthy subjects [0.5 (0.1) *vs* 0.4 (0.1) mm, $p < 0.001$]. Higher values of CCIMT were associated with HIV infection ($p < 0.001$) and male gender ($p < 0.001$). CCIMT was also associated with the duration of treatment in subjects with the longest cART exposure, *i.e.* those exposed to a PI-based and/or NNRTI-based regimen plus a single or double NRTI ($p = 0.019$).

*Conclusion*: HIV infection and longer duration of cART are associated with higher CCIMT in adolescents and young adults.

**Keywords:** Cross-sectional study, children, HIV, antiretroviral therapy, intima-media thickness.

## INTRODUCTION

The life expectancy and quality of life of HIV-infected pediatric patients have greatly improved since the introduction of combination antiretroviral therapy (cART) [1]. On the other hand, proatherogenic metabolic abnormalities have been associated with antiretroviral therapy (ART) in HIV-infected children [2]. So far, it is unclear whether HIV infection or ART or their combination is responsible for an increased risk of atherosclerosis [2]. Moreover, the role of traditional cardiovascular risk factors, as opposed to HIV-related and ART-related factors, is not known.

Although the clinical manifestations of atherosclerosis do not appear until middle and late adulthood, the process of atherogenesis starts much earlier [3]. Therefore, the identification of early signs of atherosclerosis is of primary importance in the management of HIV-infected children. Carotid intima-media thickness (IMT) as measured by Echo-Doppler ultrasound is an index of subclinical atherosclerosis which is especially suitable for children because of its low invasiveness [4, 5].

Hyperhomocysteinemia is a risk factor for cardiovascular diseases, stroke and venous thromboembolism [6]. Only sparse data are available on homocysteine in HIV-infected children and adolescents and its relationship with IMT is not known [7].

CD36 is a multifactorial transmembrane glycoprotein widely distributed among tissues and with many functions in lipid metabolism [8]. Physiologic and pathologic consequences of CD36 engagement depend on the involved tissues [9, 10]. Conflicting data have been reported on the role of CD36 in the pathogenesis of ART-related dyslipidemia in HIV-infected adults [11, 12]. Moreover, the association between CD36 expression on monocytes and IMT has never been investigated in HIV-infected individuals.

We performed a cross-sectional study of HIV-infected and healthy adolescents matched by gender, age, and body mass index (BMI) to evaluate the relationship between IMT, HIV infection, cART, traditional and novel cardiovascular risk factors, and CD36 expression on monocytes.

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## MATERIALS AND METHODS

### Study Design

This is a cross-sectional study of IMT predictors performed in HIV-infected cART-treated adolescents and young adults. The data reported here were obtained from the baseline evaluation of HIV-infected subjects participating to an ongoing 3-year follow-up study.

Twenty-three HIV-infected adolescents and young adults followed at the Department of Pediatrics of the "L. Sacco" Hospital were selected on the basis of the following criteria: 1) Caucasian ethnicity, 2) vertical HIV infection, 3) 15 ≤ age ≤ 24 years, 4) treatment with cART for at least 6 consecutive months prior to the enrollment and, 5) viral load < 5000 copies/mL at enrollment. Allowed ART at enrollment included two nucleoside analogue reverse transcriptase inhibitors (NRTI) in combination with either a protease inhibitor (PI) or a non-nucleoside reverse transcriptase inhibitor (NNRTI). Concomitant opportunistic infections, hypertension, diabetes, renal failure, and family history of premature cardiovascular disease were reasons for exclusion. The ART history was obtained from clinical charts specifying the start and end date for any treatment. The HIV-infected subjects were matched to 19 healthy adolescents and young adults without HIV infection who were recruited among the medical students and the family members of the personnel of the Department of Pediatrics. Matching was performed by age (within 1 year), gender (same) and body mass index (BMI) (within 1 kg/m$^2$). The study was approved by the Ethical Committee of the "L. Sacco" Hospital and informed consent was obtained from the subjects when aged ≥ 18 years or from parents or legal guardians.

### Clinical Evaluation

Weight and height were measured following the *Anthropometric Standardization Reference Manual* [13]. Waist circumference was measured midline between the last rib and the iliac crest [14]. Systolic and diastolic blood pressure was measured following the *Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents* [15]. Mean blood pressure (MBP) was calculated as [(2 X diastolic blood pressure) + systolic blood pressure)/3] [16].

### Laboratory Evaluation

Total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, glucose and insulin were measured after an overnight fast. Insulin was measured using a chemoluminescence immunometric assay (Immunolite 2000, Medical Systems, Genoa, Italy). The homeostasis model assessment (HOMA-R) was used as surrogate marker of insulin resistance [17]. Homocysteine was measured using an enzymatic immunoassay (Homocysteine EIA, Axis Shield, Dundee, Scotland). Folate was measured using an automatic analyzer (Elecsys 2010, Roche Diagnostics, Mannheim, Germany). CD36 antigen expression on peripheral blood monocytes (CD14+) was measured by flow cytometry. 50 μL of fresh whole blood were stained with directly labeled fluorescein (FITC) and phycoerythrin (PE) monoclonal antibodies (anti-CD36 FITC, anti-CD14 PE; Beckman Coulter Immunotech, Marseille, France). Appropriate isotypic controls were used for CD36 and CD14 antibodies. Stained specimens were fixed with the Immunoprep Reagent Kit (Beckman Coulter Immunotech, Marseille, France) using a Q-prep workstation (Beckman Coulter Immunotech, Marseille, France) and analyzed using an EPICS XL flow cytometer (IL Coulter, Milan, Italy). 5000 monocytes gated on side-scatter PE parameters were evaluated for CD36 antigen expression. Because most CD14+ antibodies are CD36+, we measured the channel of mean fluorescence intensity (MFI) of the CD36+ peak on a linear scale. To minimize measurement errors, instrument calibration was performed daily using Flow-Set Fluorospheres (Beckman Coulter Immunotech, Marseille, France) with a MFI comparable to that of the monoclonal antibodies of interest. When needed, the photomultiplier tube (PMT) voltage was changed in order to acquire the MFI of Flow-set Fluorospheres in the same channel; PMT voltage changes never exceeded 5 mV.

### Echo-Doppler Ultrasound

Common carotid intima-media thickness (CCIMT) measurements were performed at the Angiology Unit, Research Center on Vascular Diseases, University of Milan. The same trained operator, who was blinded to the HIV status of the subjects, performed CCIMT measurements on all the children. The Echo-Doppler apparatus (ATL HDI 5000, Philips, Milan, Italy) was equipped with a multi-frequency linear array probe (5-12 MHz) and a 3-electrode electrocardiographer. IMT was measured while the subject was lying supine by visualizing a longitudinal cross-section of the branching point of the artery at one end of the image. B-mode scans were performed using the least possible depth and no enlargement of the image. The intima-media distance, i.e. IMT at the point of bifurcation and at 1 and 2 cm from the bifurcation on the rear part of the common carotid artery, was measured in synchrony with the R-wave of the electrocardiogram. The procedure was repeated to obtain antero-posterior, lateral and postero-anterior scans. Each measurement was performed in triplicate and the mean value was used for analysis. The intra-operator coefficient of variation for CCIMT measurement in our laboratory is 2.3%, which is in agreement with available data [18-20].

### Statistical Analysis

Normally distributed variables are reported as mean, standard deviation (SD) and minimum and maximum values. Variables that were not normally distributed (LDL-cholesterol, triglycerides, homocysteine, insulin, HOMA-R, systolic blood pressure, diastolic blood pressure and CD36 expression) were transformed using natural logarithms to achieve or better approximate the normal distribution (Shapiro-Wilk test) and are reported as geometric mean and minimum and maximum values. Between-group comparisons of continuous variables were performed using the unpaired Student's *t*-test on untransformed values for normally distributed variables and on log-transformed values for not normally distributed variables [21]. Log-transformation was performed using natural logarithms. Between-group comparisons of categorical variables were

performed using Fisher's exact test. The agreement between CCIMT measurements on the left and right side was evaluated using Lin's concordance correlation coefficient (CCC) [22, 23]. Because there was good concordance between the two sides, the average of left and right CCIMT measurements was used for further analysis. In order to identify predictors of CCIMT among the variables of interest we used bootstrapped multiple linear regression with backward elimination [24]. The outcome variable was IMT (continuous). Among the predictors, HIV status (1 = HIV-infected; 0 = healthy) and gender (1 = male; 0 = female) were evaluated as (naturally) dichotomous while the remaining ones (age, BMI, waist circumference, HDL-cholesterol, LDL-cholesterol, triglycerides, folate, homocysteine, HOMA-R, MBP and CD36 expression) were evaluated as continuous. Waist circumference, HDL-cholesterol, triglycerides and MBP were included in the model as they are 4 of the 5 parameters of the metabolic syndrome (MS) [25]. We preferred HOMA-R to glucose for modeling carbohydrate metabolism for the purpose of the MS definition [25]. Virtually identical results were obtained by modeling glucose instead of HOMA-R (data not shown). MBP was used instead of separate values of systolic and diastolic blood pressure to avoid problems of collinearity. Bootstrap resampling was performed on 1000 random samples of 42 subjects. The predictors with the greatest bootstrap inclusion fraction (BIF) were selected for inclusion in a definitive regression model. The 95% confidence intervals of the regression coefficients and of the measures of model fit [root mean squared error (RMSE) and adjusted coefficient of determination ($R^2_{adj}$)] of the definitive model were calculated using bias-corrected accelerated bootstrap on 1000 random samples of 42 subjects.

## RESULTS

Twenty-three HIV-infected adolescents and young adults and 19 healthy subjects matched by gender, age and BMI were enrolled into the study between May 2007 and March 2008.

At enrollment, 18 HIV-infected subjects were receiving a NNRTI-based regimen and 5 a ritonavir (RTV)-boosted PI-based regimen. Among the subjects undergoing the NNRTI-based regimen, 2 were naive to ART, 12 had been exposed to a single PI-based regimen, 2 to two PI-based regimens, and 2 to three PI-based regimens; none of these PI-based regimens included RTV as booster. Among the 5 subjects receiving the RTV-boosted PI-based regimen, 3 had been exposed to a single PI-based regimen unboosted with RTV and 2 to a single RTV-boosted PI-based regimen. The reverse transcriptase inhibitors included in the NNRTI- and PI-based regimens were zidovudine, stavudine, lamivudine, tenofovir disoproxil fumarate and emtricitabine. At the beginning of ART, 11 subjects had undergone a regimen based on single or double NRTI (zidovudine in 6 cases and zidovudine + didanosine in 5 cases). The mean (SD) duration of NNRTI-based and PI-based regimens were 3 (2) and 6 (3) years respectively. Overall, the mean (SD) duration of ART in subjects exposed ($n$ = 11) and not exposed ($n$ = 12) to a single or double NRTI was 16 (3) and 6 (4) years respectively. All treatments were performed without interruption.

Because of the study design, HIV-infected and healthy subjects had a similar age, BMI and gender distribution (male/female ratio: 12/11 *vs* 10/9, $p$ = 1.000 and Table **1**). The values of total cholesterol, LDL cholesterol, triglycerides, homocysteine, glucose, insulin, HOMA-R and systolic and diastolic blood pressure were similar in the two groups. HDL cholesterol was lower in HIV-infected children ($p$ = 0.016). Also folate was lower ($p$ < 0.001) in HIV-infected children even if only one of them had a value below the lower normal limit of 3 ng/mL. CD36 antigen expression on peripheral blood monocytes was higher in HIV-infected children ($p$ < 0.002). The mean (SD) CD4+ cell number was 690 (373)/mm$^3$ and the viral load was < 50 copies/mL in 21 patients (91%) and equal to 458 and 2532 copies/mL in 2 patients. The right and left CCIMT measurements were highly concordant in both HIV-infected (CCC = 0.90, 95% CI 0.81 to 0.98, $p$ < 0.001) and healthy subjects (CCC = 0.93, 95% CI 0.86 to 0.99, $p$ < 0.001). Because of this finding, right and left measurements were averaged for further analysis. The mean (SD) CCIMT was higher in HIV-infected than in healthy subjects [0.5 (0.1) *vs* 0.4 (0.1) mm, $p$ < 0.001].

The BIF of the potential predictors of IMT is given in Table **2** (see *Statistical analysis* for details). HIV status was the most frequently chosen predictor (97%), followed by gender (71%). No other predictor was selected in more than 50% of bootstrap samples.

The regression model based on the two predictors identified at bootstrap analysis is given in Table **3**. HIV infection was associated with an increase of 0.13 (95%CI 0.09 to 0.17) mm of IMT and male gender with one of 0.08 (95%CI 0.03 to 0.12) mm. These two predictors were able to explain a substantial proportion of IMT variance ($R^2_{adj}$ = 54%, 95%CI 36 to 73%) with a RMSE of 0.07 (95%CI 0.06 to 0.08) mm.

A recent study developed age-specific standard deviation scores (SDS) of CCIMT for Caucasians aged 10-20 years [26]. When we applied these SDS to our subjects aged ≤ 20 years ($n$ = 30), we found that the mean (SD) CCIMT was higher in HIV than in healthy subjects [2.1 (1.2) SDS, $n$ = 17 *vs* - 0.4 (1.9) SDS, $n$ = 13, unpaired $t$-test < 0.001]. The mean SDS correspond to the 98$^{th}$ percentile in HIV subjects and to the 46$^{th}$ percentile in controls. Although reference data were available only for subjects aged 20 years and less [26], this finding confirms that the difference between HIV and controls reported in Table **1** is clinically meaningful.

The duration of exposure to different antiretroviral regimens was not the same in HIV-infected subjects. The mean (SD) duration of NNRTI-based and PI-based regimens was in fact 3 (2) and 6 (3) years respectively and the mean (SD) duration of ART in subjects exposed ($n$ = 11) and not exposed ($n$ = 12) to a single or double NRTI was 16 (3) and 6 (4) years respectively. We found an association between CCIMT and the duration of ART in subjects exposed to a PI-based and/or NNRTI-based regimen plus a single or double NRTI ($R^2_{adj}$ = 0.42, $p$ = 0.019). However, this association was no longer present after consideration of the duration of PI- and/or NNRTI-based regimen ($p$ = 0.308), NNRTI-based regimen ($p$ = 0.389) or PI-based regimen ($p$ = 0.640) (Fig. **1**). These associations persisted virtually unmodified after correction for age (data not shown). As shown in Fig. (**1**),

**Table 1. Measurements of the Children**

| | HIV (*n* = 23) | | | | Healthy (*n* = 19) | | | | Unpaired *t*-test |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Min | Max | Mean | SD | Min | Max | *p*-value |
| Age (years) | 20 | 2 | 17 | 23 | 20 | 1 | 18 | 22 | 0.675 |
| Weight (kg) | 54.2 | 12.0 | 38.2 | 85.5 | 61.1 | 11.6 | 42.0 | 83.0 | 0.070 |
| Height (m) | 1.60 | 0.10 | 1.40 | 1.90 | 1.70 | 0.10 | 1.50 | 1.80 | 0.024 |
| BMI (kg/m$^2$) | 19.9 | 2.5 | 16.0 | 25.2 | 20.7 | 2.5 | 16.9 | 25.6 | 0.289 |
| Waist circumference (cm) | 73 | 9 | 61 | 92 | 72 | 8 | 57 | 85 | 0.661 |
| Cholesterol (mg/dL) | 160 | 33 | 107 | 261 | 173 | 31 | 126 | 241 | 0.196 |
| HDL-cholesterol (mg/dL) | 53 | 15 | 20 | 85 | 66 | 18 | 32 | 100 | 0.016 |
| LDL-cholesterol (mg/dL) | 83* | — | 55 | 165 | 88* | — | 58 | 163 | 0.504 |
| Triglycerides (mg/dL) | 91* | — | 33 | 260 | 74* | — | 46 | 162 | 0.137 |
| Folate (ng/mL) | 4.7 | 1.5 | 2.6 | 8.5 | 6.9 | 1.7 | 4.4 | 10.5 | <0.001 |
| Homocysteine (μmol/L) | 11* | — | 5 | 33 | 9* | 5 | 5 | 25 | 0.233 |
| Glucose (mg/dL) | 80 | 8 | 66 | 101 | 81 | 7 | 73 | 96 | 0.501 |
| Insulin (mg/dL) | 7* | — | 2 | 23 | 6* | — | 2 | 19 | 0.736 |
| HOMA-R | 1.3* | — | 0.4 | 4.3 | 1.3* | — | 0.4 | 4.2 | 0.819 |
| Systolic blood pressure (mm Hg) | 112* | — | 90 | 126 | 110* | — | 95 | 128 | 0.989 |
| Diastolic blood pressure (mm Hg) | 67* | — | 60 | 75 | 68 | — | 58 | 80 | 0.996 |
| CD36+ cells (fluorescence units) | 519 | — | 217 | 952 | 352 | — | 211 | 762 | 0.002 |
| CCIMT (mm) | 0.5 | 0.1 | 0.4 | 0.7 | 0.4 | 0.1 | 0.3 | 0.5 | <0.001 |
| CD4+ cells (fluorescence units) | 690 | 373 | 176 | 1446 | — | — | — | — | — |

*Geometric mean (between-group comparison performed on log-transformed variable).
Abbreviations: min = minimum; max = maximum; SD = standard deviation; BMI = body mass index.

CCIMT may be similar at the beginning and at the end of exposure to a NNRTI-based regimen, a PI-based regimen or a NNRTI- and/or PI-based regimen.

**Table 2. Bootstrap Selection of Predictors**

| Predictor | BIF (Out of 1000) |
|---|---|
| HIV status | 972 |
| Gender | 708 |
| Mean blood pressure* | 325 |
| Triglycerides* | 292 |
| Homocysteine* | 245 |
| HDL-cholesterol | 264 |
| BMI | 343 |
| Waist circumference | 289 |
| CD36+ cells* | 240 |
| Age | 221 |
| Folate | 219 |
| LDL* | 265 |
| HOMA-R* | 205 |

*Selection performed on log-transformed variable.
Abbreviations: BIF = bootstrap inclusion fraction; other abbreviations as in Table **1**.

**Table 3. Prediction of CCIMT from HIV Status and Gender**

| | Regression Coefficient or Measure of Model Fit [Bootstrapped 95%CI] |
|---|---|
| HIV (1 = yes; 0 = no) | 0.13** [0.09 to 0.17] |
| Male gender (1 = yes; 0 = no) | 0.08* [0.03 to 0.12] |
| Intercept | 0.36 [0.33 to 0.39] |
| $R^2_{adj}$ | 0.54* [0.36 to 0.73] |
| RMSE (mm) | 0.07** [0.06 to 0.08] |

* $p < 0.001$.
Abbreviations: $R^2_{adj}$ = adjusted coefficient of determination; RMSE = root mean squared error.

## DISCUSSION

In the present study, CCIMT was higher in HIV-infected adolescents and young adults as compared to healthy subjects matched for gender, age and BMI. Moreover, HIV

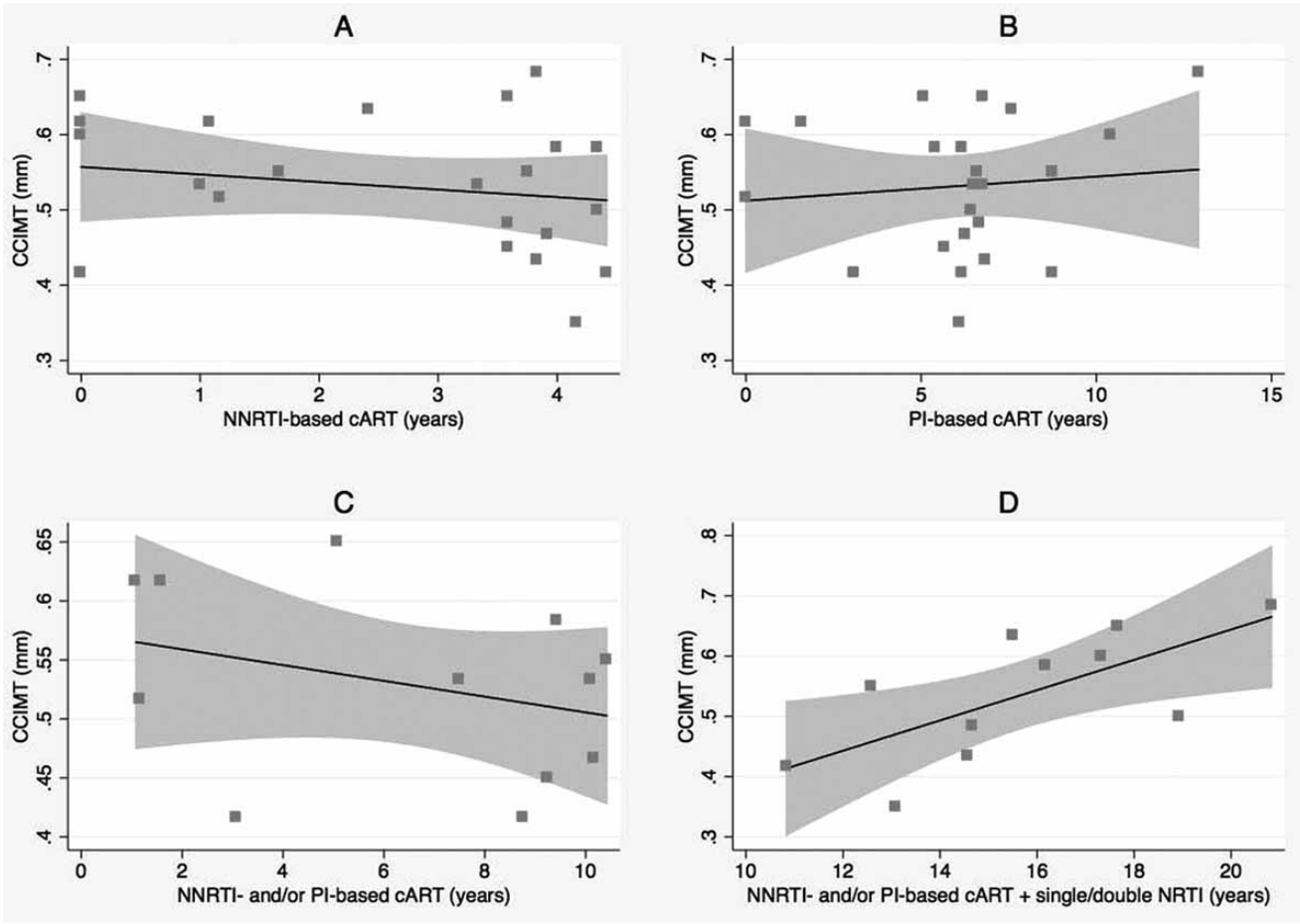


**Fig. (1).** Relationship between common carotid intima media thickness and duration of antiretroviral therapy. Abbreviations: CCIMT = common carotid intima media thickness; cART = combination antiretroviral therapy; Panel **A**: duration of therapy in subjects exposed to NNRTI-based cART regimen; Panel **B**: duration of therapy in subjects exposed to PI-based regimen; Panel **C**: duration of therapy in subjects exposed to NNRTI- and/or PI-based HAART regimen; Panel **D**: duration of therapy in subjects exposed to NNRTI- and/or PI-based cART regimen plus single/double NRTIs. Shaded areas are 95% confidence intervals of the regression line.

infection and male gender were associated with higher values of CCIMT. The relationship of CCIMT with HIV status was expected on the basis of previous studies [28] and that with male gender may simply reflect the tendency of males to have higher values of IMT than females [29].

Atherosclerosis begins in childhood and adolescence and progresses during young adulthood to cause cardiovascular diseases in middle-aged and older individuals [30]. Autopsy studies of children and adolescents have shown that the frequency and severity of atherosclerosis is positively associated with LDL-cholesterol, hypertension, impaired glucose tolerance and obesity, and negatively associated with HDL-cholesterol [30]. Thus, it is important to control for these confounders when studying the cardiovascular risk of HIV-infected children [2]. Our HIV-infected and healthy subjects were matched for gender, age and BMI by study design. Moreover, they had similar values of total cholesterol, LDL-cholesterol, triglycerides, glucose, insulin, HOMA-R and systolic and diastolic blood pressure. However, HDL-cholesterol was lower in HIV-infected than in healthy subjects. As far as non-traditional risk factors are concerned [17], homocysteine was similar in the two groups while folate was lower in HIV-subjects.

Our outcome measure was CCIMT, which has been widely employed as a marker of subclinical atherosclerosis in children at risk of cardiovascular disease [31, 32]. We measured CCIMT on the left and right side and because the measurements were highly concordant we averaged them as done by other researchers [32]. Limited data are available on the relationship between carotid IMT at different sites and cardiovascular outcomes. However, recent data show that the hazard rate of myocardial infarction and stroke increases to the same extent for increasing values of IMT at common, bifurcation, and internal carotid sites [4].

In HIV-infected adults, subclinical atherosclerosis has been linked to ART by some studies [33, 34] and to traditional and novel cardiovascular risk factors by other studies [35, 36]. However, recent data show that HIV infection and cART are independent risk factors for early carotid atherosclerosis [37]. After correction for multiple confounders, HIV-infection was associated with a higher IMT at the common carotid (+5%) and carotid bifurcation (+25%) levels. On the other hand, IMT at the carotid

bifurcation was 20% higher in subjects treated with cART for more than two years than in those who were cART-naive. The association between CCIMT, HIV infection and ART in children has been investigated by three studies [28, 38, 39]. In agreement with such studies, we found that HIV infection is associated with an higher CCIMT. Two of these studies evaluated the relationship between CCIMT and ART duration with opposite results [28, 39]. In the PERI study, children treated with cART for a mean (SD) of 86 (40) months had an IMT similar to that of the children treated for 80 (41) months [39]. In the study of McComsey and colleagues, the cumulative ART duration but not the duration of PI exposure was associated with higher common and internal carotid IMT in HIV-infected children treated with ART for a median of 64 months [28]. In the present study, CCIMT was associated with the duration of exposure to a PI-based and NNRTI-based regimen plus single or double NRTI (11 to 20 years), but not to that of a NNRTI-based regimen (0 to 5 years), PI-based regimen (0 to 12 years), or NNRTI-based and PI-based regimen (1 to 11 years). The longer duration of ART in our subjects may partly explain the different findings from the PERI study where the mean duration of ART was 7 years. Moreover, our subjects had fewer cardiovascular risk factors than those studied by McComsey *et al.* [28] and this may partially explain why a longer ART exposure was associated with CCIMT. Finally the D:A:D study has reported a greater risk of myocardial infarction in HIV-infected adults exposed to didanosine but this risk was no longer present 6 months after drug suspension [27]. Of the eleven patients with the longest exposure to ART, five were exposed to zidovudine plus didanosine. Although we cannot determine the separate contribution of didanosine to the IMT of these patients, on the basis of the available evidence, we believe that such a contribution is unlikely.

The generation of lipid-laden macrophages is a key event in atherogenesis [40]. Aberrant sterol accumulation is influenced by scavenger receptors such as CD36 [9, 10]. Studies of CD36-null mice have shown that CD36 promotes the development of atheromas [9, 10]. An increased expression of CD36 on circulating monocytes has been reported in both HIV-infected adults and children [41, 42]. Our study, the first to look at the association between CD36 and IMT, shows that although CD36 is overexpressed in HIV-infected children, it is not associated with CCIMT. The lack of association between CD36 and CCIMT does not however negate the pathophysiological relevance of CD36 overexpression on monocytes and does not exclude an association with hard cardiovascular outcomes, which should be investigated by further studies.

Our study is one of the very few that has investigated the association of IMT with HIV infection and traditional and non-traditional cardiovascular risk factors in children. However, it is not without limitations. First, our findings cannot be extrapolated outside adolescence and young adulthood because our subjects were aged from 17 to 23 years. Second, all our subjects were Caucasians so that we cannot control for the possibly confounding effect of race on the IMT-HIV relationship [43]. Third, our healthy subjects were a convenience sample of children and adolescents enrolled among the families of the personnel and the medical students and this may have introduced some bias in the comparison. Fourth and most important, a cross-sectional study cannot be used to infer any cause-effect relationship. We plan, however, to study the changes of IMT and their relationship with HIV status and treatment during the ongoing 3-year follow-up of which the data described here represent the baseline evaluation. In order to increase the available knowledge, further studies in children should have frequent follow-ups and consider measurement of IMT at multiple sites such the internal/bulb region as well the common carotid.

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