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ORIGINAL RESEARCH

Cardiovascular disease risk and its determinants in people living with HIV across different settings in South Africa

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Objectives

Socio-economic factors and lifestyle are known to differ across geographies and populations, which may result in distinct risk profiles for cardiovascular disease (CVD). This study assessed carotid intima-media thickness (CIMT), a proxy for CVD, and its determinants in two groups of people living with HIV (PLHIV) in two different settings in South Africa.

Methods

A cross-sectional analysis was conducted comparing data from the Ndlovu Cohort Study in the Limpopo Province (group 1) and from three clinical trials in Johannesburg (group 2). The association between demographics, conventional CVD risk factors, HIV-related factors and CIMT in groups 1 and 2 was analysed with two separate multivariable linear regression models.

Results

Group 1 consisted of 826 participants (mean age 42.2 years) and mean (± standard deviation) CIMT was 0.626 ± 0.128 mm. In this group, sex, age, body mass index (BMI), cholesterol, glucose and antiretroviral therapy (ART) duration (β = 0.011 mm per 5 years; P = 0.02) were associated with higher CIMT. There were positive interactions between age and ART duration and age and cholesterol. Group 2 consisted of 382 participants (mean age 39.5 years) and mean (± standard deviation) CIMT was 0.560 ± 0.092 mm. In this group, only sex, education level, BMI and cholesterol were associated with higher CIMT, albeit with weaker associations than in group 1.

Conclusions

Conventional CVD risk factors were the main drivers of CIMT. The impact of some of these risk factors appeared to increase with age. Differences in sample size, age and viral suppression might explain why an effect of ART was observed in group 1 but not in group 2.

Keywords: cardiovascular, carotid intima-media thickness, HIV, South Africa, sub-Saharan Africa

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Introduction

Infection with HIV has been the main cause of death in South Africa in the past two decades [1]. Current estimates indicate that approximately 7.2 million people live with HIV in South Africa, of whom about 5 million are being treated with combination antiretroviral therapy (ART) [2].

Noncommunicable diseases, particularly cardiovascular disease (CVD), are now responsible for a substantial burden of disease [3]. In 2016, one in five deaths was due to CVD in South Africa [4]. ART has transformed HIV infection into a chronic treatable condition [5]. This results in a near-normal life expectancy of people living with HIV (PLHIV), who as a result will experience more age-related diseases like CVD [6]. Research from high-income countries suggests that PLHIV are twice as likely to develop CVD and both HIV infection and ART have been shown to be risk factors for the development of CVD [7,8].

The mechanisms by which HIV affects CVD risk are, however, not yet fully understood. HIV infection is associated with activation of the immune system, even in virally suppressed PLHIV [9]. Immune activation is a key factor in the formation of atherosclerosis [10,11]. Furthermore, ART, especially the use of protease inhibitors and efavirenz, may increase CVD risks by causing alterations in lipid and glucose metabolism [12,13]. Finally, conventional CVD risk factors such as obesity, diabetes and hypertension are common in PLHIV in South Africa [14].

Lifestyle, socio-economic factors and access to health care services vary across different regions of South Africa, and the country is experiencing rapid economic change and urbanization. Therefore, the contribution of conventional CVD risk factors to the occurrence of CVD may also differ across settings. Those differences are particularly pronounced when contrasting rural and urban settings. In the last few decades, lifestyle and dietary patterns in sub-Saharan Africa (SSA), and particularly in South Africa, have changed substantially [15]. People have become less physically active, while dietary fat and sugar consumption has increased [16]. These lifestyle changes seemingly happen first in urban areas before they occur in rural areas [17]. Urban residents have been shown to have a higher body mass index (BMI), higher blood pressure and more instances of diabetes than rural residents [18]. In South Africa, considerable differences in socio-demographics exist between rural and urban populations; lower educational attainment and high rates of unemployment contribute to lower incomes in rural areas [17]. These differences in socio-economic and CVD risk factors could create distinct CVD risk profiles across the country [19]. In addition, inequity in access to health care may aggravate these differences [20].

Data on the occurrence of clinical CVD in the HIV-infected population in South Africa are scarce. As longitudinal studies are awaited, surrogate markers like carotid intima-media thickness (CIMT) can be used to estimate CVD risk. CIMT is associated with the risk of myocardial infarction and stroke [21,22], and it has been used in Caucasian and African populations [23,24]. In studies in high-income countries, higher CIMT values have been found in PLHIV compared to HIV-negative people, even after adjusting for conventional CVD risk factors [25].

This study aimed to assess the burden of CVD risk using CIMT as a surrogate CVD marker in groups of PLHIV from two different settings in South Africa. In addition, we investigated determinants of CIMT in these two groups, focussing on conventional CVD and HIV-specific factors.

Methods

Study setting

In this cross-sectional analysis, we included ART-naïve and treated HIV-positive participants who were ≥ 18 years old from a rural and an urban site in South Africa.

The first group of participants was selected from the Ndlovu Cohort Study (NCS), a longitudinal study of which the design and methodology have been described previously [26]. In brief, the NCS is conducted in Elandsdoorn, a rural township in the Limpopo Province, South Africa. Between December 2014 and July 2016, 887 HIV-positive participants were recruited from a public HIV clinic and the community around the HIV clinic. Study approval was obtained from the Human Research Ethics Committee at the University of Pretoria, Pretoria, South Africa, and the Limpopo Department of Health Ethics Committee (ethics clearance 227-2014). This group will be referred to as group 1. The majority of participants in this group received an ART regimen according to the South African national guideline. For first-line ART, this regimen consisted of tenofovir, either emtricitabine or lamivudine and efavirenz. Second-line ART included a lopinavir-based regimen with either emtricitabine or lamivudine and either zidovudine or tenofovir.

Participants in group 2 were selected from three randomized controlled trials (RCTs) that recruited participants from public HIV treatment centres in the inner city of Johannesburg, South Africa. This group will be referred to as group 2.

Urban HIV-positive, ART-naïve participants (n = 104) were recruited from an open-label RCT comparing the efficacies of two dolutegravir-containing regimens with that of the current standard of care first-line regimen in ART-naïve participants (ClinicalTrials.gov identifier NCT03122262) [27]. Participants were eligible for enrolment in our study before the initiation of ART or at the latest within 3 months after initiation of ART.

Participants from group 2 on stable first-line ART (n = 94) were selected from an RCT that aimed to demonstrate noninferiority of low-dose stavudine compared to tenofovir disoproxil fumarate in the period 2012 to 2016

[28]. Participants were ART-naïve upon enrolment in the RCT. Upon completion of the RCT, all participants were switched to the standard first-line ART regimen consisting of emtricitabine, tenofovir and efavirenz. To be eligible for inclusion in our study, participants had to be on a regimen of tenovofir, lamivudine and efavirenz for at least 2.5 years before enrolment in our study.

Participants from group 2 on second-line ART (n = 197) were selected from an RCT that aimed to demonstrate non-inferiority of ritonavir-boosted low-dose darunavir compared with boosted second-line therapy [29]. Participants were virally suppressed on second-line ART for at least 6 months prior to enrolment in the RCT. Their ART regimen consisted of either darunavir or lopinavir with either tenofovir and lamivudine or emtricitabine and zidovudine. They were eligible for inclusion in our study at any moment of follow-up in the RCT. All participants who attended the RCT site in the time frame of our study were approached for participation. Between July 2016 and November 2017, 395 HIV-positive participants were recruited.

Study approval was obtained from the Medical Human Research Ethics Committee of the University of Witwatersrand, Johannesburg, South Africa (M160130).

Data collection

Data were collected in identical ways at both sites unless stated otherwise. Counsellors or nurses collected information on participants' lifestyle, medical history and medication use (both HIV-related and for other medical conditions). Information on demographics (including employment status and education level), smoking, alcohol use and medical history was assessed with a modified version of the World Health Organization (WHO) STEPs instrument [30]. Participants who reported having quit smoking < 1 month ago were considered current smokers. Students, retirees, disabled people and volunteers were considered unemployed. Information on physical activity was assessed using the International Physical Activity Questionnaire and accordingly patients were categorized as having a high, intermediate or low level of activity [31]. Family history was considered positive for a cardiovascular event when participants reported a history of stroke and/or heart attack in a first-degree family member (parent or sibling) before the age of 60 years. ART duration was the time between the initiation of ART and the inclusion date. Participants were considered ART-naïve when they did not use ART or when they had initiated ART < 3 months prior to inclusion.

Blood pressure was measured with an electronic blood pressure device, in a seated position after 5 min at rest. Blood pressure was measured on both arms and a third measurement was taken on the side with the highest value; subsequently, the average of the last two measurements was used. Waist circumference was measured halfway between the lower rib and the iliac crest during expiration in a standing position.

Blood was taken for measurement of total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, random glucose, viral load and CD4 cell count. For urban participants, laboratory data from the last RCT visit were used. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula [32].

Hypertension, abdominal obesity, dyslipidaemia and metabolic syndrome were defined according to the National Cholesterol Education Program, Adult Treatment Panel III (NCEP ATP III) [33]. Accordingly, hypertension was defined as systolic blood pressure (SBP) of > 130 mm Hg, diastolic blood pressure (DBP) of > 85 mm Hg or use of antihypertensive medication. Abdominal obesity was defined as a waist circumference ≥ 102 cm for men and ≥ 88 cm for women. Diabetes mellitus was defined as random glucose of > 11 mmol/L or the use of blood glucose-lowering medication. Dyslipidaemia was defined as elevated triglycerides (≥ 1.7 mmol/L) and/or reduced HDL cholesterol (< 1.0 mmol/L for men and < 1.3 mmol/L for women). Metabolic syndrome was defined as at least three of: diabetes, hypertension, elevated triglycerides, lowered HDL cholesterol or abdominal obesity.

For all participants, the Framingham 10-year CVD risk score [34], and the 5-year CVD risk score from the Data Collection on Adverse Effects of Anti-HIV Drugs Study (D:A:D) [35] were calculated. As a consequence of their calibration populations, the Framingham score was only calculated for participants aged ≥ 30 years. The D:A:D risk score was only calculated for patients with an ART duration of ≤ 10 years. The likelihood of a CVD event occurring in the next 10 or 5 years, respectively, was reported as a percentage.

Ultrasound measurements of CIMT were performed by trained research staff using an ultrasound (Acuson, Siemens, Johannesburg, South Africa) (P300 for the rural site and P500 for the urban site) with a linear probe of ≥ 7.0 MHz. End-diastolic images were collected of the right common carotid segment at angles of 90°, 120° and 150° and of the left common carotid segment at angles of 210°, 240° and 270° using a Meijer Carotid Arc [36]. Both the near wall and the far wall were measured. For the carotid bifurcation, similar approaches were used at the best visible angle on both sides while focusing on the far wall only. Performance reviews were carried out to ensure quality of measurements.

Common carotid artery (CCA) and bifurcation (BIF) intima-media thickness (IMT) were measured semi-automatically with the ARTERY MEASUREMENT SYSTEM software

(Chalmers University of Technology, Göteborg, Sweden). A uniform reading protocol was used to ensure standardized settings across reading stations. Images were read in batch fashion by trained readers who were blinded to the participant's HIV status.

The following CIMT measurements were reported: the mean IMT of the near and far walls across all angles of the CCA (mean CCA-IMT), the mean of the maximum IMT of the near and the far walls of the CCA across all angles of the CCA (max CCA-IMT), and the mean of the maximum IMT at the far wall of the bifurcation at both sides (max BIF-IMT). Mean CCA-IMT was used as the outcome variable in the multivariable linear regression models.

A mean IMT > 1.0 mm anywhere in one of the measured angles in the far wall of the CCA was considered a plaque [37].

Statistical analysis

Groups 1 and 2 were not compared directly in the statistical analysis as they represent different populations. Group 1 had been recruited from the general population, whereas group 2 had been recruited from RCTs. Hence, in group 2, the proportions of ART-naïve participants, participants on first-line ART and participants on second-line ART are not a representation of the ART coverage and treatment regimens in the general HIV-positive population. In addition, the different recruitment strategies may have led to unmeasured confounding between groups. Hence, our statistical analyses were performed for the two groups in two separate models.

Demographics, CVD risk factors and CIMT of both groups are reported as mean and standard deviation (SD) for normally distributed continuous variables, median and interquartile range (IQR) for nonnormally distributed continuous variables and frequency counts with percentages for categorical variables.

For group 1, 52% of the blood pressure readings were regarded as missing data as these measurements had been taken with a nonvalidated blood pressure device. These data were missing completely at random, and therefore we decided not to exclude those observations from the analysis and instead we imputed the missing data. Observations were stratified by HIV and treatment status and multiple imputations were used, following a Markov chain Monte Carlo method to estimate the missing values. Imputations were repeated 20 times generating 20 different data sets. Subsequently, a singly imputed data set was created by selecting a random draw from the 20 data sets for the final imputed blood pressure values.

To assess associations between conventional CVD and HIV-related risk factors and mean CCA-IMT or max BIF-IMT, a multivariable linear regression model was created for the two groups separately. First, we tested the association of all socio-demographic, CVD and HIV-related factors with CIMT in a univariable linear regression model. The association between ART status (i.e. ART-naïve, on first-line ART or on second-line ART) and mean CCA-IMT was tested using the ART-naïve participants as the reference group. Secondly, all variables with a P-value < 0.20 in univariable regression and variables that are known determinants of CIMT (i.e. sex, age, smoking, BMI, SBP, total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides [36]) were entered in a multivariable linear regression model. The assumptions of linear regression models were met for all models. Variables were excluded from the multivariable model if multicollinearity occurred.

Thirdly, possible interactions between age and HIV infection duration (per 5 years), age and ART duration (per 5 years), and age and conventional CVD risk factors (smoking, BMI, SBP, blood lipids and glucose) were tested in relation to mean CCA-IMT [38]. First, an analysis restricted to the main effects and the interaction term was tested in a multivariable linear regression. Secondly, the interaction terms with a P-value < 0.20 were added all at once to the multivariable model.

A two-sided P-value < 0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS STATISTICS software, version 25 (IBM, Armonk, NY).

Results

Study characteristics

Of the 887 eligible HIV-positive participants in group 1, CIMT data were available for 826 (93.1%) participants. In group 1, 39.7% (n = 328) were men and the mean (± SD) age was 42.2 ± 10.2 years. The majority of the participants were in a relationship. Approximately 70% had completed at least secondary school. The unemployment rate was > 70%. More than 30% of the participants were overweight/obese, had hypertension or had dyslipidaemia. On average, participants had known about their HIV infection for about 5 years, and 77% of the participants were on ART, of whom 11% were on second-line ART. The median Framingham 10-year CVD risk for patients aged ≥ 30 years was 2.9% (IQR 1.6–6.1%) and the median D:A:D: 5-year CVD risk for patients with an ART duration of < 10 years was 0.7% (IQR 0.3–1.5%).

For group 2, of the 395 eligible HIV-positive participants, CIMT data were available for 382 (96.7%)

Table 1 Noncomparative presentation of characteristics of both groups

	Group 1 (*n* = 826)	Group 2 (*n* = 382)
Demographics		
Male sex [*n* (%)]	328 (39.7)	130 (34.0)
Age (years) [mean (SD)]	42.2 (10.3)	39.5 (8.8)
Age category [*n* (%)]		
18–29 years	100 (12.1)	51 (13.4)
30–49 years	530 (64.2)	282 (73.8)
≥ 50 years	196 (23.7)	49 (12.8)
Partnership status: single [*n* (%)]	369 (45.0)	140 (36.8)
Highest level of completed education [*n* (%)]		
None or primary school	232 (28.1)	50 (13.3)
Secondary school or matric	542 (65.7)	298 (79.0)
College or university	52 (6.3)	29 (7.7)
Employment status: unemployed [*n* (%)][a]	604 (73.1)	126 (33.2)
Lifestyle [*n* (%)]		
Physical activity[b]		
Low	382 (46.2)	161 (42.3)
Moderate	267 (32.3)	159 (41.7)
High	177 (21.5)	61 (16.0)
Smoking		
Current smoker[c]	194 (23.5)	55 (14.4)
Previous smoker	108 (13.1)	25 (6.5)
Never smoked	524 (63.4)	302 (79.1)
Heavy alcohol drinker[d]	20 (2.7)	5 (1.3)
Chronic medication use		
Antihypertensive medication	30 (3.6)	29 (7.6)
Blood glucose-lowering medication	8 (1.0)	6 (1.6)
Cholesterol-lowering medication	0 (0.0)	10 (2.6)
HIV-related factors		
Known duration of HIV infection (years) [median (IQR)]	4.9 (1.2-8.3)	6.0 (2.0-10.0)
ART status [*n* (%)]		
ART-naïve[e]	192 (23.2)	107 (28.0)
On first-line ART	564 (68.3)	84 (22.0)
On second-line ART	70 (8.5)	191 (50.0)
Total ART duration (first + second lines) (years) [median (IQR)]	5.3 (2.4-8.3)	6.0 (3.5-9.0)
Duration of first-line ART (years) [median (IQR)]	4.9 (2.0-8.1)	4.0 (3.0-7.0)
Duration of second-line ART (years) [median (IQR)]	3.4 (1.3-4.6)	1.0 (0.3-4.0)
Last CD4 cell count (cells/μL) [median (IQR)]	472 (323-658)	458 (294-693)
Last CD4 cell count < 200 cells/μL [*n* (%)]	86 (10.6)	34 (10.1)
Last viral load of patients on ART [*n* (%)]		
< 50 copies/mL	509 (80.3)	246 (93.5)
50–1000 copies/mL	52 (8.2)	7 (2.7)
> 1000 copies/mL	73 (11.5)	10 (3.8)
Anthropometric measurements		
BMI (kg/m^2) [mean (SD)]	23.5 (5.7)	26.6 (6.2)
BMI category [*n* (%)]		
Underweight: < 18.5 kg/m^2	133 (16.1)	12 (3.1)
Normal: 18.5–25 kg/m^2	437 (53.0)	176 (46.1)
Overweight: > 25–30 kg/m^2	145 (17.6)	94 (24.6)
Obese: > 30 kg/m^2	110 (13.3)	100 (26.2)
Abdominal obesity [*n* (%)][f]	251 (30.4)	176 (46.2)
Cardiovascular measurements [mean (SD)]		
Systolic blood pressure (mm Hg)	119 (22)	122 (18)
Diastolic blood pressure (mm Hg)	75 (14)	78 (11)
Heart rate (beats/min)	75 (13)	71 (11)
Biochemical measurements [median (IQR)]		
Total cholesterol (mmol/L)	4.2 (3.6-4.9)	4.4 (3.7-5.1)
HDL cholesterol (mmol/L)	1.4 (1.2-1.6)	1.3 (1.0-1.5)
LDL cholesterol (mmol/L)	2.2 (1.7-2.8)	2.6 (2.1-3.2)
Triglycerides (mmol/L)	1.0 (0.7-1.4)	1.1 (0.8-1.6)
Glucose (mmol/L)	4.7 (4.3-5.1)	4.6 (4.3-5.0)
Cardiovascular risk factors [*n* (%)]		
Hypertension[g]	274 (33.2)	118 (31.0)
Diabetes[h]	11 (1.3)	7 (2.0)
Dyslipidaemia[i]	304 (37.0)	180 (49.5)
Metabolic syndrome[j]	83 (10.1)	62 (17.1)
Positive family history[k]	11 (1.3)	27 (7.1)
CVD prediction models [median (IQR)]		
Framingham[l], 10-year CVD risk (%)	2.9 (1.6-6.1)	2.5 (1.5-4.3)
D:A:D[m], 5-year CVD risk (%)	0.7 (0.3-1.5)	0.6 (0.3-1.1)

ART, antiretroviral therapy; BMI, body mass index; CVD, cardiovascular disease; D:A:D, Data Collection of Adverse Events on Anti-HIV Drugs; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; SD, standard deviation.
[a]Also includes students, retirees, disabled people and volunteers (rural, *n* = 29; urban, *n* = 6).
[b]Based on the International Physical Activity Questionnaire (IPAQ).
[c]Participants who quit smoking < 1 month ago were also considered current smokers.
[d]Heavy alcohol drinker: ≥ 5 days of drinking a week in the past month.
[e]Participants who initiated ART within 3 months before inclusion were also considered ART-naïve.
[f]Abdominal obesity: waist circumference ≥ 102 cm for men and ≥ 88 cm for women.
[g]Hypertension: systolic blood pressure > 130 mm Hg, diastolic blood pressure > 85 mm Hg and/or use of antihypertensive medication.
[h]Diabetes mellitus: random glucose > 11 mmol/L and/or using blood glucose-lowering medication.
[i]Dyslipidaemia: elevated triglycerides (≥ 1.7 mmol/L) and/or reduced HDL cholesterol (< 1.0 mmol/L for men and < 1.3 mmol/L for women).
[j]Metabolic syndrome: at least three out of: diabetes, hypertension, elevated triglycerides, lowered HDL cholesterol or abdominal obesity.
[k]Calculated for participants aged ≥ 30 years.
[l]Positive family history: self-reported stroke and/or heart attack of parent and/or sibling before the age of 60 years.
[m]Calculated for participants with an ART duration of a maximum of 10 years.

participants. In total, 34% (*n* = 130) were men and the mean (± SD) age was 39.5 ± 8.8 years. More than 85% had completed at least secondary school. About 50% of the participants were overweight or obese. Thirty-one per cent of the participants had hypertension and 50% had dyslipidaemia. On average, participants had known about their HIV infection for 6 years and 72% were on ART, of whom 69% were on second-line ART. The median Framingham 10-year CVD risk for patients aged ≥ 30 years was 2.5% (IQR 1.5–4.3%) and the median D:A:D: 5-year CVD risk for patients with an ART duration of < 10 years was 0.6% (IQR 0.3–1.1%) (Table 1).

CIMT

Participants from group 1 had a mean (± SD) CCA-IMT of 0.626 ± 0.128 mm and 39 participants (5%) had a

plaque in the common carotid artery. In group 2, the mean (± SD) CCA-IMT was 0.527 ± 0.092 mm and six participants (2%) had a plaque in the CCA (Table 2).

In group 1, a higher mean CCA-IMT was associated with male sex, older age, longer ART duration, higher BMI, higher total cholesterol, and higher glucose following multivariable analysis. HDL cholesterol was inversely associated with CCA-IMT (Table 3). In group 2, older age, higher education level, higher BMI and higher total cholesterol were associated with a higher mean CCA-IMT in multivariable analysis (Table 4).

Table 5 shows the contribution of the interaction terms that were added to the multivariable models. In group 1, there was a significant interaction between age and ART duration (per 5 years) (β = 0.002 mm; $P < 0.001$), age and total cholesterol (β = 0.002 mm; $P < 0.001$), and age and HDL cholesterol (β = −0.003 mm; $P < 0.001$) in relation to mean CCA-IMT. In group 2, the only significant interaction was between age and total cholesterol (β = 0.001 mm; $P = 0.01$), whereby the effect of total cholesterol on CCA-IMT was accentuated with age.

In a sensitivity analysis in group 1, only participants with a real blood pressure measurement were included (n = 390; 47.2%). Results had the same magnitude and direction, except for the contribution of SBP, as this showed no association in the full data set, but showed a trend towards an association between higher SBP and a higher mean CCA-IMT in the sensitivity analysis (β = 0.001; $P = 0.05$).

All analyses were repeated using the maximum thickness of the bulb far wall as outcome. Results had the same magnitude and direction.

Discussion

In this analysis of PLHIV in South Africa, the main drivers of mean CCA-IMT in both group 1 and group 2 were conventional CVD risk factors, and the effect of these conventional risk factors increased with age, especially in participants from group 1. In group 1, longer duration of ART use was associated with higher CCA-IMT. No HIV-related factors were associated with mean CCA-IMT in group 2.

Table 2 Noncomparative presentation of carotid intima-media thickness in both groups

Intima-media thickness outcomes	Group 1	Group 2
Mean CCA (mm) [mean (SD)]	0.626 (0.128)	0.560 (0.092)
Max CCA (mm) [mean (SD)]	0.712 (0.157)	0.644 (0.113)
Max BIF (mm) [mean (SD)]	0.870 (0.249)	0.797 (0.287)
Plaque† [*n* (%)]	39 (4.7)	6 (1.6)

BIF, carotid artery bifurcation; CCA, common carotid artery; SD, standard deviation; Max, maximum; Min, minimum.
†Plaque: a mean intima-media thickness > 1.0 mm anywhere in one of the measured angles in the far wall of the CCA.

The finding that conventional CVD risk factors contribute significantly to mean CCA-IMT is in line with research from both high-income countries and countries in SSA [25,39]. It is surprising that we did not find a correlation between blood pressure and CIMT. Blood pressure is known to be lower in PLHIV compared to the HIV-negative population [40]. It could be that the contribution of CVD risk factors to CIMT differs between HIV-positive and HIV-negative populations. In a sensitivity analysis in group 1, using only actual blood pressure outcomes (excluding participants with imputed values), we did find a trend towards an association between higher SBP and higher CIMT. This sensitivity analysis may have lacked power to find a significant association, and hence the high proportion of participants with imputed values in the full analysis might have obscured a real association between blood pressure and CCA-IMT in this group.

We observed that the effects of total cholesterol and HDL cholesterol on CCA-IMT increased with age, in line with recent results from a meta-analysis by Hanna *et al*., on determinants of CIMT in an HIV-positive population. In addition, Hannah *et al*. reported that the influence of SBP on CIMT also increased with age [38], a finding that we could not confirm in our analysis.

In group 1, longer ART duration was associated with higher mean CCA-IMT in an adjusted analysis. In addition, there was a positive interaction between age and ART duration in their effects on mean CCA-IMT, which implies that the effect of ART on CIMT increases with age. There is no consensus in the literature yet regarding the effects of HIV and ART on CIMT, with some studies reporting that HIV infection and/or ART is associated with a higher CIMT [41–43], whereas other studies did not find an effect of HIV infection or ART on CIMT [39,44–46]. Possibly, an HIV-related increase in CIMT only occurs after years of living with HIV. This is supported by the study of Fourie *et al*. [47], who reported that CIMT was similar in HIV-positive and HIV-negative participants, despite the fact that HIV-positive participants had higher levels of soluble vascular cell adhesion molecule-1 (sVCAM-1), a strong indicator of endothelial damage. It might also be that CIMT does not fully summarize the influence of HIV and ART on the arterial wall. HIV likely influences CVD risk through mechanisms beyond the regular process of atherosclerosis. CVD in HIV infection has also been found to be related to disturbances in the immune system and coagulation system, and direct viral infiltration of the endothelium [47].

Inconsistencies in the effects of ART on CIMT were also observed in our results: we did not find any association

Table 3 Factors associated with mean common carotid artery intima-media thickness (CCA-IMT): group 1

Factor	Univariable β (95% CI) (mm)	*P*	Multivariable† β (95% CI) (mm)	*P*
Demographic factors				
Male sex	**0.057 (0.039, 0.074)**	**< 0.001**	**0.048 (0.031, 0.065)**	**< 0.001**
Age, per year	**0.008 (0.007, 0.009)**	**< 0.001**	**0.007 (0.006, 0.008)**	**< 0.001**
Single (versus having partner)	**−0.019 (−0.036, −0.001)**	**0.04**	−0.008 (−0.021, 0.006)	0.26
No/primary education (versus secondary/higher education)	**0.085 (0.067, 0.104)**	**< 0.001**	0.002 (−0.015, 0.019)	0.83
Employed (versus unemployed)	0.001 (−0.019, 0.021)	0.90		
HIV-related factors				
Known duration of HIV infection, per 5 years	**0.027 (0.017, 0.037)**	**< 0.001**	*	
On first-line ART (versus ART-naïve)	**0.055 (0.034, 0.076)**	**< 0.001**	*	
On second-line ART (versus ART-naïve)	**0.053 (0.018, 0.088)**	**< 0.01**	*	
Total ART duration, per 5 years	**0.043 (0.032, 0.054)**	**< 0.001**	**0.011 (0.002, 0.005)**	**0.02**
Last CD4 cell count, per 100 cells/μL	0.001 (−0.003, 0.004)	0.73		
Last viral load, per 1 $\log_{10}$ copies/mL	**−0.012 (−0.18, −0.006)**	**< 0.001**	0.001 (−0.004, 0.007)	0.64
Cardiovascular risk factors				
Low physical activity (versus moderate/high)	0.025 (0.007, 0.042)	0.06	0.007 (−0.006, 0.020)	0.28
Ever smoked (versus never smoked)	0.014 (−0.004, 0.032)	0.14	−0.013 (−0.028, 0.003)	0.11
Heavy alcohol drinker	−0.023 (−0.079, 0.034)	0.43		
Positive family history	−0.052 (−0.129, 0.024)	0.18	−0.019 (−0.076, 0.037)	0.50
BMI, per kg/m^2	**0.003 (0.001, 0.004)**	**< 0.01**	**0.003 (0.002, 0.005)**	**< 0.001**
Abdominal obesity	0.012 (−0.007, 0.031)	0.22		
SBP, per mm Hg	**0.001 (0.000, 0.001)**	**< 0.001**	0.000 (0.000, 0.001)	0.64
DBP, per mm Hg	**0.001 (0.000, 0.002)**	**0.01**	0.000 (−0.001, 0.001)	0.68
Heart rate, per beat/min	0.000 (−0.001, 0.000)	0.20		
Total cholesterol, per mmol/L	**0.030 (0.022, 0.038)**	**< 0.001**	**0.019 (0.011, 0.027)**	**< 0.001**
HDL cholesterol, per mmol/L	−0.011 (0.032, 0.010)	0.30	**−0.034 (−0.052, −0.016)**	**< 0.001**
LDL cholesterol, per mmol/L	**0.030 (0.020, 0.040)**	**< 0.001**	*	
Triglycerides, per mmol/L	**0.033 (0.022, 0.044)**	**< 0.001**	−0.004 (−0.014, 0.005)	0.40
Glucose, per mmol/L	**0.017 (0.010, 0.024)**	**< 0.001**	**0.006 (0.000, 0.011)**	**0.04**

Significant results ($P < 0.05$) are in bold font.
ART, antiretroviral therapy; BMI, body mass index; CI, confidence interval; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure.
*Excluded from multivariable regression because of collinearity.
†A total of 811 participants (98.2%) were included.

between ART duration and mean CCA-IMT in group 2, in contrast to our findings in group 1. As there is no reason to expect that the influence of ART use on mean CCA-IMT would differ between the two settings, we presume that the following differences between the two groups may be responsible for these findings.

First, the participants in group 1 were substantially older than the urban population, with a larger proportion of participants aged ≥ 50 years (23.7% in group 2 versus 12.8% in group 2). The participants in group 2 might not have been old enough to reflect the influence of ART on CIMT in an aging population. Another explanation to consider is that participants in group 1 had been on first-line ART for longer than participants in group 2 (median duration 4.9 versus 4.0 years, respectively), with a greater spread (the 75% percentile of ART duration was 8.1 years in group 1 versus 7.0 years in group 2). Possibly, participants from group 1 were exposed for longer to older stavudine-containing ART regimens. Stavudine was part of South African first-line regimens until health care professionals started to switch patients to new regimens after the guidelines recommended against stavudine in 2010, as stavudine has been associated with enhanced CVD risk [48]. A third possibility is that the virological control differed between sites: in group 1, 80.3% of participants on ART were virally suppressed, whereas in group 2, 93.5% of the participants on ART showed viral suppression. Ongoing viraemia is associated with immune activation [49], higher CIMT [50], and an increased risk of CVD [51]. Finally, as group 2 had a considerably lower sample size than group 1, the lack of an association between ART use and CCA-IMT might be attributable to a lack of power.

To summarize, the main drivers for CIMT in an HIV-positive population in SSA remained conventional CVD risk factors. ART use was associated with CIMT, but only in group 1 (sampled from a rural setting), which had a substantial number of participants over the age of 50 years and had suboptimal virological control. This suggests that immune-related mechanisms may add to CVD risk in an older treated HIV-positive population.

To our knowledge, this is the first study to report CIMT and its determinants in PLHIV from different settings in SSA. Some limitations need to be considered. Our ability to investigate whether location of residence (a more rural

Table 4 Factors associated with mean common carotid artery intima-media thickness (CCA-IMT): group 2

Factor	Univariable β (95% CI) (mm)	*P*	Multivariable† β (95% CI) (mm)	*P*
Demographic factors				
Male sex	**0.020 (0.001, 0.040)**	**0.04**	0.005 (−0.017, 0.027)	0.62
Age, per year	**0.007 (0.006, 0.008)**	**< 0.001**	**0.006 (0.005, 0.008)**	**< 0.001**
Single (versus having partner)	−0.010 (−0.030, 0.009)	0.29		
No/primary education (versus secondary/higher education)	**0.046 (0.019, 0.073)**	**< 0.1**	**0.025 (0.001, 0.049)**	**< 0.05**
Employed (versus unemployed)	−0.005 (−0.024, 0.015)	0.65		
HIV-related factors				
Known duration of HIV infection, per 5 years	**0.028 (0.020, 0.036)**	**< 0.001**	*	
On first-line ART (versus ART-naïve)	0.015 (−0.010, −0.040)	0.24	*	
On second-line ART (versus ART-naïve)	**0.067 (0.047, 0.088)**	**< 0.001**	*	
Total ART duration, per 5 years	**0.034 (0.023, 0.044)**	**< 0.001**	−0.005(−0.020, 0.010)	0.54
Last CD4 cell count, per 100 cells/μL	**0.007 (0.004, 0.011)**	**< 0.001**	0.000 (0.000, 0.00)	0.24
Last viral load, per 1 $\log_{10}$ copies/mL	**−0.016 (−0.021, −0.010)**	**< 0.001**	0.001 (−0.006, 0.008)	0.78
Cardiovascular risk factors				
Low physical activity (versus moderate/high)	0.017 (−0.002, 0.036)	0.07	0.009 (−0.007, 0.025)	0.29
Ever smoked (versus never smoked)	−0.006 (−0.029, 0.016)	0.58	−0.011 (−0.032, 0.011)	0.32
Heavy alcohol drinker: yes (versus no)	−0.006 (−0.149, 0.076)	0.88		
Positive family history	0.013 (−0.014, 0.049)	0.49		
BMI, per kg/m^2	**0.003 (0.001, 0.004)**	**< 0.01**	**0.002 (0.000, 0.004)**	**0.03**
Abdominal obesity	**0.023 (0.005, 0.042)**	**0.02**	−0.018 (−0.041, 0.006)	0.14
SBP, per mm Hg	**0.001 (0.000, 0.001)**	**< 0.01**	0.000 (0.000, 0.001)	0.39
DBP, per mm Hg	**0.001 (0.000, 0.002)**	**0.01**	0.000 (−0.001, 0.001)	0.91
Heart rate, per beat/min	**−0.001 (−0.002, 0.000)**	**0.04**	0.000 (−0.001, 0.001)	0.60
Total cholesterol, per mmol/L	**0.031 (0.021, 0.040)**	**< 0.001**	**0.011 (0.001, 0.021)**	**0.04**
HDL cholesterol, per mmol/L	**0.028 (0.004, 0.052)**	**0.02**	−0.002 (−0.027, 0.023)	0.88
LDL cholesterol, per mmol/L	**0.029 (0.018, 0.040)**	**< 0.001**	*	
Triglycerides, per mmol/L	**0.021 (0.009, 0.034)**	**< 0.01**	0.000 (−0.012, 0.012)	0.96
Glucose, per mmol/L	0.007 (0.000, 0.014)	0.07	0.000 (−0.006, 0.006)	0.89

Significant results ($P < 0.05$) are in bold font.
ART, antiretroviral therapy; BMI, body mass index; CI, confidence interval; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure.
*Excluded from multivariable regression because of collinearity.
†A total of 319 participants (83.5%) were included.

Table 5 Multivariable model including interaction terms which had $P < 0.2$ in univariable analysis

Interaction terms	β (95% CI) (mm)	*P*
Group 1		
Age × ART duration (per 5 years)	**0.002 (0.001, 0.003)**	**< 0.001**
Age × BMI	0.000 (0.000, 0.000)	0.40
Age × total cholesterol	**0.002 (0.001, 0.003)**	**< 0.001**
Age × HDL cholesterol	**−0.003 (−0.005, −0.001)**	**< 0.001**
Age × triglycerides	−0.001 (−0.002, 0.000)	0.19
Age × glucose	0.001 (0.000, 0.001)	0.07
Group 2		
Age × ART duration (per 5 years)	0.000 (−0.002, 0.001)	0.43
Age × BMI	0.000 (0.000, 0.000)	0.58
Age × total cholesterol	**0.001 (0.000, 0.002)**	**0.01**
Age × triglycerides	0.001 (0.000, 0.002)	0.17

Significant results ($P < 0.05$) are in bold font.
ART, antiretroviral therapy; BMI, body mass index; CI, confidence interval; HDL, high-density lipoprotein.

setting versus a more urban setting) influences CVD risk was limited as we could not directly compare the burdens of subclinical CVD according to CIMT between the rural and the urban sites because of differences in population characteristics and recruitment methods. Group 1 had a relatively high number of participants without viral suppression. This might impact the generalizability of our findings to populations with adequate virological control. Subclinical atherosclerosis assessment did not include internal carotid IMT as this outcome was not available; this information would have been useful, as plaque in this region is more strongly related to CVD than an increase in CCA-IMT. Finally, our imputation method (a random draw out of 20 imputed data sets) may have resulted in a too narrow confidence interval for the imputed blood pressure readings.

Further research including an older HIV-positive population with better virological control is recommended to further elucidate the associations of HIV infection and ART use with CVD risk. Ideally, studies with a prospective design, including HIV-negative controls, could provide more insight into causal relationships between HIV infection, conventional CVD risk factors, and CVD. Our results suggest that CVD prevention in PLHIV should be directed at conventional CVD risk factors alongside optimizing HIV care.

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