

**Cross-sectional comparison of the prevalence of age-associated comorbidities and their risk factors between HIV-infected and uninfected individuals: the AGE<sub>n</sub>IV Cohort Study**

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**Short summary:**

Age-associated comorbidities (cardiovascular and renal disease) were more prevalent among HIV-positives compared to HIV-negatives. Comorbidity was associated with cardiovascular risk factors, but also with HIV infection, immune deficiency, and (to a lesser extent) systemic inflammation, and prior high-dose ritonavir use.

**Abstract:****Background:**

HIV-infected individuals may be at increased risk of age-associated non-communicable comorbidity (AANCC).

**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-1-infected individuals and HIV-uninfected controls, aged  $\geq 45$  and comparable regarding most lifestyle and demographic factors.

**Results:**

HIV-positives (n=540) had a significantly higher mean number of AANCC than HIV-negatives (n=524) (1.3 (SD 1.14) vs. 1.0 (SD 0.95),  $p < 0.001$ ), with significantly more HIV-positives having  $\geq 1$  AANCC (69.4% vs. 61.8%,  $p = 0.009$ ).

Hypertension, myocardial infarction, peripheral arterial disease, and impaired renal function were significantly more prevalent among HIV-positives.

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 (OR 1.58, 95% CI 1.23-2.03,  $p < 0.001$ ). In those with HIV, longer exposure to CD4-counts  $< 200$  cells/mm<sup>3</sup>, and to a lesser extent higher levels of hsCRP and sCD14, and longer prior use of high-dose ritonavir ( $\geq 400$ mg/24hrs) were each also associated with a higher risk of AANCC.

**Conclusions:**

All AANCC were numerically more prevalent, with peripheral arterial, cardiovascular disease, and impaired renal function significantly so, among HIV-positives compared to HIV-uninfected controls.

Besides recognised cardiovascular risk factors, HIV infection and longer time spent with severe immune deficiency 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.

## Background

AIDS-associated morbidity and mortality has dramatically declined with combination antiretroviral therapy (cART).[1–3] Life expectancy of HIV-infected individuals however 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 toxicity, HIV infection, immune dysfunction/dysregulation, and chronic immune activation/inflammation associated with the infection.[11–16]

By 2015, half the HIV-infected population in the United States will be over 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 further, 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 non-communicable comorbidities (AANCC) and organ dysfunction among HIV-1-infected individuals and HIV-uninfected controls. We report a cross-sectional comparison at the time of enrolment of AANCC prevalence between the HIV-infected and HIV-uninfected group, 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, The Netherlands, and HIV-uninfected controls from the sexual health clinic of the Amsterdam Public Health Service or amongst uninfected participants in the existing Amsterdam Cohort Studies on HIV/AIDS.[19] To ensure comparability of the HIV-infected and HIV-uninfected studygroups, we regularly monitored age, gender, and ethnicity in both studygroups, and adjusted enrollment of underrepresented categories amongst HIV-uninfected participants accordingly.

All participants were aged  $\geq 45$  with laboratory-confirmed presence or absence of HIV-1 infection.

All subjects who provided written informed consent within the two-year enrolment period were included. Of 1100 eligible patients from the HIV outpatient clinic, between 600-800 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 AANCC and potential risk factors.

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

Participants are requested to complete a standardized questionnaire concerning demographics, (family) medical history, use of (prescribed and over-the-counter) medications, participation in population screening-programs, substance use, quality of life, depression, sexual orientation/behaviour/dysfunction, cognitive complaints, calcium/vitamin D intake, physical exercise, social behaviour, 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 endproducts 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 present if diastolic blood pressure  $\geq 90$  mmHg and/or systolic blood pressure  $\geq 140$  mmHg in all three measurements (Omron 705IT) with a one-minute interval, and if on antihypertensive medication[21];

*Diabetes mellitus type 2* if HbA1c (IFCC)  $\geq 48$  mmol/mol and/or elevated blood glucose (non-fasting  $\geq 11.1$  mmol/L or fasting)  $\geq 7.0$  mmol/L), and if on antidiabetic medication[22];

*Obstructive pulmonary disease* if one second forced expiratory volume (FEV<sub>1</sub>) to forced vital capacity (FVC) ratio  $< 0.7$  in all three 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 (eGFR)  $< 60$  mL/min using the Chronic Kidney Disease Epidemiology Collaboration formula (CKD-EPI);

*Atraumatic fractures/osteoporosis* in case of a dual-energy X-ray absorptiometry (DXA-scan: Hologic QDR 4500W and Hologic Discovery A densitometers, software versions 12.4 en 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 non-melanoma skin cancers) were diagnosed in participants self-reporting these diseases by questionnaire. All self-reported diagnoses were validated using hospital records for HIV-positives, and, general practitioners' records for HIV-negatives, provided the latter had consented to contact 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 AANCC among HIV-negatives.

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

Studygroups were compared using the Chi-square, Wilcoxon rank-sum, nonparametric test for trend, or Student's 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 towards AANCC. The outcome measure was the total number of AANCC per participant. All models were adjusted for age, gender, 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 co-infection. 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

packyears for non-smokers. All models used data from both studygroups (including those exploring HIV-related risk factors), except where explicitly stated otherwise.

Analyses were performed using SAS version 9.2.

## Results

598 HIV-infected and 550 HIV-uninfected participants 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 studygroups were very comparable, with a median age of around 52 years, the majority being male, men who have sex with men (MSM), and of Dutch origin. Significantly fewer HIV-positives were of Dutch, and more of African origin (72.2% vs. 81.3%,  $p<0.001$ , and 7.4% vs. 1.3%,  $p<0.001$ , respectively). Significantly fewer HIV-negatives were hepatitis B/C co-infected (0.6% vs. 3.9%,  $p<0.001$  and 0.8% vs. 2.8%,  $p=0.029$ , respectively) (Table 1). No statistically significant difference in age-distribution was found between both studygroups.

On average HIV-positives 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 plasmaviral load. The majority had experienced immune recovery on treatment, with a median nadir CD4-count of 180 cells/mm<sup>3</sup> and current median CD4-count of 565 cells/mm<sup>3</sup>.

Significantly more HIV-positives were current smokers (32.0% vs. 24.6%,  $p=0.007$ ), whereas ecstasy use was significantly more prevalent among HIV-negatives (4.3% vs. 8.6%,  $p=0.004$ ) (Table 2).

Body mass index (BMI) was lower (24.2 (IQR 22.3-26.6) vs. 24.5 (IQR 22.8-27.0) kg/m<sup>2</sup>,  $p=0.019$ ) and above-normal waist-to-hip ratio was significantly more prevalent (84.0% vs. 62.4%,  $p<0.001$ ) among

HIV-positives. Systolic (135 (IQR 126-147) vs. 133 (IQR 125-143) mmHg,  $p=0.006$ ) and diastolic blood pressure (81 (IQR 75-89) vs. 79 (IQR 72-85) mmHg,  $p<0.001$ ) were significantly higher among HIV-positives. Significantly fewer HIV-positives were physically active (44.3% vs. 53.0%,  $p=0.005$ ) and they had significantly lower levels of 25-OH vitamin D2+D3 (47 (IQR 29-71) vs. 54 (39-72) nmol/L,  $p<0.001$ ).

#### 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-positives: of the total 155 self-reported diagnoses, 100 were confirmed and 55 rejected. Fourteen HIV-negative participants 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. HIV-positives had a significantly higher mean number of AANCC than HIV-uninfected controls (1.3 (SD 1.14) vs. 1.0 (SD 0.95),  $p<0.001$ ). The proportion of participants with  $\geq 1$  AANCC was also significantly higher among those with HIV (69.4% vs. 61.8%,  $p=0.009$ ).

The mean number of AANCC within the 50-55, 60-65, and 65+ age-categories was significantly higher among HIV-infected than HIV-uninfected participants (Figure 1). Furthermore, the distribution of the number of AANCC for HIV-positives in each 5-year age-stratum resembled the distribution for those without HIV who are 5 years older.

Each individual AANCC was numerically more prevalent among HIV-positives, with hypertension (45.4% vs. 30.5%,  $p<0.001$ ), myocardial infarction (3.9% vs. 1.5%,  $p=0.018$ ), peripheral arterial disease (2.6% vs. 0.6%,  $p=0.008$ ), and impaired renal function (4.3% vs. 2.1%,  $p=0.044$ ) being significantly more prevalent among HIV-positives (Figure 2).



Factors contributing to the risk of AANCC

*HIV-related risk factors*

After adjustment for age, gender, 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 co-infection, HIV infection remained independently associated with a higher number of AANCC (OR 1.58, 95% CI 1.23-2.03,  $p < 0.001$ ). Age, positive family history, and smoking were also strongly independently associated with AANCC. Analyzing the HIV-infected and HIV-uninfected studygroups separately, the odds ratio for age was higher in the HIV-infected studygroup (OR 1.60, 95% CI 1.41-1.81,  $p < 0.001$ ) compared to the uninfected controls (OR 1.41, 95% CI 1.25-1.60,  $p < 0.001$ ), the difference being borderline significant ( $p$ -value for interaction 0.06).

In “univariable” analyses (adjusting conform previous models) several HIV-related variables were significantly associated with AANCC: time since HIV diagnosis (OR 1.03 per additional year, 95% CI 1.02-1.05,  $p < 0.001$ ), duration of ART-use (OR 1.04 per additional year, 95% CI 1.02-1.06,  $p < 0.001$ ), and duration of CD4-count  $< 200$  cells/mm<sup>3</sup> (OR 1.30 per additional year, 95% CI 1.17-1.45,  $p < 0.001$ ). In multivariable analysis only duration of having CD4-counts  $< 200$  cells/mm<sup>3</sup> remained an independent risk factor for AANCC.

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 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 (hsCRP), coagulation (D-dimer) and monocyte activation (sCD14 and sCD163).

The median levels of each of these biomarkers, except D-dimer, were significantly higher among HIV-positives versus HIV-negatives (Table 3).

Adding hsCRP and sCD14 to the abovementioned regression model (analyzing both studygroups jointly) showed both markers to be (borderline) significantly associated with AANCC (hsCRP: OR 1.03 per mg/L higher, 95% CI 1.00-1.07,  $p=0.037$ ; sCD14: OR 1.02 per 100 ng/mL higher, 95% CI 1.00-1.03,  $p=0.057$ ), whereas this was not the case for hsCRP >10 mg/L, D-dimer, D-dimer >0.5 mg/L, and sCD163. Analyzing the effect of hsCRP and sCD14 in the two studygroups separately, both were independent risk factors for AANCC in the HIV-infected, but not the HIV-uninfected cohort. 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 AANCC, both in the cohorts combined (OR 1.49 per 0.1 higher ratio, 95% CI 1.23-1.80,  $p<0.001$ ) and in the HIV-infected (OR 1.35 per 0.1 higher ratio, 95% CI 1.04-1.76,  $p=0.024$ ) and HIV-uninfected groups separately (OR 1.78 per 0.1 higher ratio, 95% CI 1.34-2.37,  $p<0.001$ ). 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 AANCC.

#### *Specific ART and the risk of AANCC*

Current or cumulative use of abacavir, stavudine, and didanosine were not significantly associated with risk of AANCC, whereas cumulative use of ritonavir was identified as independent risk factor for AANCC (OR 1.29 per 5 years of ritonavir-use, 95% CI 1.04-1.60,  $p=0.018$ ). Exploring this further, only cumulative duration of high ( $\geq 400\text{mg}/24\text{hrs}$ ) but not of lower doses of ritonavir remained borderline significantly associated with risk of AANCC (OR 1.08 per year "high-dose" ritonavir-use, 95% CI 0.99-1.18,  $p=0.083$ ) (Table 4).

## Discussion

### Key results

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

HIV infection was independently associated with a higher total number of AANCC, as were age, smoking, and positive family history for myocardial infarction, hypertension, diabetes mellitus type 2, or hypercholesterolemia. These traditional risk factors, as well as higher waist-to-hip ratio, independently also contributed to risk of AANCC in each of the studygroups. A borderline significant interaction between age and HIV infection suggested a stronger age-effect among HIV-positives.

A longer time spent at a CD4-count < 200 cells/mm<sup>3</sup> and to a lesser extent more systemic inflammation and innate immune activation, as reflected in higher hsCRP and sCD14 levels, as well as longer prior use of “high-dose” ritonavir (≥400mg/24hrs) were additional factors contributing to AANCC burden.

### Interpretation and limitations

Our finding that comorbidity was significantly more prevalent among HIV-positives (the majority having sustained suppression of viremia on cART) compared to uninfected controls of similar age is compatible with earlier reports.[8,27–36] Earlier studies however either did not include a comparable uninfected controlgroup, 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 sexual risk taking behaviour as HIV-infected study-participants. Although smoking and hepatitis B/C were more prevalent in HIV-positives and ecstasy use in controls (which also consisted of more native Dutch persons), overall the differences between both studygroups were relatively minor.

Our findings thus add robustness to the notion that AANCC indeed are more prevalent among those living with HIV, including in those with a sustained response to antiretroviral treatment.

Unravelling underlying mechanisms and risk factors for this increased comorbidity burden among HIV-positives is the subject of ongoing research. A central question concerns the contributions of HIV infection itself (by viral- and immune-related mechanisms), co-infections (including cytomegalovirus and chronic viral hepatitis), and anti-retroviral therapy.[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 (i.e. duration of having CD4-counts  $<200$  cells/mm<sup>3</sup>) were each univariably associated with AANCC, these associations were all confounded by duration of immune deficiency.

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 hsCRP, but not of sCD163 or D-dimer, were borderline significantly associated with increased risk for AANCC. 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/24hrs) in our analyses was borderline significantly associated with risk for AANCC. 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 AANCC may differ in (the magnitude of) their effect when addressing specific comorbidities separately. Although the HIV-infected and HIV-uninfected studygroups 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 AANCC 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 immune deficiency 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 towards a stronger association between age and AANCC burden amongst 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].

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**Conflicts of interest:**

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Table 1: Baseline demographic and HIV-related characteristics.

	<b>HIV-uninfected participants (n=524)</b>	<b>HIV-infected participants (n=540)</b>	<b>P-value</b>
Age (years)	52.1 (47.9-58.3)	52.9 (48.3-59.6)	0.200 <sup>a</sup>
Male gender (%)	85.1%	88.1%	0.146 <sup>b</sup>
Dutch origin (%)	81.3%	72.2%	<0.001 <sup>b</sup>
African origin (%)	1.3%	7.4%	<0.001 <sup>b</sup>
MSM (%) <sup>1</sup>	69.7%	73.9%	0.125 <sup>b</sup>
Hepatitis C RNA positive (%)	0.8%	2.8%	0.029 <sup>b</sup>
Hepatitis B antigen and/or hepatitis B DNA positive (%)	0.6%	3.9%	<0.001 <sup>b</sup>
Time since HIV-1 diagnosis (years)	-	12.1 (6.2-17.1)	-
Diagnosed with HIV-1 prior to 1996 (%)	-	32.8%	-
CD4-count at enrollment (cells/mm <sup>3</sup> )	-	565 (435-745)	-

Nadir CD4-count (cells/mm <sup>3</sup> )	-	180 (78-260)	-
Known cumulative duration of CD4-count < 200 cells/mm <sup>3</sup> (months)	-	0.8 (0.0-9.6)	-
Plasma viral load > 200 c/mL among cART-treated participants within 4 months before or at enrollment (%) <sup>2</sup>	-	1.5%	-
Last plasma viral load within 4 months before or at enrollment (log <sub>10</sub> c/mL) <sup>2</sup>	-	1.6 (1.6-1.6)	-
Duration of plasma viral load ≤ 200 c/mL (years) <sup>3</sup>	-	5.8 (2.4-10.2)	-
Prior clinical AIDS (%) <sup>4</sup>	-	31.3%	-
On cART (%) <sup>5</sup>	-	95.7%	-
Time since ART was first initiated (years)	-	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 (months)	-	0.0 (0.0-6.3)	-
Cumulative exposure among participants that used high-dose ritonavir (months)	-	17.6 (7.6-40.4)	-

Data presented as median (IQR) or percentage as appropriate.

Test type used: <sup>a</sup> Wilcoxon rank-sum test, <sup>b</sup> Chi-square test.

<sup>1</sup> The term “MSM” (Men having Sex with Men) applied to male participants that stated in the questionnaire to feel mostly or exclusively sexually attracted to men.

<sup>2</sup> Only a plasma HIV-1 viral load that was measured  $\leq 4$  months prior to enrolment was used. If such a recent test result was not available, plasma HIV-1 viral load was measured at enrolment.

<sup>3</sup> Duration since last plasma viral load  $>200$  c/mL.

<sup>4</sup> The term “prior clinical AIDS” was used in case of a previous AIDS-defining condition following the United States Centers for Disease Control and Prevention (CDC) classification.

<sup>5</sup> The term “cART” was used for a combination of  $\geq 3$  antiretroviral drugs, other than ritonavir used as a booster.

Table 2: Prevalence of comorbidity risk factors.

	HIV-uninfected participants (n=524)	HIV-infected participants (n=540)	P-value
Smokingstatus			
Never smoked (%)	36.5%	33.0%	0.028 <sup>a</sup>
Ever smoked (%)	38.9%	35.0%	
Currently smoking (%) <sup>1</sup>	24.6%	32.0%	
Packyears of smoking among ever-smokers (packyears)	15.0 (4.5-28.8)	22.2 (7.8-36.8)	0.001 <sup>b</sup>
Severe alcohol use (%) <sup>2</sup>	7.3%	4.8%	0.098 <sup>c</sup>
Daily to monthly use of cannabis (%)	11.6%	13.5%	0.356 <sup>c</sup>
Daily to monthly use of cocaine (%)	2.9%	3.7%	0.442 <sup>c</sup>
Daily to monthly use of ecstasy (%)	8.6%	4.3%	0.004 <sup>c</sup>
BMI (kg/m <sup>2</sup> )	24.5 (22.8-27.0)	24.2 (22.3-26.6)	0.019 <sup>b</sup>
BMI categories			
<20 kg/m <sup>2</sup>	3.3%	8.2%	0.121 <sup>a</sup>
20-<25 kg/m	54.1%	50.7%	
25-<30 kg/m	32.7%	33.2%	
≥30 kg/m	9.9%	8.0%	
Waist-to-hip ratio higher than normal (%) <sup>3</sup>	62.4%	84.0%	<0.001 <sup>c</sup>

Blood pressure systolic (mmHg)	133 (125-143)	135 (126-147)	0.006 <sup>d</sup>
Blood pressure diastolic (mmHg)	79 (72-85)	81 (75-89)	<0.001 <sup>d</sup>
Positive family history for myocardial infarction, hypertension, diabetes mellitus type 2, or hypercholesterolemia (%) <sup>4</sup>	66.5%	70.8	0.139 <sup>c</sup>
Physical activity <sup>5</sup>	53.0%	44.3%	0.005 <sup>c</sup>
25-OH vitamin D2+D3 (nmol/L)	54 (39-72)	47 (29-71)	<0.001 <sup>a</sup>

Data presented as median (IQR) or percentage as appropriate.

Test type used: <sup>a</sup> Nonparametric test for trend, <sup>b</sup> Wilcoxon rank-sum test, <sup>c</sup> Chi-square test, <sup>d</sup> Student's t-test.

<sup>1</sup> The term "currently smoking" applied if participants stated in the questionnaire to have smoked during the last month before completing the questionnaire.

<sup>2</sup> Severe alcohol use was diagnosed in participants that stated in the questionnaire to have an alcohol intake >4 units (for men) or >2 units (for women) daily or almost daily.

<sup>3</sup> The waist-to-hip ratio was considered higher than normal if it was  $\geq 0.9$  in males and  $\geq 0.85$  in females.

<sup>4</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 that 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>5</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]



Table 3: Values of several markers of systemic inflammation, compared between the two studygroups.

	<b>HIV-uninfected participants (n=524)</b>	<b>HIV-infected participants (n=540)</b>	<b>P-value</b>
hsCRP (mg/L)	1.0 (0.6-1.9)	1.5 (0.7-3.5)	<0.001 <sup>a</sup>
hsCRP >10 mg/L (%)	1.6%	6.7%	<0.001 <sup>b</sup>
D-dimer (mg/L)	0.24 (0.20-0.38)	0.23 (0.20-0.36)	0.078 <sup>a</sup>
D-dimer>0.5 mg/L (%)	14.1%	13.2%	0.659 <sup>b</sup>
sCD14 (ng/mL)	1356 (1080-1738)	1576 (1305-2011)	<0.001 <sup>a</sup>
sCD163 (ng/mL)	252 (182-342)	289 (207-419)	<0.001 <sup>a</sup>

Data presented as median (IQR) or percentage as appropriate.

Test type used: <sup>a</sup> Wilcoxon rank-sum test, <sup>b</sup> Chi-square test.

Table 4: Risk factors for AANCC, multivariably analyzed using the HIV-infected and HIV-uninfected studygroups jointly.

	Odds ratio	95% CI lower	95% CI upper	P-value
Age	1.39	1.27	1.52	<0.001
(per 5 years)	(1.50)	(1.39)	(1.63)	(<0.001)
Smoking	1.08	1.05	1.12	<0.001
(per 5 packyears)	(1.10)	(1.07)	(1.13)	(<0.001)
Positive family history <sup>1</sup>	1.88	1.45	2.44	<0.001
(yes/no)	(1.57)	(1.23)	(2.01)	(<0.001)
HIV infection	1.07	0.80	1.42	0.661
(yes/no)	(1.68)	(1.34)	(2.10)	(<0.001)
Known cumulative duration of immune deficiency	1.23	1.10	1.38	<0.001
(per year with a CD4-count<200 cells/mm <sup>3</sup> )	(1.33)	(1.20)	(1.48)	(<0.001)
Waist-to-hip ratio	1.49	1.23	1.80	<0.001
(per 0.1)	(1.94)	(1.67)	(2.25)	(<0.001)

hsCRP	1.03	0.99	1.06	0.107
(per mg/mL)	(1.06)	(1.03)	(1.09)	(<0.001)
sCD14	1.02	1.00	1.03	0.074
(per 100 ng/mL)	(1.02)	(1.01)	(1.04)	(0.007)
Cumulative duration of ritonavir use in high dosages ( $\geq 400$ mg/daily) (per year)	1.08 (1.19)	0.99 (1.10)	1.18 (1.28)	0.083 (<0.001)

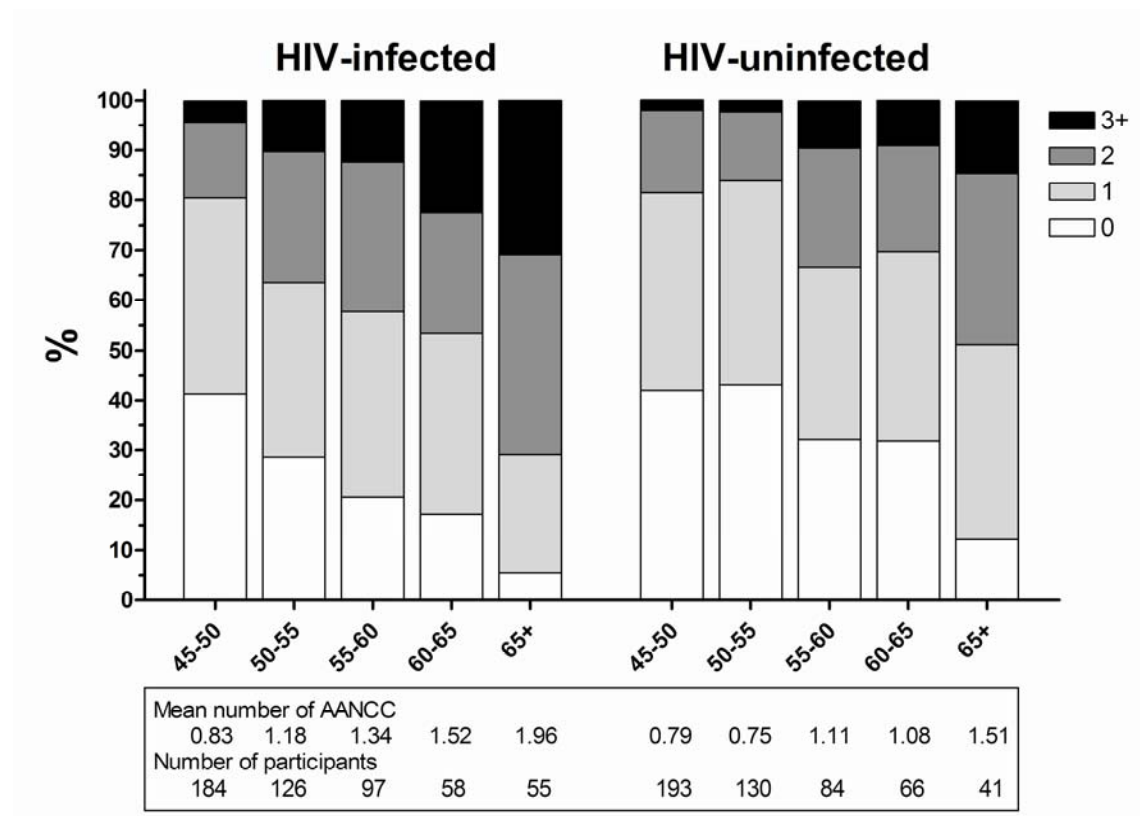
Abbreviations: AANCC=age-associated non-communicable comorbidity; CI=confidence interval.

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

In brackets the univariable odds ratios, 95% confidence intervals, and P-values of each of the variables.

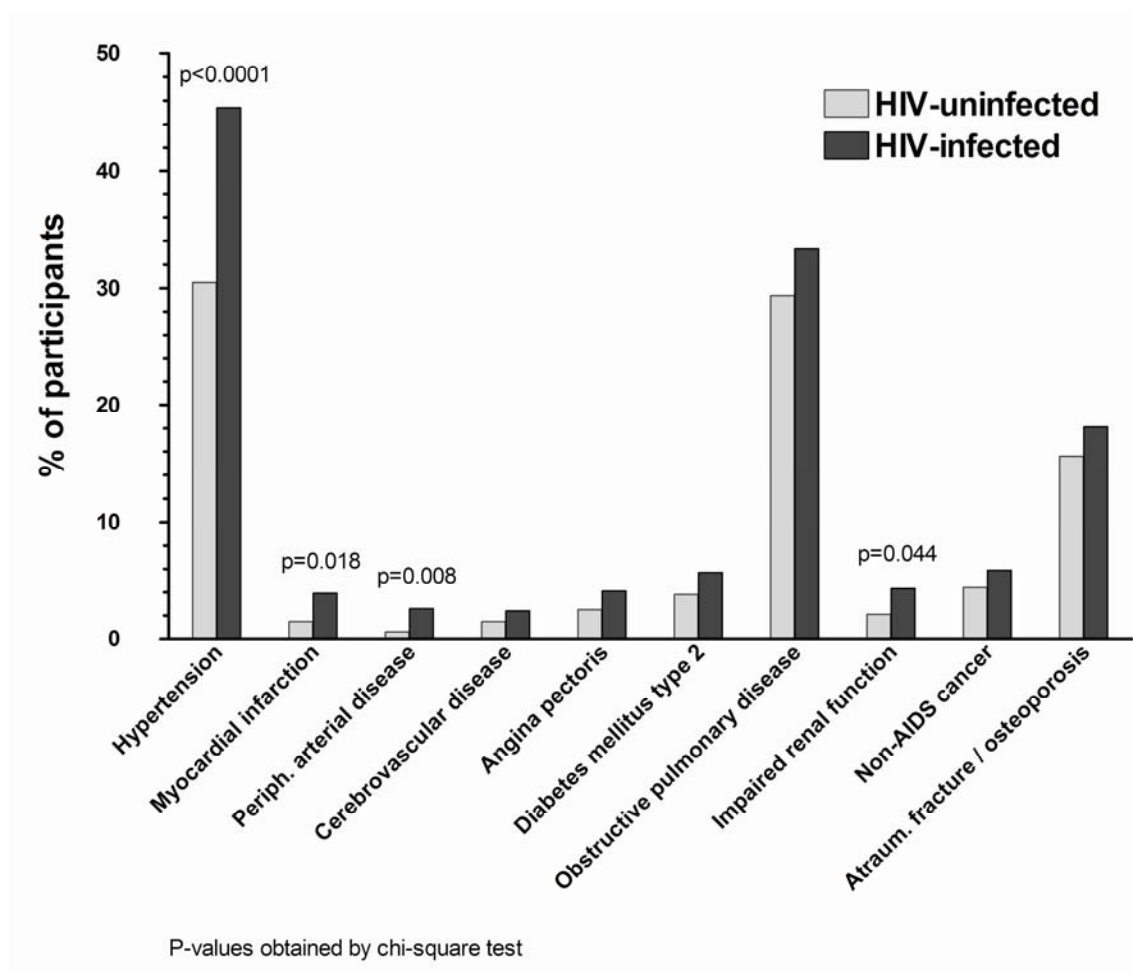
<sup>1</sup> Positive family history: a first degree family member suffering from myocardial infarction, hypertension, diabetes mellitus type 2, or hypercholesterolemia.

Figure 1: Distribution of the number of AANCC stratified by age across both studygroups.



Accepted

Figure 2: Prevalences of each of the different AANCC over the two studygroups.



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