

# Cross-sectional Comparison of the Prevalence of Age-Associated Comorbidities and Their Risk Factors Between HIV-Infected and Uninfected Individuals: The AGE<sub>h</sub>IV Cohort Study

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**Background.** Human immunodeficiency virus (HIV)-infected individuals may be at increased risk of age-associated noncommunicable comorbidities (AANCCs).

**Methods.** Cross-sectional analyses of AANCC prevalence (including cardiovascular, metabolic, pulmonary, renal, bone, and malignant disease) and risk factors in a prospective cohort study of HIV type 1-infected individuals and HIV-uninfected controls, who were aged  $\geq 45$  years and comparable regarding most lifestyle and demographic factors.

**Results.** HIV-infected participants ( $n = 540$ ) had a significantly higher mean number of AANCCs than controls ( $n = 524$ ) (1.3 [SD, 1.14] vs 1.0 [SD, 0.95];  $P < .001$ ), with significantly more HIV-infected participants having  $\geq 1$  AANCC (69.4% vs 61.8%;  $P = .009$ ). Hypertension, myocardial infarction, peripheral arterial disease, and impaired renal function were significantly more prevalent among HIV-infected participants. Risk of AANCC by ordinal logistic regression was independently associated with age, smoking, positive family history for cardiovascular/metabolic disease, and higher waist-to-hip ratio, but also with HIV infection (odds ratio, 1.58 [95% confidence interval, 1.23–2.03];  $P < .001$ ). In those with HIV, longer exposure to CD4 counts  $< 200$  cells/ $\mu$ L, and, to a lesser extent, higher levels of high-sensitivity C-reactive protein and soluble CD14, and longer prior use of high-dose ritonavir ( $\geq 400$  mg/24 hours) were each also associated with a higher risk of AANCCs.

**Conclusions.** All AANCCs were numerically more prevalent, with peripheral arterial, cardiovascular disease, and impaired renal function significantly so, among HIV-infected participants compared with HIV-uninfected controls. Besides recognized cardiovascular risk factors, HIV infection and longer time spent with severe immunodeficiency increased the risk of a higher composite AANCC burden. There was a less pronounced contribution from residual inflammation, immune activation, and prior high-dose ritonavir use.

**Keywords.** HIV infection; aging; comorbidity.

AIDS-associated morbidity and mortality have dramatically declined with the advent of combination

antiretroviral therapy (cART) [1–3]. However, the life expectancy of individuals infected with human immunodeficiency virus (HIV) on average remains shorter than for the general population [3–5], and non-AIDS comorbidities have gained increasing importance as causes of death in cART-treated patients [2, 3, 6, 7]. As HIV-infected individuals on cART age, they increasingly experience non-AIDS comorbidities [7, 8], which in HIV may be both accentuated and/or accelerated, thereby possibly occurring at younger ages [8–10]. Potential contributors may include a higher prevalence of recognized risk factors, as well as ART exposure and

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toxicity, HIV infection, immune dysfunction/dysregulation, and chronic immune activation/inflammation associated with the infection [11–16].

By 2015, half of the HIV-infected population in the United States will be aged >50 years, with similar trends observed in Europe and resource-limited settings [7, 9, 17]. More insight into prevalence, incidence, and risk factors of non-AIDS comorbidity among HIV-infected individuals is therefore essential to optimize policy for prevention and management [18]. Most published studies thus far did not include a comparable uninfected control group. Whether different comorbidities occur more often and possibly at a younger age among HIV-infected individuals therefore remains unclear.

To clarify these issues, the AGE<sub>HIV</sub> Cohort Study was implemented in 2010 in Amsterdam, the Netherlands, to compare the prevalence, incidence and risk factors of aging-associated noncommunicable comorbidities (AANCCs) and organ dysfunction among HIV type 1 (HIV-1)-infected individuals and HIV-uninfected controls. We report a cross-sectional comparison at the time of enrollment of AANCC prevalence between the HIV-infected and HIV-uninfected groups, and analyzed both recognized and potential HIV-associated risk factors.

## METHODS

### Study Design and Data Collection

HIV-1-infected participants were recruited from the HIV outpatient clinic of the Academic Medical Center in Amsterdam, and HIV-uninfected participants (controls) were recruited from the sexual health clinic of the Amsterdam Public Health Service or among uninfected participants in the existing Amsterdam Cohort Studies on HIV/AIDS [19]. To ensure comparability of the HIV-infected and HIV-uninfected study groups, we regularly monitored age, sex, and ethnicity in both study groups, and adjusted enrollment of underrepresented categories among HIV-uninfected participants accordingly.

All participants were aged  $\geq 45$  years with laboratory-confirmed presence or absence of HIV-1 infection. All subjects who provided written informed consent within the 2-year enrollment period were included. Of 1100 eligible patients from the HIV outpatient clinic, between 600 and 800 were expected to be enrolled, and we therefore aimed to include a similar number of uninfected controls. This sample size would provide sufficient statistical power to investigate associations between a broad range of AANCCs and potential risk factors.

At baseline, 2 years later, and depending on sufficient resources every 2 years thereafter, participants undergo standardized screening for AANCCs and organ dysfunction.

Participants are requested to complete a standardized questionnaire concerning demographics, (family) medical history,

use of medications (both prescribed and over-the-counter), participation in population screening programs, substance use, quality of life, depression, sexual orientation/behavior/dysfunction, cognitive complaints, calcium/vitamin D intake, physical exercise, social behavior, and work participation/income. All participants undergo measurements of blood pressure, height, weight, and hip/waist circumference, as well as electrocardiography, measurement of vascular elasticity, spirometry, screening cognitive tests, frailty, bone densitometry, and quantification of advanced glycation end products in the skin. Blood and urine samples are obtained for extensive laboratory testing, and cryopreserved for future analyses.

Detailed information concerning HIV and ART history is obtained from the Dutch HIV Monitoring Foundation, formally responsible for capturing detailed HIV/ART-related data from all individuals in care for HIV at an HIV treatment facility in the Netherlands [20]. The study protocol was approved by the local ethics review committee and registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) (identifier NCT01466582). All participants provided written informed consent.

### Study Participants

All study participants who underwent baseline assessments (between 1 October 2010 and 30 September 2012), and completed a study questionnaire were included in the analyses.

### Definitions

Data were available on hypertension, angina pectoris, myocardial infarction, peripheral arterial disease, ischemic cerebrovascular disease, diabetes mellitus type 2, obstructive pulmonary disease, impaired renal function, non-AIDS cancer, and atraumatic fractures/osteoporosis.

Hypertension was considered to be present if diastolic blood pressure  $\geq 90$  mm Hg and/or systolic blood pressure  $\geq 140$  mm Hg in all 3 measurements (Omron 705IT) with a 1-minute interval, and if on antihypertensive medication [21]; diabetes mellitus type 2 if hemoglobin A1c (HbA1c) (International Federation of Clinical Chemistry and Laboratory Medicine [IFCC]) was  $\geq 48$  mmol/mol and/or elevated blood glucose (nonfasting  $\geq 11.1$  mmol/L or fasting  $\geq 7.0$  mmol/L), and if on antidiabetic medication [22]; obstructive pulmonary disease if 1-second forced expiratory volume (FEV<sub>1</sub>) to forced vital capacity (FVC) ratio was  $<0.7$  in all 3 forced expiratory spirometric measurements (MicroDirect SpiroUSB) without bronchodilation, in those on bronchodilators, or in those self-reporting obstructive pulmonary disease by questionnaire [23]; impaired renal function if estimated glomerular filtration rate was  $<60$  mL/minute using the Chronic Kidney Disease Epidemiology Collaboration formula; atraumatic fractures/osteoporosis in case of a dual-energy X-ray absorptiometry (DXA) (Hologic QDR 4500 W and Hologic Discovery A densitometers, software versions

12.4–13.3)  $T$  score  $\leq -2.5$  standard deviations (postmenopausal women and men aged  $\geq 50$ ) or  $z$  score  $\leq -2$  standard deviations (premenopausal women and men aged  $< 50$ ), or in those reporting atraumatic fracture by questionnaire [24, 25].

Angina pectoris, myocardial infarction, peripheral arterial disease, ischemic cerebrovascular disease, and non-AIDS cancer (including nonmelanoma skin cancers) were diagnosed in participants self-reporting these diseases by questionnaire. All self-reported diagnoses were validated using hospital records for HIV-infected participants, and, general practitioners' records for controls, provided the latter had consented to contact with their general practitioner. If not, unvalidated diagnoses were used. This may result in a conservative estimate of the difference in AANCC prevalence between the HIV-infected and -uninfected cohorts by likely overestimating the true number of AANCCs among controls.

Physical activity was defined according to Dutch healthy physical activity guidelines ("Combinorm"): moderate physical activity  $\geq 5$  days per week for  $\geq 30$  minutes, or heavy physical activity at least twice a week for  $\geq 20$  minutes [26].

### Statistical Analysis

Study groups were compared using the  $\chi^2$ , Wilcoxon rank-sum, nonparametric test for trend, or Student  $t$  test as appropriate. All reported  $P$  values are 2-sided.

Multivariable ordinal logistic regression analysis (proportional odds model) was performed to assess the contribution of HIV and recognized risk factors toward AANCCs. The outcome measure was the total number of AANCCs per participant. All models were adjusted for age, sex, Dutch origin, sexual orientation, positive family history (for myocardial infarction, hypertension, diabetes mellitus type 2, or hypercholesterolemia), smoking, use of cocaine/ecstasy/cannabis, severe alcohol use, and hepatitis B/C coinfection. Biologically plausible determinants of AANCC (including HIV/ART-related factors, and markers of systemic inflammation/monocyte activation/coagulation) were explored using a stepwise model selection. Continuous variables were transformed or categorized when necessary. Exposure to HIV-related factors was set to zero for HIV-uninfected participants, as were pack-years for nonsmokers. All models used data from both study groups (including those exploring HIV-related risk factors), except where explicitly stated otherwise.

Analyses were performed using SAS version 9.2.

## RESULTS

A total of 598 HIV-infected participants and 550 HIV-uninfected controls completed a baseline visit between 1 October 2010 and 30 September 2012. Data from 540 HIV-infected and 524 HIV-uninfected participants were available for analysis, after excluding 58 HIV-infected and 26 HIV-uninfected participants with a

missing questionnaire. Age, DXA results, glucose/HbA1c, blood pressure, FEV<sub>1</sub>/FVC ratio, and renal function were not significantly different between HIV-infected and -uninfected participants with or without a completed questionnaire.

### Baseline Characteristics of Participants

Participants in both study groups were very comparable in terms of baseline characteristics; the median age was around 52 years, and the majority were male, men who have sex with men (MSM), and of Dutch origin. Significantly fewer HIV-infected participants were of Dutch, and more of African, origin (72.2% vs 81.3%;  $P < .001$  and 7.4% vs 1.3%;  $P < .001$ , respectively). Significantly fewer controls were hepatitis B/C coinfect-ed (0.6% vs 3.9%;  $P < .001$  and 0.8% vs 2.8%;  $P = .029$ , respectively) (Table 1). No statistically significant difference in age distribution was found between the two study groups.

On average, HIV-infected participants were known to be infected for a prolonged period of time, and 30% had prior AIDS. Virtually all were on cART for many years, and currently had undetectable HIV-1 plasma viral loads. The majority had experienced immune recovery on treatment, with a median nadir CD4 count of 180 cells/ $\mu$ L and current median CD4 count of 565 cells/ $\mu$ L.

Significantly more HIV-infected participants were current smokers (32.0% vs 24.6%;  $P = .007$ ), whereas ecstasy use was significantly more prevalent among controls (4.3% vs 8.6%;  $P = .004$ ) (Table 2).

Body mass index (BMI) was lower (24.2 [interquartile range {IQR}, 22.3–26.6] vs 24.5 [IQR, 22.8–27.0] kg/m<sup>2</sup>;  $P = .019$ ) and above-normal waist-to-hip ratio was significantly more prevalent among HIV-infected participants (84.0% vs 62.4%;  $P < .001$ ). Systolic (135 [IQR, 126–147] vs 133 [IQR, 125–143] mm Hg;  $P = .006$ ) and diastolic blood pressure (81 [IQR, 75–89] vs 79 [IQR 72–85] mm Hg;  $P < .001$ ) were significantly higher among HIV-infected participants. Significantly fewer HIV-infected participants were physically active (44.3% vs 53.0%;  $P = .005$ ) and they had significantly lower levels of 25-hydroxy vitamin D2 + D3 (47 [IQR, 29–71] vs 54 [IQR, 39–72] nmol/L;  $P < .001$ ).

### AANCC Prevalence

All self-reported diagnoses of angina pectoris, myocardial infarction, peripheral arterial disease, ischemic cerebrovascular disease, and non-AIDS cancer could be validated among HIV-infected participants: of the total 155 self-reported diagnoses, 100 were confirmed and 55 rejected. Fourteen controls did not consent to contact their general practitioner for validation of 16 self-reported diagnoses, which accounted for 21.6% of 74 self-reported diagnoses among controls. Of the remaining 58 self-reported diagnoses that could be validated, 39 were confirmed and 19 rejected.

**Table 1. Baseline Demographic and HIV-Related Characteristics**

Characteristic	HIV-Uninfected Participants (n = 524)	HIV-Infected Participants (n = 540)	P Value
Age, y	52.1 (47.9–58.3)	52.9 (48.3–59.6)	.200*
Male sex	85.1%	88.1%	.146**
Dutch origin	81.3%	72.2%	<.001**
African origin	1.3%	7.4%	<.001**
MSM <sup>a</sup>	69.7%	73.9%	.125**
Hepatitis C RNA positive	0.8%	2.8%	.029**
Hepatitis B antigen and/or hepatitis B DNA positive	0.6%	3.9%	<.001**
Time since HIV-1 diagnosis, y	...	12.1 (6.2–17.1)	...
Diagnosed with HIV-1 prior to 1996	...	32.8%	...
CD4 count at enrollment, cells/μL	...	565 (435–745)	...
Nadir CD4 count, cells/μL	...	180 (78–260)	...
Known cumulative duration of CD4 count <200 cells/μL, mo	...	0.8 (0.0–9.6)	...
Plasma viral load >200 copies/mL among cART-treated participants within 4 mo before or at enrollment <sup>b</sup>	...	1.5%	...
Last plasma viral load within 4 mo before or at enrollment, log <sub>10</sub> copies/mL <sup>b</sup>	...	1.6 (1.6–1.6)	...
Duration of plasma viral load ≤200 copies/mL, y <sup>c</sup>	...	5.8 (2.4–10.2)	...
Prior clinical AIDS <sup>d</sup>	...	31.3%	...
On cART <sup>e</sup>	...	95.7%	...
Time since ART was first initiated, y	...	10.4 (4.4–14.5)	...
Naive at start of cART	...	79.1%	...
High-dose ritonavir (≥400 mg daily) use			
Prior exposure among all non-ART-naive participants	...	31.5%	...
Cumulative exposure among all non-ART-naive participants, mo	...	0.0 (0.0–6.3)	...
Cumulative exposure among participants that used high-dose ritonavir, mo	...	17.6 (7.6–40.4)	...

Data are presented as median (interquartile range) or percentage.

Abbreviations: ART, antiretroviral therapy; cART, combination antiretroviral therapy; HIV-1, human immunodeficiency virus type 1; MSM, men who have sex with men.

<sup>a</sup> Male participants who stated in the questionnaire to feel mostly or exclusively sexually attracted to men.

<sup>b</sup> Only a plasma HIV-1 load that was measured ≤4 months prior to enrollment was used. If such a recent test result was not available, plasma HIV-1 load was measured at enrollment.

<sup>c</sup> Duration since last plasma viral load >200 copies/mL.

<sup>d</sup> Previous AIDS-defining condition following the US Centers for Disease Control and Prevention classification.

<sup>e</sup> Combination of ≥3 antiretroviral drugs, other than ritonavir used as a booster.

\* Wilcoxon rank-sum test.

\*\*  $\chi^2$  test.

HIV-infected individuals had a significantly higher mean number of AANCCs than controls (1.3 [SD, 1.14] vs 1.0 [SD, 0.95];  $P < .001$ ). The proportion of participants with ≥1 AANCCs was also significantly higher among those with HIV (69.4% vs 61.8%;  $P = .009$ ).

The mean number of AANCCs within the 50–54, 60–64, and ≥65 age categories was significantly higher among HIV-infected than HIV-uninfected participants (Figure 1). Furthermore, the distribution of the number of AANCCs for HIV-infected participants in each 5-year age stratum resembled the distribution for controls who were 5 years older.

Each individual AANCC was numerically more prevalent among HIV-infected participants, with hypertension (45.4% vs 30.5%;  $P < .001$ ), myocardial infarction (3.9% vs 1.5%;  $P = .018$ ), peripheral arterial disease (2.6% vs 0.6%;  $P = .008$ ), and impaired renal function (4.3% vs 2.1%;  $P = .044$ ) being significantly more prevalent among HIV-infected participants (Figure 2).

### Factors Contributing to the Risk of AANCC

#### HIV-Related Risk Factors

After adjustment for age, sex, Dutch origin, sexual orientation, positive family history (for myocardial infarction, hypertension,

**Table 2. Prevalence of Comorbidity Risk Factors**

Characteristic	HIV-Uninfected Participants (n = 524)	HIV-Infected Participants (n = 540)	P Value
<b>Smoking status</b>			
Never smoked	36.5%	33.0%	.028*
Ever smoked	38.9%	35.0%	
Currently smoking <sup>a</sup>	24.6%	32.0%	
Pack-years of smoking among ever-smokers	15.0 (4.5–28.8)	22.2 (7.8–36.8)	.001**
Severe alcohol use <sup>b</sup>	7.3%	4.8%	.098***
Daily to monthly use of cannabis	11.6%	13.5%	.356***
Daily to monthly use of cocaine	2.9%	3.7%	.442***
Daily to monthly use of ecstasy	8.6%	4.3%	.004***
BMI, kg/m <sup>2</sup>	24.5 (22.8–27.0)	24.2 (22.3–26.6)	.019**
<b>BMI categories, kg/m<sup>2</sup></b>			
<20	3.3%	8.2%	.121*
20 to <25	54.1%	50.7%	
25 to <30	32.7%	33.2%	
≥30	9.9%	8.0%	
Waist-to-hip ratio higher than normal <sup>c</sup>	62.4%	84.0%	<.001***
Blood pressure, systolic, mm Hg	133 (125–143)	135 (126–147)	.006****
Blood pressure, diastolic, mm Hg	79 (72–85)	81 (75–89)	<.001****
Positive family history for myocardial infarction, hypertension, diabetes mellitus type 2, or hypercholesterolemia <sup>d</sup>	66.5%	70.8	.139***
Physical activity <sup>e</sup>	53.0%	44.3%	.005***
25-hydroxy vitamin D2 + D3, nmol/L	54 (39–72)	47 (29–71)	<.001**

Data are presented as median (interquartile range) or percentage.

Abbreviations: BMI, body mass index; HIV, human immunodeficiency virus.

<sup>a</sup> Smoked during the last month before completing the questionnaire.

<sup>b</sup> Alcohol intake >4 units (for men) or >2 units (for women) daily or almost daily.

<sup>c</sup> If ≥0.9 in males and ≥0.85 in females.

<sup>d</sup> Participants were considered to have a positive family history for myocardial infarction/hypertension/diabetes mellitus type 2/hypercholesterolemia when they stated in the questionnaire to have a first-degree family member who experienced a myocardial infarction before the age of 60, or to have a first-degree family member suffering from hypertension, diabetes mellitus type 2, or hypercholesterolemia.

<sup>e</sup> Physical activity was defined following the Dutch guidelines for healthy physical activity ("Combinorm"): at least 5 days per week at least 30 minutes of moderate physical activity or at least twice per week at least 20 minutes of heavy physical activity [26].

\* Nonparametric test for trend.

\*\* Wilcoxon rank-sum test.

\*\*\*  $\chi^2$  test.

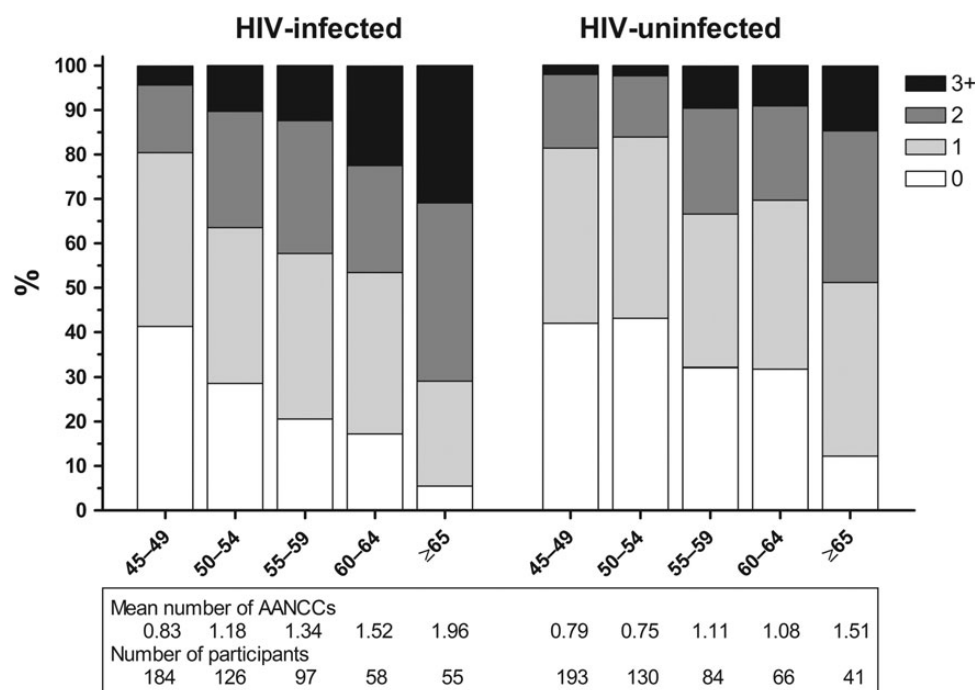
\*\*\*\* Student *t* test.

diabetes mellitus type 2, or hypercholesterolemia), smoking, use of cocaine/ecstasy/cannabis, severe alcohol use, and hepatitis B/C coinfection, HIV infection remained independently associated with a higher number of AANCCs (odds ratio [OR], 1.58; 95% confidence interval [CI], 1.23–2.03;  $P < .001$ ). Age, positive family history, and smoking were also strongly independently associated with AANCCs. Analyzing the HIV-infected and HIV-uninfected study groups separately, the OR for age was higher in the HIV-infected study group (OR, 1.60; 95% CI, 1.41–1.81;  $P < .001$ ) compared with the uninfected controls (OR, 1.41; 95% CI, 1.25–1.60;  $P < .001$ ), the difference being borderline significant ( $P$  value for interaction = .06).

In univariable analyses (though adjusting conform previous models), several HIV-related variables were significantly associated with AANCCs: time since HIV diagnosis (OR, 1.03 per additional year; 95% CI, 1.02–1.05;  $P < .001$ ), duration of ART use (OR, 1.04 per additional year; 95% CI, 1.02–1.06;  $P < .001$ ), and duration of CD4 count <200 cells/ $\mu$ L (OR, 1.30 per additional year; 95% CI, 1.17–1.45;  $P < .001$ ). In multivariable analysis, only duration of having CD4 counts <200 cells/ $\mu$ L remained an independent risk factor for AANCCs.

In multivariable analyses nadir CD4-count, prior AIDS, (cumulative) duration of undetectable plasma HIV-1 viral load, being diagnosed before 1996, and being pretreated with





**Figure 1.** Distribution of the number of age-associated noncommunicable comorbidities stratified by age across both study groups. Abbreviations: AANCC, age-associated noncommunicable comorbidities; HIV, human immunodeficiency virus.

mono-/dual therapy before starting cART were not significantly associated with risk of AANCC.

### **Inflammation, Coagulation, and Innate Immune Activation**

We subsequently analyzed the potential contribution of markers of systemic inflammation (high-sensitivity C-reactive protein [hs-CRP]), coagulation (D-dimer), and monocyte activation (soluble CD14 [sCD14] and soluble CD163 [sCD163]).

The median levels of each of these biomarkers, except D-dimer, were significantly higher among HIV-infected vs HIV-uninfected participants (Table 3). Adding hs-CRP and sCD14 to the above-mentioned regression model (analyzing both study groups jointly) showed both markers to be (borderline) significantly associated with AANCCs (hs-CRP: OR, 1.03/mg/L higher; 95% CI, 1.00–1.07;  $P = .037$ ; sCD14: OR, 1.02 per 100 ng/mL higher; 95% CI, 1.00–1.03;  $P = .057$ ), whereas this was not the case for hs-CRP >10 mg/L, D-dimer, D-dimer >0.5 mg/L, and sCD163. Analyzing the effect of hs-CRP and sCD14 in the 2 study groups separately, both were independent risk factors for AANCC in the HIV-infected cohort, but not in controls. None of these differences, however, reached statistical significance.

### **Other (Lifestyle-Related) Risk Factors**

An above-normal waist-to-hip ratio was an independent risk factor for AANCCs, both in the cohorts combined (OR, 1.49 per 0.1 higher ratio; 95% CI, 1.23–1.80;  $P < .001$ ) and in the

HIV-infected (OR, 1.35 per 0.1 higher ratio; 95% CI, 1.04–1.76;  $P = .024$ ) and HIV-uninfected groups separately (OR, 1.78 per 0.1 higher ratio; 95% CI, 1.34–2.37;  $P < .001$ ). No significant interaction between waist-to-hip ratio and HIV infection was found.

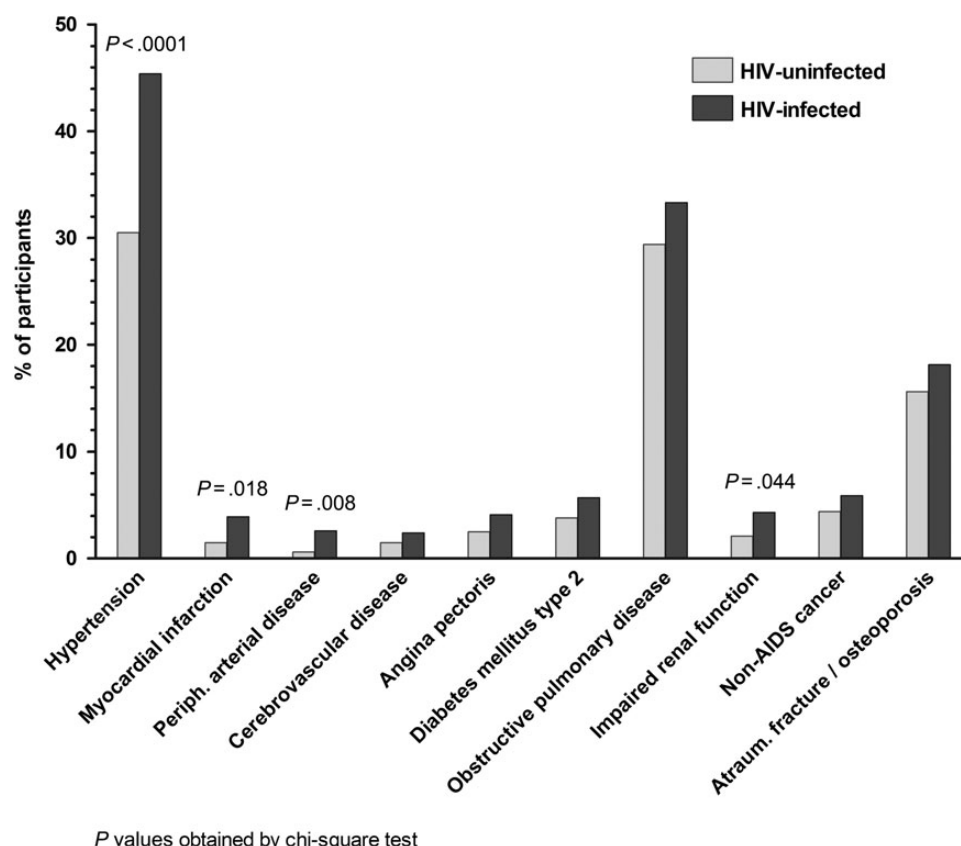
Level of physical activity and vitamin D status were not associated with risk of AANCCs.

### **Specific ART and the Risk of AANCCs**

Current or cumulative use of abacavir, stavudine, and didanosine were not significantly associated with risk of AANCCs, whereas cumulative use of ritonavir was identified as an independent risk factor for AANCC (OR, 1.29 per 5 years of ritonavir use; 95% CI, 1.04–1.60;  $P = .018$ ). Exploring this further, only cumulative duration of high ( $\geq 400$  mg/24 hours) but not of lower doses of ritonavir remained borderline significantly associated with risk of AANCCs (OR, 1.08 per year high-dose ritonavir use; 95% CI, .99–1.18;  $P = .083$ ; Table 4).

## **DISCUSSION**

HIV-infected participants, compared with uninfected controls of similar age, had a significantly higher prevalence of AANCCs, both in terms of composite comorbidity burden, and more specifically of hypertension, cardiovascular and peripheral vascular disease, and impaired renal function.



**Figure 2.** Prevalences of each of the different age-associated noncommunicable comorbidities over the 2 study groups. Abbreviation: HIV, human immunodeficiency virus.

HIV infection was independently associated with a higher total number of AANCCs, as were age, smoking, and positive family history for myocardial infarction, hypertension, diabetes mellitus

**Table 3. Values of Several Markers of Systemic Inflammation, Compared Between the 2 Study Groups**

Marker	HIV-Uninfected Participants (n = 524)	HIV-Infected Participants (n = 540)	P Value
hs-CRP, mg/L	1.0 (0.6–1.9)	1.5 (0.7–3.5)	<.001*
hs-CRP >10 mg/L	1.6%	6.7%	<.001**
D-dimer, mg/L	0.24 (0.20–0.38)	0.23 (0.20–0.36)	.078*
D-dimer >0.5 mg/L	14.1%	13.2%	.659**
sCD14, ng/mL	1356 (1080–1738)	1576 (1305–2011)	<.001*
sCD163, ng/mL	252 (182–342)	289 (207–419)	<.001*

Data are presented as median (interquartile range) or percentage.

Abbreviations: HIV, human immunodeficiency virus; hs-CRP, high-sensitivity C-reactive protein; sCD14, soluble CD14; sCD163, soluble CD163.

\* Wilcoxon rank-sum test.

\*\*  $\chi^2$  test.

type 2, or hypercholesterolemia. These traditional risk factors, as well as higher waist-to-hip ratio, independently also contributed to risk of AANCCs in each of the study groups. A borderline significant interaction between age and HIV infection suggested a stronger age effect among HIV-infected participants.

A longer time spent at a CD4 count <200 cells/ $\mu$ L and, to a lesser extent, more systemic inflammation and innate immune activation, as reflected in higher hs-CRP and sCD14 levels, as well as longer prior use of high-dose ritonavir ( $\geq 400$  mg/24 hours), were additional factors contributing to AANCC burden.

Our finding that comorbidity was significantly more prevalent among HIV-infected individuals (the majority having sustained suppression of viremia on cART) compared with uninfected controls of similar age is compatible with earlier reports [8, 27–36]. Earlier studies, however, either did not include a comparable uninfected control group but used general population [8, 29–32, 35] or patient registry data for comparison [27, 28, 33, 34, 36], or were not designed a priori to prospectively capture data on comorbidity and comorbidity risk factors with similar detail and rigor [35]. To try and overcome these limitations, we purposely recruited our HIV-uninfected participants from a setting where they were expected to exhibit similar lifestyle and

**Table 4. Risk Factors for Age-Associated Noncommunicable Comorbidities, Multivariably Analyzed Using the HIV-Infected and HIV-Uninfected Study Groups Jointly**

Risk Factor	Univariable Analysis			Multivariable Analysis		
	Odds Ratio	95% CI	P Value	Odds Ratio	95% CI	P Value
Age (per 5 years)	1.50	1.39–1.63	<.001	1.39	1.27–1.52	<.001
Smoking (per 5 pack-years)	1.10	1.07–1.13	<.001	1.08	1.05–1.12	<.001
Positive family history <sup>a</sup> (yes/no)	1.57	1.23–2.01	<.001	1.88	1.45–2.44	<.001
HIV infection (yes/no)	1.68	1.34–2.10	<.001	1.07	0.80–1.42	.661
Known cumulative duration of immunodeficiency (per year with a CD4 count <200 cells/ $\mu$ L)	1.33	1.20–1.48	<.001	1.23	1.10–1.38	<.001
Waist-to-hip ratio (per 0.1)	1.94	1.67–2.25	<.001	1.49	1.23–1.80	<.001
hs-CRP (per mg/mL)	1.06	1.03–1.09	<.001	1.03	0.99–1.06	.107
sCD14 (per 100 ng/mL)	1.02	1.01–1.04	.007	1.02	1.00–1.03	.074
Cumulative duration of ritonavir use in high dosages ( $\geq 400$ mg/daily) (per year)	1.19	1.10–1.28	<.001	1.08	0.99–1.18	.083

The outcome variable is the number of age-associated noncommunicable comorbidities (AANCCs) per participant. Analyses were performed using ordinal logistic regression. This model was adjusted for sex, Dutch origin, sexual orientation, smoking, use of cocaine/ecstasy/cannabis, severe alcohol use, and hepatitis B/C coinfection (all of which were not significantly associated with risk for AANCC).

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; hs-CRP, high-sensitivity C-reactive protein; sCD14, soluble CD14.

<sup>a</sup> Positive family history: a first-degree family member suffering from myocardial infarction, hypertension, diabetes mellitus type 2, or hypercholesterolemia.

sexual risk-taking behavior as HIV-infected study participants. Although smoking and hepatitis B/C were more prevalent in HIV-infected participants and ecstasy use was more prevalent in controls (which also consisted of more native Dutch persons), overall the differences between both study groups were relatively minor. Our findings thus add robustness to the notion that AANCCs indeed are more prevalent among those living with HIV, including in those with a sustained response to ART.

Unraveling underlying mechanisms and risk factors for this increased comorbidity burden among HIV-infected individuals is the subject of ongoing research. A central question concerns the contributions of HIV infection itself (by viral- and immune-related mechanisms), coinfections (including cytomegalovirus and chronic viral hepatitis), and ART [18]. A study by Guaraldi et al identified longer ART exposure and lower nadir CD4 count as independent risk factors for non-AIDS comorbidities [8].

We found that although HIV infection status, duration of HIV infection, duration of ART use, and duration of immune deficiency (ie, duration of having CD4 counts <200 cells/ $\mu$ L) were each univariably associated with AANCCs, these associations were all confounded by duration of immunodeficiency.

HIV infection is associated with inflammation, innate immune activation, and altered coagulation [37–39], which are generally considered important drivers for comorbidity in both HIV-uninfected and HIV-infected individuals [15, 16, 40, 41]. Higher levels of sCD14 and hs-CRP, but not of sCD163 or D-dimer, were borderline significantly associated with increased risk for AANCCs. Additional work is needed

to determine which specific inflammatory, innate and adaptive immune system, and coagulatory pathways are driving comorbidity risk, and to which extent this differs for individual comorbidities. Innate immune and particularly monocyte activation have recently been reported to be more relevant than T-cell activation in enhancing cardiovascular disease risk in HIV [42, 43].

Duration of exposure to high-dose ritonavir ( $\geq 400$  mg/24 hours) in our analyses was borderline significantly associated with risk for AANCCs. Currently, ritonavir is almost exclusively used at lower doses, and exposure to higher doses in this cohort therefore occurred many years previously. Although identified in cross-sectional analyses and potentially driven by bias, plausible mechanisms by which ritonavir may contribute to AANCC risk include its known dose-dependent effect on lipids, induction of endothelial dysfunction [44, 45], and cellular accumulation of prelamin A, which may result in premature cellular senescence similar to what is observed in some genetically determined premature aging syndromes [46, 47].

Our results being those of cross-sectional analyses, we are merely able to demonstrate associations rather than causality. Of note, risk factors identified for the presence of the composite number of different AANCCs may differ in (the magnitude of) their effect when addressing specific comorbidities separately. Although the HIV-infected and HIV-uninfected study groups were largely comparable, differences in some demographic and lifestyle-related factors were present, which was addressed by adjusting all regression analyses for a broad range of demographic and lifestyle-related factors. Nonetheless, differences in



remaining unmeasured confounders potentially influencing our results cannot be excluded.

In conclusion, all AANCCs were numerically more prevalent, and peripheral arterial, cardiovascular disease, and impaired renal function significantly so, in this cohort of HIV-infected individuals with largely sustained suppressed viremia on cART. Besides cardiovascular risk factors, HIV infection and longer time spent with severe immunodeficiency increased the risk of higher AANCC burden. Less pronounced contributions were identified from residual inflammation, immune activation, and prior high-dose ritonavir use. The trend toward a stronger association between age and AANCC burden among HIV-infected participants might support the hypothesis of premature or accelerated aging in HIV [8–10]. Whether this reflects HIV acting as an additive risk factor for comorbidity development in conjunction with traditional risk factors, or includes HIV impacting on and accelerating the biology of aging itself, remains to be elucidated [18, 48].

## Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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## APPENDIX

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