Coronary Aging in HIV-Infected Patients

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Background. Human immunodeficiency virus (HIV)–infected patients often demonstrate accelerated aging processes. We investigated whether the vascular age of a cohort of stable HIV-infected patients receiving antiretroviral therapy (ART) was increased and sought out predictors of increased vascular age.

Methods. In this cross-sectional study, 400 HIV-infected patients (mean age, 48 years) attending a cardiometabolic clinic underwent cardiac computed tomography imaging to identify coronary artery calcium (CAC). Vascular age was estimated on the basis of the extent of CAC by means of previously published equations.

Results. Increased vascular age was observed in 162 patients (40.5%), with an average increase of 15 years (range, 1–43 years) over the chronological age. In univariable analyses, chronological age, male sex, systolic blood pressure, duration of ART, fasting glucose level, fasting serum triglyceride level, total cholesterol level, low-density and high-density lipoprotein cholesterol levels, hypertension, and the presence of the metabolic syndrome were associated with increased vascular age. In multivariable linear regression analyses, current $CD4^+$ cell count was the only predictor of increased vascular age ($\beta = 0.51$; $P = .005$).

Conclusions. Increased vascular age is frequent among HIV-infected patients and appears to be associated with $CD4^+$ cell count. If these findings were to be confirmed in prospective trials, a positive response to ART with an increase in $CD4^+$ cell count may become a marker of increased risk of atherosclerosis development.

Since the introduction of highly active antiretroviral therapy (ART), the life expectancy of patients infected with human immunodeficiency virus (HIV) has significantly increased [1]. This has been accompanied by noticeable changes in the array of clinical morbidities associated with ART-treated chronic HIV infection. Atherosclerosis, dyslipidemia, diabetes mellitus, changes in body fat distribution, loss of renal function, osteopenia, and non–AIDS-defining cancers have increasingly been described as occurring prematurely in several HIV observational cohorts. Rather than occurring merely as a consequence of extended survival among ART recipients, it has been suggested that the increased rate of these morbidities may result from accelerated biological aging imposed by HIV itself and/or antiretroviral treatments. Several hypotheses have been formulated in an attempt to provide a justification for the premature aging. They include an accelerated immune senescence secondary to the exhaustion of immunological resources [2], ART toxicity (mitochondrial dysfunction and oxidative stress induced by thymidine analogues) [3], or even the accumulation of abnormal laminin A associated with HIV protease inhibitor (PI) toxicity [4–6]. Coronary artery calcium (CAC) has been used to describe the biological age of an individual, which may be very different from his chronological age [7]. In fact, since CAC accumulation is closely linked with atherosclerosis development and aging, a middle-aged man with extensive CAC has the coronary age of a much older individual. Previous studies have examined the importance of age- and sex-specific calcium score percentiles as predictors of cardiovascular events [7–9]. More recently, using data from the Multi-Ethnic Study of Atherosclerosis (MESA), age-specific curves of CAC scores appropriate for sex and ethnicity have been generated [10–12]. On the basis of these curves, an

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individual's coronary age can be calculated by matching his CAC score to the closest median CAC score of people of the same sex and race [13]. Coronary age may thus be considered a surrogate marker of the true biological age of the individual under investigation. The objective of the present study was to assess the biological age of a cohort of HIV-infected patients by means of CAC equations as recently described [13] and to identify factors associated with increased coronary age. Such an objective measure of biological age could be used as a surrogate marker of premature aging in HIV-infected patients and, potentially, as a predictor of comorbid events.

METHODS

Study population. This was a cross-sectional observational study of 400 consecutive HIV-infected patients recruited at an outpatient clinic of the University of Modena and Reggio Emilia in Italy from January 2006 through June 2007. The multidisciplinary team caring for these patients included infectious disease specialists, cardiologists, endocrinologists, radiologists, nutritionists, personal trainers, psychologists, and plastic surgeons [14]. Inclusion criteria were serologically documented HIV-1 infection, age >18 years, at least 18 months of ART exposure, and, for patients with established diagnoses of hyperlipidemia and hyperglycemia, stable lipid-lowering and diabetes therapy for at least 6 months. A signed informed-consent form to participate in this study was obtained from each patient. Patients were excluded if they reported or had documented evidence of any of the following cardiovascular conditions: previous myocardial infarction, stroke, percutaneous coronary angioplasty, coronary artery bypass surgery, or peripheral vascular disease. Demographic and clinical data, including duration of HIV infection, prior opportunistic diseases (Centers for Disease Control and Prevention classification), ART history, and lifestyle were obtained by medical chart review. Diabetes was defined as actively taking drugs to lower serum glucose level or a fasting glucose level >126 mg/dL. Smoking, alcohol consumption, and physical activity were assessed at entry according to the following arbitrarily chosen criteria. If the patient reported active smoking, the habit was classified as heavy if he smoked $\geqslant$10 cigarettes per day or light if he smoked <10 cigarettes per day. Alcohol consumption was defined as heavy when >20 g of ethanol per day was consumed. Physical activity was defined as mild or intense when <4 or $\geqslant$4 h per week of exercise, respectively, were reported. Insulin resistance (IR) was calculated using the homeostasis model assessment equation (HOMA-IR = [fasting insulin mU/mL $\times$ fasting glucose mmol/L)/22.5] [14, 15]. $CD4^+$ cell counts (most recent value and nadir), plasma HIV-1 RNA levels, and cumulative exposure to nonnucleoside reverse-transcriptase inhibitors (NNRTI), nucleoside reverse-transcriptase inhibitors (NRTIs), and PIs were recorded. HIV load was categorized as undetectable if <40 copies/mL were present. Previous AIDS diagnosis was defined according to the Centers for Disease Control and Prevention category C [16].

Total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, apolipoprotein A and B, glucose, and insulin levels were measured at entry after an overnight fast. The following anthropometrical data were assessed: weight and body mass index (BMI) (calculated as weight in kilograms divided by the square of height in meters). The presence of metabolic syndrome was defined according to the criteria proposed by the Adult Treatment Panel III (ATP-III) [17]. Finally, the Framingham risk score for each patient was calculated according to the equations proposed by the ATP-III [17].

Imaging of CAC. All subjects underwent cardiac computed tomography (CT) imaging with a Volume CT 64-slice scanner (GE Medical Systems). All images were obtained during a single breath hold, using 320 mA and 140 kV. Image acquisition was prospectively triggered at 80% of the R-R interval on the surface electrocardiogram. A section thickness of 2.5 mm, a field of view of 20 cm^2, and a matrix of 512 by 512 were used to reconstruct the raw image data, yielding a nominal pixel size of 0.39 mm^2 and a voxel volume of 0.4 mm^3. The estimated radiation dose was 1.1 mSv. Images were then transferred to an offline workstation that enabled CAC quantification using Smart Score software (GE Medical Systems). The CAC score was calculated according to the Agatston method, as described elsewhere [18].

Statistical analysis. The primary end point of interest was coronary artery age in subjects with a CAC score >0. To calculate the coronary age of our patients, we applied the equations published by Sirineni et al [13], which include the following variables: age, race, sex, and CAC score. Vascular age is calculated on the basis of tables of CAC scores published by the investigators of the North American MESA study [11]. We used the method proposed by Sirineni et al [13] despite the fact that it is based on American white subjects because calcium data are readily available on the Internet and the method has already been published. However, a population-based study similar to MESA is ongoing in Germany, and the published evidence confirms that the calcium scores of North American whites and Germans do not differ [19].

The formula for calculation of coronary age for white women is

$$y = 1\text{E-}09x^5 - 5\text{E-}07x^4 + 8\text{E-}05x^3 - 0.006x^2 + 0.376x + 65.89 ,$$

and the formula for white men is

$$y = 7\text{E-}12x^5 - 1\text{E-}08x^4 + 6\text{E-}06x^3 - 0.001x^2 + 0.248x + 53.65 ,$$

Table 1. Clinical Characteristics of the Patient Population Identified, by Expected or Decreased and Increased Coronary Age

Characteristic	Coronary age		*P*
	Expected or decreased	Increased	
Population size	238 (59.50)	162 (40.50)	…
Demographics			
Men	156 (65.55)	140 (86.42)	**<.001**
Age, mean years ± SD	46.97 ± 8.83	49.95 ± 6.73	**<.001**
BMI, mean ± SD	23.23 ± 3.49	24.60 ± 3.74	**<.001**
Physical activity			
None	136 (57.14)	93 (57.41)	
Mild	73 (30.67)	47 (29.01)	.63
Intense	26 (10.92)	17 (10.49)	
Missing	3 (1.26)	5 (3.09)	
Smoking			
No	141 (59.24)	84 (51.85)	
Mild	31 (13.03)	22 (13.58)	.33
Intense	63 (26.47)	51 (31.48)	
Missing	3 (1.26)	5 (3.09)	
Alcohol consumption			
No	147 (61.76)	89 (54.94)	.12
Mild	88 (36.97)	66 (40.74)	
Intense	0 (0)	2 (1.23)	
Missing	3 (1.26)	5 (3.09)	
Diabetes mellitus	35 (14.71)	33 (20.37)	.175
HOMA-IR, median (range)	2.94 (0.13 to 28.94)	3.46 (0.97 to 99.73)	.18
HIV history			
Risk group			
IDU	77 (30.43)	41 (27.70)	.83
Homosexual/bisexual	84 (33.20)	52 (35.14)	
Heterosexual	92 (36.36)	55 (37.16)	
Duration of infection, median years (range)	15.79 (1.60 to 23.55)	15.67 (2.39 to 23.49)	.29
Previous AIDS diagnosis	71 (29.83)	42 (25.93)	.63
$CD4^+$ cell count, median cells/μL (range)			
Nadir	138.5 (0 to 605)	167 (3 to 870)	.25
Current	510.5 (14 to 2352)	536 (33 to 1494)	.112
Reconstitution	326 (−60 to 972)	342.5 (−171 to 1303)	.29
HIV load, mean $\log_{10}$ copies/mL ± SD	2.12 ± 1.01	2.11 ± 0.98	.908
Undetectable HIV load	166 (69.75)	119 (73.46)	.42
Duration of exposure to ART, median years (range)	10.88 (1.5 to 15.85)	11.47 (1.5 to 15.27)	**<.001**
Duration of PI exposure, median months (range)	34 (0 to 131)	37 (0 to 172)	.242
Duration of NNRTI exposure, median months (range)	27 (0 to 124)	24 (0 to 137)	.49
Duration of NRTI exposure, median months (range)	118 (18 to 234)	118.5 (19 to 227)	.459
Cardiovascular			
Hypertension	47 (19.75)	62 (38.27)	**<.001**
Framingham risk, median (range)	3 (1 to 25)	8 (1 to 30)	**<.001**
Metabolic syndrome			
ATP-III	44 (18.49)	40 (24.69)	**.033**
Lipid serum levels, mg/dL			
Triglycerides, median (range)	151 (32 to 1077)	178.5 (30 to 1644)	**.004**
Total cholesterol, mean ± SD	184.25 ± 41.05	197.42 ± 43.84	**.003**
HDL cholesterol, mean ± SD	46.02 ± 14.02	41.95 ± 11.96	**.004**
LDL cholesterol, mean ± SD	111.42 ± 34.14	120.71 ± 37.46	**.017**
ApoA1, mean ± SD	141.94 ± 24.99	138.34 ± 23.71	.196
ApoB, mean ± SD	97.72 ± 24.88	108.25 ± 24.37	**<.001**

NOTE. Data are no. (%), unless otherwise indicated. Boldface type indicates statistically significant differences. Apo, apolipoprotein; ART, antiretroviral therapy; ATP-III, adult treatment panel III; BMI, body mass index; IDU, injection drug user; HDL, high-density lipoprotein; HIV, human immunodeficiency virus; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein; NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; PI, protease inhibitor; SD, standard deviation.

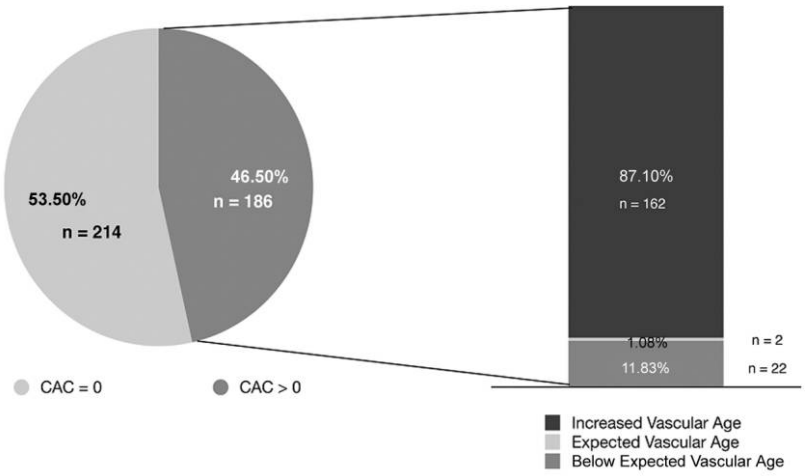

Figure 1. Prevalence of coronary artery calcium (CAC) in the entire cohort and coronary age distribution in the subpopulation with a CAC score >0.

as described in Sirineni et al [13].

The algorithm we used suggests assigning an age of 40 years to individuals with a chronological age <40 years with evidence of CAC on CT. In our database, only 4 patients with a chronological age <40 years had CAC, and their coronary age was therefore considered to be greater than their chronological age. The difference between chronological age and coronary age was categorized as decreased coronary age, expected coronary age, and increased coronary age. Normally distributed continuous variables were compared using the *t* test, and nonnormally distributed variables were compared using the Mann-Whitney *U* test. Differences in categorical variables were analyzed using the χ^2 test.

To better interpret increased coronary age in HIV infected patients, we analyzed possible associations with immunological parameters that characterize this population. We performed linear regression analyses between coronary age and current $CD4^+$ cell count, $CD4^+$ cell count nadir, $CD4^+$ cell count reconstitution (defined as the change in $CD4^+$ cell count from nadir to current value), respectively.

Multivariable linear regression analyses were performed to find independent predictors of increased coronary age. Variables included in the model were BMI; plasma levels of LDL and HDL cholesterol; HOMA-IR; systemic hypertension; duration (in months) of treatment with PI-, NRTI-, and NNRTI-containing ART; and current $CD4^+$ cell count. HIV load was not included in the model because we considered it to be colinear with $CD4^+$ cell count. As confirmation, we constructed

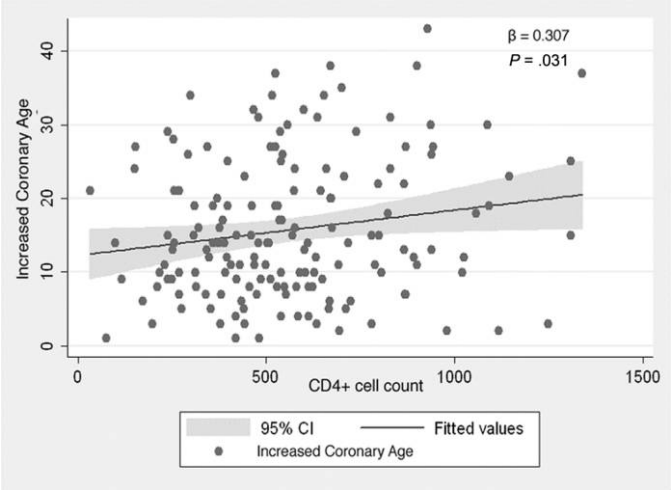

Figure 2. Univariable linear regression analysis of increased vascular age and $CD4^+$ cell count in the group with increased vascular age. CI, confidence interval.

Table 2. Multivariable Linear Regression of Predictors of Increased Coronary Age

Variable	β (95% CI)	*P*
BMI	−0.23 (−0.74 to 0.28)	.376
LDL cholesterol level	−0.03 (−0.08 to 0.02)	.212
HDL cholesterol level	−0.03 (−0.18 to 0.11)	.668
HOMA-IR	−0.09 (−0.26 to 0.09)	.328
Hypertension	1.47 (−2.39 to 5.33)	.452
Months of PI exposure	−0.04 (−0.09 to 0.01)	.122
Months of NRTI exposure	−0.0005 (−0.04 to 0.04)	.980
Months of NNRTI exposure	−0.03 (−0.09 to 0.03)	.367
$CD4^+$ cell count (50 cell/μL arbitrary scale)	0.51 (0.16 to 0.86)	**.005**

NOTE. Boldface type indicates statistically significant differences. BMI, body mass index; CI, confidence interval; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein; NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; PI, protease inhibitor.

a second model that included both $CD4^+$ cell count and viral load using a backward stepwise analysis, and the results were not different from those presented here.

A third backward stepwise linear regression model was performed including in the analyses $CD4^+$ cell count reconstitution; results did not vary, and this covariate was not significant (data not shown).

In each model, $CD4^+$ cell count was included using an arbitrary scale of 50 cells/μL because of the clinical significance attributed to such a change in $CD4^+$ cell count. Statistical significance was defined as $P < .05$. Stata software package, version 9 (Intercooled; StataCorp), was used for statistical analyses.

RESULTS

Participant baseline characteristics are presented in Table 1; patients with expected or decreased coronary age are grouped together. The mean age of the entire patient cohort was 48 years (range, 20–76 years). The mean BMI was 23.8 (standard deviation, 3.7). Diabetes mellitus was present in 68 patients (17%), and 94 (23.50%) were receiving stable lipid lowering therapy (91 were receiving statins, and 5 were receiving fibrates). Women's median CAC score was 0 (range, 0–1230), and men's median CAC score was 5.25 (range, 0–1766). The prevalence of CAC in the entire cohort and the coronary age distribution in subjects with a CAC score >0 are shown in Figure 1. Of the 186 patients with a CAC score >0, 162 (87%) had an increased coronary age, with an average increase of 15 years (range, 1–43 years) over their chronological age.

Variables that were significantly associated with increase coronary age were male sex, chronological age, BMI, years of exposure to ART, hypertension, Framingham risk score, presence of the metabolic syndrome, serum triglyceride level, total cholesterol level, LDL cholesterol level, ApoB level, and low HDL cholesterol level.

Univariable linear regression analysis seeking an association between coronary age and immunological characteristics among the 162 patients with increased coronary age was significant for current $CD4^+$ cell count, as shown in Figure 2. The linear tendency was verified by robust locally weighted regression [20] (data not shown).

This result was confirmed in the multivariable linear regression model, where $CD4^+$ cell count was the only variable associated with increased coronary age (Table 2). For each 100 cell/μL increase in $CD4^+$ cell count, a loss of 1 year of life was predicted.

DISCUSSION

CAC scores have been reported in 8 studies of HIV-infected patients [8, 9, 21–26]. Each of them described a high prevalence of CAC that was usually associated with traditional atherosclerosis risk factors, including age, sex, and changes in atherogenic serum lipid levels. In none of the prior studies was CAC used to determine the coronary age of an individual patient. In the present study, we used an objective method to assess the coronary age of HIV-infected patients, a population considered more likely to experience an acceleration of the normal aging process. Increased coronary age was used as a surrogate marker of premature biological aging, and we found evidence of accelerated aging in nearly half our cohort. The definition of "older age" in HIV-infected patients has traditionally been reserved for individuals age 50 or older [27]. The age cutoff of 50 years seems particularly appropriate, given that the median chronological age of individuals with an increased coronary age in our cohort was 49.95 years. Increased biological age may result in a significant increase in risk of cardiovascular events. For example, using the MESA calculator [28] (last accessed 30 April 2009), the 10-year cardiovascular risk for a 50-year-old man with a CAC score of 56 is comparable to that of

a 68-year-old (95% confidence interval, 67–70-year-old) person. Indeed, taking into consideration his increased biological age based on the unexpectedly high calcium score, his 10-year risk of cardiovascular events changed from 4% to 12%. In view of the demonstrated underestimation of risk with the Framingham risk equations in HIV-infected patients [28], it may be advisable to consider adjusting the equations using a modifier such as calculated biological age. Although this approach may refine cardiovascular risk predictions, future studies should address the association between coronary age and other premature aging events (cancer, osteoporotic fractures, diabetes mellitus, and others) in HIV-infected patients.

The second main finding of our study was the identification of absolute $CD4^+$ cell count as the sole independent predictor of increased coronary age in multivariable analyses. This was an unexpected result and one seemingly counterintuitive, because large databases demonstrated a better outcome (in terms of HIV-related and non–HIV-related diseases) with a higher $CD4^+$ cell count. However, our finding may highlight an interesting pathophysiologic mechanism. Atherosclerosis is an inflammatory process of the subintimal layer of the arterial wall in which lymphocytes and macrophages play a major role. The $CD4^+$ type 1 T helper (Th1) lymphocyte is the predominant subtype of T cells in atherosclerotic plaques of humans [29, 30] and animals, such as $apoE^{-/-}$ and $LDLR^{-/-}$ mice [31]. In one study, when $CD4^+$ cells were transferred to $CD4^+$ cell–deprived mice, the atherosclerotic lesions enlarged significantly [32], suggesting a pathogenic role for $CD4^+$ cells in atherosclerosis. An increase in $CD4^+$ cell count during ART is accompanied by an increase in serum levels of interferon γ, the principal Th1 cytokine. Although this has been regarded as an indicator of the effectiveness of ART, interferon γ is also a mediator of inflammation, and it is highly atherogenic [33]. Therefore, it is conceivable—albeit speculative—that the ART-induced increase in $CD4^+$ cell count may be coresponsible for the development of atherosclerosis in HIV-infected patients. In apparent contrast with this hypothesis, 2 recent publications described an association between low $CD4^+$ cell counts and cardiovascular risk in HIV-infected patients. Lichtenstein et al [34] found that a baseline $CD4^+$ cell count <350 cells/mL was independently associated with incident cardiovascular disease ($P < .05$) in 1697 HIV-infected subjects. In a second study, Kaplan et al [35] described an increased adjusted hazard ratio for carotid plaques and increased carotid intimamedia thickness among HIV-infected patients with a $CD4^+$ cell count <200 cell/μL. In neither study, however, the described associations were linked with ART. Although apparently contradicting our findings, the discrepancy between these studies may be explained by a U-shaped curve effect. The left arm of the curve (low $CD4^+$ cell count) may be associated with increased cardiovascular risk due to acute inflammatory processes promoting cardiovascular events as well as opportunistic infections involving increased cytokine expression and vascular damage. On the other end of the curve, an increased $CD4^+$ cell count may be associated with chronic ongoing inflammatory processes (possibly related to immune activation) and could promote atherosclerosis development. In our study, the most recent $CD4^+$ cell count was representative of the right arm of this putative U-shaped curve.

Study limitations include the following. Ours was a cross-sectional study, and thus it does not provide proof of causality between higher $CD4^+$ cell counts and development of coronary atherosclerosis. Although we measured the number of $CD4^+$ lymphocytes, we did not assess $CD4^+$ cell functionality, and a dysfunctional lymphocyte (ie, more proinflammatory) may potentially provide a link between $CD4^+$ cell count and atherosclerosis development in HIV-infected patients. Increased coronary age was calculated using the white MESA database, and a potential bias cannot be excluded. In the absence of Italian data, we were able to find evidence in the literature that the calcium scores of German and North American white subjects are very similar [19]. We were unable to find an association between increased coronary age and $CD4^+$ cell count reconstitution rather than absolute $CD4^+$ cell count. The hypothesis of an association between immune reconstitution and coronary disease needs to be confirmed in future studies addressing $CD4^+$ cell count recovery in association with levels of soluble and tissue inflammatory markers.

In conclusion, we believe that this study has useful clinical implications in that it provides an objective tool to assess premature biological aging in HIV-infected patients. Additionally, a risk score that includes CAC and age may be an easily understandable measure of risk for patients ("you are 50 years old but your heart's age is 65") and may be an attractive tool for physicians to use for education of patients and for implementation of primary prevention strategies. Future studies will need to demonstrate an association between increased coronary age and cardiovascular events or other premature aging events among HIV-infected patients.

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