Int J Cardiovasc Imaging (2012) 28:935–941
DOI 10.1007/s10554-011-9898-y

ORIGINAL PAPER

Progression of coronary artery calcium in men affected by human immunodeficiency virus infection

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Received: 1 March 2011 / Accepted: 20 May 2011 / Published online: 5 June 2011

Abstract Cardiovascular risk is increased in HIV infected patients. We assessed progression of coronary artery calcium (CAC) in patients with HIV infection to identify factors that may help explain progression of atherosclerosis. Prospective, observational study of 132 HIV-infected men receiving chronic antiretroviral therapy (ART); we measured traditional atherosclerosis risk factors and assessed progression of CAC on sequential 64-slice CT scans at an average interval of 11 months (range 6–36). CAC score progression was defined as absolute and percentage change from baseline. During follow-up 45 patients (34%) showed absolute progression of CAC and 34 of them showed >15% yearly progression, a threshold previously associated with a high risk of myocardial infarction. Age, LDL cholesterol, visceral abdominal fat and current T-helper (CD4+) cell count were significantly associated with absolute CAC progression. Progression of subclinical atherosclerosis in HIV patients is associated with traditional coronary risk factors as well as HIV related factors such as the CD4+ cell count. Therefore, immunologic perturbations secondary to HIV infection may contribute to atherosclerosis progression.

Keywords Coronary artery calcium · HIV · Atherosclerosis · Cardiovascular disease · Progression of atherosclerosis

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Introduction

In areas of the world where effective antiretroviral therapy (ART) is available, cardiovascular diseases (CVD) are becoming important contributors to morbidity and mortality in HIV-infected patients [1, 2]. A sizeable body of evidence indicates that the risk of CVD is increased in HIV-infected compared with uninfected individuals, and has stimulated a considerable scientific debate on the pathogenesis and natural history of atherosclerosis in HIV patients [3, 4]. By means of multidetector computed tomography imaging (MDCT) coronary artery calcium (CAC), a sensitive marker of atherosclerosis, can be accurately quantified. Prior extensive literature has

shown that CAC improves prediction of cardiovascular events and mortality in the general population probably because of its strong association with total coronary atherosclerotic disease burden, as shown in pathologic specimens [5, 6]. Progression of CAC, on the other hand, has been associated with an adverse outcome [6, 7]. In this study we assessed progression of CAC in patients with HIV infection.

Methods

Study design

In an observational study, we recruited 132 consecutive HIV-infected men attending a tertiary level outpatient clinic. Patients underwent measurement of risk factors for atherosclerosis and assessment of subclinical coronary atherosclerosis by means of baseline and repeat CAC measurements (median of 2 scans per patient, range 2–4). The study was approved by the University of Modena and Reggio Emilia Ethical Committee and was performed in accordance to the ethical standards described in the 1964 Declaration of Helsinki and its amendments. All patients gave informed consent to participate.

Participant selection

Inclusion criteria were: serologically documented HIV-1 infection, age >18 years, male gender and at least two CAC measurements at a minimum interval of 6 months. For patients with established diagnoses of hyperlipidemia and hyperglycemia, stable lipid-lowering and diabetes mellitus therapies for at least 6 months were required. Patients were excluded if they reported or had documented evidence of any of the following cardiovascular conditions: previous myocardial infarction, stroke, coronary artery by-pass surgery, angioplasty and peripheral vascular disease.

Demographic and clinical data, including duration of HIV infection, prior opportunistic diseases, antiretroviral therapy history were recorded. More in detail we recorded: CD4+/μL (most recent value and nadir), plasma HIV-1 RNA levels, cumulative exposure to non-nucleoside reverse transcriptase inhibitors (NNRTI), nucleoside reverse transcriptase inhibitors (NRTI) and protease inhibitors (PI). HIV viral load was categorized as undetectable if <40 HIV mRNA copies/mL were present. Previous AIDS diagnosis was defined according to the Center for Disease Control group "C" category [8].

Lifestyle information was obtained during patients' interview. Smoking, alcohol consumption, and physical activity were assessed at entry by questionnaire.

Diabetes mellitus was diagnosed according to the American Diabetes Association criteria, past medical history of diabetes or use of glucose lowering therapy [9].

Insulin resistance (IR) was calculated using the homeostasis model assessment equation (HOMA-IR = [fasting insulin (mU/mL) × fasting glucose mmol/L)/22.5] [10]. Total cholesterol, low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL), triglycerides, apolipoprotein A and B, glucose and insulin levels were measured at entry after an overnight fast. Dyslipidemia was defined as one or more of the following criteria: total cholesterol >240 mg/dL, LDL >160 mg/dL, HDL <40 mg/dL, triglycerides >200 mg/dL, or use of lipid lowering medications. Hypertension was defined as a systolic pressure ≥140 mmHg or a diastolic pressure ≥90 mmHg or use of anti-hypertensive agents. The presence of the metabolic syndrome was defined according to the criteria proposed by the Adult Treatment Panel III (ATP-III) [11]. A Framingham risk assessment estimating 10 year risk of developing hard coronary heart disease for each patient was calculated according to the equations proposed by the ATP-III (http://hp2010.nhlbihin.net/atpiii/calculator.asp?usertype=prof, last accessed 17th May 2011).

The following anthropometric data were obtained on the same day the serum chemistries were drawn: weight and body mass index (calculated as weight in kilograms divided by the square of height in meters), waist circumference, and visceral abdominal adipose tissue volume (VAT) measured using a single-CT-scan slice of the abdomen obtained at the level of the 4th lumbar vertebra.

Imaging of coronary artery calcium

All subjects underwent cardiac MDTC imaging with a Volume CT 64-slice scanner (GE Medical Systems, Milwaukee, WI). All images were obtained during a

single breath hold using 320 mAs 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 × 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 off-line workstation that enabled CAC score quantification using the "Smart Score" software (GE Medical Systems, Milwaukee, WI). The CAC score was calculated according to the Agatston method, as previously described [12].

Statistical analysis

The primary end point of the study was CAC progression as continuous variable defined as absolute calcium score change from baseline. Patients were then grouped in CAC progressors and non-progressors including in the latter group patients with a CAC score unchanged or reduced from baseline at the time of follow-up. A percentage change in CAC score was also calculated for patients with a baseline CAC > 0; a percentage increase >15%/year has been shown to be a marker of enhanced risk of cardiovascular complications and was therefore chosen as a clinically relevant end-point [5, 6].

Continuous, normally distributed variables were compared using the T-test, while non-normally distributed variables were compared by medians using the Mann–Whitney test; differences in categorical variables were analyzed using the χ^2-test or Fisher's exact test where appropriate.

A longitudinal multivariable generalized estimating equations (GEE) model was applied to find predictors associated with CAC progression. Variables statistically associated with CAC progression on univariate analysis were included in the model. Variables included in the model were age, LDL-cholesterol, HDL-cholesterol, triglycerides, HOMA-IR, hypertension, months of exposure to antiretroviral drug classes, VAT, and current CD4+ cell/μL count. VAT, rather than BMI and waist ircumference, was included in this analysis because it is a more accurate measurement of abdominal obesity [13].

LDL-cholesterol, HDL-cholesterol, triglycerides, cumulative exposure to antiretroviral therapies were forced into the GEE model because they were considered clinically relevant although they were not statistically associated with CAC progression on univariate analysis. We chose to use HOMA-IR rather than the diagnosis of diabetes mellitus in the model to increase the likelihood to detect an association between impaired glucose metabolism and CAC progression. The Framingham risk score was not included in the GEE model since it is derived from variables already included in the model.

The GEE model allows to analyze multiple longitudinal data collected at different time intervals between measurements [14].

All analyses were conducted using STATA software package version 10.1 Intercooled for Mac (StataCorp LP, College Station, TX, USA); a 2-sided P-values < 0.05 was considered significant.

Results

Clinical characteristics of study population

One hundred thirty-two HIV-infected men previously exposed to antiretroviral therapy (ART) participated in this prospective observational study. The median interval between scans was 13 months (range 6; 36). Patients had a long duration of HIV infection (14.2 ± 5.6 years), good current CD4+ cell count (mean 504/μL, range 350–695) and undetectable HIV viral load in the majority of cases (83%). Their mean age was 49 ± 9 years, mean BMI was 25 ± 3 and 9 patients (6%) were obese (BMI ≥30). Ten-year risk of developing hard events calculated according to the Framingham equations was ≤10% in 86 patients (65%), between 10 and 20% in 43 patients (33%) and ≥20% in 3 patients (2%).

The median baseline CAC score was zero (range: 0; 974), and the median follow-up CAC score was zero (range: 0; 1115). Figure 1 shows the proportion of patients with and without CAC progression and their baseline CAC score.

Eighty-seven patients (67%) had a stable CAC score, most of them (82%) with CAC = 0 both at baseline and at follow up. Forty-five patients (34%) showed progression of CAC, 34 (75%) of whom had a CAC score increase ≥15% per year, linked with an increased risk of myocardial infarction in the general population [6]. The median interval between scans

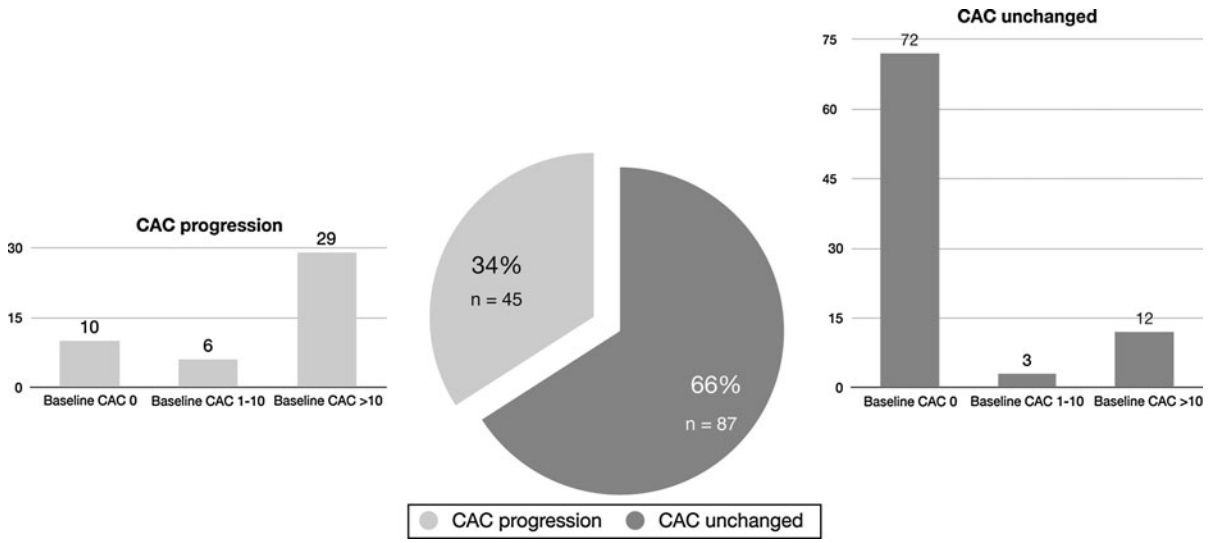

Fig. 1 Proportion of patients with and without CAC progression and the baseline CAC score

was similar between groups, 14 months (range 6–36 months) in the stable CAC group and 13 months (range 6–30 months) in the CAC progression group ($P = 0.540$), respectively.

Demographic and clinical characteristics of patients showing absolute CAC progression or no progression are described in Table 1. Baseline variables associated with CAC progression on univariate analyses were: age, BMI, waist, VAT, years of HIV infection, current CD4+ count, systolic blood pressure, Framingham risk score and baseline CAC score. Table 2 lists independent predictors of absolute CAC progression using a longitudinal multivariable GEE regression analysis. Age, LDL cholesterol, VAT per 10 cm^3 and CD4+ cell count per 50 cells/μL (arbitrary scale) were significantly associated with CAC progression.

Discussion

This study shows that progression of subclinical atherosclerosis in HIV infected patients is associated with traditional atherosclerosis risk factors as well as the CD4+ cell count, suggesting that therapeutic interventions directed at increasing the T-lymphocyte count may indirectly contribute to atherosclerosis development. An increased burden of subclinical atherosclerosis in HIV has been reported by several investigators. Hsue et al. demonstrated that carotid intima media thickness (IMT) is greater in HIV patients than in age-matched control subjects and progresses more rapidly than previously reported in non-HIV cohorts [15].

Our results confirm the role of traditional cardiovascular risk factors as independent predictors of atherosclerosis progression, namely age, LDL-cholesterol and VAT. VAT is an excellent objective measure of abdominal adiposity and it is associated with cardiovascular disease. Furthermore, VAT is increased in HIV patients affected by dystrophic fat accumulations known as lipodystrophy syndromes [16]. Besides traditional risk factors, however, we also found that a high CD4+ cell count was associated with CAC progression suggesting that there are additional mechanisms that influence atherosclerosis inception and/or progression in HIV patients compared to the general population. One autopsy study demonstrated accelerated atherosclerosis in young HIV-1 infected patients with mixed features of small-vessel vasculitis and common coronary artery disease [17]. In a large cross-sectional study in HIV+ patients, we previously reported that a high CD4+ cell count was associated with higher CAC scores, again pointing at the role of potentially dysfunctional T-lymphocytes in HIV atherosclerosis. However, Lo et al. recently showed that a low CD4+/CD8+ cell ratio was associated with evidence of non-calcified coronary plaque [18], and Hsue et al. demonstrated that a nadir CD4+ cell count $\leq$200/μL was associated with progression of IMT [15]. We speculate that this apparent discrepancy may be

Table 1 Baseline characteristics of subgroups and univariate analysis of factors associated with absolute progression of calcium artery calcium score

	CAC Unchanged	Progression of CAC	*P* value
n	87 (65.91%)	45 (34.09%)	–
Demographics and Anthropometrics			
Age years, mean (SD)	45.8 (7.3)	54.2 (8.7)	<0.001
BMI kg/m^2, mean (SD)	24.2 (3.1)	25.7 (3.9)	0.017
Waist cm, mean (SD)	86.8 (8.9)	91.7 (9.9)	0.005
VAT cm^3, median (IQR)	122 (89; 169)	177 (125; 223)	<0.001
Smoking, n (%)	41 (47%)	16 (35.6%)	0.441
Alcohol use	33 (38%)	22 (49%)	0.226
HIV history			
Risk group			
IDU n (%)	23 (26.4%)	4 (8.9%)	0.051
MSM n (%)	41 (47.1%)	24 (53.3%)	
Heterosex n (%)	23 (26.4%)	17 (37.8%)	
Years of HIV infection median (IQR)	15.05 (11.4; 20.0)	11.99 (8.2; 17.6)	0.039
Previous AIDS diagnosis n (%)	21 (24.1%)	14 (31.1%)	0.401
CD4+ Nadir median (IQR)	150 (59; 265)	165 (60; 290)	0.5233
CD4+ Current median (IQR)	474 (349; 600)	586 (428.5; 808.5)	0.009
VL undetectable	72 (82.7%)	39 (86.7%)	0.561
Months of NRTI exposure median (IQR)	104 (61; 134)	110 (84; 133)	0.325
Months of NNRTI exposure median (IQR)	25 (7; 57)	27 (6; 64)	0.647
Months of PI exposure median (IQR)	36 (13; 66)	33 (3; 51)	0.317
Cardiovascular			
Diastolic blood pressure mmHg, median (IQR)	80 (70; 90)	80 (70; 90)	0.539
Systolic blood pressure mmHg, median (IQR)	120 (110; 130)	130 (115; 140)	0.011
Hypertension diagnosis n (%)	35 (40.23)	28 (62.22)	0.016
Framingham risk %, median (IQR)	6 (3; 12)	12 (8; 16)	<0.001
Metabolic syndrome			
ATP-III n (%)	21 (25.3%)	13 (31.7%)	0.522
Triglycerides mg/dL, median (IQR)	169.5 (118; 269.5)	189 (141; 272)	0.382
Total cholesterol mg/dL, median (IQR)	181 (157; 217.5)	188 (161; 219)	0.596
HDL-chol mg/dL, median (IQR)	40 (31; 47)	40 (33; 49)	0.761
LDL-chol mg/dL, median (IQR)	115 (92; 144)	114 (90; 132)	0.719
Glucose metabolism			
Glucose mg/dL, median (IQR)	93 (85; 101.5)	95 (84; 106)	0.578
HOMA-IR median (IQR)	3.15 (2.1; 5.5)	3.48 (2.2; 4.9)	0.721
Diabetes mellitus n (%)	12 (13.8)	9 (20)	0.455
Calcium score			
CAC score median (range)	0 (0; 61)	25 (0; 974)	<0.001

ATP-III adult treatment panel III, *BMI* body mass index, *CAC* coronary artery calcium, *HAART* highly active anti-retroviral therapy, *HOMA-IR* homeostasis model assessment of insulin resistance, *IDU* intravenous drug user, *IQR* inter-quartile range, *MSM* Men who have Sex with Men, *NNRTI* non-nucleoside reverse transcriptase inhibitor, *NRTI* nucleoside reverse transcriptase inhibitor, *PI* protease inhibitor, *VL* viral load

explained by a U-shaped effect [19], whereby patients with a low CD4+ count may be exposed to inflammatory processes and opportunistic infections with increased cytokine expression and lymphocytes infiltration of the vessel wall. On the other hand, a high CD4+ cell count may hide immune activation and abnormal lymphocyte activity, that may promote and sustain atherosclerosis plaque progression and calcium

Table 2 Longitudinal multivariable GEE regression model to identify factors associated with progression of coronary artery calcium

	O.R.	Confidence interval	*P* value
Age	1.10	1.05; 1.16	<0.001
LDL per 10 mg/dL	1.15	1.02; 1.30	0.021
HDL per 10 mg/dL	1.08	0.77; 1.50	0.637
Triglycerides per 10 mg/dL	1.00	0.98; 1.02	0.604
HOMA-IR	0.98	0.89; 1.08	0.710
Hypertension	0.49	0.21; 1.14	0.101
VAT per 10 cm^3	1.06	1.01; 1.11	0.016
Months of PI exposure	0.99	0.98; 1.01	0.623
Months of NRTI exposure	1.01	0.99; 1.01	0.087
Months of NNRTI exposure	1.01	0.99; 1.02	0.155
CD4+ per 50 cell/mL (arbitrary scale)	1.08	1.01; 1.15	0.041

HOMA-IR homeostasis model assessment of insulin resistance, *NNRTI* non-nucleoside reverse transcriptase inhibitor, *NRTI* nucleoside reverse transcriptase inhibitor, *PI* protease inhibitor, *VAT* visceral adipose tissue

deposition [20]. In our study, the most recent CD4+ cell count was representative of the right arm (high count) of this putative U-shaped curve. It might be of interest to measure the activity of reconstituted T-lymphocytes after initiation of ART. In fact, there is preliminary evidence that activated or senescent CD4+ and CD8+ T-lymphocytes may promote atherosclerosis in HIV infected patients [21].

There were a few limitations in our study that include its observational nature, precluding the establishment of a cause and effect relationship between the events described, and the inclusion of men alone, although men are known to have more CAC than women until late stages in life.

In conclusion, this study provides evidence of rapid progression of subclinical atherosclerosis in HIV-infected patients and points at the potential role of HIV and its associated immunologic perturbations on atherosclerosis progression.

Acknowledgment This study was partially supported by an independent grant from Theratechnologies Inc. (Montreal, Quebec, Canada) and from Gilead Sciences (Gilead Italy).

Conflict of interest None.

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