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# Head and heart in treated HIV infection

Judith Schouten



## HEAD AND HEART IN TREATED HIV INFECTION



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## **Colofon**

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# HEAD AND HEART IN TREATED HIV INFECTION

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# 1

## General introduction

### Historical background

In 1981, the Centers for Disease Control (CDC) Morbidity and Mortality Weekly Report published the case histories of five young, previously healthy, homosexual men with *Pneumocystis carinii* pneumonia, cytomegalovirus infection, and mucosal candidiasis.<sup>1</sup> Soon after, several reports followed on additional young, mostly homosexual, men suffering and dying from similar opportunistic infections and Kaposi's sarcoma, an otherwise rare malignancy (**Figure 1.1**).<sup>2</sup> As these opportunistic infections and malignancies are very rare among individuals with an intact immune system, the possibility of an underlying immune dysfunction was soon recognized.<sup>1</sup> In 1982, the term 'acquired immune deficiency syndrome' (AIDS) was introduced and defined by CDC as "a disease at least moderately predictive of a defect in cell-mediated immunity, occurring in a person with no known cause for diminished resistance to that disease".<sup>3</sup> Although, at that time, the cause of AIDS was still unclear, by 1983 it was discovered to be human immunodeficiency virus, or HIV.<sup>4</sup>

HIV is a retrovirus that is transmitted by sexual contact, via contaminated blood, and from mother to child. HIV infects its target cells by bind-



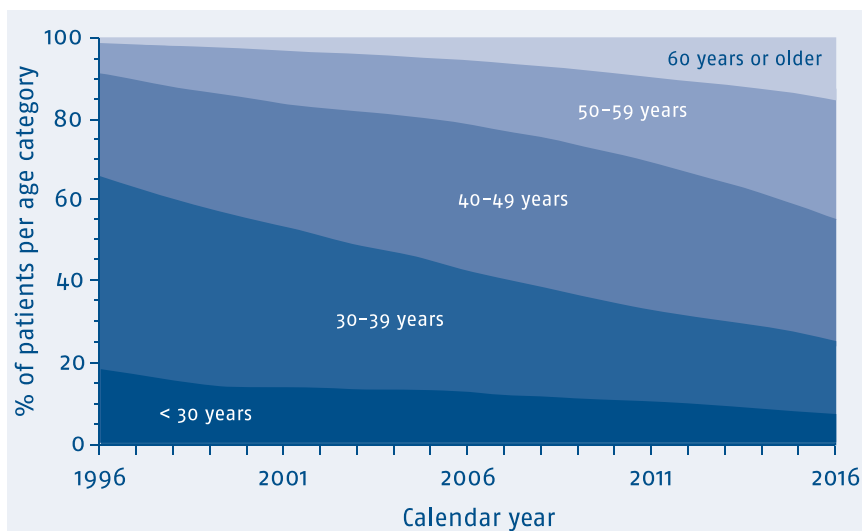
antiretroviral treatment (cART) as it is called nowadays, has been proven to provide efficient, potent, and long term suppression of HIV replication. Since then, cART has become the cornerstone of successful HIV treatment and, with its introduction in 1996, HIV infection changed from an inevitably fatal condition into a chronic manageable disease.

## Ageing of the HIV-infected population

Wherever access to cART has been secured, AIDS-related morbidity and mortality have decreased significantly, resulting in prolonged survival of HIV-infected individuals.<sup>14</sup> As a consequence, since 1996, the proportion of adults living with HIV aged 50 years and older has been increasing in all regions of the world.<sup>14</sup> In 2013, UNAIDS reported that 10% of the adult HIV-infected population in low and middle-income countries and 30% of the adult HIV-infected population in high-income countries were 50 years of age and above. Worldwide, over 3.6 million people aged 50 years and older were estimated to be living with HIV.<sup>14</sup> In The Netherlands, a similar trend has been observed with the proportion of HIV-infected individuals aged 50 years or above increasing from 9% in 1996 to 42% in 2015 (**Figure 1.2**).<sup>15</sup>

## Non-AIDS comorbidities

After the initial optimism that followed the cART triumph, concerns have been raised during the last decade about the increasingly important role of non-AIDS comorbidities as causes of death in cART-treated patients.<sup>16–18</sup> The life expectancy of HIV-infected individuals still remained shorter than that of the general population<sup>17,19,20</sup> and several publications reported an excess prevalence of non-AIDS comorbidities among people living with HIV.<sup>21,22</sup> These non-AIDS comorbidities included cardiovascular and cerebrovascular disease, hypertension, renal dysfunction, osteoporosis, diabetes mellitus, liver failure, non-AIDS



**Figure 1.2: Age distribution of the HIV-infected population in clinical care in The Netherlands over calendar time (1996-2015)**

Reprinted from Monitoring Report 2015. Human immunodeficiency virus (HIV) infection in The Netherlands, by van Sighem A, Gras L, Smit C, Stolte I, Reiss P. Retrieved from <http://www.hiv-monitoring.nl>. Copyright 2015 Stichting HIV Monitoring. Reprinted with permission.

malignancies, and cognitive impairment. Several potential contributors were considered: a higher prevalence of recognized (lifestyle-related) risk factors among those infected with HIV, ART exposure and drug toxicity, HIV infection itself, immune dysfunction/dysregulation, and chronic immune activation/inflammation associated with HIV infection.<sup>23-28</sup> As many of the reported non-AIDS comorbidities are associated with older age in the general population, ageing of the HIV-infected population was also considered an important contributing factor.<sup>21-23,26</sup> With some papers reporting non-AIDS/ageing-associated comorbidities to be occurring at younger ages among HIV-infected individuals compared with the general population, this fostered the hypothesis that the ageing process in the context of HIV infection might be accentuated and/or accelerated.<sup>22,29,30</sup> However, as most published studies did not include a

comparable HIV-uninfected control group, it remained unclear whether or not certain comorbidities were truly occurring more often and/or at a younger age in the HIV-infected population, and if so, whether this excess risk was attributable to HIV-related factors.

## The AGE<sub>h</sub>IV Cohort Study

To obtain more insight into these issues, the AGE<sub>h</sub>IV Cohort Study was set up in 2010 in Amsterdam, The Netherlands. This prospective cohort study was designed to compare the prevalence, incidence and risk factors of non-AIDS/ageing-associated comorbidities and organ dysfunction among HIV-type-1-infected (hereafter referred to as HIV-infected) individuals and HIV-uninfected controls.

HIV-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 and among uninfected participants in the existing Amsterdam Cohort Studies on HIV/AIDS.<sup>31</sup> These specific controls were chosen as they were likely to have a similar lifestyle and risk behaviour pattern as the HIV-infected participants. In other words, even though they were not HIV infected, they were at risk of becoming infected with HIV. To ensure comparability of the HIV-infected and HIV-uninfected studygroups, age, sex, and ethnicity in both studygroups were monitored regularly, and enrolment of under-represented categories among HIV-uninfected participants was adjusted accordingly. All participants were aged  $\geq 45$  years and had laboratory-confirmed presence or absence of HIV infection. All subjects who met these criteria and provided written informed consent were included within a 2-year enrolment period. This resulted in the inclusion of 598 HIV-infected and 550 uninfected participants between 1 October 2010 and 30 September 2012. The efforts to achieve optimal comparability between the HIV-infected and HIV-uninfected participants resulted in two well-characterized studygroups, highly similar in terms of demographics, lifestyle, and risk factors.

Every two years, all participants undergo extensive standardized screening for non-AIDS/ageing-associated comorbidities and organ dysfunction. All study procedures are listed in **Table 1.1**.

Detailed information concerning HIV and ART history was 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.<sup>32</sup> A biobank was initiated for the cryopreservation of blood (including storage of peripheral blood mononuclear cells, plasma and serum), urine, and stool samples, allowing for future analyses and investigation of pathogenic mechanisms.

## HIV-associated cognitive impairment

In the pre-cART era approximately 30% of HIV-infected patients developed severe progressive cognitive and motor impairment in the final months of their illness. This clinical syndrome was characterized clinically and neuropathologically by Price and Navia et al. in 1986 and termed AIDS dementia complex (ADC).<sup>33,34</sup> ADC causes symptoms in three areas of functioning: cognition, motor function and behaviour. Cognitive impairment (CI) predominantly consists of mental slowing and attention/memory deficits. Motor symptoms comprise slowness and loss of balance; behavioural changes are characterized by apathy, social withdrawal and mood disturbances.

Many studies have confirmed the hypothesis that HIV itself causes dysfunction and damage in the central nervous system (CNS) by producing viral proteins and inducing an inflammatory response within the CNS.

After the introduction of ART, and especially cART in 1996, ADC (or HIV-associated dementia, HAD, as it is termed nowadays) became a rare complication of HIV infection and has largely disappeared from clinical practice.<sup>35,36</sup>

After several years of cART availability, however, long term infected and treated patients, including those with systemically well-controlled infection, started to complain of milder memory problems and slowness, difficulties in concentration, planning, and multitasking.<sup>35,37</sup> A high, but



**Table 1.1: Study procedures of the main AGE<sub>h</sub>IV Cohort Study**

Procedure	Additional details
Standardized questionnaire	Demographics (Family) medical history Use of 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 Work participation and income
Blood pressure	
Height and weight	
Waist and hip circumference	
Electrocardiography	
Vascular elasticity (Arteriograph®)	
Spirometry	
Bone densitometry	
Quantification of advanced glycation end products in the skin (AGE-reader®)	
Screening cognitive tests	
Frailty	
Extensive laboratory testing of blood and urine	HIV viral load Hematology Renal function Liver function Electrolytes Lipid profile (Para)thyroid function Calcium, phosphate, vitamin D Markers of inflammation Immunological analyses <sup>a</sup> Syphilis infection status Hepatitis infection B/C status

<sup>a</sup> Soluble CD163 and soluble CD14 levels were determined for all study participants with available blood samples. Extensive immunological testing was performed in 95 HIV-infected individuals who had an undetectable plasma HIV RNA level (<50 copies/mL) for >12 months and 94 HIV-uninfected controls who were randomly selected from the AGE<sub>h</sub>IV Cohort Study.<sup>46</sup>

- Peripheral blood mononuclear cells (PBMCs) were isolated from fresh blood specimens, and these freshly isolated cells were used for immune phenotyping by flow cytometry.
- Cytomegalovirus-specific T-cell responses were assessed in cryopreserved PBMC samples of a subgroup (24/24).
- Cytomegalovirus antibody titers, as well as high-avidity antibody titers, were measured.
- Telomere and single-joint T-cell receptor excision circle (sjTREC) polymerase chain reaction analysis was performed.

varying, prevalence of milder forms of CI was reported, ranging from 15 to 69%, including amongst those with systemically well-controlled infection.<sup>38-41</sup> To classify this broadening clinical spectrum of cognitive disorders, a new terminology and classification system was developed in 2007, commonly referred to as the Frascati criteria.<sup>42</sup> As the Frascati criteria have a low threshold for detecting milder forms of CI they may overestimate CI prevalence. As a consequence, the exact prevalence of HIV-associated CI was heavily debated.<sup>43,44</sup> In an attempt to improve the diagnostic properties and decrease the oversensitivity of the Frascati criteria, Gisslén et al. proposed a modification of these criteria (from now on referred to as the Gisslén criteria).<sup>43</sup>

To explain the underlying potential pathogenic mechanisms and risk factors for developing HIV-associated CI, many contributing factors have been considered, including HIV/ART-specific factors such as ART toxicity, low-level persistent HIV replication within the CNS, and HIV-associated immune dysfunction and inflammation, along with other potential contributors such as cardiovascular, metabolic, and additional comorbid conditions.<sup>45</sup> Moreover, since CI is associated with older age in the general population, ageing of the HIV-infected population was also believed to be an important factor. However, the relative contribution of each of these factors, as well as that of (possibly premature) ageing, remained unclear.

## Neurological nested substudy within the AGE<sub>h</sub>IV Cohort Study

To investigate the specific non-AIDS comorbidity of HIV-associated CI, a neurological nested substudy within the main AGE<sub>h</sub>IV Cohort Study was initiated in December 2011. All eligible participants from the main AGE<sub>h</sub>IV Cohort were consecutively invited to participate in the nested substudy. Additional eligibility criteria for the substudy were male gender (as the availability of Dutch-speaking women in the main AGE<sub>h</sub>IV Cohort was very limited, and a good command of the Dutch language is necessary for a reliable neuropsychological assessment) and, for the HIV-

infected group, sustained suppression of HIV viremia on antiretroviral treatment (plasma HIV RNA <40 copies/mL) for at least 12 months; the presence of so-called viral 'blips' (transient low-level viremia) was not an exclusion criterion.

Exclusion criteria for the substudy were a history of severe neurological disorder (e.g., stroke, seizure disorders, multiple sclerosis, dementia (including previous or current diagnosis of HAD)), history of traumatic brain injury with loss of consciousness for more than 30 minutes, current/past (HIV-associated) CNS infection or tumor, current severe psychiatric disorder (e.g., psychosis, major depression), current intravenous drug use, daily use of illicit drugs (with the exception of daily cannabis use), current excessive alcohol consumption (>48 units of alcohol/week), insufficient command of the Dutch language, and mental retardation.

This resulted in the inclusion of 103 HIV-infected and 74 uninfected substudy participants between December 2011 and August 2013. The substudy participants underwent additional study procedures (listed in **Table 1.2**) at baseline and after two years. Cerebrospinal fluid (CSF) samples were cryopreserved and added to the above-mentioned biobank.

## Thesis outline

This thesis focuses on two non-AIDS/ageing-associated phenomena in the context of chronic treated HIV infection: vascular complications and cognitive impairment, and is therefore divided into two parts: 'Heart' (Part I) and 'Head' (Part II). All analyses were cross-sectional and performed on baseline data gathered via the AGE<sub>h</sub>IV main study and neurological substudy.

An introduction to Part I is provided in **chapter 2**, in the form of a published review on coronary heart disease in treated HIV infection. **Chapter 3** reports prevalence and risk factors of a range of non-AIDS/ageing-associated comorbidities within the main AGE<sub>h</sub>IV Cohort at baseline, comparing the HIV-infected and uninfected studygroups. In **chapter 4**, aortic stiffness is compared between the HIV-infected and uninfected

**Table 1.2: Study procedures for the nested neurological substudy within the main AGE<sub>n</sub>IV Cohort Study**

Procedure	Additional details
Full neuropsychological assessment	Fluency Attention Information processing speed Executive function Memory
Neuroimaging	Motor function (Functional) MRI MR spectroscopy Diffusion tensor imaging Arterial spin labeling
Lumbar puncture to collect cerebrospinal fluid (CSF)	HIV viral load Cell count Glucose Protein Albumin ratio IgG index Immunological analyses <sup>a</sup> Neuronal damage markers <sup>b</sup> ART concentrations N-glycans
Optical coherence tomography	

<sup>a</sup> Neopterin, tryptophan/kynurenine, TNF- $\alpha$ , IP-10, Mip1 $\alpha$ , IL-6, MCP-1, CXCL9, RANTES, sCD14, and sCD163

<sup>b</sup> Neurofilament, A $\beta$ 1-42, t-tau, and p-tau

studygroups of the main AGE<sub>n</sub>IV Cohort, and determinants of increased aortic stiffness are investigated.

An introduction to Part II is provided in **chapter 5**, in the form of a published review on HIV-associated CI in treated HIV infection. **Chapter 6** reports the prevalence of HIV-associated CI, as diagnosed by the Frascati and Gisslén criteria, within the AGE<sub>n</sub>IV neurological substudy at baseline, and proposes a novel and more optimal method (multivariate normative comparison, or MNC) for reliably detecting CI. **Chapter 7** investigates the determinants of cognitive impairment/dysfunction as diagnosed by MNC and **chapter 8** reports on the diagnostic characteristics of four cognitive screening instruments for detecting HIV-associated CI.

Finally, in **chapter 9**, we summarize and review the main findings of

this thesis and discuss the bigger picture and future perspectives of non-AIDS/ageing-associated comorbidity in the context of chronic treated HIV infection.

## References

1. Centers for Disease Control (CDC). Pneumocystis pneumonia—Los Angeles. *MMWR Morb. Mortal. Wkly. Rep.* **30**, 250–252 (1981).
2. Rare cancer seen in 41 homosexuals. *New York Times* (1981).
3. Centers for Disease Control (CDC). Update on acquired immune deficiency syndrome (AIDS)—United States. *MMWR Morb. Mortal. Wkly. Rep.* **31**, 507–508, 513–514 (1982).
4. Barré-Sinoussi, F. *et al.* Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* **220**, 868–871 (1983).
5. *Global HIV/AIDS medicine.* (Saunders Elsevier, 2008).
6. Centers for Disease Control (CDC). Mortality attributable to HIV infection/AIDS—United States, 1981–1990. *MMWR Morb. Mortal. Wkly. Rep.* **40**, 41–44 (1991).
7. Chin, J. Global estimates of AIDS cases and HIV infections: 1990. *AIDS Lond. Engl.* **4 Suppl 1**, S277–283 (1990).
8. Fischl, M. A. *et al.* The Efficacy of Azidothymidine (AZT) in the Treatment of Patients with AIDS and AIDS-Related Complex. *N. Engl. J. Med.* **317**, 185–191 (1987).
9. Brook, I. Approval of zidovudine (AZT) for acquired immunodeficiency syndrome. A challenge to the medical and pharmaceutical communities. *JAMA* **258**, 1517 (1987).
10. Jablonowski, H. Studies of zidovudine in combination with didanosine and zalcitabine. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirology Off. Publ. Int. Retrovirology Assoc.* **10 Suppl 1**, S52–56 (1995).
11. Collier, A. C. *et al.* Treatment of human immunodeficiency virus infection with saquinavir, zidovudine, and zalcitabine. AIDS Clinical Trials Group. *N. Engl. J. Med.* **334**, 1011–1017 (1996).
12. Gulick, R. M. *et al.* Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy. *N. Engl. J. Med.* **337**, 734–739 (1997).
13. Montaner, J. S. *et al.* A randomized, double-blind trial comparing combinations of nevirapine, didanosine, and zidovudine for HIV-infected patients: the INCAS Trial. Italy, The Netherlands, Canada and Australia Study. *JAMA* **279**, 930–937 (1998).

14. *HIV and aging, a special supplement to the UNAIDS report on the global AIDS epidemic 2013.* (UNAIDS, 2013).
15. Sighem, A. van, Gras, L., Smit, C., Stolte, I. & Reiss, P. *Monitoring report 2015: monitoring of human immunodeficiencyvirus (HIV) infection in the Netherlands.* (Stichting HIV Monitoring (SHM), 2015).
16. Weber, R. *et al.* Decreasing mortality and changing patterns of causes of death in the Swiss HIV Cohort Study. *HIV Med.* **14**, 195–207 (2013).
17. Wada, N. *et al.* Cause-Specific Life Expectancies After 35 Years of Age for Human Immunodeficiency Syndrome-Infected and Human Immunodeficiency Syndrome-Negative Individuals Followed Simultaneously in Long-term Cohort Studies, 1984–2008. *Am. J. Epidemiol.* **177**, 116–125 (2013).
18. Antiretroviral Therapy Cohort Collaboration. Causes of death in HIV-1-infected patients treated with antiretroviral therapy, 1996–2006: collaborative analysis of 13 HIV cohort studies. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **50**, 1387–1396 (2010).
19. Lohse, N. *et al.* Survival of persons with and without HIV infection in Denmark, 1995–2005. *Ann. Intern. Med.* **146**, 87–95 (2007).
20. The Antiretroviral Therapy Cohort Collaboration. Life expectancy of individuals on combination antiretroviral therapy in high-income countries: a collaborative analysis of 14 cohort studies. *Lancet* **372**, 293–299 (2008).
21. Hasse, B. *et al.* Morbidity and aging in HIV-infected persons: the Swiss HIV cohort study. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **53**, 1130–1139 (2011).
22. Guaraldi, G. *et al.* Premature age-related comorbidities among HIV-infected persons compared with the general population. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **53**, 1120–1126 (2011).
23. Deeks, S. G. & Phillips, A. N. HIV infection, antiretroviral treatment, ageing, and non-AIDS related morbidity. *BMJ* **338**, a3172 (2009).
24. Deeks, S. G. HIV infection, inflammation, immunosenescence, and aging. *Annu. Rev. Med.* **62**, 141–155 (2011).
25. Justice, A. C. HIV and aging: time for a new paradigm. *Curr. HIV/AIDS Rep.* **7**, 69–76 (2010).
26. Deeks, S. G., Tracy, R. & Douek, D. C. Systemic Effects of Inflammation on Health during Chronic HIV Infection. *Immunity* **39**, 633–645 (2013).
27. Armah, K. A. *et al.* HIV Status, Burden of Comorbid Disease, and Biomarkers of Inflammation, Altered Coagulation, and Monocyte Activation. *Clin. Infect. Dis.* **55**, 126–136 (2012).
28. Capeau, J. Premature Aging and Premature Age-Related Comorbidities in HIV-Infected Patients: Facts and Hypotheses. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **53**, 1127–1129 (2011).
29. Effros, R. B. *et al.* Aging and infectious diseases: workshop on HIV infection and aging: what is known and future research directions. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **47**, 542–553 (2008).

30. Pathai, S., Bajillan, H., Landay, A. L. & High, K. P. Is HIV a Model of Accelerated or Accentuated Aging? *J. Gerontol. A. Biol. Sci. Med. Sci.* (2013).
31. <http://www.amsterdamcohortstudies.org> (27-7-2016).
32. <http://www.hiv-monitoring.nl/english> (27-7-2016).
33. Navia, B. A., Jordan, B. D. & Price, R. W. The AIDS dementia complex: I. Clinical features. *Ann. Neurol.* **19**, 517–524 (1986).
34. Navia, B. A., Cho, E. S., Petito, C. K. & Price, R. W. The AIDS dementia complex: II. Neuropathology. *Ann. Neurol.* **19**, 525–535 (1986).
35. Cysique, L. A., Maruff, P. & Brew, B. J. Prevalence and pattern of neuropsychological impairment in human immunodeficiency virus-infected/acquired immunodeficiency syndrome (HIV/AIDS) patients across pre- and post-highly active antiretroviral therapy eras: a combined study of two cohorts. *J. Neurovirol.* **10**, 350–357 (2004).
36. Portegies, P. *et al.* Declining incidence of AIDS dementia complex after introduction of zidovudine treatment. *BMJ* **299**, 819–821 (1989).
37. Simioni, S. *et al.* Cognitive dysfunction in HIV patients despite long-standing suppression of viremia: *AIDS* **1** (2009).
38. Cysique, L. A. & Brew, B. J. Prevalence of non-confounded HIV-associated neurocognitive impairment in the context of plasma HIV RNA suppression. *J. Neurovirol.* **17**, 176–183 (2011).
39. Winston, A. *et al.* Neurocognitive Function in HIV Infected Patients on Antiretroviral Therapy. *PLoS ONE* **8**, e61949 (2013).
40. Robertson, K. R. *et al.* The prevalence and incidence of neurocognitive impairment in the HAART era. *AIDS Lond. Engl.* **21**, 1915–1921 (2007).
41. Heaton, R. K. *et al.* Neurocognitive change in the era of HIV combination antiretroviral therapy: the longitudinal CHARTER study. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **60**, 473–480 (2015).
42. Antinori, A. *et al.* Updated research nosology for HIV-associated neurocognitive disorders. *Neurology* **69**, 1789–1799 (2007).
43. Gisslén, M., Price, R. W. & Nilsson, S. The definition of HIV-associated neurocognitive disorders: are we overestimating the real prevalence? *BMC Infect. Dis.* **11**, 356 (2011).
44. Meyer, A.-C. L., Boscardin, W. J., Kwasa, J. K. & Price, R. W. Is It Time to Rethink How Neuropsychological Tests Are Used to Diagnose Mild Forms of HIV-Associated Neurocognitive Disorders? Impact of False-Positive Rates on Prevalence and Power. *Neuroepidemiology* **41**, 208–216 (2013).
45. Clifford, D. B. & Ances, B. M. HIV-associated neurocognitive disorder. *Lancet Infect. Dis.* **13**, 976–986 (2013).
46. Jiménez, V. C. *et al.* T-Cell Activation Independently Associates With Immune Senescence in HIV-Infected Recipients of Long-term Antiretroviral Treatment. *J. Infect. Dis.* **214**, 216–225 (2016).



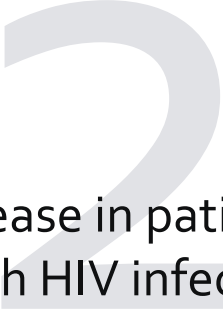


Part I

HEART







# Risk of coronary heart disease in patients with HIV infection

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Peter Reiss

\*both authors contributed equally

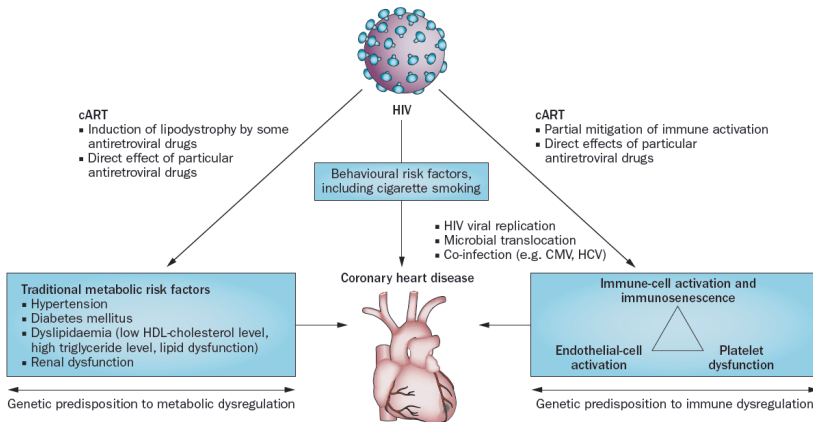
## Abstract

The lives of individuals infected with HIV who have access to combination antiretroviral therapy (cART) are substantially prolonged, which increases the risk of developing non-AIDS comorbidities, including coronary heart disease (CHD). In Europe and the USA, individuals with HIV infection have a ~1.5-fold increased risk of myocardial infarction relative to uninfected individuals. In Africa, the relative risk of myocardial infarction is unknown, but broadened access to life-extending cART suggests that rates of CHD will rise in this and other resource-constrained regions. Atherogenesis in HIV is affected by complex interactions between traditional and immune risk factors. cART has varied, regimen-specific effects on metabolic risk factors. Overall, cART seems to lessen proatherogenic immune activation, but does not eliminate it even in patients in whom viremia is suppressed. Current strategies to decrease the risk of CHD in individuals infected with HIV include early initiation of cART regimens with the fewest metabolic adverse effects, and careful management of traditional CHD risk factors throughout treatment. Future strategies to prevent CHD in patients with HIV infection might involve the use of HIV-tailored CHD risk prediction paradigms and the administration of therapies alongside cART that will further decrease proatherogenic HIV-specific immune activation.

## Introduction

Advances in the clinical management of HIV infection over the past two decades have resulted in prolonged survival and decreased rates of opportunistic infections and AIDS-related morbidity and mortality.<sup>1-3</sup> This trend has been observed wherever access to combination antiretroviral therapy (cART) - combinations of at least three antiretroviral drugs - has been secured, irrespective of whether regions are resource-rich<sup>4,5</sup> or resource-poor.<sup>6,7</sup> Presently, >35 million individuals worldwide are living with HIV infection, with an estimated 3.6 million being aged  $\geq 50$  years. In low-income and middle-income countries, older patients comprise  $\sim 10\%$  of the HIV-infected population; in high-income countries, the percentage is  $\sim 33\%$ .<sup>8-10</sup> As access to cART expands, chronic noncommunicable diseases, including cardiovascular disease (CVD), and specifically coronary heart disease (CHD), are emerging as important causes of morbidity for patients infected with HIV.<sup>11,12</sup> CVD is the third<sup>5,13</sup> to fifth<sup>14</sup> leading non-AIDS cause of death in individuals with HIV infection. Furthermore, in patients with HIV infection who have access to cART, CHD is the leading cause of CVD death.<sup>13</sup> HIV infection has been associated with up to a 50% increase in the risk of incident acute myocardial infarction (MI) beyond that explained by recognized CHD risk factors, even in patients taking cART who have sustained suppression of viral replication.<sup>15</sup>

Patients with HIV infection who are taking cART, compared with uninfected individuals, might also be at increased risk of atrial arrhythmias<sup>16</sup>, myocardial disease<sup>17</sup>, sudden cardiac death<sup>18</sup>, and stroke.<sup>19</sup> The focus of this review is on CHD risk in patients with HIV. We present epidemiological evidence of increased incidence and prevalence of CHD among individuals with HIV infection in resource-rich countries, and discuss anticipated CHD trends among those in low-income and middle-income countries. We explore the multifactorial pathogenesis of CHD in HIV and aim to address traditional and novel CHD risk pathways and to separate the effects of HIV and cART on both (**Figure 2.1**). Finally, we cover CHD prevention and risk-management strategies for patients infected with HIV and offer a perspective on potential innovative future atheroprotective interventions based on integrated views of CHD pathogenesis.



**Figure 2.1: Pathophysiology of coronary heart disease risk in patients with HIV**

Behavioural risk factors, traditional metabolic risk factors, and immune dysregulation contribute to the risk of coronary heart disease in patients with HIV. Particular antiretroviral drugs might exacerbate metabolic risk factors for coronary heart disease but, in general, cART partially mitigates HIV-specific immune activation. However, immune activation in patients with HIV persists even in those treated with cART, owing to ongoing HIV replication, microbial translocation, and co-infection with viruses such as CMV and HCV. Genetic predisposition to metabolic and immune dysregulation also contributes to risk of coronary heart disease in patients with HIV.

Abbreviations: cART=combination antiretroviral therapy, CMV=cytomegalovirus, HCV=hepatitis C virus.

## Review criteria

We identified full-text, original articles and reviews published in English from the PubMed database by searching with the term “HIV” and the following key words, individually or in combination: “cardiovascular disease”, “coronary heart disease”, “myocardial infarction”, “atherosclerosis”, “subclinical atherosclerosis”, “intima-media thickness”, “flow-mediated dilation”, “coronary artery calcification”, “non-calcified plaque”, “vulnerable plaque”, “CT angiography”, “arterial inflammation”, “FDG-PET”, “lipodystrophy”, “metabolic risk factors”, “smoking”, “hypertension”, “diabetes”, “dyslipidemia”, “lipid func-

tion", "HDL function", "cocaine", "renal dysfunction", "antiretroviral therapy", "protease inhibitors", "nucleoside reverse transcriptase inhibitors", "non-nucleoside reverse transcriptase inhibitors", "ART naive", "ART initiation", "ART interruption", "immune activation", "inflammation", "monocytes", "T cells", "endothelial cells", "platelets", "microbial translocation", "hepatitis C co-infection", "immunosenescence", "biomarkers", "genetics", "women", "cardiovascular risk prediction", "lifestyle intervention", "statins", "tesamorelin", "aspirin", "methotrexate", "CCR5 antagonist", "non-communicable disease", "global burden of disease", "low-income", and "Africa". No date restrictions were applied to the search. The reference lists of identified articles were also searched for relevant articles. Reports from UNAIDS and the WHO were searched for global statistics. Relevant abstracts from the 2014 Conference on Retroviruses and Opportunistic Infections were also reviewed.

## Epidemiology of CHD in HIV

Since 2002, data from various large-scale observational studies conducted primarily in North America and Europe have shown that the risk of MI is increased by 50-100% in patients infected with HIV compared with control individuals without HIV infection.<sup>15,20-23</sup> Freiberg et al. published data from the VACS-VC study,<sup>15</sup> which included 22,350 veterans with, and 55,109 control veterans without, HIV infection. In this study, the hazard ratio of incident acute MI in the HIV-infected group was 1.48 (95% confidence interval (CI) 1.27-1.72) even after adjustment for Framingham risk factors, medical comorbid conditions, and illicit drug use.

No similar large-scale, case-control, observational studies of the risk of MI have been conducted in sub-Saharan Africa, where 23.5 million individuals are living with HIV infection.<sup>8,10</sup> Although the incidence and prevalence of CHD among HIV-infected individuals in Africa are unknown, both are expected to rise as access to cART broadens and AIDS-related morbidity and mortality decline. Historically, the most prevalent cardiac pathologies among African individuals infected with HIV have been pericardial disease, cardiomyopathy, and pulmonary hypertension.

Contributors to these pathologies - unchecked HIV infection and, in the case of pericardial disease, co-infection with *Mycobacterium tuberculosis* - are now being more effectively addressed.<sup>24</sup>

## Traditional risk factors for CHD

### Prevalence

Prevalence estimates of traditional CHD risk factors in patients with HIV infection vary widely owing to differences in risk factor cut-offs, patients' genetic background, geographical location, socio-economic status, cultural norms, dietary habits, access to any cART, and access to cART regimens that have reduced adverse metabolic effects.

In resource-rich countries, rates of tobacco smoking among individuals with HIV infection are higher than those among uninfected individuals. For example, among 5,427 patients with HIV infection enrolled in the SMART trial<sup>25</sup>, which covered 33 predominantly high-income counties, the current smoking rate was 40.5%. The rate was even higher (51.5%) among 17,852 patients with HIV infection enrolled in the D:A:D study<sup>26</sup>, which was conducted in Australia, Europe, and the USA.

By contrast, the rate of smoking in the general population is 13-45% in European countries and 25% in the USA.<sup>27</sup> Large studies of smoking prevalence among patients infected with HIV and individuals not infected with HIV have yet to be performed in Africa. In a small South African study, the prevalence of smoking among individuals infected with HIV was 15% overall (23.2% among men and 7.4% among women).<sup>28</sup> This rate is notably lower than those among patients with HIV infection in resource-rich countries and is similar to that in the general population of Africa.<sup>27</sup>

In a retrospective cohort study of 3,851 patients with HIV infection in the US healthcare system (most of whom were taking cART) and 1,044,589 patients without HIV infection, prevalence was significantly higher in the HIV group for hypertension (21.2% vs. 15.9%), diabetes mellitus (11.5% vs. 6.6%), and dyslipidemia (23.3% vs. 17.6%).<sup>21</sup> Large, observational studies to examine the prevalence of metabolic risk fac-



tors in patients with HIV infection have also been conducted in Europe and Africa, but some have not featured control groups. Among 17,852 patients infected with HIV (81% taking cART) in the mainly European D:A:D study<sup>26</sup>, 1,517 (8.5%) had hypertension. Similarly, 1,061 (8.7%) of 12,194 patients with HIV infection (66% taking cART) had hypertension in a Kenyan study of outpatient treatment programmes.<sup>29</sup> Comparisons of the prevalence of diabetes and dyslipidemia in patients with HIV infection in Africa, Europe, and the USA are complicated by inconsistent definitions of metabolic risk factors. However, use of second-line or third-line cART, which would be likely to lead to increased rates of lipodystrophy (accumulation of visceral fat and wasting of peripheral subcutaneous fat), might increase the prevalence of these comorbidities in resource-constrained regions.<sup>24</sup>

In resource-rich countries with access to cART, renal dysfunction is more prevalent among patients infected with HIV than uninfected control individuals. In a US study of 845 patients with HIV infection receiving care in an urban outpatient clinic (63% taking cART), 35.4% had stage 2 chronic kidney disease and 5.3% had stage 3 chronic kidney disease.<sup>30</sup> These rates were significantly higher than those from 845 control individuals from the general population in the NHANES database matched for age and sex (29.3% with stage 2 and 1.7% with stage 3 chronic kidney disease).<sup>30</sup> Several large studies without control groups have also been performed to investigate the prevalence of renal dysfunction among patients with HIV infection in Europe and Africa. Among 12,155 patients in the EuroSIDA cohort (82.2% taking cART), 29.1% had stage 2 chronic kidney disease and 3.2% had stage  $\geq 3$ .<sup>31</sup> Among 25,779 Zambian individuals infected with HIV, the prevalence of stage 3 chronic kidney disease was notably higher (23.4%).<sup>32</sup> Importantly, this cohort was cART-naive<sup>32</sup>; HIV itself is a well-known contributor to nephropathy.<sup>33</sup>

### Effects of cART

cART has revolutionized the care of patients infected with HIV and led to prolonged, healthier lives. However, some cART regimens have been implicated in the induction of lipodystrophy, which in turn promotes dyslipidemia and insulin resistance<sup>34-36</sup>, and might predispose pa-

tients to hypertension through hyperactivation of the renin-angiotensin-aldosterone system.<sup>37</sup> Effects of cART on traditional (nonbehavioural) CHD risk factors vary by regimen. The earliest classes of antiretrovirals included protease inhibitors, nucleoside reverse-transcriptase inhibitors, and non-nucleoside reverse-transcriptase inhibitors. Particular agents in these classes have been heavily assessed for potential contributions to metabolic dysregulation. Many of the agents identified as having adverse metabolic effects, including the nucleoside reverse-transcriptase inhibitor stavudine, are no longer widely used in resource-rich regions. However, these drugs are still used in some resource-poor settings, albeit, fortunately, at a declining rate. New classes of antiretrovirals, including entry inhibitors (CCR5 antagonists), fusion inhibitors, and integrase strand transfer inhibitors, have been less extensively assessed for associations with metabolic CHD risk factors than the older drug classes, but no notably adverse metabolic effects have yet been identified.

Among cART-naive individuals in the D:A:D study<sup>26</sup>, the prevalence of hypertension was lower than that among patients previously treated with cART. In a prospective observational study to examine the effects of newly initiated cART on blood pressure in outpatients with HIV infection who were cART-naive, modest - but significant - rises in both systolic and diastolic blood pressure at 48 weeks were recorded.<sup>38</sup>

In the US MACS cohort, the prevalence of diabetes was significantly lower among cART-naive individuals than among patients treated with cART (7% vs. 14%).<sup>39</sup> In several studies, investigators have shown a relationship between cART and increased diabetes prevalence<sup>40</sup> and incidence.<sup>41,42</sup> In a study of 1,046 patients with HIV infection across 47 French clinics, the incidence of diabetes was significantly associated with cART use, especially with short term exposure to the nucleoside reverse-transcriptase inhibitors stavudine and didanosine and the protease inhibitor indinavir.<sup>42</sup> In the D:A:D study<sup>43</sup>, the incidence of diabetes increased with cumulative exposure to cART, particularly regimens including stavudine or zidovudine, even after controlling for lipodystrophy. Specific cART regimens are hypothesized to contribute to glucose dysregulation via induction of lipodystrophy and by lipodystrophy-independent mechanisms, such as mitochondrial toxic effects in the

case of some nucleoside reverse-transcriptase inhibitors<sup>44</sup> and glucose transporter inhibition in the case of some protease inhibitors.<sup>45</sup>

The typical pattern of dyslipidemia in cART-naive patients infected with HIV is low concentrations of HDL cholesterol and LDL cholesterol and high concentrations of triglycerides.<sup>46</sup> In a landmark study performed in men with HIV infection, some of whom had advanced disease, initiation of cART was associated with increased concentrations of LDL, but not of HDL, cholesterol.<sup>47</sup> This effect was deemed to indicate a 'return-to-health' phenomenon.<sup>36</sup> By contrast, in a contemporary cohort of women infected with HIV, initiation of cART was associated with increased concentrations of HDL cholesterol irrespective of regimen, and increased concentrations of LDL cholesterol only when the regimen included a protease inhibitor.<sup>48</sup> The protease inhibitors lopinavir and ritonavir have also been associated with raised triglyceride levels, even in healthy volunteers without HIV infection.<sup>49</sup> To add further complexity to the effects of treatment on dyslipidemia, cART might affect lipoprotein structure or function as well as lipoprotein concentrations. For instance, in patients who have started cART, partial restoration of the normal distribution of cholesterol across HDL particle sizes<sup>50</sup> and increased beneficial HDL-associated apolipoprotein A1 levels<sup>51</sup> have been observed. Whether cART exerts net positive or negative effects on lipid homeostasis in individuals infected with HIV remains uncertain.

cART-naive patients with HIV infection have a higher prevalence of chronic kidney disease, particularly stage  $\geq 3$ , than cohorts of patients with HIV infection who have access to cART.<sup>30,31,52</sup> In patients infected with HIV, starting cART is associated with slowing of the annual decline in estimated glomerular filtration rate compared with that in untreated patients.<sup>53</sup> cART has been associated with an overall reduced risk of renal dysfunction, despite tenofovir and ritonavir-boosted protease-inhibitor-based regimens being associated with increased risks.<sup>53</sup> The initiation of cART seems, therefore, to offer a net protection against progressive HIV-associated nephropathy, notwithstanding the modest decrements in estimated glomerular filtration rate reported with some antiretrovirals.

### Contribution to CHD risk

In patients infected with HIV compared with uninfected individuals, tobacco smoking seems to contribute disproportionately to the risk of acute coronary syndrome (ACS). Calvo-Sanchez and colleagues conducted two parallel case-control studies in which individuals with HIV infection who had experienced ACS were compared with uninfected individuals with ACS (control group 1) or with individuals with HIV infection who had not had an ACS (control group 2).<sup>54</sup> Cases were matched for age, sex, and date of ACS diagnosis with control group 1 or for duration of known HIV infection with control group 2. Independent risk factors for ACS were determined through logistic regression, including smoking and family history of CVD in individuals with HIV infection, and smoking, diabetes, and hypertension in individuals without HIV infection. The contribution of smoking to the risk of ACS in patients infected with HIV was almost double that in control individuals without HIV (population attributable risk 54.35% vs. 30.58%).<sup>54</sup> Helleberg et al. demonstrated in a large Danish cohort that the population-attributable risk of death associated with smoking in patients with HIV infection was double than in control individuals without HIV (61.5% vs. 34.2%).<sup>55</sup>

Published comparisons of the contributions of dyslipidemia, renal dysfunction, or both, to the risk of MI between patients with or without HIV infection are lacking. In patients with HIV infection, both factors have been shown to contribute significantly to the risk of CHD. Analysis of 33,308 patients infected with HIV in the D:A:D study<sup>56</sup> showed that levels of total and HDL cholesterol remained significantly associated with risk of MI after adjustment for other CVD and HIV-specific risk factors, whereas the association between triglyceride concentration and risk of MI was reduced to marginal significance. In this study, the relationship between LDL cholesterol level and risk of MI was not assessed.<sup>56</sup>

With respect to kidney dysfunction, abnormal creatinine clearance and albuminuria in a cohort of US veterans were associated with incident CVD in patients with HIV infection and access to cART.<sup>57</sup> In multivariate analysis adjusted for demographics, traditional CVD risk factors, medical comorbidities, and HIV-specific factors, estimated glomerular filtration rate remained associated with increased risk of incident CVD.<sup>57</sup> Compared with patients who had an estimated glomerular filtration rate

of  $\geq 60$  mL/min/1.73 m<sup>2</sup>, individuals with HIV infection and stage 3-4 chronic kidney disease had ~50-100% increase in the adjusted risk of incident CVD.<sup>57</sup> Abnormal creatinine clearance was also associated with increased risk of CVD in a study of the Johns Hopkins HIV cohort.<sup>58</sup>

Hypertension, diabetes, and dyslipidemia together have been reported to account for 25% of excess CVD risk in a cohort of US patients infected with HIV, although smoking and other important risk factors could not be fully controlled for in this analysis.<sup>21</sup> As noted above, in the US VACS-VC study<sup>15</sup>, 27,350 patients with HIV infection were compared with 55,109 patients without HIV infection receiving care in US Veterans Affairs hospitals, and the risk of MI was found to be increased ~1.5-fold in patients infected with HIV. Importantly, this analysis was adjusted for demographics (age, ethnicity, and sex), traditional CHD risk factors (diabetes, hypertension, and smoking), comorbidities (anemia, obesity, renal disease, and infection with hepatitis C virus), statin use, and illicit drug use. Although the possibility of residual confounding effects cannot be fully excluded, the findings are highly convincing. Among the patients with HIV infection in this cohort, high-level viremia and low CD4+ T-cell counts were associated with increased risk of MI after adjustment for traditional CHD risk factors, comorbidities, and illicit drug use.<sup>15</sup> Data from this and other studies suggest that heightened CHD risk in patients with HIV is associated with immune dysregulation. Interestingly, an increased risk of MI of similar magnitude has also long been recognized in other diseases characterized by immune dysregulation, including rheumatoid arthritis, systemic lupus erythematosus, psoriatic disease, and ankylosing spondylitis.<sup>59</sup> In these diseases, as in HIV infection, immune activation and traditional CHD risk factors are thought to contribute, possibly synergistically, to CHD risk.<sup>59</sup>

## Immune risk factors for CHD

### Immune dysfunction and activation in HIV

Paradoxically, HIV infection leads to immune dysfunction (low CD4+ T-cell counts) and also to potentially proatherogenic immune activation

**Table 2.1: In vitro atherogenic effects of HIV and antiretroviral therapy**

Source of effects	Monocytes and macrophages	CD4+ and CD8+ T-cells	Endothelial cells	Platelets
<b>HIV</b>				
Virus (studies in which the viral proteins below were not isolated)	Might infect monocytes directly and alter biological behaviour <sup>134,135</sup>	-	Might infect endothelial cells directly and stimulate MCP-1 secretion <sup>136,137</sup>	-
gp120	-	Induces T-cells to adhere to vascular endothelium <sup>138</sup>	Decreases eNOS production by endothelial cells <sup>139</sup> , stimulates endothelial-cell production of ICAM <sup>140</sup> , and might induce endothelial cell apoptosis <sup>141,142</sup>	-
Protein Nef	Activates macrophages, stimulating cytokine secretion <sup>143,144</sup> , and inhibits macrophage cholesterol efflux <sup>145-147</sup>	Inhibits T-cell transendothelial migration <sup>148</sup>	Induces superoxide anion formation, decreases eNOS production by endothelial cells <sup>149</sup> , and might induce endothelial cell production of MCP-1 <sup>150</sup> and apoptosis <sup>150,151</sup>	-
Protein Tat	Activates macrophages, stimulating cytokine secretion <sup>152-155</sup> , and induces chemotaxis of monocytes <sup>156,157</sup>	-	Activates endothelial cells, increasing expression of MCP-1 <sup>158</sup> , ICAM-1 <sup>159,160</sup> , VCAM-1 <sup>159,161</sup> , and E-selectin <sup>162</sup> , and encourages recruitment or adhesion of monocytes <sup>158,163</sup> and T-cells <sup>163</sup>	Activates platelets to release CD154, potentially contributing to HIV-induced autoimmune thrombocytopenia <sup>164</sup>
gp41	-	-	-	Interacts with platelets, altering patterns of protein production <sup>165</sup>
<b>cART</b>				
Protease inhibitors	Select protease inhibitors <sup>166</sup> and cART regimens containing protease inhibitors decrease cholesterol efflux from macrophages <sup>167</sup>	-	Select protease inhibitors (for example, ritonavir) induce superoxide anion formation <sup>168</sup> and decrease eNOS production by endothelial cells <sup>169</sup> and might induce endothelial cell cytotoxic effects and apoptosis <sup>170</sup>	-
NRTI	-	-	-	Select NRTI's (for example, abacavir) increase platelet reactivity <sup>171</sup>
NNRTI's	Select NNRTI's induce monocytes to adhere to vascular endothelium <sup>172</sup>	-	-	-

Abbreviations: cART=combined antiretroviral therapy, eNOS=endothelial nitric oxide synthase, ICAM-1=intercellular adhesion molecule 1, MCP-1=C-C motif chemokine 2 (also known as monocyte chemoattractant protein 1), NRTI=nucleoside reverse-transcriptase inhibitor, NNRTI=non-nucleoside reverse transcriptase inhibitor, VCAM-1=vascular cell adhesion protein 1.

**Table 2.2: In vivo atherogenic effects of HIV and antiretroviral therapy**

Source of effects	Monocytes and macrophages	CD4+ and CD8+ T-cells	Platelets
<b>HIV</b>			
HIV (± co-infection) in cART-naive patients	Increased circulating percentages of atherogenic CD14 <sup>low</sup> CD16 <sup>high</sup> monocytes <sup>373</sup> and monocytes expressing cell-surface tissue factor <sup>374</sup> , increased monocyte expression of leukocyte adhesion molecules (e.g. CD11a, CD18) <sup>375*</sup> , signs of immunosenescence <sup>376*</sup> , and accumulation of cholesterol <sup>377*</sup>	Increased circulating percentages of activated CD4+ and CD8+ T-cells (expressing CD38+HLADR+) <sup>378*</sup> , immunosenescent CD8+ T-cells <sup>379</sup>	Increased platelet activation <sup>380</sup>
HIV infection and cART treatment	Increased circulating percentages of atherogenic nonclassical (CD14+CD16++) and intermediate (CD14+CD16+) monocytes <sup>381</sup>	Increased circulating percentage of senescent CD8+ T-cells <sup>382</sup>	Activation of platelets and platelet microparticles and increased tissue-factor expression <sup>383</sup> and platelet aggregation <sup>329</sup>
<b>cART</b>			
Treatment initiation	-	-	Restores platelet count (which declines in absence of cART initiation) <sup>384</sup> , select protease inhibitors might increase platelet-leukocyte interactions without increasing platelet activation <sup>385</sup> , and the NRTI abacavir (but not tenofovir) increases platelet activation and platelet reactivity <sup>386</sup>
Treatment interruption	-	Increases T-cell activation <sup>68,387</sup>	Leads to recurrent thrombocytopenia <sup>388</sup>

\* Greatest difference versus controls and significant even versus cART-treated patients with HIV.

Abbreviations: cART=combined antiretroviral therapy, NRTI=nucleoside reverse-transcriptase inhibitor.

(endothelial cells, monocytes, platelets, and T cells; **Tables 2.1 and 2.2**). Unsurprisingly, in the context of HIV-specific immune cell activation, patients with HIV infection and uncontrolled viremia who are cART-naive have been noted to have elevated levels of circulating immune and inflammatory markers. These markers include soluble monocyte activation markers (such as soluble CD14)<sup>60</sup>, pro-inflammatory cytokines (such as IL-6)<sup>61</sup>, markers of arterial inflammation (such as platelet-activating factor acetylhydrolase)<sup>62</sup>, soluble cytokine receptors (such as soluble tumor necrosis factor (TNF)-R2)<sup>60,63</sup>, chemokines (such as C-C motif chemokine 2, also known as monocyte chemoattractant protein 1 (MCP-1))<sup>60</sup>, acute-phase proteins (such as C-reactive protein)<sup>64</sup>, soluble leuko-

cyte adhesion markers (such as soluble intercellular adhesion molecule 1 (ICAM-1) and soluble vascular cell adhesion protein 1 (VCAM-1)),<sup>61,63</sup> and fibrin degradation products (such as D-dimer).<sup>60,61</sup> Patients with HIV infection who had never received cART have more advanced subclinical atherosclerosis than control individuals without HIV infection and matched for cardiometabolic risk factors, indicated by increased carotid intima-media thickness.<sup>65</sup> Among patients with HIV infection who are cART-naive, subclinical atherosclerosis has been associated with traditional CHD risk factors<sup>66</sup> and with circulating inflammatory markers, such as IL-6.<sup>67</sup>

### Effects of cART

Data from in vitro studies suggest that some antiretroviral drugs activate immune cells, endothelial cells, and platelets (**Table 2.1**), but overall clinical studies show that the use of cART lessens, although does not completely quell, HIV-specific immune activation (**Table 2.2**). Interruption of cART administration leads to increased levels of circulating immune, inflammatory, and procoagulation markers, for example soluble TNF-R2<sup>68</sup>, IL-6<sup>69</sup>, soluble ICAM-1<sup>70</sup>, soluble VCAM-1<sup>70</sup>, and D-dimer.<sup>69</sup> By contrast, cART initiation seems to lower the levels of many of these markers,<sup>60,64,71-74</sup> albeit not down to the concentrations seen in individuals without HIV infection.<sup>60,64</sup> In line with these observations, among patients infected with HIV who are cART-naive, initiation with one of two protease-inhibitor-sparing cART regimens improves endothelial function within 4 weeks and the effects persist for up to 24 weeks.<sup>75</sup> By contrast, in patients with HIV who are cART-naive, treatment with specific protease-inhibitor-based regimens has been associated with increased carotid intima-media thickness at 24 months, despite concomitant decreases in levels of circulating leukocyte adhesion markers.<sup>74</sup> Although potential confounding by effects of ageing on carotid intima-media thickness could not be explored because of the absence of a control group, this finding illustrates the possible disjunction between concentrations of inflammatory markers and imaging surrogates of CHD risk.

Although HIV-associated immune activation is partially decreased by cART, immune activation and related changes persist even in cART-



treated patients with suppressed viremia (**Table 2.2**). Potential explanations for persistent immune activation include reservoirs of persistent HIV replication, continued microbial translocation, and co-infections (for example with cytomegalovirus, hepatitis C virus, or both).<sup>76,77</sup> Approximately 25-35% of patients had both HIV and hepatitis C virus infection in large European<sup>78</sup> and US<sup>15</sup> cohorts. Hepatitis C virus co-infection has been associated with a blunted decline in immune activation markers in response to cART in patients with HIV infection who were treatment-naïve<sup>64</sup> and has been linked to a moderate increase in the risk of MI in some studies<sup>79,80</sup>, but not in others.<sup>81</sup> Ongoing immune activation among patients with HIV infection treated with cART might also be related to increased visceral adiposity if lipodystrophy has been induced by medication.<sup>34,35</sup> Importantly, many studies have shown associations between persistent immune dysfunction and subclinical and clinical CHD in patients with HIV infection, most of whom have suppressed viremia while taking cART (**Table 2.3**).

#### Contribution to CHD risk

The degree to which immune factors contribute to CHD risk in HIV is unknown. HIV-specific immune activation could synergize, in negative fashion, with immune-modulatory behavioural CHD risk factors, such as smoking.<sup>55</sup> Moreover, immune activation in people infected with HIV might negatively synergize with metabolic CHD risk factors, for example by proatherogenic dysregulation of lipid structure or function.<sup>82</sup> Genes that have been associated with risk of CHD through genome-wide association studies in the general population also seemed to be important in a study of 1,875 patients with HIV infection in Europe and the USA.<sup>83</sup> However, in some genetics studies, variants in immune-related genes have also been associated with CHD risk in people with HIV infection. For example, polymorphisms in CCL2, which strongly influence MCP-1 levels<sup>84</sup>, have been associated with an increased risk of subclinical atherosclerosis in European individuals infected with HIV.<sup>85</sup> In people with a genetic predisposition to proatherogenic immune activation, HIV infection might constitute a 'second hit' in the pathway towards incident CHD.

**Table 2.3: Immune and inflammatory markers in HIV-related cardiovascular disease**

Subclinical atherosclerosis
<p>Immune markers: CD<sub>4</sub>+ T-cell counts</p> <ul style="list-style-type: none"> <li>Low CD<sub>4</sub>+ T-cell counts associated with carotid artery atherosclerotic lesions<sup>189</sup></li> </ul> <p>Microbial translocation or monocyte activation markers</p> <ul style="list-style-type: none"> <li>Lipopolysaccharides: associated with progressive cIMT<sup>190</sup></li> <li>Soluble CD<sub>14</sub> associated with progressive<sup>190</sup> or pathological<sup>191</sup> cIMT and calcified coronary plaque<sup>192</sup></li> <li>Soluble CD<sub>163</sub> associated with vulnerable<sup>197</sup>, noncalcified<sup>196</sup>, calcified, mixed, and total coronary plaque<sup>192</sup>, and aortic <sup>18</sup>F-FDG uptake on PET<sup>112</sup></li> </ul> <p>T-cell markers</p> <ul style="list-style-type: none"> <li>Increased activated CD<sub>4</sub>+ and CD8+ T-cells (CD38+HLA-DR+) associated with carotid artery lesions<sup>182</sup></li> <li>Increased activated memory CD8+ T-cells (CD8+CD38+CD45Ro+) associated with pathological cIMT in some<sup>191</sup>, but not all<sup>193</sup>, studies</li> <li>Increased immunosenescent CD8+ T-cells (CD28-CD57+) associated with carotid artery lesions<sup>182</sup></li> <li>Increased apoptotic CD<sub>4</sub>+ (CD<sub>4</sub>+CD95+) and CD8+ (CD8+CD95+) T-cells associated with pathological cIMT<sup>191</sup></li> <li>CMV-specific T-cell responses associated with increased<sup>193</sup> and progressive<sup>194</sup> cIMT</li> </ul> <p>Markers of general inflammation and coagulation</p> <ul style="list-style-type: none"> <li>Increased soluble VCAM-1<sup>195</sup>, MCP-1<sup>196</sup>, TNF-<math>\alpha</math><sup>195</sup>, soluble TNF-R1<sup>197</sup>, PAF acetylhydrolase<sup>198</sup>, and fibrinogen<sup>197</sup> associated with pathological cIMT</li> <li>Increased MCP-1 associated with coronary plaque burden<sup>199</sup></li> </ul> <p>Lipoprotein structural and function parameters</p> <ul style="list-style-type: none"> <li>Oxidized LDL levels associated with increased cIMT<sup>196</sup></li> <li>HDL redox activity associated with noncalcified coronary atherosclerotic plaque<sup>200</sup></li> </ul>
MI and CVD mortality
<p>Immune markers: CD<sub>4</sub>+ T-cell counts</p> <ul style="list-style-type: none"> <li>Low CD<sub>4</sub>+ T-cell counts associated with MI (incident and acute<sup>15,201-203</sup>)</li> <li>Low nadir CD<sub>4</sub>+ T-cell counts associated with MI in some<sup>204,205</sup>, but not all<sup>206</sup>, studies</li> </ul> <p>Viral load</p> <ul style="list-style-type: none"> <li>Viremia associated with incident MI risk (rises with increasing viral load)<sup>15,203,205</sup>; association with acute MI lost after adjustment for CD<sub>4</sub>+ T-cell count<sup>201</sup></li> </ul> <p>Markers of monocyte activation, general inflammation, and coagulation</p> <ul style="list-style-type: none"> <li>Highest quartile concentrations (versus lowest) of soluble CD<sub>14</sub> associated with CVD death<sup>207</sup></li> <li>Increased IL-6 level associated with incident MI<sup>208</sup></li> <li>Increased CRP level associated with MI (incident<sup>208</sup> and acute<sup>209</sup>)</li> <li>Increased D-dimer level associated with incident CVD events<sup>210</sup> and MI<sup>208</sup></li> </ul> <p>Lipoprotein structural and function parameters</p> <ul style="list-style-type: none"> <li>Total, large, and small HDL particles associated with CVD and nonfatal coronary heart disease<sup>211</sup></li> </ul>

Abbreviations: cIMT=carotid intima-media thickness, CMV=cytomegalovirus, CRP=C-reactive protein, CVD=cardiovascular disease, MCP-1=C-C motif chemokine 2 (also known as monocyte chemoattractant protein 1), MI=myocardial infarction, PAF=platelet-activating factor, TNF=tumour necrosis factor, VCAM-1=vascular cell adhesion protein 1.

## cART and incident MI, a paradigm shift

Initial analysis of the D:A:D study<sup>86</sup> data revealed an association between incident MI and exposure to cART regimens including either a non-nucleoside reverse-transcriptase inhibitor or a protease inhibitor. The adjusted relative risk of MI per additional year of cART exposure was 1.26 (95% CI 1.12-1.41).<sup>86</sup> Data analysis from the same cohort with an additional 3 years of follow-up established that, after adjustment for CVD risk factors excluding lipids, the relative risk of MI per year of exposure remained raised for protease inhibitors (relative risk (RR) 1.16, 95% CI 1.10-1.23), but not for non-nucleoside reverse-transcriptase inhibitors.<sup>87</sup> Further adjustment for lipid concentrations reduced the risk of MI conferred by exposure to a protease inhibitor, but did not eliminate it entirely (RR 1.10, 95% CI 1.04-1.18).<sup>87</sup>

With respect to the risks associated with individual antiretrovirals, data from the D:A:D study indicate an increased relative risk of MI with exposure to the nucleoside reverse-transcriptase inhibitors abacavir and didanosine (but not with lamivudine, stavudine, tenofovir, zalcitabine, or zidovudine), and with cumulative exposure to the protease inhibitors indinavir and lopinavir-boosted ritonavir (but not with atazanavir, efavirenz, nelfinavir, nevirapine, or saquinavir).<sup>88-90</sup> To address the question of whether exposure to specific antiretrovirals predisposes patients infected with HIV to MI, Lang and colleagues used a case-control design.<sup>91</sup> The investigators assessed data from the French Hospital Database on HIV and found that cumulative exposure to protease inhibitors (except saquinavir) was associated with an increased risk of MI (odds ratio (OR) 1.33-1.53 per year) whereas exposure to non-nucleoside reverse-transcriptase inhibitors was not.<sup>91</sup> Additionally, although in the overall sample, short term or recent exposure to the nucleoside reverse-transcriptase inhibitor abacavir was associated with an increased risk of MI (OR 2.01, 95% CI 1.11-3.64), this association was not observed in the subset of individuals who did not use illicit drugs.<sup>91</sup> In several studies, including two meta-analyses, no convincing association between abacavir and a heightened risk of MI has been found.<sup>92,93</sup> However, in follow-up of the D:A:D study<sup>94</sup> from 2008 to 2013, the increased risk persisted despite patients with a perceived high risk of CHD not being

prescribed abacavir. The evidence on abacavir and cardiovascular risk has been further reviewed previously.<sup>95</sup> Overall, the primary data suggest that some, but not all, cART regimens increase the risk of CHD in patients infected with HIV, partly through exacerbation of traditional CHD risk factors.

Despite the early concerns that cART might increase the risk of CHD in patients infected with HIV, increasing evidence suggests that early and continuous cART treatment can potentially reduce CHD risk, which is a major paradigm shift. In the SMART trial<sup>96,97</sup>, 5,472 patients with HIV infection and a CD4+ T-cell count >350 cells/mm<sup>3</sup> were randomly allocated to either a drug conservation strategy of episodic cART guided primarily by CD4+ T-cell count, or to a strategy of continuous cART aimed at maintaining viral suppression. The primary end point was new or recurrent opportunistic disease or death from any cause, and the secondary end point was serious CVD, renal disease, and hepatic disease (mean follow-up 16 months). In the drug conservation group, the risks of both the primary end point (hazard ratio (HR) 2.6, 95% CI 1.9-3.7) and the secondary end point (HR 1.7, 95% CI 1.1-2.5) were increased, as were the risks of each component of the secondary end point when assessed separately.<sup>96</sup> A subsequent sensitivity analysis indicated an increased risk of CVD events with drug conservation compared with viral suppression (HR 1.57, 95% CI 1.00-2.46).<sup>97</sup>

Although the apparent atheroprotective effects of cART have not yet been fully explored, the partial decrease in HIV-specific immune activation during treatment might outweigh potential adverse effects of cART on traditional CHD risk factors. Revisions in 2012 to the US Department of Health and Human Services Guidelines for care of patients with HIV infection provided a moderate recommendation for starting cART in patients with a CD4+ T-cell count >500 cell/mm<sup>3</sup>.<sup>98</sup> These guidelines were made on the basis of public health benefits of decreased infectivity and "growing evidence that untreated HIV infection or uncontrolled viremia is associated with development of non-AIDS-defining diseases, including cardiovascular disease".<sup>98</sup> Such evidence includes studies linking viremia, as well as low CD4+ T-cell counts and/or low nadir CD4+ T-cell count, to a heightened risk of MI in patients infected with HIV (**Table 2.3**). Investigators in the ongoing START trial<sup>99</sup> will determine whether

the risk of developing a serious non-AIDS disease is reduced by a strategy of immediate (CD4+ cell T-count >500 cells/mm<sup>3</sup>) versus delayed (CD4+ T-cell count <350 cells/mm<sup>3</sup>) cART initiation.

## Unique aspects of CHD risk in HIV

### Increased relative risk in women

In a large, retrospective cohort study of patients in the US healthcare system, the relative risk of MI in women infected with HIV versus female controls without HIV infection was 2.98 (95% CI 2.33-3.75) versus 1.40 (95% CI 1.16-1.67) overall for men.<sup>21</sup> Subsequently, using data from the French Hospital Database on HIV, the standardized morbidity ratio for MI among women with HIV infection was shown to be 2.7 (95% CI 1.8-3.9) versus 1.4 (95% CI 1.3-1.6) overall for men, when compared with control individuals without HIV infection.<sup>22</sup> Using data from the VACS-VC cohort, investigators found that incident CVD per 1,000 person-years was significantly higher among women with HIV infection than in female controls without HIV infection (adjusted HR 3.12, 95% CI 1.89-5.11).<sup>100</sup>

A possible explanation for the higher relative risk of CHD in women with HIV than in men with HIV is that these women have specific CHD risk-factor profiles. In a prospective study of the 4,987 patients in the ANRS CO3 Aquitaine Cohort in France, women with HIV had lower rates of traditional CHD risk factors (tobacco use, hypertension, diabetes, and dyslipidemia), but higher rates of intravenous illicit drug use and co-infection with hepatitis C virus, than men with HIV.<sup>101</sup> The higher rates of intravenous illicit drug use might be related to other CHD risk factors, such as those associated with socio-economic status and mental health. Additionally, differences in cardiac preventative care might exist between the sexes. Yet another consideration is that the immune response to infection with HIV might be greater in women than men. Indeed, women with HIV infection who are cART-naive have been shown to have more substantial CD8+ T-cell activation than men after adjustment for HIV RNA concentrations.<sup>102</sup>

### Age at time of clinical event

In an analysis of the US VACS-VC cohort, although the overall adjusted hazard ratio of incident MI was 1.48, stratification by age (10-year age-groups) indicated that the incidence rate ratio was highest among patients aged 30-39 years.<sup>15</sup> Another analysis was performed using data from 68 patients infected with HIV and 68 randomly selected control individuals without HIV infection all hospitalized for ACS in a US tertiary care centre.<sup>103</sup> Among the patients with ACS, those infected with HIV were younger and had lower risk scores for MI than control individuals. Angiography revealed fewer vessels with >50% stenosis in the group with HIV infection than in the control group.<sup>103</sup> A small case-control study conducted in Soweto, South Africa yielded similar results to the US study, despite notable differences between study populations.<sup>104</sup> In the South African study, the characteristics of the first 30 patients with ACS presenting to a single medical site who were infected with HIV and cART-naïve were compared with those of 30 controls who presented at the same time with ACS but were not infected with HIV. Patients with HIV infection were younger ( $43 \pm 7$  years vs.  $54 \pm 13$  years) and had fewer traditional CVD risk factors than those in the control group.<sup>104</sup> Although the patients with HIV infection had lower atherosclerotic burden on angiography than control individuals, the thrombotic burden was higher. This finding of increased thrombotic burden might be associated with platelet dysfunction in patients with HIV infection who have or have not received cART (**Tables 2.1 and 2.2**). Angiographic features of CHD in patients infected with HIV and the clinical course of these patients after cardiac procedures have been reviewed previously.<sup>105</sup>

### Novel phenotypes of subclinical CHD

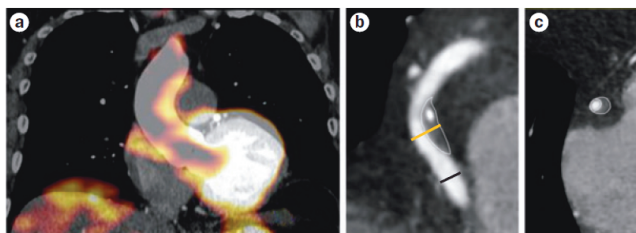
One explanation for why patients with HIV infection who present with ACS are younger than patients without HIV who present with ACS is that the HIV-infected population is younger than the general population. Alternately, the biology of atherosclerosis in people infected with HIV might differ from that in individuals without HIV infection, favouring the development of a type of coronary atherosclerotic plaque that is vulnerable to early rupture. The latter explanation is supported by several

noninvasive imaging studies conducted in patients with HIV infection, most of whom had cART-induced virological suppression.

In one set of analyses, CT angiography data on 102 patients with HIV infection without known CVD were compared with data on 41 prospectively recruited patients without HIV infection who were matched for traditional CVD risk factors.<sup>106</sup> Among the patients infected with HIV, the prevalence of subclinical noncalcified coronary atherosclerotic plaque and high-risk morphological plaque features were increased.<sup>106,107</sup> Such features include low attenuation on CT, which indicates a plaque with a prominent necrotic lipid core.<sup>108</sup> Increased prevalence of noncalcified coronary atherosclerotic plaque in patients infected with HIV was also found in 1,001 US patients in the MACS cohort.<sup>109</sup> The importance of noncalcified and high-risk morphology plaques resides in their vulnerability to plaque rupture, at least in patients in the general population.<sup>110</sup>

In a US study, 27 patients with HIV infection, treated with cART, and without known CHD underwent cardiovascular <sup>18</sup>F-FDG-PET scanning for assessment of aortic inflammation. With this technique, radio-labelled glucose administered intravenously is metabolically trapped within macrophages residing in the arterial tunica intima.<sup>111</sup> Levels of aortic inflammation were higher in the group with HIV infection than in a historical control group of individuals without HIV infection, matched for traditional risk factors.<sup>112</sup> In the general population, arterial inflammation on <sup>18</sup>F-FDG-PET is associated with the risk and timing of incident CVD.<sup>113</sup> In a study of patients infected with HIV without known CHD, the degree of aortic inflammation on cardiac <sup>18</sup>F-FDG-PET corresponded to high-risk morphology plaque parameters on coronary CT angiography (**Figure 2.2**).<sup>114</sup>

Importantly, the Framingham risk scores in patients with HIV infection in these studies of subclinical atherosclerosis and arterial inflammation were low. Therefore, patients infected with HIV but without known CHD who are taking cART and have low Framingham risk scores seem to have a novel atherosclerotic plaque phenotype that tends to be characterized by a lack of calcification, high-risk morphology, and inflammation. Subclinical atherosclerosis observed on coronary CT angiography in individuals infected with HIV has also been associated with



**Figure 2.2: Coronary atherosclerotic plaque in a patient infected with HIV**

**a** Coronal  $^{18}\text{F}$ -FDG-PET image of the aorta shows an increased aortic target to background ratio (2.36). In the same patient, CT angiography images of the right coronary artery in the **b** long-axis (lines show differences in segment diameter in regions of plaque and normal vessel) and **c** short-axis views. High-risk morphology features of low attenuation, positive remodelling, and spotty calcification are revealed.

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increased volume of epicardial adipose tissue<sup>115</sup> that is generally greater than volumes seen in control individuals without HIV.<sup>116</sup>

The degree to which traditional risk factors, immune risk factors, and nontraditional behavioural risk factors contribute to the novel atherosclerotic phenotype among individuals with HIV remains uncertain. Importantly, high-risk morphology coronary atherosclerotic plaques and arterial inflammation have been associated with the circulating monocyte activation marker sCD163.<sup>107,112</sup> Associations have been shown between sCD163 and noncalcified plaques in some studies<sup>106</sup>, but with calcified plaques in others.<sup>109</sup> Among patients with HIV infection in particular demographic areas, nontraditional behavioural risk factors, such as cocaine use (which promotes CHD via multiple mechanisms<sup>117</sup>) might also be contributory. Investigators conducting epidemiological studies in the USA, for instance, have demonstrated rates of cocaine use as high as 11.3% among patients infected with HIV.<sup>15</sup> However, cocaine use tends to be associated with calcified coronary atherosclerotic plaques<sup>118,119</sup>, which is atypical of the proposed HIV-associated novel phenotype.



## Risk assessment and management

Effective prevention of CHD in individuals infected with HIV involves recognition that traditional risk scores do not fully explain the increased risk.<sup>120</sup> HIV-specific CHD risk prediction paradigms have been developed<sup>121</sup>, but have not yet been widely adopted. As for therapeutic approaches to CHD prevention, current understanding of HIV-specific atherogenesis would suggest greatest efficacy for strategies targeting both traditional risk factors and nontraditional immune factors. Nevertheless, no evidence yet indicates that immune modulatory strategies reduce CHD risk among patients with HIV infection.

### Monitoring traditional CHD risk factors

The European AIDS Clinical Society guidelines on the prevention and management of comorbidities in people infected with HIV suggest the following parameters should be assessed at time of diagnosis, before cART is started, and annually thereafter unless otherwise indicated: historical factors related to CHD risk, body mass index, blood pressure, fasting glucose concentration (measured every 6-12 months), and fasting concentrations.<sup>122</sup> The guidelines also suggest determining estimated glomerular filtration rate every 3-12 months.<sup>122</sup> The HIV Medicine Association of the Infectious Diseases Society of America primary care guidelines for the management of HIV infection suggest close surveillance of lipid and glucose levels, with measurement of fasting lipid and fasting glucose concentrations or hemoglobin A1c concentrations before and 1-3 months after cART initiation.<sup>123</sup>

### Addressing behavioural risk factors

The disproportionate contribution of smoking to CHD risk in individuals infected with HIV<sup>54,55</sup> mandates that special attention is given to encouraging smoking cessation.<sup>122</sup> Research is being undertaken to identify feasible, effective strategies through which to accomplish this aim.<sup>124</sup> Lifestyle counselling on diet and exercise is also recommended.<sup>122</sup>

## Management of metabolic risk factors

### *Lipodystrophy*

Given that lipodystrophy predisposes individuals with HIV to metabolic dysregulation, prevention and management strategies must be considered. Avoidance of cART regimens containing stavudine and zidovudine might help to prevent the development of lipoatrophy, but no strategies are proven to prevent lipohypertrophy.<sup>36,122</sup> For patients with established lipoatrophy, switching from cART regimens containing stavudine or zidovudine to an alternative cART regimen should be tried. Tesamorelin, a synthetic growth-hormone-releasing analogue, has been approved for treatment of lipohypertrophy. This agent reduces visceral adiposity<sup>125</sup> and liver fat<sup>126</sup>, but long term effects on metabolic and CHD risks remain uncertain.

### *Blood pressure, lipids, and blood sugar*

National, regional, and expert guidelines on the management of blood pressure, lipid concentrations, and blood sugar based on general population data tend to be extrapolated to patients with HIV infection, despite no specific validation in this group. CHD-event-driven randomized controlled trials to test strategies to reduce CHD risk in patients with HIV infection are, therefore, needed.

For management of hypertension in individuals infected with HIV, angiotensin-receptor blockers are of particular interest. HIV, particular antiretroviral drugs, or both seem to contribute to hyperactivation of the renin-angiotensin-aldosterone system<sup>37</sup>, with potential adverse effects on blood pressure and glucose homeostasis. Angiotensin-receptor blockers might, therefore, improve glucose homeostasis along with blood pressure, and also prevent microalbuminuria and renal dysfunction induced by HIV infection or use of cART. Whether angiotensin-receptor blockers lead to greater reductions in CHD events than other antihypertensive drugs in patients with HIV infection and hypertension is unknown.

Treatment strategies for dyslipidemia in people infected with HIV are particularly controversial. The pattern of dyslipidemia in patients with HIV infection who are receiving cART typically involves high triglyceride and low HDL cholesterol concentrations; LDL cholesterol levels are not

generally elevated.<sup>127</sup> Guidelines from the ACC/AHA no longer recommend lipidlowering treatment for non-HDL cholesterol as a cardioprotective strategy. Rather, the guidelines recommend that candidates for statin therapy be selected on the basis of LDL cholesterol levels in conjunction with cardiovascular disease history, diabetes history, and atherosclerotic cardiovascular disease risk score.<sup>128</sup> Given the potential for traditional cardiovascular risk scores to underestimate CHD risk in individuals infected with HIV, the question arises as to whether statins would reduce CHD risk in those not meeting criteria to receive them. Indeed, the NIH has funded the large, randomized, placebo-controlled REPRIEVE trial to assess the use of statins as a preventive therapy against CVD in patients infected with HIV and not meeting criteria to receive statins according to the ACC/AHA guidelines. This trial is anticipated to determine whether the use of statins reduces CHD risk in patients with HIV infection and low traditional risk scores, as well as the mechanisms for any observed effects.

Meanwhile, in the management of blood pressure, lipids, and blood sugar among patients with HIV infection, special care must be taken to avoid drug-drug interactions when treating patients with HIV infection. Some protease inhibitors, including ritonavir, inhibit cytochrome P<sub>450</sub> 3A<sub>4</sub>, which can lead to decreased metabolism of drugs by this enzyme, including statins (particularly simvastatin and, to a lesser extent, atorvastatin) and calcium-channel blockers (such as diltiazem). By contrast, some non-nucleoside reverse-transcriptase inhibitors, including efavirenz, induce cytochrome P<sub>450</sub> 3A<sub>4</sub>, which can have the opposite effect on drug metabolism.<sup>36,105,122</sup>

### Targeting proatherogenic immune activation

Early implementation of effective cART leads to improvement of immune function and inflammation in patients infected with HIV. The effect of early cART initiation on CHD risk should be determined by forthcoming results of the ongoing START trial.<sup>99</sup> Additional theoretically appealing strategies to address traditional metabolic risk factors and exert anti-inflammatory effects include aspirin and statins. In patients infected with HIV, aspirin decreases platelet aggregation and, importantly, lessens

activation of monocytes and T cells.<sup>129</sup> Nevertheless, preliminary data indicate that aspirin is less effective as a primary CHD prevention strategy in patients with HIV infection than in individuals without HIV.<sup>130</sup> According to US Preventive Services Task Force guidelines, aspirin is underused in the population with HIV<sup>131</sup>, but whether it should be prescribed more or less frequently remains uncertain.

Various other immune modulatory agents that do not modify traditional CHD risk factors are being studied as adjunctive therapies to reduce CHD risk in patients with HIV infection who are receiving cART. For instance, a US trial to examine the effects of methotrexate on immune activation and endothelial function in patients with HIV infection being treated with cART is underway.<sup>132</sup> The challenge with these agents will be to decrease proatherogenic immune activation without diminishing the immune defence against HIV.

Finally, interest has been shown in using the CCR5 antagonist maraviroc (approved for treatment of HIV infection with select subtype virions) as an antiatherogenic agent, given its potential to decrease monocyte recruitment to incipient coronary atheromas. Studies in mice suggest that it effectively reduces ritonavir-induced atherosclerotic plaque progression.<sup>133</sup>

## Conclusions

Further research is needed to unravel the complex pathophysiology of atherogenesis in people infected with HIV, to elucidate the relationships between traditional and immune CHD risk factors, and investigate how cART alters these interactions. Investigation into the genetic predisposition to develop CHD in those infected with HIV, and into the contributions of functional lipid changes, renal dysfunction, as well as behavioural factors, such as cocaine use, to CHD risk is also warranted. Finally, clinical studies to assess methods of risk prediction and risk-reduction strategies for CHD applicable to patients with HIV infection would be of great use. Such studies should be conducted both in resource-rich and resource-constrained countries. Research in these areas, combined with ongoing

global health efforts to make cART accessible to all patients who need it, will help patients with HIV infection to live full and healthy lives unencumbered by complications of CHD.

## Authors' contributions

MZ and JS researched data for the article.

All the authors discussed the content of the article, and wrote, reviewed, and edited the manuscript before submission.

## References

1. High, K. P. *et al.* HIV and aging: state of knowledge and areas of critical need for research. A report to the NIH Office of AIDS Research by the HIV and Aging Working Group. *J. Acquir. Immune Defic. Syndr.* 1999 **60** Suppl 1, S1-18 (2012).
2. Ingle, S. M. *et al.* Impact of risk factors for specific causes of death in the first and subsequent years of antiretroviral therapy among HIV-infected patients. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **59**, 287–297 (2014).
3. Greene, M., Justice, A. C., Lampiris, H. W. & Valcour, V. Management of human immunodeficiency virus infection in advanced age. *JAMA* **309**, 1397–1405 (2013).
4. Palella, F. J. *et al.* Mortality in the highly active antiretroviral therapy era: changing causes of death and disease in the HIV outpatient study. *J. Acquir. Immune Defic. Syndr.* 1999 **43**, 27–34 (2006).
5. Lewden, C. *et al.* Changes in causes of death among adults infected by HIV between 2000 and 2005: The 'Mortalité 2000 and 2005' surveys (ANRS EN19 and Mortavic). *J. Acquir. Immune Defic. Syndr.* 1999 **48**, 590–598 (2008).
6. Bendavid, E., Ford, N. & Mills, E. J. HIV and Africa's elderly: the problems and possibilities. *AIDS Lond. Engl.* **26** Suppl 1, S85-91 (2012).
7. Hontelez, J. A. C. *et al.* Ageing with HIV in South Africa. *AIDS Lond. Engl.* **25**, 1665–1667 (2011).
8. UNAIDS. AIDS by the numbers [online], [http://www.unaids.org/en/media/unaids/contentassets/documents/unaidspublication/2013/JC2571\\_AIDS\\_by\\_the\\_numbers\\_en.pdf](http://www.unaids.org/en/media/unaids/contentassets/documents/unaidspublication/2013/JC2571_AIDS_by_the_numbers_en.pdf) (2013).
9. UNAIDS. HIV and aging [online], [http://www.unaids.org/en/media/unaids/contentassets/documents/unaidspublication/2013/20131101\\_JC2563\\_hiv-and-aging\\_en.pdf](http://www.unaids.org/en/media/unaids/contentassets/documents/unaidspublication/2013/20131101_JC2563_hiv-and-aging_en.pdf) (2013).

10. WHO. HIV/AIDS: data and statistics [online], <http://www.who.int/hiv/data/en/> (2012).
11. Hasse, B. *et al.* Morbidity and aging in HIV-infected persons: the Swiss HIV cohort study. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **53**, 1130–1139 (2011).
12. Miller, C. J. *et al.* Adjudicated morbidity and mortality outcomes by age among individuals with HIV infection on suppressive antiretroviral therapy. *PLoS One* **9**, e95061 (2014).
13. Morlat, P. *et al.* Causes of death among HIV-infected patients in France in 2010 (national survey): trends since 2000. *AIDS Lond. Engl.* **28**, 1181–1191 (2014).
14. Antiretroviral Therapy Cohort Collaboration. Causes of death in HIV-1-infected patients treated with antiretroviral therapy, 1996–2006: collaborative analysis of 13 HIV cohort studies. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **50**, 1387–1396 (2010).
15. Freiberg, M. S. *et al.* HIV infection and the risk of acute myocardial infarction. *JAMA Intern. Med.* **173**, 614–622 (2013).
16. Hsu, J. C. *et al.* Atrial fibrillation and atrial flutter in human immunodeficiency virus-infected persons: incidence, risk factors, and association with markers of HIV disease severity. *J. Am. Coll. Cardiol.* **61**, 2288–2295 (2013).
17. Holloway, C. J. *et al.* Comprehensive cardiac magnetic resonance imaging and spectroscopy reveal a high burden of myocardial disease in HIV patients. *Circulation* **128**, 814–822 (2013).
18. Tseng, Z. H. *et al.* Sudden cardiac death in patients with human immunodeficiency virus infection. *J. Am. Coll. Cardiol.* **59**, 1891–1896 (2012).
19. Marcus, J. L. *et al.* HIV infection and incidence of ischemic stroke. *AIDS Lond. Engl.* **28**, 1911–1919 (2014).
20. Klein, D., Hurley, L. B., Quesenberry, C. P. & Sidney, S. Do protease inhibitors increase the risk for coronary heart disease in patients with HIV-1 infection? *J. Acquir. Immune Defic. Syndr.* **1999** **30**, 471–477 (2002).
21. Triant, V. A., Lee, H., Hadigan, C. & Grinspoon, S. K. Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease. *J. Clin. Endocrinol. Metab.* **92**, 2506–2512 (2007).
22. Lang, S. *et al.* Increased risk of myocardial infarction in HIV-infected patients in France, relative to the general population. *AIDS Lond. Engl.* **24**, 1228–1230 (2010).
23. Durand, M., Sheehy, O., Baril, J.-G., Leloir, J. & Tremblay, C. L. Association between HIV infection, antiretroviral therapy, and risk of acute myocardial infarction: a cohort and nested case-control study using Québec's public health insurance database. *J. Acquir. Immune Defic. Syndr.* **1999** **57**, 245–253 (2011).
24. Thienemann, F., Sliwa, K. & Rockstroh, J. K. HIV and the heart: the impact of antiretroviral therapy: a global perspective. *Eur. Heart J.* **34**, 3538–3546 (2013).
25. Lifson, A. R. *et al.* Smoking-related health risks among persons with HIV in the Strategies for Management of Antiretroviral Therapy clinical trial. *Am. J. Public Health* **100**, 1896–1903 (2010).

26. Friis-Møller, N. *et al.* Cardiovascular disease risk factors in HIV patients—association with antiretroviral therapy. Results from the DAD study. *AIDS Lond. Engl.* **17**, 1179–1193 (2003).
27. WHO. Prevalence of tobacco use among adults and adolescents [online], [http://gamapserver.who.int/gho/interactive\\_charts/tobacco/use/atlas.html](http://gamapserver.who.int/gho/interactive_charts/tobacco/use/atlas.html) (2009). (2009).
28. Waweru, P. *et al.* The prevalence of smoking and the knowledge of smoking hazards and smoking cessation strategies among HIV- positive patients in Johannesburg, South Africa. *South Afr. Med. J. Suid-Afr. Tydskr. Vir Geneesk.* **103**, 858–860 (2013).
29. Bloomfield, G. S. *et al.* Hypertension and obesity as cardiovascular risk factors among HIV seropositive patients in Western Kenya. *PLoS One* **6**, e22288 (2011).
30. Overton, E. T., Nurutdinova, D., Freeman, J., Seyfried, W. & Mondy, K. E. Factors associated with renal dysfunction within an urban HIV-infected cohort in the era of highly active antiretroviral therapy. *HIV Med.* **10**, 343–350 (2009).
31. Mocroft, A. *et al.* Deteriorating renal function and clinical outcomes in HIV-positive persons. *AIDS Lond. Engl.* **28**, 727–737 (2014).
32. Mulenga, L. B. *et al.* Baseline renal insufficiency and risk of death among HIV-infected adults on antiretroviral therapy in Lusaka, Zambia. *AIDS Lond. Engl.* **22**, 1821–1827 (2008).
33. Kalayjian, R. C. Renal issues in HIV infection. *Curr. HIV/AIDS Rep.* **8**, 164–171 (2011).
34. Grinspoon, S. & Carr, A. Cardiovascular risk and body-fat abnormalities in HIV-infected adults. *N. Engl. J. Med.* **352**, 48–62 (2005).
35. Stanley, T. L. & Grinspoon, S. K. Body composition and metabolic changes in HIV-infected patients. *J. Infect. Dis.* **205 Suppl 3**, S383–390 (2012).
36. Brown, T. T. & Glesby, M. J. Management of the metabolic effects of HIV and HIV drugs. *Nat. Rev. Endocrinol.* **8**, 11–21 (2012).
37. Lo, J., Looby, S. E. D., Wei, J., Adler, G. K. & Grinspoon, S. K. Increased aldosterone among HIV-infected women with visceral fat accumulation. *AIDS Lond. Engl.* **23**, 2366–2370 (2009).
38. Palacios, R. *et al.* Impact of highly active antiretroviral therapy on blood pressure in HIV-infected patients. A prospective study in a cohort of naive patients. *HIV Med.* **7**, 10–15 (2006).
39. Brown, T. T. *et al.* Antiretroviral therapy and the prevalence and incidence of diabetes mellitus in the multicenter AIDS cohort study. *Arch. Intern. Med.* **165**, 1179–1184 (2005).
40. Butt, A. A. *et al.* HIV infection and the risk of diabetes mellitus. *AIDS Lond. Engl.* **23**, 1227–1234 (2009).
41. Tien, P. C. *et al.* Antiretroviral therapy exposure and incidence of diabetes mellitus in the Women’s Interagency HIV Study. *AIDS Lond. Engl.* **21**, 1739–1745 (2007).
42. Capeau, J. *et al.* Ten-year diabetes incidence in 1046 HIV-infected patients

- started on a combination antiretroviral treatment. *AIDS Lond. Engl.* **26**, 303–314 (2012).
43. De Wit, S. *et al.* Incidence and risk factors for new-onset diabetes in HIV-infected patients: the Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) study. *Diabetes Care* **31**, 1224–1229 (2008).
  44. Fleischman, A. *et al.* Effects of a nucleoside reverse transcriptase inhibitor, stavudine, on glucose disposal and mitochondrial function in muscle of healthy adults. *Am. J. Physiol. Endocrinol. Metab.* **292**, E1666–1673 (2007).
  45. Hresko, R. C. & Hruz, P. W. HIV protease inhibitors act as competitive inhibitors of the cytoplasmic glucose binding site of GLUTs with differing affinities for GLUT1 and GLUT4. *PLoS One* **6**, e25237 (2011).
  46. Grunfeld, C. *et al.* Lipids, lipoproteins, triglyceride clearance, and cytokines in human immunodeficiency virus infection and the acquired immunodeficiency syndrome. *J. Clin. Endocrinol. Metab.* **74**, 1045–1052 (1992).
  47. Riddler, S. A. *et al.* Impact of HIV infection and HAART on serum lipids in men. *JAMA* **289**, 2978–2982 (2003).
  48. Parrinello, C. M. *et al.* Treatment-related changes in serum lipids and inflammation: clinical relevance remains unclear. Analyses from the Women's Interagency HIV study. *AIDS Lond. Engl.* **27**, 1516–1519 (2013).
  49. Lee, G. A. *et al.* The metabolic effects of lopinavir/ritonavir in HIV-negative men. *AIDS Lond. Engl.* **18**, 641–649 (2004).
  50. Aragonès, G. *et al.* Human immunodeficiency virus-infection induces major changes in high-density lipoprotein particle size distribution and composition: the effect of antiretroviral treatment and disease severity. *Clin. Chem. Lab. Med.* **48**, 1147–1152 (2010).
  51. Baker, J. V. *et al.* Inflammation predicts changes in high-density lipoprotein particles and apolipoprotein A1 following initiation of antiretroviral therapy. *AIDS Lond. Engl.* **25**, 2133–2142 (2011).
  52. Cailhol, J. *et al.* Prevalence of chronic kidney disease among people living with HIV/AIDS in Burundi: a cross-sectional study. *BMC Nephrol.* **12**, 40 (2011).
  53. Kalayjian, R. C. *et al.* Risk factors for chronic kidney disease in a large cohort of HIV-1 infected individuals initiating antiretroviral therapy in routine care. *AIDS Lond. Engl.* **26**, 1907–1915 (2012).
  54. Calvo-Sánchez, M. *et al.* Differences between HIV-infected and uninfected adults in the contributions of smoking, diabetes and hypertension to acute coronary syndrome: two parallel case-control studies. *HIV Med.* **14**, 40–48 (2013).
  55. Helleberg, M. *et al.* Mortality attributable to smoking among HIV-1-infected individuals: a nationwide, population-based cohort study. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **56**, 727–734 (2013).
  56. Worm, S. W. *et al.* Elevated triglycerides and risk of myocardial infarction in HIV-positive persons. *AIDS Lond. Engl.* **25**, 1497–1504 (2011).



57. Choi, A. I. *et al.* Association between kidney function and albuminuria with cardiovascular events in HIV-infected persons. *Circulation* **121**, 651–658 (2010).
58. George, E. *et al.* Kidney function and the risk of cardiovascular events in HIV-1-infected patients. *AIDS Lond. Engl.* **24**, 387–394 (2010).
59. Symmons, D. P. M. & Gabriel, S. E. Epidemiology of CVD in rheumatic disease, with a focus on RA and SLE. *Nat. Rev. Rheumatol.* **7**, 399–408 (2011).
60. Kaplan, R. C. *et al.* Potential cardiovascular disease risk markers among HIV-infected women initiating antiretroviral treatment. *J. Acquir. Immune Defic. Syndr.* **60**, 359–368 (2012).
61. Baker, J. *et al.* High-density lipoprotein particles and markers of inflammation and thrombotic activity in patients with untreated HIV infection. *J. Infect. Dis.* **201**, 285–292 (2010).
62. Mangili, A. *et al.* Lipoprotein-associated phospholipase A2, a novel cardiovascular inflammatory marker, in HIV-infected patients. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **58**, 893–900 (2014).
63. Ross, A. C. *et al.* Endothelial activation markers are linked to HIV status and are independent of antiretroviral therapy and lipodystrophy. *J. Acquir. Immune Defic. Syndr.* **49**, 499–506 (2008).
64. Beltrán, L. M. *et al.* Reduced sTWEAK and increased sCD163 levels in HIV-infected patients: modulation by antiretroviral treatment, HIV replication and HCV co-infection. *PLoS One* **9**, e90541 (2014).
65. Oliviero, U. *et al.* Human immunodeficiency virus per se exerts atherogenic effects. *Atherosclerosis* **204**, 586–589 (2009).
66. De Socio, G. V. L. *et al.* Relations between cardiovascular risk estimates and subclinical atherosclerosis in naive HIV patients: results from the HERMES study. *Int. J. STD AIDS* **21**, 267–272 (2010).
67. Stein, J. H. *et al.* Ultrasonographic measures of cardiovascular disease risk in antiretroviral treatment-naive individuals with HIV infection. *AIDS Lond. Engl.* **27**, 929–937 (2013).
68. Tebas, P. *et al.* Metabolic and immune activation effects of treatment interruption in chronic HIV-1 infection: implications for cardiovascular risk. *PLoS One* **3**, e2021 (2008).
69. Kuller, L. H. *et al.* Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. *PLoS Med.* **5**, e203 (2008).
70. Papasavvas, E. *et al.* Increased soluble vascular cell adhesion molecule-1 plasma levels and soluble intercellular adhesion molecule-1 during antiretroviral therapy interruption and retention of elevated soluble vascular cellular adhesion molecule-1 levels following resumption of antiretroviral therapy. *AIDS Lond. Engl.* **22**, 1153–1161 (2008).
71. Wolf, K. *et al.* Antiretroviral therapy reduces markers of endothelial and coagulation activation in patients infected with human immunodeficiency virus type 1. *J. Infect. Dis.* **185**, 456–462 (2002).

72. Francisci, D. *et al.* HIV type 1 infection, and not short-term HAART, induces endothelial dysfunction. *AIDS Lond. Engl.* **23**, 589–596 (2009).
73. Calmy, A. *et al.* HIV increases markers of cardiovascular risk: results from a randomized, treatment interruption trial. *AIDS Lond. Engl.* **23**, 929–939 (2009).
74. van Vonderen, M. G. A. *et al.* Increase in carotid artery intima-media thickness and arterial stiffness but improvement in several markers of endothelial function after initiation of antiretroviral therapy. *J. Infect. Dis.* **199**, 1186–1194 (2009).
75. Torriani, F. J. *et al.* Endothelial function in human immunodeficiency virus-infected antiretroviral-naive subjects before and after starting potent antiretroviral therapy: The ACTG (AIDS Clinical Trials Group) Study 5152s. *J. Am. Coll. Cardiol.* **52**, 569–576 (2008).
76. Sandler, N. G. & Douek, D. C. Microbial translocation in HIV infection: causes, consequences and treatment opportunities. *Nat. Rev. Microbiol.* **10**, 655–666 (2012).
77. Hatano, H. Immune activation and HIV persistence: considerations for novel therapeutic interventions. *Curr. Opin. HIV AIDS* **8**, 211–216 (2013).
78. Peters, L. *et al.* Hepatitis C virus viremia increases the incidence of chronic kidney disease in HIV-infected patients. *AIDS Lond. Engl.* **26**, 1917–1926 (2012).
79. Freiberg, M. S. *et al.* The association between hepatitis C infection and prevalent cardiovascular disease among HIV-infected individuals. *AIDS Lond. Engl.* **21**, 193–197 (2007).
80. Gillis, J. *et al.* Risk of cardiovascular disease associated with HCV and HBV coinfection among antiretroviral-treated HIV-infected individuals. *Antivir. Ther.* **19**, 309–317 (2014).
81. Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) Study Group *et al.* HBV or HCV coinfections and risk of myocardial infarction in HIV-infected individuals: the D:A:D Cohort Study. *Antivir. Ther.* **15**, 1077–1086 (2010).
82. Khovidhunkit, W. *et al.* Effects of infection and inflammation on lipid and lipoprotein metabolism: mechanisms and consequences to the host. *J. Lipid Res.* **45**, 1169–1196 (2004).
83. Rotger, M. *et al.* Contribution of genetic background, traditional risk factors, and HIV-related factors to coronary artery disease events in HIV-positive persons. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **57**, 112–121 (2013).
84. Joven, J. *et al.* The influence of HIV infection on the correlation between plasma concentrations of monocyte chemoattractant protein-1 and carotid atherosclerosis. *Clin. Chim. Acta Int. J. Clin. Chem.* **368**, 114–119 (2006).
85. Alonso-Villaverde, C. *et al.* Atherosclerosis in patients infected with HIV is influenced by a mutant monocyte chemoattractant protein-1 allele. *Circulation* **110**, 2204–2209 (2004).
86. Friis-Møller, N. *et al.* Combination antiretroviral therapy and the risk of myocardial infarction. *N. Engl. J. Med.* **349**, 1993–2003 (2003).

87. DAD Study Group *et al.* Class of antiretroviral drugs and the risk of myocardial infarction. *N. Engl. J. Med.* **356**, 1723–1735 (2007).
88. D:A:D Study Group *et al.* Use of nucleoside reverse transcriptase inhibitors and risk of myocardial infarction in HIV-infected patients enrolled in the D:A:D study: a multi-cohort collaboration. *Lancet Lond. Engl.* **371**, 1417–1426 (2008).
89. Worm, S. W. *et al.* Risk of myocardial infarction in patients with HIV infection exposed to specific individual antiretroviral drugs from the 3 major drug classes: the data collection on adverse events of anti-HIV drugs (D:A:D) study. *J. Infect. Dis.* **201**, 318–330 (2010).
90. Monforte, A. d'Arminio *et al.* Atazanavir is not associated with an increased risk of cardio- or cerebrovascular disease events. *AIDS Lond. Engl.* **27**, 407–415 (2013).
91. Lang, S. *et al.* Impact of individual antiretroviral drugs on the risk of myocardial infarction in human immunodeficiency virus-infected patients: a case-control study nested within the French Hospital Database on HIV ANRS cohort CO4. *Arch. Intern. Med.* **170**, 1228–1238 (2010).
92. Cruciani, M. *et al.* Abacavir use and cardiovascular disease events: a meta-analysis of published and unpublished data. *AIDS Lond. Engl.* **25**, 1993–2004 (2011).
93. Ding, X. *et al.* No association of abacavir use with myocardial infarction: findings of an FDA meta-analysis. *J. Acquir. Immune Defic. Syndr.* **1999** **61**, 441–447 (2012).
94. Sabin, C. A. *et al.* Is there continued evidence for an association between abacavir and myocardial infarction risk? [online], [http://www.natap.org/2014/CROI/croi\\_191.htm](http://www.natap.org/2014/CROI/croi_191.htm) (2014). (2014).
95. Costagliola, D., Lang, S., Mary-Krause, M. & Boccard, F. Abacavir and cardiovascular risk: reviewing the evidence. *Curr. HIV/AIDS Rep.* **7**, 127–133 (2010).
96. Strategies for Management of Antiretroviral Therapy (SMART) Study Group *et al.* CD4+ count-guided interruption of antiretroviral treatment. *N. Engl. J. Med.* **355**, 2283–2296 (2006).
97. Phillips, A. N. *et al.* Interruption of antiretroviral therapy and risk of cardiovascular disease in persons with HIV-1 infection: exploratory analyses from the SMART trial. *Antivir. Ther.* **13**, 177–187 (2008).
98. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV 1 infected adults and adolescents [online], <http://aidsinfo.nih.gov/contentfiles/lvguidelines/adultandadolescentgl.pdf> (2014).
99. US National Library of Medicine. ClinicalTrials.gov [online], <https://clinicaltrials.gov/ct2/show/NCT00867048> (2014). (2014).
100. Womack J. A. *et al.* HIV infection and the risk of cardiovascular disease in women [online], [http://www.natap.org/2014/CROI/croi\\_151.htm](http://www.natap.org/2014/CROI/croi_151.htm) (2014). (2014).
101. Hessamfar, M. *et al.* Severe morbidity according to sex in the era of combined antiretroviral therapy: the ANRS CO3 Aquitaine Cohort. *PLoS One* **9**, e102671 (2014).

102. Meier, A. *et al.* Sex differences in the Toll-like receptor-mediated response of plasmacytoid dendritic cells to HIV-1. *Nat. Med.* **15**, 955–959 (2009).
103. Hsue, P. Y. *et al.* Clinical features of acute coronary syndromes in patients with human immunodeficiency virus infection. *Circulation* **109**, 316–319 (2004).
104. Becker, A. C. *et al.* Acute coronary syndromes in treatment-naïve black South africans with human immunodeficiency virus infection. *J. Intervent. Cardiol.* **23**, 70–77 (2010).
105. Boccaro, F. *et al.* HIV and coronary heart disease: time for a better understanding. *J. Am. Coll. Cardiol.* **61**, 511–523 (2013).
106. Burdo, T. H. *et al.* Soluble CD163, a novel marker of activated macrophages, is elevated and associated with noncalcified coronary plaque in HIV-infected patients. *J. Infect. Dis.* **204**, 1227–1236 (2011).
107. Zanni, M. V. *et al.* Increased coronary atherosclerotic plaque vulnerability by coronary computed tomography angiography in HIV-infected men. *AIDS Lond. Engl.* **27**, 1263–1272 (2013).
108. Voros, S. *et al.* Coronary atherosclerosis imaging by coronary CT angiography: current status, correlation with intravascular interrogation and meta-analysis. *JACC Cardiovasc. Imaging* **4**, 537–548 (2011).
109. Post, W. S. *et al.* Associations between HIV infection and subclinical coronary atherosclerosis. *Ann. Intern. Med.* **160**, 458–467 (2014).
110. Motoyama, S. *et al.* Computed tomographic angiography characteristics of atherosclerotic plaques subsequently resulting in acute coronary syndrome. *J. Am. Coll. Cardiol.* **54**, 49–57 (2009).
111. Rudd, J. H. F. *et al.* Imaging atherosclerotic plaque inflammation with [18F]-fluorodeoxyglucose positron emission tomography. *Circulation* **105**, 2708–2711 (2002).
112. Subramanian, S. *et al.* Arterial inflammation in patients with HIV. *JAMA* **308**, 379–386 (2012).
113. Figueroa, A. L. *et al.* Measurement of arterial activity on routine FDG PET/CT images improves prediction of risk of future CV events. *JACC Cardiovasc. Imaging* **6**, 1250–1259 (2013).
114. Tawakol, A. *et al.* Increased arterial inflammation relates to high-risk coronary plaque morphology in HIV-infected patients. *J. Acquir. Immune Defic. Syndr.* **1999** **66**, 164–171 (2014).
115. Longenecker, C. T. *et al.* Perivascular fat, inflammation, and cardiovascular risk in HIV-infected patients on antiretroviral therapy. *Int. J. Cardiol.* **168**, 4039–4045 (2013).
116. Brener, M. *et al.* Epicardial fat is associated with duration of antiretroviral therapy and coronary atherosclerosis. *AIDS Lond. Engl.* **28**, 1635–1644 (2014).
117. Chakko, S. & Myerburg, R. J. Cardiac complications of cocaine abuse. *Clin. Cardiol.* **18**, 67–72 (1995).

118. Lai, S. *et al.* Human immunodeficiency virus 1 infection, cocaine, and coronary calcification. *Arch. Intern. Med.* **165**, 690–695 (2005).
119. Lai, S. *et al.* Long-term combination antiretroviral therapy is associated with the risk of coronary plaques in African Americans with HIV infection. *AIDS Patient Care STDs* **23**, 815–824 (2009).
120. D'Agostino, R. B. Cardiovascular risk estimation in 2012: lessons learned and applicability to the HIV population. *J. Infect. Dis.* **205 Suppl 3**, S362–367 (2012).
121. Friis-Møller, N. *et al.* Predicting the risk of cardiovascular disease in HIV-infected patients: the data collection on adverse effects of anti-HIV drugs study. *Eur. J. Cardiovasc. Prev. Rehabil. Off. J. Eur. Soc. Cardiol. Work. Groups Epidemiol. Prev. Card. Rehabil. Exerc. Physiol.* **17**, 491–501 (2010).
122. European AIDS Clinical Society (EACS). European AIDS Clinical Society: Guidelines version 7.02 [online], [http://eacsociety.org/Portals/0/140601\\_EACS%20EN7.02.pdf](http://eacsociety.org/Portals/0/140601_EACS%20EN7.02.pdf) (2014).
123. Aberg, J. A. *et al.* Primary care guidelines for the management of persons infected with HIV: 2013 update by the HIV Medicine Association of the Infectious Diseases Society of America. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **58**, 1–10 (2014).
124. Cropsey, K. L. *et al.* A pilot study of screening, brief intervention, and referral for treatment (SBIRT) in non-treatment seeking smokers with HIV. *Addict. Behav.* **38**, 2541–2546 (2013).
125. Falutz, J. *et al.* Metabolic effects of a growth hormone-releasing factor in patients with HIV. *N. Engl. J. Med.* **357**, 2359–2370 (2007).
126. Stanley, T. L. *et al.* Effect of tesamorelin on visceral fat and liver fat in HIV-infected patients with abdominal fat accumulation: a randomized clinical trial. *JAMA* **312**, 380–389 (2014).
127. Riddler, S. A. *et al.* Impact of HIV infection and HAART on serum lipids in men. *JAMA* **289**, 2978–2982 (2003).
128. Stone, N. J. *et al.* 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J. Am. Coll. Cardiol.* **63**, 2889–2934 (2014).
129. O'Brien, M. *et al.* Aspirin attenuates platelet activation and immune activation in HIV-1-infected subjects on antiretroviral therapy: a pilot study. *J. Acquir. Immune Defic. Syndr.* **1999 63**, 280–288 (2013).
130. Suchindran, S. R. S., Meigs, J., Grinspoon, S. & Triant, V. Comparison of aspirin use and incident myocardial infarction rates in HIV+ and HIV- patients in a large US healthcare system [abstract]. Presented at the Conference on Retroviruses and Opportunistic Infections (2013). (2013).
131. Burkholder, G. A. *et al.* Underutilization of aspirin for primary prevention of cardiovascular disease among HIV-infected patients. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **55**, 1550–1557 (2012).

132. US National Library of Medicine. ClinicalTrials.gov [online], <https://clinicaltrials.gov/ct2/show/NCT01949116> (2014). (2014).
133. Cipriani, S. *et al.* Efficacy of the CCR5 antagonist maraviroc in reducing early, ritonavir-induced atherogenesis and advanced plaque progression in mice. *Circulation* **127**, 2114–2124 (2013).
134. Mengozzi, M. *et al.* Human immunodeficiency virus replication induces monocyte chemotactic protein-1 in human macrophages and U937 promonocytic cells. *Blood* **93**, 1851–1857 (1999).
135. Westhorpe, C. L. V. *et al.* Effects of HIV-1 infection in vitro on transendothelial migration by monocytes and monocyte-derived macrophages. *J. Leukoc. Biol.* **85**, 1027–1035 (2009).
136. Green, D. F., Resnick, L. & Bourgoignie, J. J. HIV infects glomerular endothelial and mesangial but not epithelial cells in vitro. *Kidney Int.* **41**, 956–960 (1992).
137. Conaldi, P. G. *et al.* Productive HIV-1 infection of human vascular endothelial cells requires cell proliferation and is stimulated by combined treatment with interleukin-1 beta plus tumor necrosis factor-alpha. *J. Med. Virol.* **47**, 355–363 (1995).
138. Takano, Y., Shimokado, K., Hata, Y. & Yoshida, M. HIV envelope protein gp120-triggered CD4+ T-cell adhesion to vascular endothelium is regulated via CD4 and CXCR4 receptors. *Biochim. Biophys. Acta* **1772**, 549–555 (2007).
139. Jiang, J. *et al.* HIV gp120 induces endothelial dysfunction in tumour necrosis factor-alpha-activated porcine and human endothelial cells. *Cardiovasc. Res.* **87**, 366–374 (2010).
140. Ren, Z., Yao, Q. & Chen, C. HIV-1 envelope glycoprotein 120 increases intercellular adhesion molecule-1 expression by human endothelial cells. *Lab. Invest. J. Tech. Methods Pathol.* **82**, 245–255 (2002).
141. Huang, M. B., Hunter, M. & Bond, V. C. Effect of extracellular human immunodeficiency virus type 1 glycoprotein 120 on primary human vascular endothelial cell cultures. *AIDS Res. Hum. Retroviruses* **15**, 1265–1277 (1999).
142. Green, L. A. *et al.* HIV envelope protein gp120-induced apoptosis in lung microvascular endothelial cells by concerted upregulation of EMAP II and its receptor, CXCR3. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **306**, L372–382 (2014).
143. Olivetta, E. *et al.* HIV-1 Nef induces the release of inflammatory factors from human monocyte/macrophages: involvement of Nef endocytotic signals and NF-kappa B activation. *J. Immunol. Baltim. Md 1950* **170**, 1716–1727 (2003).
144. Percario, Z. *et al.* Human immunodeficiency virus type 1 (HIV-1) Nef activates STAT3 in primary human monocyte/macrophages through the release of soluble factors: involvement of Nef domains interacting with the cell endocytotic machinery. *J. Leukoc. Biol.* **74**, 821–832 (2003).
145. Mujawar, Z. *et al.* Human immunodeficiency virus impairs reverse cholesterol transport from macrophages. *PLoS Biol.* **4**, e365 (2006).
146. Mujawar, Z. *et al.* Mutation of the ATP cassette binding transporter A1 (ABCA1)

- C-terminus disrupts HIV-1 Nef binding but does not block the Nef enhancement of ABCA1 protein degradation. *Biochemistry (Mosc.)* **49**, 8338–8349 (2010).
147. Cui, H. L. *et al.* HIV-1 Nef mobilizes lipid rafts in macrophages through a pathway that competes with ABCA1-dependent cholesterol efflux. *J. Lipid Res.* **53**, 696–708 (2012).
148. Park, I.-W. & He, J. J. HIV-1 Nef-mediated inhibition of T cell migration and its molecular determinants. *J. Leukoc. Biol.* **86**, 1171–1178 (2009).
149. Duffy, P., Wang, X., Lin, P. H., Yao, Q. & Chen, C. HIV Nef protein causes endothelial dysfunction in porcine pulmonary arteries and human pulmonary artery endothelial cells. *J. Surg. Res.* **156**, 257–264 (2009).
150. Wang, T. *et al.* Transfer of intracellular HIV Nef to endothelium causes endothelial dysfunction. *PLoS One* **9**, e91063 (2014).
151. Acheampong, E. A. *et al.* Human Immunodeficiency virus type 1 Nef potentially induces apoptosis in primary human brain microvascular endothelial cells via the activation of caspases. *J. Virol.* **79**, 4257–4269 (2005).
152. Zauli, G. *et al.* Human immunodeficiency virus type 1 (HIV-1) tat-protein stimulates the production of interleukin-6 (IL-6) by peripheral blood monocytes. *New Microbiol.* **16**, 115–120 (1993).
153. Gibellini, D. *et al.* Recombinant human immunodeficiency virus type-1 (HIV-1) Tat protein sequentially up-regulates IL-6 and TGF-beta 1 mRNA expression and protein synthesis in peripheral blood monocytes. *Br. J. Haematol.* **88**, 261–267 (1994).
154. Bennasser, Y., Badou, A., Tkaczuk, J. & Bahraoui, E. Signaling pathways triggered by HIV-1 Tat in human monocytes to induce TNF-alpha. *Virology* **303**, 174–180 (2002).
155. Ben Haij, N., Leghmari, K., Planès, R., Thieblemont, N. & Bahraoui, E. HIV-1 Tat protein binds to TLR4-MD2 and signals to induce TNF- $\alpha$  and IL-10. *Retrovirology* **10**, 123 (2013).
156. Mitola, S. *et al.* Tat-human immunodeficiency virus-1 induces human monocyte chemotaxis by activation of vascular endothelial growth factor receptor-1. *Blood* **90**, 1365–1372 (1997).
157. Weiss, J. M., Nath, A., Major, E. O. & Berman, J. W. HIV-1 Tat induces monocyte chemoattractant protein-1-mediated monocyte transmigration across a model of the human blood-brain barrier and up-regulates CCR5 expression on human monocytes. *J. Immunol. Baltim. Md 1950* **163**, 2953–2959 (1999).
158. Park, I. W., Wang, J. F. & Groopman, J. E. HIV-1 Tat promotes monocyte chemoattractant protein-1 secretion followed by transmigration of monocytes. *Blood* **97**, 352–358 (2001).
159. Dhawan, S. *et al.* Human immunodeficiency virus-1-tat protein induces the cell surface expression of endothelial leukocyte adhesion molecule-1, vascular cell adhesion molecule-1, and intercellular adhesion molecule-1 in human endothelial cells. *Blood* **90**, 1535–1544 (1997).

160. Duan, M. *et al.* HIV Tat induces expression of ICAM-1 in HUVECs: implications for miR-221/-222 in HIV-associated cardiomyopathy. *PLoS One* **8**, e60170 (2013).
161. Liu, K. *et al.* HIV-1 Tat protein-induced VCAM-1 expression in human pulmonary artery endothelial cells and its signaling. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **289**, L252-260 (2005).
162. Cota-Gomez, A. *et al.* The human immunodeficiency virus-1 Tat protein activates human umbilical vein endothelial cell E-selectin expression via an NF-kappa B-dependent mechanism. *J. Biol. Chem.* **277**, 14390–14399 (2002).
163. Matzen, K. *et al.* HIV-1 Tat increases the adhesion of monocytes and T-cells to the endothelium in vitro and in vivo: implications for AIDS-associated vasculopathy. *Virus Res.* **104**, 145–155 (2004).
164. Wang, J., Zhang, W., Nardi, M. A. & Li, Z. HIV-1 Tat-induced platelet activation and release of CD154 contribute to HIV-1-associated autoimmune thrombocytopenia. *J. Thromb. Haemost. JTH* **9**, 562–573 (2011).
165. Cognasse, F. *et al.* Altered release of regulated upon activation, normal T-cell expressed and secreted protein from human, normal platelets: contribution of distinct HIV-1MN gp41 peptides. *AIDS Lond. Engl.* **23**, 2057–2059 (2009).
166. Wang, X. *et al.* Human immunodeficiency virus protease inhibitor ritonavir inhibits cholesterol efflux from human macrophage-derived foam cells. *Am. J. Pathol.* **171**, 304–314 (2007).
167. Wang, X., Liao, D., Lin, P. H., Yao, Q. & Chen, C. Highly active antiretroviral therapy drugs inhibit in vitro cholesterol efflux from human macrophage-derived foam cells. *Lab. Investig. J. Tech. Methods Pathol.* **89**, 1355–1363 (2009).
168. Conklin, B. S. *et al.* HIV protease inhibitor ritonavir decreases endothelium-dependent vasorelaxation and increases superoxide in porcine arteries. *Cardiovasc. Res.* **63**, 168–175 (2004).
169. Fu, W., Chai, H., Yao, Q. & Chen, C. Effects of HIV protease inhibitor ritonavir on vasomotor function and endothelial nitric oxide synthase expression. *J. Acquir. Immune Defic. Syndr.* **39**, 152–158 (2005).
170. Zhong, D. *et al.* HIV protease inhibitor ritonavir induces cytotoxicity of human endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* **22**, 1560–1566 (2002).
171. Baum, P. D., Sullam, P. M., Stoddart, C. A. & McCune, J. M. Abacavir increases platelet reactivity via competitive inhibition of soluble guanylyl cyclase. *AIDS Lond. Engl.* **25**, 2243–2248 (2011).
172. Orden, S. *et al.* Efavirenz induces interactions between leucocytes and endothelium through the activation of Mac-1 and gp150,95. *J. Antimicrob. Chemother.* **69**, 995–1004 (2014).
173. Thieblemont, N., Weiss, L., Sadeghi, H. M., Estcourt, C. & Haeflner-Cavaillon, N. CD14<sup>low</sup>CD16<sup>high</sup>: a cytokine-producing monocyte subset which expands during human immunodeficiency virus infection. *Eur. J. Immunol.* **25**, 3418–3424 (1995).
174. Funderburg, N. T. *et al.* Increased tissue factor expression on circulating mono-




- cytes in chronic HIV infection: relationship to in vivo coagulation and immune activation. *Blood* **115**, 161–167 (2010).
175. Palmer, S. & Hamblin, A. S. Increased CD11/CD18 expression on the peripheral blood leucocytes of patients with HIV disease: relationship to disease severity. *Clin. Exp. Immunol.* **93**, 344–349 (1993).
  176. Hearps, A. C. *et al.* HIV infection induces age-related changes to monocytes and innate immune activation in young men that persist despite combination antiretroviral therapy. *AIDS Lond. Engl.* **26**, 843–853 (2012).
  177. Feeney, E. R. *et al.* The expression of cholesterol metabolism genes in monocytes from HIV-infected subjects suggests intracellular cholesterol accumulation. *J. Infect. Dis.* **207**, 628–637 (2013).
  178. Hunt, P. W. *et al.* T cell activation is associated with lower CD4+ T cell gains in human immunodeficiency virus-infected patients with sustained viral suppression during antiretroviral therapy. *J. Infect. Dis.* **187**, 1534–1543 (2003).
  179. Wolthers, K. C. *et al.* T cell telomere length in HIV-1 infection: no evidence for increased CD4+ T cell turnover. *Science* **274**, 1543–1547 (1996).
  180. Holme, P. A. *et al.* Enhanced activation of platelets with abnormal release of RANTES in human immunodeficiency virus type 1 infection. *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.* **12**, 79–89 (1998).
  181. Funderburg, N. T. *et al.* Shared monocyte subset phenotypes in HIV-1 infection and in uninfected subjects with acute coronary syndrome. *Blood* **120**, 4599–4608 (2012).
  182. Kaplan, R. C. *et al.* T cell activation and senescence predict subclinical carotid artery disease in HIV-infected women. *J. Infect. Dis.* **203**, 452–463 (2011).
  183. Mayne, E. *et al.* Increased platelet and microparticle activation in HIV infection: upregulation of P-selectin and tissue factor expression. *J. Acquir. Immune Defic. Syndr.* **1999** **59**, 340–346 (2012).
  184. Zetterberg, E. *et al.* Platelet count kinetics following interruption of antiretroviral treatment. *AIDS Lond. Engl.* **27**, 59–68 (2013).
  185. von Hentig, N. *et al.* Platelet-leucocyte adhesion markers before and after the initiation of antiretroviral therapy with HIV protease inhibitors. *J. Antimicrob. Chemother.* **62**, 1118–1121 (2008).
  186. Falcinelli, E. *et al.* In vivo platelet activation and platelet hyperreactivity in abacavir-treated HIV-infected patients. *Thromb. Haemost.* **110**, 349–357 (2013).
  187. Papasavvas, E. *et al.* Delayed loss of control of plasma lipopolysaccharide levels after therapy interruption in chronically HIV-1-infected patients. *AIDS Lond. Engl.* **23**, 369–375 (2009).
  188. Ananworanich, J. *et al.* Recurring thrombocytopenia associated with structured treatment interruption in patients with human immunodeficiency virus infection. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **37**, 723–725 (2003).
  189. Kaplan, R. C. *et al.* Low CD4+ T-cell count as a major atherosclerosis risk factor in HIV-infected women and men. *AIDS Lond. Engl.* **22**, 1615–1624 (2008).

190. Kelesidis, T., Kendall, M. A., Yang, O. O., Hodis, H. N. & Currier, J. S. Biomarkers of microbial translocation and macrophage activation: association with progression of subclinical atherosclerosis in HIV-1 infection. *J. Infect. Dis.* **206**, 1558–1567 (2012).
191. Merlini, E. *et al.* T-cell phenotypes, apoptosis and inflammation in HIV+ patients on virologically effective cART with early atherosclerosis. *PLoS One* **7**, e46073 (2012).
192. Daar, E.S. *et al.* Monocyte but not cellular activation is associated with coronary atherosclerosis in the MACS. Presented at the Conference on Retroviruses and Opportunistic Infections 2014. (2014).
193. Hsue, P. Y. *et al.* Increased carotid intima-media thickness in HIV patients is associated with increased cytomegalovirus-specific T-cell responses. *AIDS Lond. Engl.* **20**, 2275–2283 (2006).
194. Sacre, K. *et al.* A role for cytomegalovirus-specific CD4+CX3CR1+ T cells and cytomegalovirus-induced T-cell immunopathology in HIV-associated atherosclerosis. *AIDS Lond. Engl.* **26**, 805–814 (2012).
195. Ross, A. C. *et al.* Relationship between inflammatory markers, endothelial activation markers, and carotid intima-media thickness in HIV-infected patients receiving antiretroviral therapy. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **49**, 1119–1127 (2009).
196. Parra, S. *et al.* Nonconcordance between subclinical atherosclerosis and the calculated Framingham risk score in HIV-infected patients: relationships with serum markers of oxidation and inflammation. *HIV Med.* **11**, 225–231 (2010).
197. Longenecker, C. T. *et al.* Markers of inflammation and CD8 T-cell activation, but not monocyte activation, are associated with subclinical carotid artery disease in HIV-infected individuals. *HIV Med.* **14**, 385–390 (2013).
198. Ross Eckard, A. *et al.* Lipoprotein-associated phospholipase A2 and cardiovascular disease risk in HIV infection. *HIV Med.* **15**, 537–546 (2014).
199. Lo, J. *et al.* Increased prevalence of subclinical coronary atherosclerosis detected by coronary computed tomography angiography in HIV-infected men. *AIDS Lond. Engl.* **24**, 243–253 (2010).
200. Zanni, M. V. *et al.* HDL redox activity is increased in HIV-infected men in association with macrophage activation and noncalcified coronary atherosclerotic plaque. *Antivir. Ther.* <http://dx.doi.org/10.3851/IMP2756>. (2014).
201. Triant, V. A. *et al.* Association of immunologic and virologic factors with myocardial infarction rates in a US healthcare system. *J. Acquir. Immune Defic. Syndr.* **55**, 615–619 (2010).
202. Lichtenstein, K. A. *et al.* Low CD4+ T cell count is a risk factor for cardiovascular disease events in the HIV outpatient study. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **51**, 435–447 (2010).
203. Drozd D. R. *et al.* Lower CD4 count and higher viral load are associated with

- increased risk of myocardial infarction. Presented at the Conference on Retroviruses and Opportunistic Infections 2014. (2014).
204. Silverberg, M. J. *et al.* Immunodeficiency and risk of myocardial infarction among HIV-positive individuals with access to care. *J. Acquir. Immune Defic. Syndr.* 1999 **65**, 160–166 (2014).
  205. Lang, S. *et al.* HIV replication and immune status are independent predictors of the risk of myocardial infarction in HIV-infected individuals. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **55**, 600–607 (2012).
  206. Sabin, C. A. *et al.* Associations between immune depression and cardiovascular events in HIV infection. *AIDS Lond. Engl.* **27**, 2735–2748 (2013).
  207. Sandler, N. G. *et al.* Plasma levels of soluble CD14 independently predict mortality in HIV infection. *J. Infect. Dis.* **203**, 780–790 (2011).
  208. Duprez, D. A. *et al.* Inflammation, coagulation and cardiovascular disease in HIV-infected individuals. *PLoS One* **7**, e44454 (2012).
  209. Triant, V. A., Meigs, J. B. & Grinspoon, S. K. Association of C-reactive protein and HIV infection with acute myocardial infarction. *J. Acquir. Immune Defic. Syndr.* 1999 **51**, 268–273 (2009).
  210. Ford, E. S. *et al.* Traditional risk factors and D-dimer predict incident cardiovascular disease events in chronic HIV infection. *AIDS Lond. Engl.* **24**, 1509–1517 (2010).
  211. Duprez, D. A. *et al.* Lipoprotein particle subclasses, cardiovascular disease and HIV infection. *Atherosclerosis* **207**, 524–529 (2009).





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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## Abstract

### 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 (standard deviation (SD), 1.14) vs. 1.0 (SD, 0.95),  $P<0.001$ ), with significantly more HIV-infected participants 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-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 (OR) 1.58, 95% confidence interval (CI) 1.23-2.03,  $P<0.001$ ). In those with HIV, longer exposure to CD4+ T-cell counts  $<200$  cells/mm<sup>3</sup>, 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.

## Background

AIDS-associated morbidity and mortality have dramatically declined with the advent of combination antiretroviral therapy (cART).<sup>1-3</sup> However, the life expectancy of individuals infected with human immunodeficiency virus (HIV) on average remains shorter than for the general population<sup>3-5</sup>, and non-AIDS comorbidities have gained increasing importance as causes of death in cART-treated patients.<sup>2,3,6,7</sup> As HIV-infected individuals on cART age, they increasingly experience non-AIDS comorbidities<sup>7,8</sup>, which in HIV may be both accentuated and/or accelerated, thereby possibly occurring at younger ages.<sup>8-10</sup> 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.<sup>11-16</sup>

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.<sup>7,9,17</sup> 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.<sup>18</sup> 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 ageing-associated noncommunicable comorbidities (AANCCs) and organ dysfunction among HIV-type-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 groups, and analysed 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.<sup>19</sup> To ensure comparability of the HIV-infected and HIV-uninfected studygroups, we regularly monitored age, sex, and ethnicity in both studygroups, and adjusted enrolment of underrepresented categories among HIV-uninfected participants accordingly.

All participants were aged  $\geq 45$  years with laboratory-confirmed presence or absence of HIV infection. All subjects who provided written informed consent within the 2-year enrolment 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, two years later, and depending on sufficient resources every two 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/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 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.<sup>20</sup> 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  $\geq 90$  mmHg and/or systolic blood pressure  $\geq 140$  mmHg in all three measurements (Omron 705IT) with a 1-minute interval, or if on antihypertensive medication<sup>21</sup>; diabetes mellitus type 2 if hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) (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), or if on antidiabetic medication<sup>22</sup>; obstructive pulmonary disease if 1-second forced expiratory volume (FEV<sub>1</sub>) to forced vital capacity (FVC) ratio was  $< 0.7$  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<sup>23</sup>; impaired renal function if estimated glomerular filtration rate (eGFR) 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 densit-

ometers, 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.<sup>24,25</sup>

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-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.<sup>26</sup>

### Statistical analysis

Study groups were compared using the  $\chi^2$ , 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 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 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 nonsmokers. 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

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 studygroups 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%,  $P < 0.001$  and 7.4% vs. 1.3%,  $P < 0.001$ , respectively). Significantly fewer controls 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 3.1**). No statistically significant difference in age distribution was found between the two studygroups.

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 plasma viral loads. The majority had experienced immune recovery on treatment, with a median nadir CD4<sup>+</sup> T-cell count of 180 cells/mm<sup>3</sup> and current median CD4<sup>+</sup> T-cell count of 565 cells/mm<sup>3</sup> (**Table 3.1**).

Significantly more HIV-infected participants were current smokers

**Table 3.1: Baseline demographic and HIV-related characteristics**

Characteristic	HIV-uninfected participants (n=524)	HIV-infected participants (n=540)	P value
Age, years	52.1 (47.9-58.3)	52.9 (48.3-59.6)	0.200*
Male gender	85.1%	88.1%	0.146**
Dutch origin	81.3%	72.2%	<0.001**
African origin	1.3%	7.4%	<0.001**
MSM <sup>a</sup>	69.7%	73.9%	0.125**
Hepatitis C RNA positive	0.8%	2.8%	0.029**
Hepatitis B antigen and/or hepatitis B DNA positive	0.6%	3.9%	<0.001**
Time since HIV diagnosis, years	-	12.1 (6.2-17.1)	-
Diagnosed with HIV prior to 1996	-	32.8%	-
CD4+ T-cell count at enrolment, cells/mm <sup>3</sup>	-	565 (435-745)	-
Nadir CD4+ T-cell count, cells/mm <sup>3</sup>	-	180 (78-260)	-
Known cumulative duration of CD4+ T-cell count <200 cells/mm <sup>3</sup> , months	-	0.8 (0.0-9.6)	-
Plasma viral load >200 copies/mL among cART-treated participants within 4 months before or at enrolment <sup>b</sup>	-	1.5%	-
Last plasma viral load within 4 months before or at enrolment, log <sub>10</sub> copies/mL <sup>b</sup>	-	1.6 (1.6-1.6)	-
Duration of plasma viral load ≤200 copies/mL, years <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, 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 are presented as median (interquartile range) or percentage.

Abbreviations: ART=antiretroviral therapy, cART=combination antiretroviral therapy, HIV=human immunodeficiency virus, MSM=men who have sex with men.

*a* Male participants who stated in the questionnaire to feel mostly or exclusively sexually attracted to men.

*b* Only a plasma HIV load that was measured ≤4 months prior to enrolment was used. If such a recent test result was not available, plasma HIV load was measured at enrolment.

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

*d* Previous AIDS-defining condition following the US Centers for Disease Control and Prevention classification.

*e* Combination of ≥3 antiretroviral drugs, other than ritonavir used as a booster.

\* Wilcoxon rank-sum test

\*\*  $\chi^2$  test

(32.0% vs. 24.6%,  $P=0.007$ ), whereas ecstasy use was significantly more prevalent among controls (4.3% vs. 8.6%,  $P=0.004$ ) (**Table 3.2**). Body mass index (BMI) was lower (24.2 (interquartile range (IQR), 22.3-26.6) vs. 24.5 (IQR, 22.8-27.0)  $\text{kg/m}^2$ ,  $P=0.019$ ) and above-normal waist-to-hip ratio was significantly more prevalent among HIV-infected participants (84.0% vs. 62.4%,  $P<0.001$ ). 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-infected participants. Significantly fewer HIV-infected participants were physically active (44.3% vs. 53.0%,  $P=0.005$ ) and they had significantly lower levels of 25-hydroxy vitamin D<sub>2</sub>+D<sub>3</sub> (47 (IQR, 29-71) vs. 54 (IQR, 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-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.

HIV-infected individuals had a significantly higher mean number of AANCCs than controls (1.3 (SD, 1.14) vs. 1.0 (SD, 0.95),  $P<0.001$ ). The proportion of participants with  $\geq 1$  AANCCs was also significantly higher among those with HIV (69.4% vs. 61.8%,  $P=0.009$ ).

The mean number of AANCCs within the 50-54, 60-64, and  $\geq 65$  age categories was significantly higher among HIV-infected than HIV-uninfected participants (**Figure 3.1**). 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 five years older.

Each individual AANCC was numerically more prevalent among HIV-infected participants, with hypertension (45.4% vs. 30.5%,  $P<0.001$ ), myocardial infarction (3.9% vs. 1.5%,  $P=0.018$ ), peripheral arterial disease

**Table 3.2: Prevalence of comorbidity risk factors**

Characteristic	HIV-uninfected participants (n=524)	HIV-infected participants (n=540)	P value
Smoking status			
Never smoked	36.5%	33.0%	0.028*
Ever smoked	38.9%	35.0%	
Currently smoking <sup>a</sup>	24.6%	32.0%	
Packyears of smoking among ever-smokers	15.0 (4.5-28.8)	22.2 (7.8-36.8)	0.001**
Severe alcohol use <sup>b</sup>	7.3%	4.8%	0.098***
Daily to monthly use of cannabis	11.6%	13.5%	0.356***
Daily to monthly use of cocaine	2.9%	3.7%	0.442***
Daily to monthly use of ecstasy	8.6%	4.3%	0.004***
BMI, kg/m <sup>2</sup>	24.5 (22.8-27.0)	24.2 (22.3-26.6)	0.019**
BMI categories, kg/m <sup>2</sup>			
<20	3.3%	8.2%	0.121*
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%	<0.001***
Blood pressure, systolic, mmHg	133 (125-143)	135 (126-147)	0.006****
Blood pressure, diastolic, mmHg	79 (72-85)	81 (75-89)	<0.001****
Positive family history for myocardial infarction, hypertension, diabetes mellitus type 2, or hypercholesterolemia <sup>d</sup>	66.5%	70.8%	0.139***
Physical activity <sup>e</sup>	53.0%	44.3%	0.005***
25-hydroxy vitamin D <sub>2</sub> +D <sub>3</sub> , nmol/L	54 (39-72)	47 (29-71)	<0.001**

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

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

*a* Smoked during the last month before completing the questionnaire.

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

*c* If ≥0.9 in males and ≥0.85 in females.

*d* 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.

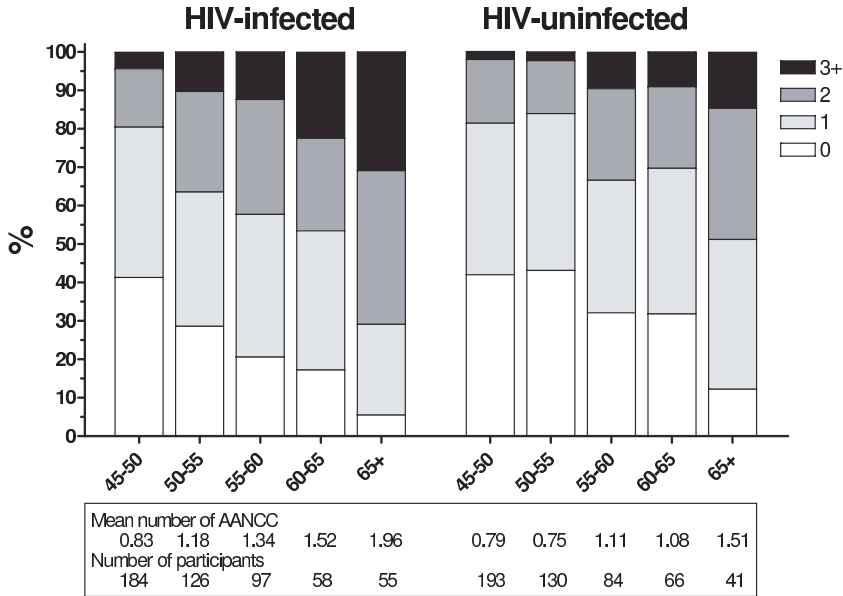
*e* Physical activity was defined following the Dutch guidelines for healthy physical activity ('Combinorm'): at least 5 days per week at least 30 minutes of moderate physical activity or at least twice per week at least 20 minutes of heavy physical activity.

\* Nonparametric test for trend

\*\* Wilcoxon rank-sum test

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

\*\*\*\* Student's t test



**Figure 3.1: Distribution of the number of age-associated noncommunicable comorbidities stratified by age across both studygroups**

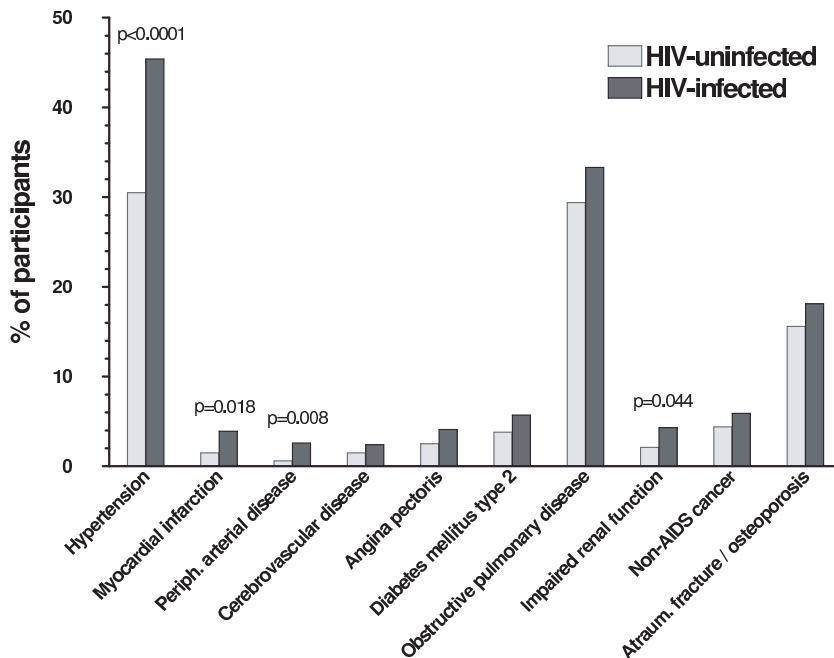
AANCC=age-associated noncommunicable comorbidities.

(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-infected participants (**Figure 3.2**).

### 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, 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 AANCCs (odds ratio (OR) 1.58, 95% confidence interval (CI) 1.23-2.03,



**Figure 3.2: Prevalences of each of the different age-associated noncommunicable comorbidities over the two studygroups**

P values obtained by  $\chi^2$  test

$P < 0.001$ ). Age, positive family history, and smoking were also strongly independently associated with AANCCs. Analysing the HIV-infected and HIV-uninfected studygroups separately, the OR for age was higher in the HIV-infected studygroup (OR 1.60, 95% CI 1.41-1.81,  $P < 0.001$ ) compared with 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 (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,  $P < 0.001$ ), duration of ART use (OR 1.04 per additional year, 95% CI 1.02-1.06,  $P < 0.001$ ), and duration of CD4+ T-cell 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+ T-cell counts  $< 200$  cells/mm<sup>3</sup> remained an independent risk factor for AANCCs.

In multivariable analyses nadir CD4+ T-cell count, prior AIDS, (cumulative) duration of undetectable plasma HIV 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 analysed the potential contribution of markers of systemic inflammation (high-sensitivity C-reactive protein (hsCRP)), 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 3.3**). Adding hsCRP and sCD14 to the above-mentioned regression model (analysing both studygroups jointly) showed both markers to be (borderline) significantly associated with AANCCs (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. Analysing the effect of hsCRP and sCD14 in the two studygroups 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,  $P < 0.001$ ) 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 AANCCs.

**Table 3.3: Values of several markers of systemic inflammation, compared between the two studygroups**

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

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

Abbreviations: HIV=human immunodeficiency virus, hsCRP=high-sensitivity C-reactive protein, sCD14=soluble CD14, sCD163=soluble CD163.

\* Wilcoxon rank-sum test

\*\*  $\chi^2$  test

### *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 five years of ritonavir use, 95% CI 1.04-1.60,  $P=0.018$ ). 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 0.99-1.18,  $P=0.083$ ) (Table 3.4).

## Discussion

### Key results

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.

HIV infection was independently associated with a higher total num-

**Table 3.4: Risk factors for age-associated noncommunicable comorbidities, multivariably analysed using the HIV-infected and HIV-uninfected studygroups 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	<0.001	1.39	1.27-1.52	<0.001
Smoking (per 5 packyears)	1.10	1.07-1.13	<0.001	1.08	1.05-1.12	<0.001
Positive family history <sup>a</sup> (yes/no)	1.57	1.23-2.01	<0.001	1.88	1.45-2.44	<0.001
HIV infection (yes/no)	1.68	1.34-2.10	<0.001	1.07	0.80-1.42	0.661
Known cumulative duration of immunodeficiency (per year with a CD <sub>4</sub> <sup>+</sup> T-cell count <200 cells/mm <sup>3</sup> )	1.33	1.20-1.48	<0.001	1.23	1.10-1.38	<0.001
Waist-to-hip ratio (per 0.1)	1.94	1.67-2.25	<0.001	1.49	1.23-1.80	<0.001
hsCRP (per mg/L)	1.06	1.03-1.09	<0.001	1.03	0.99-1.06	0.107
sCD14 (per 100 ng/mL)	1.02	1.01-1.04	0.007	1.02	1.00-1.03	0.074
Cumulative duration of ritonavir use in high dosages (≥400 mg/daily) (per year)	1.19	1.10-1.28	<0.001	1.08	0.99-1.18	0.083

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 co-infection (all of which were not significantly associated with risk for AANCC).

Abbreviations: CI=confidence interval, HIV=human immunodeficiency virus, hsCRP=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.

ber of AANCCs, 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 AANCCs in each of the studygroups. A borderline significant interaction between age and HIV infection suggested a stronger age effect among HIV-infected participants.

A longer time spent at a CD<sub>4</sub><sup>+</sup> T-cell 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 ( $\geq 400$  mg/24 hours), were additional factors contributing to AANCC burden.

#### Interpretation, limitations, and conclusion

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.<sup>8,27–36</sup> Earlier studies, however, either did not include a comparable uninfected control group but used general population<sup>8,29–32,35</sup> or patient registry data for comparison<sup>27,28,33,34,36</sup>, or were not designed a priori to prospectively capture data on comorbidity and comorbidity risk factors with similar detail and rigor.<sup>35</sup> 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-infected participants and ecstasy use was more prevalent 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 AANCCs indeed are more prevalent among those living with HIV, including in those with a sustained response to ART.

Unravelling 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), co-infections (including cytomegalovirus and chronic viral hepatitis), and ART.<sup>18</sup> A study by Guaraldi et al. identified longer ART exposure and lower nadir CD<sub>4</sub><sup>+</sup> T-cell count as independent risk factors for non-AIDS comorbidities.<sup>8</sup>

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 CD<sub>4</sub><sup>+</sup> T-cell counts  $< 200$  cells/mm<sup>3</sup>) 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<sup>37–39</sup>, which are generally considered important drivers for comorbidity in both HIV-uninfected and HIV-infected individuals.<sup>15,16,40,41</sup> Higher levels of sCD14 and hsCRP, 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.<sup>42,43</sup>

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<sup>44,45</sup>, and cellular accumulation of prelamin A, which may result in premature cellular senescence similar to what is observed in some genetically determined premature ageing syndromes.<sup>46,47</sup>

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 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 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 pre-mature or accelerated ageing in HIV.<sup>8,10</sup> 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 ageing itself, remains to be elucidated.<sup>18,48</sup>

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## Authors' contributions

JS contributed to data collection, data analysis and interpretation, writing of all drafts of the manuscript, and was responsible for producing and submitting the final manuscript. FW contributed to the study design, data analysis and interpretation, and writing of all versions of the manuscript.

IS contributed to the study design, data collection, data interpretation, and writing of the manuscript.

NK contributed to logistics, data collection, data interpretation, and writing of the manuscript.

MV contributed to inclusion of study participants, support of the study at the HIV outpatient department at the Academic Medical Center, to data interpretation, and writing of the manuscript.

SG contributed to inclusion of study participants, support of the study at the HIV outpatient department at the Academic Medical Center, and to writing of the manuscript.

MP contributed to the study design, data interpretation, and writing of the manuscript.

PR conceived the study, contributed to the study design, to data interpretation, and writing and supervision of all versions of the manuscript, including the final submitted version.

## References


1. Palella, F. J. *et al.* Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. *N. Engl. J. Med.* **338**, 853–860 (1998).
2. Weber, R. *et al.* Decreasing mortality and changing patterns of causes of death in the Swiss HIV Cohort Study. *HIV Med.* **14**, 195–207 (2013).
3. Wada, N. *et al.* Cause-specific life expectancies after 35 years of age for human immunodeficiency syndrome-infected and human immunodeficiency syndrome-negative individuals followed simultaneously in long-term cohort studies, 1984–2008. *Am. J. Epidemiol.* **177**, 116–125 (2013).
4. Lohse, N. *et al.* Survival of persons with and without HIV infection in Denmark, 1995–2005. *Ann. Intern. Med.* **146**, 87–95 (2007).
5. Antiretroviral Therapy Cohort Collaboration. Life expectancy of individuals on combination antiretroviral therapy in high-income countries: a collaborative analysis of 14 cohort studies. *Lancet Lond. Engl.* **372**, 293–299 (2008).
6. Antiretroviral Therapy Cohort Collaboration. Causes of death in HIV-1-infected patients treated with antiretroviral therapy, 1996–2006: collaborative analysis of 13 HIV cohort studies. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **50**, 1387–1396 (2010).
7. Hasse, B. *et al.* Morbidity and aging in HIV-infected persons: the Swiss HIV cohort study. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **53**, 1130–1139 (2011).
8. Guaraldi, G. *et al.* Premature age-related comorbidities among HIV-infected persons compared with the general population. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **53**, 1120–1126 (2011).

9. Effros, R. B. *et al.* Aging and infectious diseases: workshop on HIV infection and aging: what is known and future research directions. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **47**, 542–553 (2008).
10. Pathai, S., Bajillan, H., Landay, A. L. & High, K. P. Is HIV a model of accelerated or accentuated aging? *J. Gerontol. A. Biol. Sci. Med. Sci.* **69**, 833–842 (2014).
11. Deeks, S. G. & Phillips, A. N. HIV infection, antiretroviral treatment, ageing, and non-AIDS related morbidity. *BMJ* **338**, a3172 (2009).
12. Deeks, S. G. HIV infection, inflammation, immunosenescence, and aging. *Annu. Rev. Med.* **62**, 141–155 (2011).
13. Justice, A. C. HIV and aging: time for a new paradigm. *Curr. HIV/AIDS Rep.* **7**, 69–76 (2010).
14. Deeks, S. G., Tracy, R. & Douek, D. C. Systemic effects of inflammation on health during chronic HIV infection. *Immunity* **39**, 633–645 (2013).
15. Capeau, J. Premature Aging and Premature Age-Related Comorbidities in HIV-Infected Patients: Facts and Hypotheses. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **53**, 1127–1129 (2011).
16. Armah, K. A. *et al.* HIV status, burden of comorbid disease, and biomarkers of inflammation, altered coagulation, and monocyte activation. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **55**, 126–136 (2012).
17. Negin, J., Wariero, J., Cumming, R. G., Mutuo, P. & Pronyk, P. M. High rates of AIDS-related mortality among older adults in rural Kenya. *J. Acquir. Immune Defic. Syndr. 1999* **55**, 239–244 (2010).
18. High, K. P. *et al.* HIV and aging: state of knowledge and areas of critical need for research. A report to the NIH Office of AIDS Research by the HIV and Aging Working Group. *J. Acquir. Immune Defic. Syndr. 1999* **60 Suppl 1**, S1-18 (2012).
19. Amsterdam Cohort Studies. Available at: <http://www.amsterdamcohortstudies.org>. Accessed 20 December 2013. (2013).
20. HIV Monitoring Foundation. Available at: <http://www.hiv-monitoring.nl/english>. Accessed 20 December 2013. (2013).
21. Pickering, T. G. *et al.* Recommendations for blood pressure measurement in humans: an AHA scientific statement from the Council on High Blood Pressure Research Professional and Public Education Subcommittee. *J. Clin. Hypertens. Greenwich Conn* **7**, 102–109 (2005).
22. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* **35 Suppl 1**, S64-71 (2012).
23. Global Initiative for Chronic Obstructive Lung Disease (GOLD). Available at: [http://www.goldcopd.org/uploads/users/files/GOLD\\_Spirometry\\_2010.pdf](http://www.goldcopd.org/uploads/users/files/GOLD_Spirometry_2010.pdf). Accessed 20 December 2013. (2013).
24. The International Society for Clinical Densitometry (ISCD). Available at: <http://www.iscd.org/official-positions>. Accessed 20 December 2013. (2013).
25. Kanis, J. A. *et al.* A reference standard for the description of osteoporosis. *Bone* **42**, 467–475 (2008).



26. Chow, F. C. *et al.* Comparison of ischemic stroke incidence in HIV-infected and non-HIV-infected patients in a US health care system. *J. Acquir. Immune Defic. Syndr.* 1999 **60**, 351–358 (2012).
27. Goulet, J. L. *et al.* Aging and infectious diseases: do patterns of comorbidity vary by HIV status, age, and HIV severity? *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **45**, 1593–1601 (2007).
28. Hansen, A.-B. E. *et al.* Incidence of low and high-energy fractures in persons with and without HIV infection: a Danish population-based cohort study. *AIDS Lond. Engl.* **26**, 285–293 (2012).
29. Lang, S. *et al.* Increased risk of myocardial infarction in HIV-infected patients in France, relative to the general population. *AIDS Lond. Engl.* **24**, 1228–1230 (2010).
30. Obel, N. *et al.* Ischemic heart disease in HIV-infected and HIV-uninfected individuals: a population-based cohort study. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **44**, 1625–1631 (2007).
31. Rasmussen, L. D. *et al.* Risk of cerebrovascular events in persons with and without HIV: a Danish nationwide population-based cohort study. *AIDS Lond. Engl.* **25**, 1637–1646 (2011).
32. Triant, V. A., Brown, T. T., Lee, H. & Grinspoon, S. K. Fracture prevalence among human immunodeficiency virus (HIV)-infected versus non-HIV-infected patients in a large U.S. healthcare system. *J. Clin. Endocrinol. Metab.* **93**, 3499–3504 (2008).
33. Triant, V. A., Lee, H., Hadigan, C. & Grinspoon, S. K. Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease. *J. Clin. Endocrinol. Metab.* **92**, 2506–2512 (2007).
34. Seaberg, E. C. *et al.* Cancer incidence in the multicenter AIDS Cohort Study before and during the HAART era: 1984 to 2007. *Cancer* **116**, 5507–5516 (2010).
35. Freiberg, M. S. *et al.* HIV infection and the risk of acute myocardial infarction. *JAMA Intern. Med.* **173**, 614–622 (2013).
36. Sandler, N. G. *et al.* Plasma levels of soluble CD14 independently predict mortality in HIV infection. *J. Infect. Dis.* **203**, 780–790 (2011).
37. Neuhaus, J. *et al.* Markers of inflammation, coagulation, and renal function are elevated in adults with HIV infection. *J. Infect. Dis.* **201**, 1788–1795 (2010).
38. Cassol, E. *et al.* Persistent microbial translocation and immune activation in HIV-1-infected South Africans receiving combination antiretroviral therapy. *J. Infect. Dis.* **202**, 723–733 (2010).
39. Luc, G. *et al.* C-reactive protein, interleukin-6, and fibrinogen as predictors of coronary heart disease: the PRIME Study. *Arterioscler. Thromb. Vasc. Biol.* **23**, 1255–1261 (2003).
40. Kuller, L. H. *et al.* Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. *PLoS Med.* **5**, e203 (2008).
41. Baker J, Huppler Hullsiek K, Singh A, *et al.* Monocyte activation, but not T cell activation, predicts progression of coronary artery calcium in a contemporary

- HIV cohort. In: 20th Conference on Retroviruses and Opportunistic Infections, Atlanta, GA, 3–6 March 2013. Abstract 66LB. (2013).
42. Tenorio A, Zheng E, Bosch R, *et al.* Soluble markers of inflammation and coagulation, but not T cell activation, predict non-AIDS-defining events during suppressive ART. In: 20th Conference on Retroviruses and Opportunistic Infections, Atlanta, GA, 3–6 March 2013. Abstract 790. (2013).
  43. Stein, J. H. *et al.* Use of human immunodeficiency virus-1 protease inhibitors is associated with atherogenic lipoprotein changes and endothelial dysfunction. *Circulation* **104**, 257–262 (2001).
  44. Johnsen, S. *et al.* Carotid intimal medial thickness in human immunodeficiency virus-infected women: effects of protease inhibitor use, cardiac risk factors, and the metabolic syndrome. *J. Clin. Endocrinol. Metab.* **91**, 4916–4924 (2006).
  45. Hudon, S. E. *et al.* HIV-protease inhibitors block the enzymatic activity of purified Ste24p. *Biochem. Biophys. Res. Commun.* **374**, 365–368 (2008).
  46. Barrowman, J., Wiley, P. A., Hudon-Miller, S. E., Hrycyna, C. A. & Michaelis, S. Human ZMPSTE24 disease mutations: residual proteolytic activity correlates with disease severity. *Hum. Mol. Genet.* **21**, 4084–4093 (2012).
  47. Perrin, S. *et al.* HIV protease inhibitors do not cause the accumulation of prelamin A in PBMCs from patients receiving first line therapy: the ANRS EP45 'aging' study. *PLoS One* **7**, e53035 (2012).
  48. Martin, J. & Volberding, P. HIV and premature aging: A field still in its infancy. *Ann. Intern. Med.* **153**, 477–479 (2010).



Difference in aortic stiffness between  
treated middle-aged HIV-infected and  
uninfected individuals largely explained  
by traditional cardiovascular risk factors,  
with an additional contribution of prior  
advanced immunodeficiency

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## Abstract

### Background

Patients with HIV, even with suppressed viremia on combination antiretroviral therapy, are at increased risk for cardiovascular disease. The underlying pathophysiology remains to be clarified. Aortic stiffness, known to be associated with cardiovascular disease in the general population, was investigated in a cohort of HIV-infected and similar but uninfected individuals.

### Methods

Aortic stiffness was assessed by measuring pulse wave velocity with an Arteriograph. Five hundred and seven HIV-uninfected and 566 HIV-infected individuals, predominantly with suppressed viremia on combination antiretroviral therapy, aged  $\geq 45$  years, participating in the ongoing AGE<sub>HIV</sub> Cohort Study were included in the analysis. Multivariable linear regression was used to investigate whether HIV was independently associated with aortic stiffness, adjusting for traditional cardiovascular risk factors.

### Results

Studygroups were comparable in terms of demographics; smoking and hypertension were more prevalent in HIV-infected participants. Pulse wave velocity was higher in the HIV-infected group (7.9 vs. 7.7 m/s,  $P=0.004$ ). After adjustment for mean arterial pressure, age, gender and smoking, HIV status was not significantly associated with aortic stiffness. In HIV-infected participants, having a nadir CD<sub>4</sub><sup>+</sup> T-cell count  $\leq 100$  cells/mm<sup>3</sup> was independently associated with a higher pulse wave velocity.

### Conclusions

The increased aortic stiffness in HIV-infected participants was largely explained by a higher prevalence of traditional cardiovascular risk factors, particularly smoking. Though HIV itself was not independently associated with higher aortic stiffness, a prior greater degree of immunodeficiency was. This suggests a detrimental effect of immunodeficiency on the aortic wall, possibly mediated by inflammation.

## Introduction

HIV infection has been associated with an increased risk of adverse cardiovascular outcomes.<sup>1-3</sup> The pathogenesis of cardiovascular disease (CVD) in the context of HIV infection is not fully clarified. It is likely that lifestyle factors, including smoking, contribute to the increased cardiovascular risk in HIV patients. Chronic immune activation and inflammation, partly driven by gut microbial translocation and persistent low-level viral replication, and exposure to particular antiretroviral agents may also be involved.<sup>4,5</sup>

Aortic stiffening is a degenerative process, associated with loss of elastin and increased deposition of collagen and other structural proteins within the extracellular matrix of the arterial wall. This process typically occurs with ageing and is accelerated by hypertension, metabolic changes, and inflammation.<sup>6-9</sup> Aortic stiffness, assessed by measuring aortic pulse wave velocity (PWV), is independently associated with cardiovascular events and mortality in the general population.<sup>10-12</sup>

Previous studies on the association between HIV and aortic stiffness are inconsistent<sup>13-18</sup>, possibly due to small sample sizes and suboptimal control groups. In addition, several of these studies included untreated or inadequately treated HIV-infected patients.

We cross-sectionally compared aortic stiffness in a well-characterized cohort of HIV-type-1-infected individuals predominantly with suppressed viremia on long term combination antiretroviral therapy (cART), and HIV-uninfected individuals with similar behavioural and demographic characteristics, all aged  $\geq 45$  years. We assessed whether HIV infection was associated with higher aortic PWV, independent of traditional cardiovascular risk factors. In addition, we explored possible determinants of aortic PWV in the whole cohort, including behavioural, metabolic and inflammatory markers, as well as HIV-related virological, immunological and clinical factors in the HIV-infected group.

## Methods

### Subjects and clinical variables

The AGE<sub>h</sub>IV Cohort Study, an ongoing prospective comparative cohort study, aims to assess and compare prevalence, incidence of and risk factors for age-associated comorbidities and organ dysfunction among HIV-1-infected individuals and HIV-uninfected controls. Between 2010 and 2012, 598 HIV-infected individuals were recruited at the HIV outpatient clinic of the Academic Medical Center (AMC) in Amsterdam, the Netherlands. 550 HIV-uninfected individuals were recruited from the sexual health clinic and the Amsterdam Cohort Studies on HIV/AIDS at the Amsterdam Public Health Service, with similar socio-demographic and behavioural (risk) factors.<sup>19</sup> Inclusion criteria were: age  $\geq 45$  years, laboratory-confirmed presence (HIV-infected participants) or absence of HIV infection (HIV-uninfected controls). Written informed consent was obtained from all participants; the study was approved by the local ethics review board (ClinicalTrials.gov identifier NCT01466582).

Patients and controls underwent standardized screening for age-associated comorbidities and organ dysfunction. Details concerning study procedures have been reported previously.<sup>20</sup> In short, participants completed an extensive standardized questionnaire. Self-reported comorbidities were validated using AMC hospital records of HIV-infected participants and general practitioners' records of HIV-uninfected participants, provided that the latter gave consent to contact their general practitioner. Data were available on the following CVD: angina pectoris, myocardial infarction, peripheral arterial disease, and cerebrovascular disease. Participants were asked whether they had used recreational drugs in the past six months. If so, they were asked to select the types of drugs they had used from a list and indicate the frequency of usage for each drug type. Physical activity was defined according to Dutch guidelines for healthy physical activity (Combinorm).<sup>21</sup> Height, weight and waist/hip-circumference were measured. Hypertension was defined as a mean systolic blood pressure (SBP)  $\geq 140$  mmHg, mean diastolic blood pressure (DBP)  $\geq 90$  mmHg (three measurements, recorded by the Arteriograph), or use of antihypertensive medication. For both HIV-infected and uninfected participants all laboratory tests were performed centrally

in the AMC. Diabetes mellitus type 2 was defined as a hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) (International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)) level  $\geq 48$  mmol/mol and/or blood glucose (fasting/non-fasting)  $\geq 7.0/\geq 11.1$  mmol/L, or use of antidiabetic medication. We assessed levels of high and low density lipoprotein (HDL and LDL) cholesterol, total cholesterol and triglycerides and use of statins as markers of dyslipidemia. Hepatitis B virus (HBV) and hepatitis C virus (HCV) infection status, CD<sub>4</sub><sup>+</sup> T-cell counts (CD<sub>4</sub> counts) and markers of inflammation (high-sensitivity (hs)CRP), monocyte activation (soluble (s)CD163, sCD14) and coagulation (D-dimer) were determined for all participants, as well as plasma HIV viral load for HIV-infected participants.

Detailed information concerning HIV infection and ART history was obtained from the HIV Monitoring Foundation registry.<sup>22</sup>

#### Aortic stiffness as measured by PWV

Blood pressure (BP) and aortic PWV was measured at both study sites using the Arteriograph (Tensiomed Kft., Budapest, Hungary) in a standardized manner by trained study staff. The Arteriograph uses an oscillometric method to register pressure curves by an upper arm BP cuff. PWV measurements with the Arteriograph are highly correlated with invasive PWV measurements, and correlate well with aortic PWV obtained by MRI and carotid-femoral PWV.<sup>23–25</sup> PWV is measured after an oscillometric BP reading is taken and the pressure cuff is overinflated to 35–40 mmHg above SBP to occlude the brachial artery. The technology makes use of the two pressure waves during the cardiac cycle; the first is generated by the ejection of blood into the aorta during systole and the second by the reflection of the first wave from the aortic bifurcation. The Arteriograph registers these pressure fluctuations through the upper arm cuff. Aortic PWV is calculated using the time difference between the beginning of the first and the second wave and the estimated distance from the heart to the aortic bifurcation, which is estimated by measuring the distance from the jugulum to the pubic symphysis using a tape measure. PWV measurements were performed in a quiet room after at least 15 minutes of supine rest. Measurements were discarded if the standard deviation (SD) of the analysed pulse waves exceeded the cut-off ( $>1.0$  m/s and  $>15\%$  of

the measured PWV value) or if the PWV value differed >15% from the other two PWV measurements. The average of three consecutive PWV and BP (DBP, SBP and mean arterial pressure (MAP)) measurements was used for analysis.

### Statistical analysis

Characteristics of HIV-infected and uninfected groups were compared using  $\chi^2$ , Student's t and Wilcoxon rank-sum tests where appropriate.

The association between HIV status and aortic PWV, adjusted for possible confounders, was assessed by multivariable linear regression; a covariate was considered a significant confounder if its introduction into the model changed the regression coefficient of HIV by  $\geq 10\%$ . Possible determinants of aortic PWV were explored; these included demographic, behavioural and metabolic CVD risk factors, including those potentially influenced by exposure to HIV and/or ART. High sensitivity CRP, D-dimer, sCD163 and sCD14 were explored to assess the contribution of inflammatory and coagulation parameters on differences in aortic PWV. Covariates with a P value <0.1 as well as significant confounders were kept in the model. A second model, only including HIV infected individuals explored the role of HIV and ART-related clinical variables. In an additional third analysis we compared the HIV-infected group, stratified according to nadir CD4+ T-cell count (cut-off:  $\leq 100$  cells/mm<sup>3</sup>), with the entire HIV-uninfected group.

We used multiple imputation to handle missing observations of independent variables, generating 5 sets with complete covariate values. We assessed non-linearity of relationships using categorization and transformation of continuous covariates. To investigate whether associations differed significantly according to HIV status, we explored biologically plausible interactions with HIV infected status. All models were adjusted for MAP, since aortic stiffness is directly affected by BP at the time of PWV measurement.<sup>26</sup> Statistical analysis was performed using STATA version 12 (StataCorp LP, 2011, Texas, USA). All reported P values are 2-sided.



## Results

### Subject characteristics

Of the 598 HIV-infected and 550 HIV-uninfected participants of the AGE<sub>HIV</sub> Cohort Study, 32 and 43 participants, respectively, were excluded from this analysis either because of missing PWV measurements (16 HIV-infected, 22 HIV-uninfected), or because the SD or variation between measurements exceeded the predefined limits (16 HIV-infected, 21 HIV-uninfected). Individuals with missing or invalid PWV data were more often females (16.4% vs. 4.9%,  $P < 0.001$ ), but did not significantly differ regarding age or BP. Variance between the three measurements of each participant did not differ between study sites ( $P = 0.96$ ).

Age and gender distribution of the 566 HIV-infected and 507 HIV-uninfected individuals included in the analysis did not differ significantly. The majority were male and men who have sex with men.

Compared to the HIV-uninfected group, a larger proportion of the HIV-infected group was of African descent. HIV-infected individuals were more often diagnosed with hypertension, had generally less favourable lipid profiles and were more often smokers. A history of CVD was more prevalent in HIV infected compared to HIV-uninfected individuals (10.3 vs. 4.7%,  $P = 0.001$ ). Levels of hsCRP, sCD163 and sCD14 were significantly higher in HIV-infected individuals, and D-dimer in HIV-uninfected individuals (**Table 4.1**). Ninety-five percent of the HIV-infected participants was currently on cART; 92.6% of those had suppressed viremia to levels  $< 200$  copies/mL in the year prior to enrolment (**Table 4.2**).

### Determinants of aortic PWV

Unadjusted, aortic PWV was significantly higher in the HIV-infected (7.9 m/s, interquartile range (IQR) 7.2-9.0) compared to the HIV-uninfected group (7.7 m/s, IQR 7.0-8.8) ( $P = 0.004$ ). After adjusting for age, MAP and gender, the association between HIV and aortic PWV remained statistically significant (+0.20 m/s, 95% confidence interval (CI) 0.02-0.38 m/s,  $P = 0.03$ ). Further adjustment for the number of packyears of smoking attenuated the regression coefficient of HIV-infected status (adjusted coefficient: +0.12 m/s, 95% CI -0.06-0.29,  $P = 0.18$ ). Race/ethnicity, use of

**Table 4.1: Characteristics of HIV-infected and uninfected individuals**

	HIV-infected (n=566) n (%) / median (IQR)	HIV-uninfected (n=507) n (%) / median (IQR)	P value <sup>a</sup>
Age, years	52.7 (48.3-59.5)	51.9 (47.9-58.0)	0.17
Male gender	506 (89.4%)	436 (86.0%)	0.09
Men who have sex with men <sup>b</sup>	388 (75.1%)	344 (70.6%)	0.12
African descent	75 (13.5%)	31 (6.1%)	<0.001
Family history of MI <sup>b</sup>	104 (20.6%)	89 (18.4%)	0.38
Hepatitis (co)-infection			
HBsAg positive	37 (6.5%)	3 (0.6%)	<0.001
HCV RNA positive	20 (3.5%)	5 (1.0%)	0.006
Smoking <sup>b</sup>			
Current	168 (32.9%)	120 (24.8%)	0.02
Past	176 (34.5%)	185 (38.2%)	
Packyears (ex-)smokers	22.5 (7.8-36.0)	14.7 (4.5-28.5)	<0.001
Heavy alcohol intake in past 6 months <sup>bc</sup>	26 (5.1%)	32 (6.6%)	0.31
Injecting drug use (ever) <sup>b</sup>	16 (3.2%)	5 (1.0%)	0.02
≥1x/month use in past 6 months <sup>b</sup>			
Cocaine	20 (4.0%)	15 (3.1%)	0.46
Ecstasy	24 (4.8%)	39 (8.1%)	0.03
Meeting Dutch physical activity standards <sup>bd</sup>	227 (45.3%)	260 (53.8%)	0.008
Body mass index	24.1 (22.3-26.6)	24.5 (22.9-27.1)	<0.001
<18.5 kg/m <sup>2</sup>	13 (2.3%)	3 (0.6%)	0.05
18.5-25 kg/m <sup>2</sup>	328 (58.0%)	287 (56.6%)	
25-30 kg/m <sup>2</sup>	187 (33.0%)	169 (33.3%)	
≥30 kg/m <sup>2</sup>	38 (6.7%)	48 (9.5%)	
Waist-to-hip ratio	0.97 (0.92-1.01)	0.92 (0.88-0.96)	<0.001
≥0.85 (females)/≥0.9 (males)	480 (84.8%)	320 (63.1%)	<0.001
Lipid spectrum			
Total cholesterol, mmol/L	5.3 (4.6-6.0)	5.5 (4.9-6.1)	0.07
HDL, mmol/L	1.3 (1.0-1.6)	1.4 (1.1-1.7)	0.002
LDL, mmol/L	3.1 (2.4-3.7)	3.3 (2.7-3.9)	<0.001
Triglycerides, mmol/L	1.6 (1.1-2.6)	1.4 (0.9-2.1)	<0.001
Using statins <sup>b</sup>	72 (14.4%)	33 (6.8%)	<0.001

<sup>a</sup> P values obtained by  $\chi^2$ , Student's t test and Wilcoxon rank-sum test where applicable.

<sup>b</sup> Questionnaire derived data, available for 513 HIV-infected and 485 HIV-uninfected individuals.

<sup>c</sup> Consumption of ≥3/≥5 (females/males) alcohol units per day.

<sup>d</sup> ≥5 days/week ≥30 minutes of moderate physical activity or ≥2x/week 20 minutes of heavy physical activity; Dutch guidelines for healthy physical activity (Combinorm).

<sup>e</sup> HbA<sub>1c</sub> (IFCC) ≥48 mmol/mol and/or blood glucose (fasting/non-fasting) ≥7.0 or ≥11.1 mmol/L, or taking antidiabetic medication.

<sup>f</sup> Cardiovascular disease includes: angina pectoris, myocardial infarction, peripheral arterial disease, and cerebrovascular disease.

Abbreviations: MI=myocardial infarction, HBsAg=hepatitis B virus surface antigen, HCV=hepatitis C Virus, HDL=high-density lipoprotein, LDL=low-density lipoprotein, SBP=systolic blood pressure, DBP=diastolic blood pressure, hsCRP=high-sensitivity C-reactive protein, ACEi=angiotensin-converting enzyme inhibitor, ATII blocker=angiotensin II receptor antagonist, eGFR=estimated glomerular filtration rate, calculated by Chronic Kidney Disease Epidemiology Collaboration formula.

**Table 4.1: Characteristics of HIV-infected and uninfected individuals (continued)**

	HIV-infected (n=566) n (%) / median (IQR)	HIV-uninfected (n=507) n (%) / median (IQR)	P value <sup>a</sup>
Hypertension			
SBP, mmHg	129.3 (120.3-139.7)	126.5 (120.0-137.0)	0.06
DBP, mmHg	83.8 (77.3-90.0)	82.3 (76.3-88.3)	0.003
SBP $\geq$ 140 and/or DBP $\geq$ 90	185 (32.7%)	133 (26.2%)	0.02
Using antihypertensive drugs <sup>b</sup>	109 (21.8%)	61 (12.6%)	<0.001
Using ACEi or AT-II blocker <sup>b</sup>	80 (16.0%)	30 (6.2%)	<0.001
Diabetes mellitus type 2 <sup>e</sup>	33 (6.0%)	23 (4.9%)	0.46
Renal function			
eGFR 30-60 mL/min/m <sup>2</sup>	25 (4.4%)	10 (2.0%)	
eGFR <30 mL/min/m <sup>2</sup>	2 (0.4%)	0 (0%)	0.03
History of cardiovascular disease <sup>bf</sup>	53 (10.3%)	23 (4.7%)	0.001
Markers of inflammation/immune activation			
hsCRP, mg/L	1.5 (0.7-3.5)	1.0 (0.6-1.9)	<0.001
D-dimer mg/L	0.22 (0.20-0.35)	0.25 (0.20-0.38)	0.03
sCD163, ng/mL	287 (207-417)	251 (183-342)	<0.001
sCD14, ng/mL	1589 (1311-2011)	1355 (1081-1736)	<0.001

<sup>a</sup> P values obtained by  $\chi^2$ , Student's t test and Wilcoxon rank-sum test where applicable.

<sup>b</sup> Questionnaire derived data, available for 513 HIV-infected and 485 HIV-uninfected individuals.

<sup>c</sup> Consumption of  $\geq 3/\geq 5$  (females/males) alcohol units per day.

<sup>d</sup>  $\geq 5$  days/week  $\geq 30$  minutes of moderate physical activity or  $\geq 2$  times/week 20 minutes of heavy physical activity; Dutch guidelines for healthy physical activity (Combinorm).

<sup>e</sup> HbA<sub>1c</sub> (IFCC)  $\geq 4.8$  mmol/mol and/or blood glucose (fasting/non-fasting)  $\geq 7.0$  or  $\geq 11.1$  mmol/L, or taking antidiabetic medication.

<sup>f</sup> Cardiovascular disease includes: angina pectoris, myocardial infarction, peripheral arterial disease, and cerebrovascular disease.

Abbreviations: MI=myocardial infarction, HBSAg=hepatitis B virus surface antigen, HCV=hepatitis C Virus, HDL=high-density lipoprotein, LDL=low-density lipoprotein, SBP=systolic blood pressure, DBP=diastolic blood pressure, hsCRP=high-sensitivity C-reactive protein, ACEi=angiotensin-converting enzyme inhibitor, ATII blocker=angiotensin II receptor antagonist, eGFR=estimated glomerular filtration rate, calculated by Chronic Kidney Disease Epidemiology Collaboration formula.

**Table 4.2: HIV-related clinical variables of HIV-infected individuals**

	HIV-infected (n=566) n (%) / median (IQR)
Years since HIV diagnosis	12.1 (6.7-17.1)
CD4+ T-cell count, cells/mm <sup>3</sup>	
Mean value in year prior to enrolment	565 (430-740)
Nadir	170 (70-260)
Cumulative known duration of CD4+ T-cell count <200, months	0.9 (0-9.7)
History of AIDS	175 (30.9%)
Using cART at enrolment	537 (95.0%)
Cumulative duration of exposure to ART, years	10.0 (4.4-14.2)
Treated with mono/dual antiretroviral therapy before cART	111 (20.7%)
Past/current exposure to abacavir	74 (13.8%) / 73 (13.6%)
Past/current exposure to protease inhibitors	177 (33.0%) / 230 (42.8%)
Suppressed HIV viral load (of those on cART)	
HIV viral load <200 copies/mL in year prior to enrolment	497 (92.6%)
Duration of HIV viral load <200 copies/mL, years	6.5 (3.1-10.2)

(c)ART=(combination) antiretroviral therapy

substances (ecstasy, cocaine, alcohol or injecting drugs), chronic HCV infection, family history of myocardial infarction, and level of physical activity were not independently associated with PWV, nor did they significantly affect the association between HIV and PWV. Subsequent adjustment for use of antihypertensive drugs attenuated the regression coefficient of HIV-infected status further (+0.09 m/s, 95% CI -0.09-0.26, P=0.33).

Compared to a body mass index (BMI) between 18.5 and 25 kg/m<sup>2</sup>, both a BMI ≥25 kg/m<sup>2</sup> and a BMI <18.5 kg/m<sup>2</sup> were associated with a higher PWV, while waist-to-hip ratio was not. Lower HDL cholesterol levels, as well as higher triglycerides and hsCRP levels were positively associated with aortic PWV, whereas levels of LDL and total cholesterol, D-dimer, sCD163 or sCD14 and the use of statins were not independently associated with PWV (**Table 4.3**).

We found no statistically significant interactions between any of the investigated covariates and HIV status.

**Table 4.3: Determinants of pulse wave velocity, multivariable linear regression**

	Difference in PWV, m/s (95% CI)	P value
HIV-infected status	-0.004 (-0.18-0.17)	0.96
Age (per 5 years)	0.23 (0.17-0.30)	<0.001
Packyears of smoking (per 5 years)	0.08 (0.06-0.10)	<0.001
Use of antihypertensive drugs	0.39 (0.15-0.63)	0.002
Body mass index		
<18.5 kg/m <sup>2</sup>	0.70 (-0.04-1.43)	0.06
18.5-25 kg/m <sup>2</sup> (ref)	-	-
25-30 kg/m <sup>2</sup>	0.12 (-0.07-0.32)	0.21
>30 kg/m <sup>2</sup>	0.29 (-0.06-0.64)	0.10
HDL cholesterol (per mmol/L)	-0.21 (-0.43-0.01)	0.06
Triglycerides (per mmol/L)	0.09 (0.02-0.15)	0.01
hsCRP (per mg/L)	0.03 (0.01-0.05)	0.004

Adjusted for MAP and gender.

Not independently associated: African descent, family history of MI, physical activity, heavy alcohol intake, intravenous drug use,  $\geq 1$ /month use of ecstasy or cocaine, chronic HCV infection, waist-to-hip ratio, diabetes mellitus type 2, D-dimer, sCD163 and sCD14.

Abbreviations: CI=confidence interval, PWV=pulse wave velocity, hsCRP=high-sensitivity C-reactive protein, MI=myocardial infarction.

#### HIV and ART-related covariates

Including only HIV-infected individuals in the multivariable model, after adjustment for MAP, gender, age and smoking, a lower nadir CD4+ T-cell count was significantly associated with a higher aortic PWV (+0.12 m/s per 100 cells/mm<sup>3</sup> lower CD4 count, 95% CI 0.03-0.22, P=0.01). We explored several cut-off values of the nadir CD4+ T-cell count (100, 200, 350 and 500 cells/mm<sup>3</sup>); a cut-off of  $\leq 100$  cells/mm<sup>3</sup> was most strongly and significantly associated with aortic PWV (+0.33 m/s, 95% CI 0.06-0.61, P=0.02). This association was not attenuated when use of antihypertensive drugs, BMI and HDL cholesterol were added to the model, but slightly attenuated when the level of triglycerides (after adjusting: +0.31 m/s, 95% CI 0.03-0.59, P=0.03) and hsCRP were added to the model (after adjusting: +0.28 m/s, 95% CI 0.00-0.56, P=0.05). The association was not affected by sCD163, sCD14 or D-dimer. Explored, but not significantly associated with PWV were the cumulative duration of having a reduced CD4+ T-cell count (using cut-offs of 50, 100, 200 and 350 CD4 cells/mm<sup>3</sup>),

the known duration of HIV infection, a history of AIDS, the CD<sub>4</sub><sup>+</sup> T-cell count and the HIV viral load in the year prior to enrolment. No associations were observed between PWV and being treated with mono/dual antiretroviral therapy before cART initiation, (cumulative) exposure to ART, abacavir, or any drug from the protease inhibitor (PI) class.

Additionally, we constructed a multivariable model comparing both a lower nadir HIV-infected group (nadir CD<sub>4</sub><sup>+</sup> T-cell count  $\leq 100$  cells/mm<sup>3</sup>, n=190) and a higher nadir HIV-infected group (nadir CD<sub>4</sub><sup>+</sup> T-cell count  $>100$  cells/mm<sup>3</sup>, n=376) with the entire HIV-uninfected group (**Table 4.4**). In model 1 we adjusted for MAP, gender, age and smoking: aortic PWV of the subgroup with a lower nadir CD<sub>4</sub><sup>+</sup> T-cell count was significantly higher than PWV of the HIV-uninfected group (+0.34 m/s, 95% CI 0.09-0.58, P=0.007), while there was no difference in PWV between the group with higher CD<sub>4</sub><sup>+</sup> T-cell count and the HIV-uninfected group. After additional adjustment for the use of antihypertensive drugs, BMI, HDL cholesterol and triglyceride level (model 2), the coefficient of the lower nadir CD<sub>4</sub> group was attenuated (+0.24 m/s, 95% CI -0.01-0.49, P=0.06). The coefficient was further attenuated and was no longer statistically significant by adding hsCRP to the model (+0.18 m/s, 95% CI -0.07-0.43, P=0.16, model 3), but not by addition of sCD163, sCD14 or D-dimer.

### Sensitivity analyses

Multivariable models, adjusted for MAP, gender, age and packyears of smoking, were repeated excluding individuals with a history of clinical CVD. HIV-infected status was not associated with PWV in this model (+0.01 m/s, 95% CI -0.17-0.19, P=0.91). The association between being HIV-infected with a nadir CD<sub>4</sub><sup>+</sup> T-cell count below 100 cells/mm<sup>3</sup> and PWV was no longer statistically significant (+0.20 m/s, 95% CI -0.06-0.45, P=0.13). Repeating the multivariable models excluding all individuals with renal disease (an estimated glomerular filtration rate below 60 mL/min/1.73m<sup>2</sup>) showed similar results as models including these individuals. In order to explore a possible confounding effect of the use of angiotensin-converting-enzyme inhibitors or angiotensin II receptor antagonists, drugs that may affect arterial stiffness<sup>27</sup>, we repeated multivariable models separately adjusting for the use of antihypertensive regi-

**Table 4.4: Multivariable linear regression analysis comparing the HIV-infected group with a low ( $\leq 100$  cells/mm<sup>3</sup>) or higher ( $> 100$ ) nadir CD<sub>4</sub><sup>+</sup> T-cell count with the HIV-uninfected control group**

	Model 1 <sup>a</sup>		Model 2 <sup>a</sup>		Model 3 <sup>a</sup>	
	Diff. in PWV, m/s (95% CI)	P value	Diff. in PWV, m/s (95% CI)	P value	Diff. in PWV, m/s (95% CI)	P value
<b>HIV-infected</b>						
<b>Nadir CD<sub>4</sub> <math>\leq 100</math></b>	0.34 (0.09-0.58)	0.007	0.24 (-0.01-0.49)	0.06	0.18 (-0.28-0.11)	0.16
<b>Nadir CD<sub>4</sub> <math>&gt; 100</math></b>	0.01 (-0.19-0.20)	0.92	-0.06 (-0.25-0.14)	0.58	-0.08 (-0.28-0.11)	0.40
<b>HIV-uninfected (ref)</b>	-	-	-	-	-	-

<sup>a</sup> Model 1: adjusted for age, MAP, gender and smoking; Model 2: adjusted for age, MAP, gender, smoking, use of antihypertensive drugs, BMI, HDL cholesterol and triglycerides; Model 3: adjusted for age, MAP, gender, smoking, use of antihypertensive drugs, BMI, HDL cholesterol, triglycerides and hsCRP. Abbreviations: Diff.=difference, PWV=pulse wave velocity, CI=confidence interval.

mens containing any of these drugs and for use of other antihypertensive regimens. These models showed similar results as models adjusting for antihypertensive drugs in general. Repeating multivariable regression models, only including individuals with complete data, showed similar results as the analyses with multiple imputed data.

## Discussion

### Key results

Aortic stiffness (aortic PWV) was higher in middle-aged predominantly virologically suppressed HIV-infected individuals than uninfected controls of similar demographic and behavioural background. HIV, however, was not independently associated with higher aortic stiffness. Traditional cardiovascular risk factors, mainly smoking and hypertension, appeared to be the most important determinants of aortic PWV in both HIV-infected and uninfected participants; the higher prevalence of smoking in the HIV-infected subgroup largely explained the observed difference in aortic stiffness.

### Interpretation, limitations, and conclusion

Within the HIV-infected cohort, having experienced a lower nadir CD<sub>4</sub><sup>+</sup> T-cell count was significantly associated with a higher aortic PWV. This confirms previous reports on the association between immunodeficiency and aortic stiffness.<sup>13,28,29</sup> Furthermore, HIV-infected individuals with a nadir CD<sub>4</sub><sup>+</sup> T-cell count  $\leq 100$  cells/mm<sup>3</sup> had a significantly higher aortic PWV than HIV-uninfected individuals, while adjusting for behavioural and metabolic risk factors. These results suggest a lasting effect of advanced immunodeficiency on aortic PWV. A higher hsCRP level, associated with higher aortic stiffness in the general population<sup>30,31</sup> as well as in our cohort (both in HIV-infected and uninfected participants), attenuated the coefficient of the group with the lowest nadir CD<sub>4</sub><sup>+</sup> T-cell count. This suggests a role for ongoing inflammation in the pathogenesis of aortic stiffness, particularly in HIV-infected individuals with low nadir CD<sub>4</sub><sup>+</sup> T-cell counts. Possibly, cytomegalovirus infection may contribute to this pro-inflammatory state.<sup>32,33</sup> In contrast, markers of monocyte/macrophage activation (sCD14 and sCD163), previously associated with atherosclerotic disease in the context of HIV<sup>34,35</sup>, were not significantly associated with aortic PWV and did not attenuate the association between the nadir CD<sub>4</sub><sup>+</sup> T-cell count and PWV.

Although HIV-infected individuals with a nadir CD<sub>4</sub><sup>+</sup> T-cell count below 100 had significantly higher aortic PWV than HIV-uninfected controls, this was not the case for HIV-infected individuals with a higher nadir CD<sub>4</sub><sup>+</sup> T-cell count. Furthermore, although in unadjusted analysis HIV-infected individuals had a higher aortic PWV than uninfected controls, being HIV-infected was no longer significantly associated with a higher PWV after adjusting for traditional cardiovascular risk factors. Our observations corroborate results of several smaller studies comparing aortic PWV in treated HIV-infected patients to uninfected controls.<sup>15,18,29</sup> Discrepancies with some other studies may be explained by their relatively small sample size (maximum sample size was 50)<sup>13,14,16,17</sup>, which increases the risk for type I errors and limits the ability to adjust for potential confounders. Moreover, some of the earlier studies recruited hospital staff as a control group, which was likely suboptimal as they did not share many of the characteristics and lifestyle factors with the patients studied.<sup>13,14,16</sup> Our findings suggest a relatively small role for



aortic stiffening in the observed increased CVD risk in well-treated HIV infection.

PI's (particularly lopinavir and ritonavir) strongly affect lipid metabolism, thereby potentially contributing to aortic stiffening. In our study we found the levels of HDL cholesterol and triglycerides, both markers of lipid metabolism, to be associated with aortic PWV. However, we did not confirm earlier findings associating PI exposure with PWV.<sup>13</sup> This may be because a large proportion (72.5%) of PI-based regimens used in our study population contained (boosted) atazanavir or darunavir, both PI's with a relatively favourable lipid profile.<sup>36</sup> Furthermore, the usual ritonavir boosting dose in these regimens is lower than in ritonavir-boosted lopinavir.

To ensure the robustness of our conclusions, we performed several sensitivity analyses. Excluding all individuals with a history of overt CVD resulted in smaller estimates of the association between being HIV-infected with a nadir CD<sub>4</sub>+ T-cell count below 100 cells/mm<sup>3</sup> and PWV, and a decrease in the level of statistical significance. This may in part be due to a loss of power, resulting from a decrease in group size, and in part to the exclusion of the individuals with the most extreme PWV values. However, a near-significant trend towards a higher PWV in HIV-infected with the lowest nadir CD<sub>4</sub>+ T-cell count remained present, suggesting that the high PWV in this subgroup is not driven solely by individuals with overt CVD.

This study is subject to several limitations. Inherent to its cross-sectional and observational design, it does not allow us to draw conclusions regarding causality. Although we collected data on many possible confounders, effects of any residual unmeasured confounders cannot be excluded. We cannot exclude the possibility that the selection of our controls may have led to an underestimation of the effect of HIV on aortic stiffness. The HIV-infected patients included in this study were regularly monitored at the HIV outpatient clinic of our hospital, while the healthy controls were generally not monitored regularly by a physician. Conditions potentially affecting aortic stiffness, such as dyslipidemia and hypertension, may have been diagnosed and treated at an earlier stage in the HIV-infected patients, thereby limiting their negative effect on aortic stiffness. Furthermore, to include controls with a similar behavioural and

demographic background as the patients, we recruited them from a sexual health clinic. As a result, they may have recently suffered from sexually transmitted diseases associated with a pro-inflammatory state. However, the lifetime incidence of sexually transmitted diseases is likely at least as high in the HIV-infected group.

In conclusion, we show a higher aortic stiffness in HIV-infected individuals on antiretroviral therapy.

The observed higher aortic PWV in the HIV-infected participants was largely explained by a higher prevalence of traditional risk factors. Overall, the factors most strongly associated with higher aortic stiffness in this population include both traditional (and modifiable) risk factors: smoking, hypertension and dyslipidemia, each of which is highly prevalent among HIV-infected individuals.

Being HIV-infected by itself was not independently associated with a higher aortic PWV, but a prior greater degree of immunodeficiency, particularly having experienced a nadir CD4<sup>+</sup> T-cell count less than 100 cells/mm<sup>3</sup>, was. The relation between immunodeficiency and aortic stiffness should optimally be investigated in the context of a randomized controlled trial, such as the arterial elasticity substudy within the Strategic Timing of AntiRetroviral Treatment (START) trial.<sup>37</sup> Results from that study as well as longitudinal follow-up of the AGE<sub>h</sub>IV Cohort Study will hopefully provide more insight in the effect of HIV infection and ART on age-related changes in aortic stiffness, as well as on the predictive value of aortic stiffness for clinical CVD in the HIV-infected population.

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## Authors' contributions

KK contributed to data collection, data analysis and interpretation, writing of all drafts of the manuscript, and was responsible for producing and submitting the final manuscript. JS contributed to data collection, data analysis and interpretation, and writing of the manuscript.

FW contributed to the study design, data analysis and interpretation, and writing of the manuscript.

MV contributed to inclusion of study participants, support of the study at the HIV outpatient department at the Academic Medical Center, to data interpretation, and writing of the manuscript.

NK contributed to logistics, data collection, data interpretation, and writing of the manuscript.

IS contributed to the study design, data collection, data interpretation, and writing of the manuscript.

JM contributed to inclusion of study participants, support of the study at the HIV outpatient department at the Academic Medical Center, and to writing of the manuscript.

MP contributed to the study design, data interpretation, and writing of the manuscript.

DG contributed to data interpretation, and writing of the manuscript.

BB contributed to data analysis and interpretation, and writing of the manuscript.

PR conceived the study, contributed to the study design, to data interpretation, and writing and supervision of all versions of the manuscript, including the final submitted version.

## References

1. Triant, V. A., Lee, H., Hadigan, C. & Grinspoon, S. K. Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease. *J. Clin. Endocrinol. Metab.* **92**, 2506–2512 (2007).

2. Lang, S. *et al.* Increased risk of myocardial infarction in HIV-infected patients in France, relative to the general population. *AIDS Lond. Engl.* **24**, 1228–1230 (2010).
3. Freiberg, M. S. *et al.* HIV infection and the risk of acute myocardial infarction. *JAMA Intern. Med.* **173**, 614–622 (2013).
4. Hsue, P. Y., Deeks, S. G. & Hunt, P. W. Immunologic basis of cardiovascular disease in HIV-infected adults. *J. Infect. Dis.* **205 Suppl 3**, S375–382 (2012).
5. Bavinger, C. *et al.* Risk of cardiovascular disease from antiretroviral therapy for HIV: a systematic review. *PloS One* **8**, e59551 (2013).
6. Zieman, S. J., Melenovsky, V. & Kass, D. A. Mechanisms, pathophysiology, and therapy of arterial stiffness. *Arterioscler. Thromb. Vasc. Biol.* **25**, 932–943 (2005).
7. Nilsson, P. M. *et al.* Early vascular ageing in translation: from laboratory investigations to clinical applications in cardiovascular prevention. *J. Hypertens.* **31**, 1517–1526 (2013).
8. Sun, Z. Aging, arterial stiffness, and hypertension. *Hypertens. Dallas Tex* **1979** **65**, 252–256 (2015).
9. Jain, S., Khera, R., Corrales-Medina, V. F., Townsend, R. R. & Chirinos, J. A. Inflammation and arterial stiffness in humans. *Atherosclerosis* **237**, 381–390 (2014).
10. Sehestedt, T. *et al.* Risk prediction is improved by adding markers of subclinical organ damage to SCORE. *Eur. Heart J.* **31**, 883–891 (2010).
11. Vlachopoulos, C., Aznaouridis, K. & Stefanadis, C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. *J. Am. Coll. Cardiol.* **55**, 1318–1327 (2010).
12. Ben-Shlomo, Y. *et al.* Aortic pulse wave velocity improves cardiovascular event prediction: an individual participant meta-analysis of prospective observational data from 17,635 subjects. *J. Am. Coll. Cardiol.* **63**, 636–646 (2014).
13. Schillaci, G. *et al.* Impact of treatment with protease inhibitors on aortic stiffness in adult patients with human immunodeficiency virus infection. *Arterioscler. Thromb. Vasc. Biol.* **25**, 2381–2385 (2005).
14. Schillaci, G. *et al.* Aortic stiffness in untreated adult patients with human immunodeficiency virus infection. *Hypertens. Dallas Tex* **1979** **52**, 308–313 (2008).
15. van Vonderen, M. G. A. *et al.* Carotid intima-media thickness and arterial stiffness in HIV-infected patients: the role of HIV, antiretroviral therapy, and lipodystrophy. *J. Acquir. Immune Defic. Syndr.* **1999** **50**, 153–161 (2009).
16. Vlachopoulos C, Sambatakou H, Tsiachris D, *et al.* Impact of human immunodeficiency virus infection on arterial stiffness and wave reflections in the early disease stages. *Artery Research.* 2009;3(3):104-110. (2009).
17. Lekakis, J. *et al.* Association of highly active antiretroviral therapy with increased arterial stiffness in patients infected with human immunodeficiency virus. *Am. J. Hypertens.* **22**, 828–834 (2009).
18. Echeverría, P. *et al.* Pulse wave velocity as index of arterial stiffness in HIV-infected patients compared with a healthy population. *J. Acquir. Immune Defic. Syndr.* **1999** **65**, 50–56 (2014).

19. Amsterdam cohort Studies on HIV/AIDS. <http://www.amsterdamcohortstudies.org>. Accessed March 21, 2016. (2016).
20. Schouten, J. *et al.* Cross-sectional comparison of the prevalence of age-associated comorbidities and their risk factors between HIV-infected and uninfected individuals: the AGEHIV cohort study. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **59**, 1787–1797 (2014).
21. V.H. Hildebrandt, A.M.J. Chorus, J.H. Stubbe. *Trendrapport Beweging en Gezondheid 2008 - 2009. 2010.* (2010).
22. Stichting HIV Monitoring. <http://www.hiv-monitoring.nl/english/>. Accessed March 21, 2016. (2016).
23. Horváth, I. G. *et al.* Invasive validation of a new oscillometric device (Arteriograph) for measuring augmentation index, central blood pressure and aortic pulse wave velocity. *J. Hypertens.* **28**, 2068–2075 (2010).
24. Rezaei, M.-R. *et al.* A magnetic resonance perspective of the pulse wave transit time by the Arteriograph device and potential for improving aortic length estimation for central pulse wave velocity. *Blood Press. Monit.* **18**, 111–118 (2013).
25. Baulmann, J. *et al.* A new oscillometric method for assessment of arterial stiffness: comparison with tonometric and piezo-electronic methods. *J. Hypertens.* **26**, 523–528 (2008).
26. Cecelja, M. & Chowienczyk, P. Dissociation of aortic pulse wave velocity with risk factors for cardiovascular disease other than hypertension: a systematic review. *Hypertens. Dallas Tex 1979* **54**, 1328–1336 (2009).
27. Karalliedde, J. *et al.* Valsartan improves arterial stiffness in type 2 diabetes independently of blood pressure lowering. *Hypertens. Dallas Tex 1979* **51**, 1617–1623 (2008).
28. Ho, J. E. *et al.* Initiation of antiretroviral therapy at higher nadir CD4+ T-cell counts is associated with reduced arterial stiffness in HIV-infected individuals. *AIDS Lond. Engl.* **24**, 1897–1905 (2010).
29. Monteiro, P. *et al.* Is arterial stiffness in HIV-infected individuals associated with HIV-related factors? *Braz. J. Med. Biol. Res. Rev. Bras. Pesqui. Médicas E Biológicas Soc. Bras. Biofísica Al* **45**, 818–826 (2012).
30. Mattace-Raso, F. U. S. *et al.* C-reactive protein and arterial stiffness in older adults: the Rotterdam Study. *Atherosclerosis* **176**, 111–116 (2004).
31. Nakhai-Pour, H. R., Grobbee, D. E., Bots, M. L., Muller, M. & van der Schouw, Y. T. C-reactive protein and aortic stiffness and wave reflection in middle-aged and elderly men from the community. *J. Hum. Hypertens.* **21**, 949–955 (2007).
32. Hsue, P. Y. *et al.* Increased carotid intima-media thickness in HIV patients is associated with increased cytomegalovirus-specific T-cell responses. *AIDS Lond. Engl.* **20**, 2275–2283 (2006).
33. Parrinello, C. M. *et al.* Cytomegalovirus immunoglobulin G antibody is associated with subclinical carotid artery disease among HIV-infected women. *J. Infect. Dis.* **205**, 1788–1796 (2012).

34. Burdo, T. H. *et al.* Soluble CD163, a novel marker of activated macrophages, is elevated and associated with noncalcified coronary plaque in HIV-infected patients. *J. Infect. Dis.* **204**, 1227–1236 (2011).
35. Fitch, K. V. *et al.* Noncalcified coronary atherosclerotic plaque and immune activation in HIV-infected women. *J. Infect. Dis.* **208**, 1737–1746 (2013).
36. Overton, E. T., Arathoon, E., Baraldi, E. & Tomaka, F. Effect of darunavir on lipid profile in HIV-infected patients. *HIV Clin. Trials* **13**, 256–270 (2012).
37. Baker, J. V. *et al.* Assessment of arterial elasticity among HIV-positive participants with high CD4 cell counts: a substudy of the INSIGHT Strategic Timing of AntiRetroviral Treatment (START) trial. *HIV Med.* **16 Suppl 1**, 109–118 (2015).


Part II

HEAD









# HIV infection and cognitive impairment in the combination antiretroviral therapy era

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## Abstract

With the introduction of combination antiretroviral therapy AIDS dementia complex or HIV-associated dementia, as it was termed later, largely disappeared in clinical practice. However, in the past few years, patients, long term infected and treated, including those with systemically well controlled infection, started to complain about milder memory problems and slowness, difficulties in concentration, planning, and multitasking. Neuropsychological studies have confirmed that cognitive impairment (CI) occurs in a substantial (15-50%) proportion of patients. Among HIV-infected patients CI was and is one of the most feared complications of HIV infection. In addition, CI may affect adherence to treatment and ultimately result in increased morbidity for systemic disease. So what may be going on in the central nervous system after so many years of apparently controlled HIV infection is an urgent and important challenge in the field of HIV medicine. In this review we summarize the key currently available data. We describe the clinical neurological and neuropsychological findings, the preferred diagnostic approach with new imaging techniques and cerebrospinal fluid analysis. We try to integrate data on pathogenesis and finally discuss possible therapeutic interventions.

## Introduction

Within years after the start of the epidemic it became clear that many HIV-infected patients developed severe progressive cognitive and motor impairment in the final months of their illness. This clinical syndrome was characterized clinically and neuropathologically by Price<sup>1</sup> and Navia et al.<sup>2</sup> in 1986 and termed AIDS dementia complex (ADC). ADC causes symptoms in three areas: cognition, motor function and behaviour. Cognitive impairment (CI) predominantly consists of mental slowing and attention/memory deficits. Motor symptoms comprise slowness and loss of balance; behavioural changes are characterized by apathy, social withdrawal and mood disturbances.

Many studies confirmed the hypothesis that HIV itself was causing dysfunction and damage in the central nervous system (CNS). Shortly after the primary infection HIV enters the brain in mononuclear cells, and settles in perivascular macrophages and microglial cells. Replication of HIV in these cells leads to immune activation and the production of viral and inflammatory proteins that eventually leads to cognitive decline and motor dysfunction in a subset of patients.

With the introduction of combination antiretroviral therapy (cART), ADC or HIV-associated dementia (HAD), as it was termed later, largely disappeared in clinical practice. Many clinical, pathological, and cerebrospinal fluid (CSF) studies showed that antiretroviral drugs inhibit local virus replication in the brain and in doing so limit local damage. Even severely impaired patients could improve after the initiation of treatment. Some drugs likely did better than others, but in general most combinations prevented the development of HAD. HAD became a rare complication, occurring occasionally in late-presenting as yet untreated patients, in patients on treatment but with poor adherence, or in patients in whom systemic and CNS infection had an unparalleled course.

In the past few years, however, patients, long term infected and treated, including those with systemically well controlled infection, started to complain about milder memory problems and slowness, difficulties in concentration, planning, and multitasking.

In recent years a new terminology has been developed to classify a broadening clinical spectrum of HIV-associated CI, including milder

**Table 5.1: Terminology**

HAND	HIV-associated neurocognitive disorder	Umbrella definition comprising ANI, MND and HAD
ANI	Asymptomatic neurocognitive impairment	Cognitive impairment (at least 1 standard deviation below the mean), involving at least 2 cognitive domains. The cognitive impairment does not interfere with everyday functioning.
MND	Mild neurocognitive disorder	Cognitive impairment (at least 1 standard deviation below the mean), involving at least 2 cognitive domains. The cognitive impairment produces at least mild interference in daily functioning.
HAD	HIV-associated dementia	Marked cognitive impairment (at least 2 standard deviations below the mean), involving at least 2 cognitive domains. The cognitive impairment produces marked interference with day-to-day functioning.
ADC	AIDS dementia complex	Former term of HAD
MCMD	Minor cognitive motor disorder	Former term of ANI and MND combined

abnormalities (**Table 5.1**).<sup>3</sup> Neuropsychological studies have confirmed that CI occurs in a substantial (15-50%) proportion of patients.<sup>4,5</sup> It has, however, to be noted that there is a current discussion about the prevalence of cognitive dysfunction, which might be overestimated because of very sensitive criteria when applying the new terminology. In addition, compared to the first decade of the epidemic, a shift has occurred in certain demographic variables and risk factors, for example increased age and lower transmission among drug users, which might affect the proportion of patients with CI.

Among HIV-infected patients CI was and is one of the most feared complications of HIV infection. In addition, CI may affect adherence to treatment and ultimately result in increased morbidity for systemic disease.<sup>6</sup> So what may be going on in the CNS after so many years of apparently controlled HIV infection is an urgent and important challenge in the field of HIV medicine.

In this review we summarize the key currently available data. We describe the clinical neurological and neuropsychological findings, the preferred diagnostic approach with new imaging techniques and CSF analysis. We try to integrate data on pathogenesis and finally discuss

possible therapeutic interventions. In doing so it will become clear that there remains a broad research agenda in this field for the years ahead.

## Neuropsychology

The neuropsychological profile of post-cART HIV-associated neurocognitive disorders (HAND) and its similarity to the pre-cART subcortical profile is a subject of debate. In recent years, abnormalities of greater or lesser extent have been demonstrated in many different cognitive domains, resulting in an expanding phenotype of HAND and a broadening neuropsychological profile.<sup>5,7,8</sup> Despite this heterogeneity the strongest impaired cognitive domains in HAND still fit the subcortical profile with the core deficits being: mental slowness, attention/memory deficits and impaired executive functioning.<sup>8</sup>

The following cognitive domains are recommended to be surveyed if HAND is suspected (as these are most commonly associated with HAND)<sup>3</sup>: speed of information processing, attention/working memory, executive functioning, memory, verbal/language, sensory-perceptual and motor skills (**Table 5.2**).

### Neuropsychological testing

Many different tests are available to evaluate each of these cognitive domains (**Table 5.2**) and most of the large HIV cohort studies have developed their own neuropsychological test battery. As these test batteries are usually extensive and time-consuming, there is a need for a rapid screening tool for cognitive deficits.

The Mini Mental State Examination (MMSE) is the most well-known cognitive bedside test but has a limited usefulness for the detection of HAND as it mainly detects cortical (as in Alzheimer's disease) instead of subcortical dysfunction.<sup>9</sup>

The HIV Dementia Scale (HDS) tests four cognitive domains (verbal memory recall, psychomotor speed, visual construction and response inhibition) and has originally been designed to detect HAD.<sup>10</sup> The use-

**Table 5.2: Frequently used tests to examine different cognitive domains**

Cognitive domain	Cognitive domain and HIV-associated cognitive impairment	Tests
Speed of information processing	Slowing of mental processes continues to be one of the most frequent cognitive abnormalities in HIV infection. <sup>4,8</sup> As mental speed facilitates most if not all cognitive and motor processes, slowness is by some authors even regarded as the key deficit which in turn leads to defects in other cognitive domains. <sup>145</sup>	Trail making test A Stroop colour-word Symbol digit modalities test Digit symbol (WAIS/WAIS-R) Simple reaction time Choice reaction time
Attention/working memory	Attention and working memory are two closely related cognitive functions, with the working memory (the ability to create a memory for temporary processing and storage of information) being highly dependent on attentional function. As a result of this close functional relationship, attentional deficits frequently occur simultaneously with working memory deficits. <sup>8,146,147</sup>	Digit span (WAIS-R) Paced auditory serial addition test Letter-number sequencing (WAIS-III)
Executive functioning	Many different aspects of executive dysfunction (such as reasoning, planning, complex problem solving, and set shifting between tasks and strategies) are reported in HIV-associated CI. <sup>8,146</sup>	Wisconsin card sorting test Trail making test B Stroop colour-word Halstead category test
Memory	The episodic memory (storing personally experienced episodes and events) is one of the various components of the memory as a whole. The episodic memory is divided in a retrospective (experienced events in the past) and a prospective part (the ability to execute a future intention or 'remembering to remember'), the latter requiring intact executive functions as well (e.g. planning and set shifting). In HIV-associated CI especially the prospective episodic memory and learning of new information are reported to be impaired. <sup>8,148</sup>	California verbal learning test Hopkins verbal learning test Brief visuospatial memory test Rey-Osterrieth complex figure Visual reproduction WMS Logical memory WMS Story learning Halstead-Reitan battery Rey auditory verbal learning test Memory for intentions screening test
Verbal/language	The most frequently identified language defect in HIV-associated CI is fluency impairment, although this may be the result of other cognitive impairments such as slowness or executive dysfunction. <sup>8,146</sup>	Boston naming test Category fluency (animals) Letter fluency Action/verbal fluency
Sensory/perceptual	The interpretation and integration of visual, auditory or sensory stimuli takes place in this cognitive domain. Abnormalities in this predominantly cortical domain are less frequently observed in HIV-associated CI.	Tactile form recognition right and left Speech sound perception test
Motor skills	Severe motor abnormalities (e.g. chorea, myoclonus, dyskinesia, dystonia) were seen frequently in the pre-cART era and formed one of the three cardinal symptoms in HAD. Although severe deficits are rare in the post-cART era, milder impairments (slowing, incoordination) are still prevalent in HIV-associated CI. <sup>8,146,149</sup>	Finger tapping dom/nondom hand Grooved pegboard dom/nondom hand Grip strength Timed gait Unified Parkinson's disease rating scale

Computerized testing (such as CogState or CalCap) is another possibility to evaluate different cognitive domains.<sup>150,151</sup>

Abbreviations: CI=cognitive impairment, cART=combination antiretroviral therapy, WAIS=Wechsler adult intelligence scale, WAIS-R=Wechsler adult intelligence scale, revised version, WAIS-III=Wechsler adult intelligence scale, third version, WMS=Wechsler memory scale, HAD=HIV-associated dementia.

fulness of the HDS for detecting milder cognitive deficits is under investigation.<sup>4,11</sup>

The HDS requires a certain amount of literacy and language comprehension, which limits the usefulness of this test. For this reason, the International HIV Dementia Scale has been developed (IHDS), testing three cognitive domains (psychomotor speed, motor speed and verbal memory recall).<sup>12</sup> The usefulness of the IHDS for detecting milder cognitive deficits is still under investigation.

Standardized and regularly administered symptom questionnaires likely also have a role in clinical screening.

Of note, these screening tests are no substitute for performing a complete neuropsychological evaluation which remains required for the diagnosis of HAND.

## Neuroimaging

Many studies during the course of the HIV epidemic have proven neuroimaging to be both an essential diagnostic tool in clinical HIV neurology and useful in enlarging insight in the pathogenesis of HIV infection of the CNS.

### Computed tomography and magnetic resonance imaging

Cerebral atrophy and white matter abnormalities are the two most common findings in HAD in early imaging studies. Whereas atrophy can be disclosed by both computed tomography (CT) and magnetic resonance imaging (MRI), the latter is largely superior for the identification and characterization of white matter abnormalities. Atrophy is seen most often in the basal ganglia (especially the caudate nucleus) and frontal white matter although cortical regions have also been reported to be atrophic.<sup>1,13</sup> Atrophy has been associated with advanced disease stage and (to a lesser extent) with cognitive dysfunction in early studies.<sup>13,14</sup> Post-cART studies demonstrate stronger correlations between atrophy and cognitive dysfunction.<sup>13,15,16</sup> Whereas one pre-cART prospective

study reports cerebral atrophy to be progressive<sup>14</sup>, no increase of atrophy has been described in a 7-year follow-up study in post-cART years (likely indicating a beneficial effect of cART).<sup>17</sup>

MRI in HAD frequently reveals patchy or diffuse, usually symmetrical, periventricular white matter abnormalities.<sup>18</sup> Small white matter abnormalities, however, have been found in non-demented HIV patients as well and even in HIV-negative non-demented controls. Therefore, earlier MRI studies report a controversial relationship between cognition and white matter abnormalities and consider the latter to be nonspecific.<sup>13,19,20</sup> Later MRI studies though using advanced and more sensitive MRI techniques do demonstrate a relationship between white matter abnormalities and cognition. MRI white matter abnormalities also correspond to a histopathological diagnosis of HIV encephalitis.<sup>21</sup>

Several more advanced MRI techniques are hereby discussed in detail.

#### Magnetic resonance spectroscopy

Magnetic resonance spectroscopy (MRS) measures metabolite concentrations in different brain regions. MRS studies (pre and post-cART) in patients with cognitive dysfunction have demonstrated reduced levels of N-acetyl aspartate (NAA), a marker for neuronal integrity, and increased levels of the glial activation markers myoinositol (MI) and choline (CHO). Glial activation (MI/CHO increase) indicates an inflammatory process and precedes neuronal loss (NAA decrease). Abnormalities are found mainly in the frontal white matter and basal ganglia.<sup>13,22–25</sup> The abnormal metabolite profile is reported to improve with cART.<sup>26–28</sup> MRS has proven to be more sensitive for early CI than single photon emission computed tomography (SPECT) or MRI.<sup>29,30</sup>

#### Diffusion tensor imaging

Diffusion tensor imaging (DTI), used in HIV research since 2001<sup>31</sup>, is an MRI technique that measures water diffusion in tissues and enables to visualize distribution and orientation of white matter tracts. This technique is especially useful in demonstrating subtle white matter abnormalities.



DTI studies in HIV patients report white matter abnormalities diffusely in the brain and more specific in the frontal white matter and corpus callosum, despite cART. Abnormalities are correlated strongly with cognitive deficits<sup>13,26,32-34</sup>, but the sensitivity for early changes is controversial.

#### Functional magnetic resonance imaging

Functional MRI (fMRI) measures neuronal activity during specific neuropsychological tasks (which are performed while in the scanner). This technique is used in HIV research since 1998<sup>35</sup> and most studies demonstrate increased neuronal activation in cognitively impaired patients, which is regarded as a compensatory mechanism resulting from decreased cerebral efficiency.<sup>13,26,36</sup> fMRI abnormalities correlate strongly with increased glial markers (MI/CHO) in frontal white matter and basal ganglia, indicating a subcortical inflammatory process.<sup>37</sup>

#### Perfusion magnetic resonance imaging

Perfusion MRI (pMRI) measures cerebral blood flow and volume and is used in HIV research since 2000.<sup>38</sup> Most studies report decreased cerebral blood flow or volume in cognitively unimpaired and especially impaired patients.<sup>13,26,39</sup>

#### Magnetization transfer imaging

Magnetization transfer imaging (MTI) has appeared useful for the detection of damage in normal appearing white matter (on regular MRI), having the potential to visualize subtle abnormalities. It has been proven useful in neurodegenerative diseases such as multiple sclerosis.<sup>13</sup> MTI is used in HIV research since 1997<sup>40</sup> and has demonstrated white matter abnormalities diffusely in different brain regions, correlating with CI.<sup>41</sup>

Nuclear medicine techniques: single photon emission computed tomography and positron emission tomography

Single photon emission computed tomography (SPECT) measures the uptake of radiotracers (usually  $^{99}\text{Tc}$ ) in the brain, which reflects the cerebral blood flow. Early SPECT studies show cortical and subcortical areas of hypoperfusion. Although the correlation with cognitive function is controversial<sup>13,22,26,42</sup>, these changes in perfusion seem to precede abnormalities found with CT or MRI.<sup>26</sup> In later SPECT studies similar findings of reduced blood flow are reported. Remarkable is the report of increased cerebral blood flow among patients with severe cognitive deficits, which is thought to reflect active inflammation.<sup>26,43</sup>

Positron emission tomography (PET) measures (glucose) metabolism in different brain regions. Early PET studies showed hypometabolism in the basal ganglia, which was relatively specific for HAD.<sup>13,22,26</sup> Later studies revealed a characteristic time course with hypermetabolism in early cognitive disease developing into hypometabolism during advanced disease.<sup>13,26,44</sup>

In conclusion, as white matter abnormalities have in many studies been related to cognitive impairment, promising techniques are those visualizing white matter in detail, such as DTI.

## Cerebrospinal fluid markers

Many potential CSF markers have been studied in HIV-infected patients for management of CNS HIV infection (including diagnosis, prediction, assessment of disease activity and response to treatments) and provide insight into underlying pathogenic mechanisms.

Cerebrospinal fluid markers can practically be classified into virological, host response and CNS tissue damage markers (given below).

Potentially useful CSF markers of HIV CNS infection<sup>152</sup>:

- 1) **Virological markers:**
  - a) HIV RNA
  - b) Env/pol sequence analysis for compartmentalization
  
- 2) **Host response markers:**
  - a) Markers of inflammation:
    - i) White blood cells
    - ii) Cytokines and chemokines:
      - tumour necrosis factor- $\alpha$
      - monocyte chemotactic protein-1 (MCP-1)/ CCL2
      - interferon- $\gamma$  inducible protein-10 (IL-10)/ CXCL10
      - macrophage inflammatory protein- $\alpha$  (MIP1- $\alpha$ )/CCL3, MIP1- $\beta$ /CCL4, RANTES/ CCL5
      - fractalkine/CX3CL1
    - iii) Neopterin
    - iv) Beta-2 microglobulin
  - b) Proteases:
    - i) Matrix metalloproteases
    - ii) Urokinase plasminogen activator (uPA) and soluble receptor (sUPAr)
  - c) Neurotoxic host factors:
    - i) Quinolinic acid and tryptophan
    - ii) Glutamate
    - iii) Nitric oxide
  - d) Markers of apoptosis
  - e) Fas and Fas-ligand
  
- 3) **Markers of CNS damage:**
  - a) Neuronal markers:
    - i) Neurofilament protein light chain (NFL)
    - ii) Tau protein
    - iii) Soluble amyloid protein precursor (sAPP)  $\alpha/\beta$
    - iv) 14-3-3 protein

However, no single marker has so far proved to be reliable for practical purposes. The mechanisms leading from HIV infection of the CNS to tissue dysfunction and CI are not straightforward and abnormal levels of CSF markers are often also present in patients with asymptomatic HIV infection or other CNS pathological conditions. Nonetheless, the use of several CSF markers in combination could be useful to recognize HIV-related cognitive dysfunction, including milder forms, both in untreated and treated patients.

*In the absence of treatment*, CSF HIV RNA levels usually remain stable in neurologically asymptomatic patients over several years<sup>45</sup>, but tend to increase with clinical disease progression. Levels are highest in patients with HAD or HIV encephalitis<sup>46,47</sup>, irrespective of plasma viremia, supporting the view that, in these conditions, CSF virus is mainly derived from productive infection of macrophages and microglial cells within the CNS. Infection of these cells leads to the release of soluble factors which can be measured in CSF.

Among these factors, the chemokine CCL2 (or monocyte chemoattractant protein-1, MCP-1), and neopterin, a product of the guanosine triphosphate metabolism (both produced by activated macrophages and other mononuclear phagocytes) have been well characterized for their potential to serve as disease marker. In HIV-positive, neurologically asymptomatic patients, CSF CCL2 levels are similar to or slightly higher than those found in HIV-negative controls<sup>48</sup>, whereas levels of neopterin are already abnormally elevated.<sup>47</sup> Significantly higher CSF levels of both CCL2 and neopterin are found in patients with HAD and HIV encephalitis.<sup>47,49</sup> CSF concentrations of both markers correlate with CSF HIV RNA levels, but less with their respective levels in plasma, strongly arguing for intrathecal origin.<sup>47,49,50</sup>

Among markers of tissue damage, NFL, the light chain of neurofilament, a major structural component of axons, appears one of the most promising.<sup>51</sup> The highest levels are found in patients with HAD or opportunistic CNS infections. However, concentrations can also be increased in neurologically asymptomatic patients with advanced systemic disease stage, suggesting subclinical axonal injury already at this stage.

*Untreated patients initiating cART* show a decrease of all these markers within weeks after starting therapy in both asymptomatic and

neurologically impaired patients. Different dynamics of HIV RNA decay is observed between CSF and plasma: either parallel or slower in CSF<sup>52-55</sup>, reflecting the principal source of virus replication (systemic vs. intrathecal).<sup>52</sup> CCL2, neopterin and NFL levels decrease upon treatment (more markedly in HAD patients, with higher baseline concentrations) in parallel with CSF HIV RNA<sup>47,56</sup> (personal observation<sup>PC</sup>), suggesting that, by reducing viral replication in the brain, treatment interferes locally with the inflammatory process and consequent tissue damage.

*In patients on cART* with sustained systemic HIV RNA suppression to undetectable levels, the relationship between CSF HIV RNA and neurological status does not seem to be maintained<sup>57</sup>, with low or undetectable CSF HIV RNA levels frequently observed in neurologically impaired patients on cART.<sup>58</sup> Indeed, suppression of CSF replication is observed not only in patients showing full systemic responses, but also in a large proportion of patients failing to respond systemically<sup>59</sup> and it seems to be maintained for years, also when ultrasensitive methods, that is, with limit of detection of less than 2-2.5 copies/mL, are used.<sup>60,61</sup> The opposite scenario, CSF 'escape' in patients with suppressed plasma replication occurs in approximately 10%<sup>62</sup>, and may disclose an active brain process and be associated with neurological symptoms and CI.<sup>63</sup> CSF markers of macrophage activation may remain abnormally elevated in treated patients with suppressed replication in both CSF and plasma.<sup>64,65</sup>

One of the current challenges is to understand the principal cause of this persistent intrathecal immune activation, whether it is ongoing low-grade viral replication in brain tissue, rather than systemic immune activation, chronically established tissue damage or presence of other CNS conditions.

Historically, CSF markers of HIV-induced CI were studied to differentiate HAD from opportunistic infections. However, current differential diagnosis involves many novel potential causes of CI, such as ageing, with its physiological changes and associated pathological conditions, primarily Alzheimer's disease<sup>66-68</sup>, HCV co-infection<sup>69</sup>, metabolic complications<sup>70</sup>, and possible toxicity of treatments.<sup>71</sup> In this new scenario it is essential that CSF markers can recognize whether HIV replication and consequent immune activation is the main cause of CI, in order to optimize management.

Other CSF markers, in addition to those described, appear promising both for patient management and pathogenesis studies (listed above), including the soluble urokinase plasminogen activator receptor (suPAR), a novel marker of immune activation<sup>72</sup>; the tau protein and the soluble forms of the amyloid precursor protein (sAPP alpha and beta), all markers of tissue damage.<sup>68</sup> In addition, the use of new, high-throughput technologies, such as proteomics and metabolomics, may enable to search for known or unknown molecules, possibly present at abnormal concentrations in the CSF of patients with HAND.<sup>73,74</sup>

## Neuropathology

Two specific neuropathological conditions that result from HIV infection of the brain were defined in 1991: HIV encephalitis (HIVE) and HIV leukoencephalopathy (HIVL).<sup>75</sup> The hallmark of HIVE and HIVL is the presence of multinucleated giant cells (MGCs) that are formed by fusion of infected and activated macrophages. Other features of HIVE and HIVL are activated macrophages and microglial cells, the latter sometimes forming microglial noduli (MGN), reactive astrogliosis and white matter pallor.<sup>75,76</sup> HIVE and HIVL are overlapping entities but in HIVL white matter damage is the dominating feature. In most HIV-infected brains the virus itself is detected in various regions<sup>77</sup>, though preferentially in the basal ganglia, hippocampus, white matter and frontal cortex.<sup>75-77</sup> Further and probably resultant neuropathologic findings include injury and loss of dendrites, synapses and neurons, resulting macroscopically in brain atrophy.<sup>76,78,79</sup>

To define the neuropathological substrate of CI, efforts were made to correlate cognitive functioning with these neuropathological abnormalities. In pre-cART studies, the strongest correlations were demonstrated between cognitive dysfunction and increased expression of brain tissue markers of macrophage/microglial activation and between cognitive dysfunction and signs of neurodegeneration. Abnormalities such as HIV viral load, HIVE and HIVL were only loosely correlated with CI.<sup>78-86</sup> The pathological correlate of HAND in the cART-era has not been

defined; however, high-level macrophage/ microglial activation, similar to that observed in pre-cART studies, is also observed in cART-treated patients.<sup>87</sup>

Brain tissue viral load is significantly lower among cART-treated patients with HIVE.<sup>88</sup> Otherwise, post-cART autopsy studies (although sparse and probably subjected to post-mortem exam selection bias) still report an HIVE incidence of 11-30%.<sup>84,89,90</sup> Compared with a pre-cART incidence of 10-54% this is only a moderate decrease, if any at all. So called minimal nonspecific abnormalities are even reported more frequently in post-cART studies, but their significance remains to be clarified.<sup>84,89,91</sup> In two large post-cART cohort autopsy studies the CNS remains the second most frequently affected organ.<sup>89,92</sup> These observations are remarkable, considering that other neuropathologic abnormalities such as opportunistic infections and malignancies within the CNS have decreased significantly since the introduction of cART.

## Pathogenesis

The pathogenic mechanisms behind CNS dysfunction in HIV infection remain to a certain extent unclear. There are discrepancies between the distribution and number of HIV-infected cells and the severity of the clinical course and brain tissue pathology which support other mechanisms than direct viral cytotoxicity as a cause of CNS damage.

HIV enters the CNS early following infection<sup>93,94</sup>, primarily by means of monocytes and lymphocytes infected before trafficking across the blood-brain barrier (BBB). After entry, a chronic productive HIV infection of macrophages and microglial cells is established. Normally, microglial cells express CD4-antigen at low levels, but they are likely to up-regulate the expression during cellular activation.<sup>95</sup> Apart from CD4, macrophages and microglia also express CCR5 on their surface.

The CNS infection leads to a chronic intrathecal immune activation that is present during the entire infectious course<sup>96</sup> and whereas viral products may have direct toxic effects against neurons or astrocytes, the primary mechanism of neuronal damage is likely a result of the

inflammatory process initiated by HIV-infected cells.<sup>97</sup> Macrophages and microglia act as both the major targets for HIV replication and a source of neurotoxins.<sup>98</sup> Secreted cellular products such as cytokines, quinolinic and arachidonic acids and nitric oxide can have neurotoxic effects, and chemokines and pro-inflammatory cytokines promote further cell activation and recruitment of additional macrophages and T-cells, thereby amplifying HIV-induced neurotoxicity.<sup>99</sup>

Astrocytes can be infected (and perhaps even more extensively than previously thought<sup>100</sup>) by HIV, but the infection is generally non-productive with restricted viral gene expression.<sup>101</sup> However, astrocytes may indirectly contribute to the neuropathogenesis by activation and/or dysfunction leading to increased cytokine production, reduced uptake of neurotoxins and impairment of the BBB.<sup>102</sup> Dysfunction of the BBB may be the priming event in the pathogenesis of HAD<sup>103</sup>; increased BBB permeability is a consistent finding in HAD<sup>104</sup> but also commonly found in early HIV disease in which it correlates to the degree of intrathecal immune activation.<sup>105</sup>

Neurons are not infected, but neuronal loss and decreased synaptic and dendritic density are, together with microglial and astrocyte proliferation and activation, commonly found and closely associated with HAD.<sup>106</sup>

HIV-related neurodegeneration is also linked to intrathecal immune activation<sup>51</sup> and signs of axonal disruption forecast the development of HAD.<sup>107</sup> Some reports suggest a similarity between pathogenesis of HIV brain injury and Alzheimer's disease, because of the deposition of amyloid plaques and precursor proteins in both conditions. However, the plaques observed in Alzheimer's disease are typically intra-neuronal, whereas these are both intra-neuronal and extra-neuronal in HIV infection.<sup>108</sup> Patients with HAD have abnormal CSF biomarkers of amyloid and tau metabolism<sup>109</sup> (like in Alzheimer's disease), but the pattern differs from Alzheimer's disease.<sup>68</sup> These differences imply separate underlying pathogenetic pathways of brain injury in HIV-associated neurodegeneration and Alzheimer's disease. Chronic immune activation has an essential part in HIV neuropathogenesis and it is commenced and driven by the CNS viral infection.

Combination antiretroviral therapy often has a dramatic beneficial



effect on neurological and cognitive dysfunction in patients with HAD supporting that a substantial share of symptoms relates to active, reversible toxic processes.<sup>110</sup> However, symptoms are not always totally reversed and persistent intrathecal immune activation<sup>65</sup> and detectable CSF viral load<sup>59,62</sup> also after several years of otherwise effective treatment indicate an ongoing active process within the brain as well during successful antiretroviral therapy.

Compelling evidence from several studies demonstrate that HIV infection in the CNS is compartmentalized from the systemic infection, although to varying degrees at different stages of the infection.<sup>111–113</sup> It is important not to overlook the CNS when discussing HIV persistence and eradication strategies, as the brain may act as a sanctuary for latent or slowly replicating virus.

The consequences of the chronic, low-grade, CNS immune activation have not yet been elucidated although concerns have been raised about an increased risk of HAND and/or other cognitive complaints, such as Alzheimer's disease and vascular dementia, in the ageing HIV-infected population.

## Risk factors and comorbidities

Several risk factors and associated physiological and pathological conditions have been identified in patients with CI (**Table 5.3**). In particular, the consistent association with low nadir CD4+ T- cell count suggests that previous CNS damage might be relevant in the pathogenesis of HAND.<sup>114</sup> On the contrary, both physiological ageing and several current pathological conditions may themselves be associated with cognitive, neurological or psychiatric dysfunction and thus contribute to a various extent to sustain the picture of CI. Practically, the presence of any of these conditions may confound the diagnosis of HAND.

In addition, individuals may also genetically be more susceptible to develop cognitive problems. For example, the E<sub>4</sub> isoform of apolipoprotein E (APOE) has been linked, especially in the elderly, to an increased risk of HAD<sup>115</sup>, and polymorphisms of CCL2 and its receptor CCR2

**Table 5.3: Risk factors and conditions/comorbidities associated with cognitive impairment in HIV infection**

Risk factors	Conditions/comorbidities
Nadir CD <sub>4</sub> + T-cell count <sup>114</sup>	HCV co-infection <sup>153-156</sup>
Ageing <sup>157,158</sup>	Substance or alcohol abuse <sup>153,159</sup>
Microbial translocation <sup>153,160</sup>	Cardiovascular disease and metabolic disorder <sup>153,161,162</sup>
Anemia <sup>163</sup>	Depression and other psychiatric conditions <sup>164</sup>
Thrombocytopenia <sup>165</sup>	
Host genetic factors <sup>115,116</sup>	Alzheimer's disease and other neurodegenerative diseases of the central nervous system
Viral genetic factors <sup>117</sup>	

seem associated with neuropsychological abnormalities.<sup>116</sup> Finally, viral (genetic) factors might also affect neurotoxicity; the influence of viral subtypes on cognitive functioning is under investigation but not yet elucidated.<sup>117-119</sup>

## Interventions

### Combination antiretroviral therapy

Zidovudine (the first approved antiretroviral drug for HIV) has been proven to have beneficial effects on cognitive functioning.<sup>120,121</sup> After the introduction of cART in 1996 many studies have reported additional improvements on cognitive functioning.<sup>122-128</sup> Some patients, however, only stabilize or show incomplete recovery on cART<sup>124,127</sup> and a small proportion of patients even deteriorate cognitively despite cART.<sup>128</sup>

Combination antiretroviral therapy and the central nervous system cART entry into the CNS is hampered by the BBB. Drugs easily passing the BBB and affecting (macrophages in) the CNS are so called neuroactive drugs.

Several studies have shown an association between the use of neuroactive drugs (defined in different ways) and good cognitive performance.<sup>123,129</sup> To better define and quantify CNS effectiveness, a CNS penetration effectiveness (CPE) score, based on individual drug ranking, was more recently proposed.<sup>130-132</sup> Each individual antiretroviral drug has been given a score between 1 and 4; summing up the individual scores results in the CPE score, with higher scores indicating more CNS effectiveness. Regimens with higher CPE scores have been correlated with neuropsychological improvement and better neuropsychological performance.<sup>128,133,134</sup> Conversely, a low CPE ranking is associated with an 88% increase in the odds of detectable CSF viral load.<sup>130</sup> One contrasting smaller study reports less cognitive improvement in patients with high CPE rankings.<sup>135</sup>

Though efforts have been made to develop this tool to optimize treatment, there are limitations of the CPE score and it is not yet validated for clinical use. The most important limitation is the amount of data available for each drug; inevitably, only some have been classified based on clinical information; others have been classified based only on pharmacokinetic or chemical features. Secondly, other factors may be of importance for the efficacy of cART in the CNS, such as genotypic resistance.<sup>63</sup> Furthermore, since it was first reported, several adjustments have been made to the CPE scoring system, resulting in renewed versions. The CPE scoring system will probably evolve further in the coming years, and large prospective studies will be required to establish the full value of this approach for clinical management.

#### Adverse effects of combination antiretroviral therapy

Direct evidence for cART-related neurotoxicity is sparse.<sup>136</sup> Some nucleoside reverse transcriptase inhibitors are known (as is HIV infection itself) to cause mitochondrial dysfunction in peripheral tissues (liver, heart, muscles).<sup>136</sup> Whether or not neuronal damage as a result of mitochon-

drial dysfunction occurs is unknown. One MRS study reports a decrease in NAA (a marker for neuronal integrity) in patients using didanosine and/or stavudine<sup>137</sup>, indicating neuronal damage possibly as a result of mitochondrial dysfunction. In addition, in-vitro research showed protease inhibitors to cause proteasome dysfunction resulting in intracellular accumulation of toxic proteins, possibly causing cell damage.<sup>138</sup>

The non-nucleoside reverse transcriptase inhibitor efavirenz frequently causes neuropsychiatric side effects such as bad dreams, sleep disorders, dizziness, and anxiety. These effects usually subside after the first few weeks of therapy, but may persist in a minority of cases.<sup>139</sup> However, a negative effect of efavirenz on cognitive functioning in both short- and long term has not been demonstrated.<sup>140</sup>

Structured treatment interruptions (STIs) lead to viral rebound, deteriorating immune function and worsening CSF markers<sup>141</sup> and subsequently to increased incidence of opportunistic infections and death. However, the effect of STIs on cognitive function is controversial, which is interesting in the context of cART neurotoxicity. One study investigating treatment interruptions reported cognitive stability for six months, despite worsening immunosuppression and viral rebound.<sup>142</sup> Another study investigating patients with high pre-entry and nadir CD<sub>4</sub><sup>+</sup> T-cell counts who discontinued cART reported a modest neuropsychological improvement following interruption.<sup>71</sup> These results could support a degree of cART neurotoxicity.

In the long term, as nadir CD<sub>4</sub><sup>+</sup> T-cell count has been recognized as a risk factor for developing HAND, STIs (resulting in decreasing CD<sub>4</sub> counts) nevertheless might cause cognitive decline. Of note, intermittent ART has clearly been associated with a higher risk of mortality from non-AIDS morbidity and mortality than continuous cART.<sup>143</sup>

### Adjunctive agents

Several non-cART agents have been investigated in vitro, in animal models and in humans (**Table 5.4**). However, so far the results of these trials are disappointing (as is the case in trials for other neurodegenerative diseases). None of them have offered a substantial solution in treating cognitive disorders in HIV infection.<sup>136,144</sup>

**Table 5.4: Adjunctive agents**

Psychostimulants	HIV infection of the central nervous system causes hypoactivation of the dopaminergic system. <sup>166</sup> Psychostimulants are known to stimulate the dopaminergic system and have thus been investigated in patients with HIV-related cognitive impairment. Methylphenidate and dextroamphetamine have shown to improve cognitive function though this effect seems short-lived and may be a result of relieving depressive symptoms. <sup>167,168</sup> As these agents are known to cause dependence, it might not be appropriate to prescribe these agents to patients with a history or a risk of substance abuse.
Selegiline	Selegiline is a monoamine oxidase B (MAO-B) inhibitor and speculated to reduce oxidative stress and to have neuroprotective properties. Though two small studies report selegiline to improve cognitive functioning <sup>169,170</sup> , three larger studies report no significant effect. <sup>171,172</sup>
Valproic acid	Valproic acid is supposed to have neuroprotective properties by inhibiting neuronal loss, stimulating neurogenesis and reducing neurotoxicity of HIV-infected macrophages. <sup>173</sup> On the contrary, has valproic acid shown to induce microglial apoptosis and activate HIV replication in microglial cells. <sup>174,175</sup> One small study demonstrated a trend toward cognitive improvement and a significant improvement in magnetic resonance spectroscopy brain metabolite profile. <sup>176</sup> A negative effect of valproic acid on cognitive functioning though has been reported in HIV patients using valproic acid for a longer period of time and in higher dosages. <sup>177</sup>
Lexipafant	Lexipafant supposedly inhibits platelet-activating factor, which is an inflammatory mediator contributing to neuronal injury. A randomized trial reports merely a trend towards cognitive improvement. <sup>178</sup>
Calcium channel blocker	Preventing excitotoxicity using a calcium channel blocker has been investigated in HIV-associated cognitive impairment, showing only a trend towards cognitive improvement. <sup>179</sup>
Memantine	Memantine, an N-methyl-D-aspartate (NMDA) antagonist, supposedly has neuroprotective properties <sup>180,181</sup> , but two trials have shown no significant cognitive improvement. <sup>182,183</sup>
Minocycline	Minocycline is a broad-spectrum antibiotic and a member of the tetracycline family. Aside from antimicrobial properties it is supposed to have the ability to inhibit microglial activation and HIV replication and to exhibit antioxidative and neuroprotective properties. <sup>184-187</sup> A trial in humans is being conducted but not yet published.
Lithium	Lithium is used for depression and bipolar disorder and is supposed to have neuroprotective properties. <sup>188</sup> One small study reports cognitive improvement (though this effect may also be the result of improving depressive symptoms); another small study solely reports improvements on neuroimaging. <sup>189,190</sup>
Antioxidants	Inflammation results in free radicals leading to oxidative stress and cell damage. Antioxidants, inhibiting this oxidative stress, have been investigated and are still under investigation in treating HIV-associated cognitive impairment. Examples are CPI-1189, OPC-14117, thioctic acid and nutritional components such as vitamin C and E, green tea derived epigallocatechin gallate, and curcumin. <sup>191-196</sup> The few agents that have been studied on humans have not shown convincing improvements on cognitive functioning. Thioctic acid has even shown a negative effect on cognition. <sup>170</sup>
Serotonin reuptake inhibitor	In a cohort study, serotonin reuptake inhibitors (in particular sertraline, citalopram and trazodone) are associated with lower cerebrospinal fluid viral load and better cognitive performance, but this effect may also be the result of improving depressive symptoms. <sup>197</sup>
Nanoparticles	Nanoparticles or nanocarriers may increase the penetration of antiretroviral drugs through the blood-brain barrier and facilitate drug transport into the brain. Subsequently they may increase the bioavailability of antiretroviral therapy in the brain. The use of nanotechnology for the treatment of HIV and the central nervous system is yet to be further investigated. <sup>198,199</sup>

## Discussion

Do we see new cognitive problems in HIV-infected individuals?

Yes, studies from different parts of the world, including large cohorts, report abnormal scores on neuropsychological assessments in 15-50% of patients.

Neuropsychological test batteries differ between studies and there is discussion on what is an abnormal test result. Patients with cognitive complaints show worse test results than those without.

What is the character of the abnormalities found?

The abnormalities found on neuropsychological assessments are milder than in full-blown HAD. In essence the core abnormality is slowness; patients do poor on all tests with a time factor in it. The longer-term course of these cognitive deficits, however, is not known yet.

Which diagnostic tests are useful in the clinical setting?

Neuropsychological assessment, CSF examination and MRI of the brain are important tools and accessible in many clinical settings. Neuropsychological examination will more reliably reveal the presence and character of cognitive disturbances. Virological, host response and CNS tissue damage CSF markers may be helpful to diagnose CNS immune activation and HIV RNA load in particular reflects more directly to what extent the process is HIV-driven. DTI, providing detailed information of the integrity of the white matter, may become an important marker in the future.

What is going on in the brain?

Chronic immune activation, HIV-driven or caused by other conditions such as ageing (or a combination), might be the mechanism behind the cognitive problems we see today. But many uncertainties remain. The clinical course of the cognitive deficits is unknown; it might be the result of a process that has been going on for years or there could be a subacute deterioration in which viral control in the CNS is suddenly

lost. We do not know whether this will in the end happen in all HIV-infected individuals or in a subset of patients at risk. We may have missed an ongoing CNS replication in systemically well controlled patients. We still do not know how to manage patients with low-level detectable HIV RNA in the CSF. CSF, MRI and neuropathological studies have provided information about the localization, the character and the magnitude of the pathology that is present in CI though this information is fragmented and so far unable to elucidate and interconnect all aspects related to CI.

#### The HIV-neurology research agenda

In the coming years the clinical course of these impairments should be followed closely in large cohorts of patients. Risk factors and conditions other than HIV that could lead to cognitive dysfunction need to be defined more accurately. More CSF parameters need to be assessed and new MRI techniques will hopefully provide us with more information on the white matter pathology, blood flow and vascular changes. For all antiretroviral drugs, CNS/CSF penetration studies should be performed, as well as clinical trials comparing different antiretroviral regimens. Promising adjunctive treatments should also selectively be studied. These efforts are essential to understand what is going on in the brain in longstanding HIV infection and to prevent dysfunction and provide optimal management and cure.

## Authors' contributions

JS researched data, has written all drafts, and was responsible for submitting the final version of the manuscript.

PC has written the section on cerebrospinal fluid markers.

MG has written the section on pathogenesis.

PR discussed and reviewed the final drafts of the manuscript.

PP initiated and supervised the writing of this review.

All the authors discussed the content of the article, and wrote, reviewed, and edited the manuscript before submission.

## References

1. Navia, B. A., Jordan, B. D. & Price, R. W. The AIDS dementia complex: I. Clinical features. *Ann. Neurol.* **19**, 517–524 (1986).
2. Navia, B. A., Cho, E. S., Petito, C. K. & Price, R. W. The AIDS dementia complex: II. Neuropathology. *Ann. Neurol.* **19**, 525–535 (1986).
3. Antinori, A. *et al.* Updated research nosology for HIV-associated neurocognitive disorders. *Neurology* **69**, 1789–1799 (2007).
4. Simioni, S. *et al.* Cognitive dysfunction in HIV patients despite long-standing suppression of viremia. *AIDS Lond. Engl.* **24**, 1243–1250 (2010).
5. Cysique, L. A., Maruff, P. & Brew, B. J. Prevalence and pattern of neuropsychological impairment in human immunodeficiency virus-infected/acquired immunodeficiency syndrome (HIV/AIDS) patients across pre- and post-highly active antiretroviral therapy eras: a combined study of two cohorts. *J. Neurovirol.* **10**, 350–357 (2004).
6. Hinkin, C. H. *et al.* Medication adherence among HIV+ adults: effects of cognitive dysfunction and regimen complexity. *Neurology* **59**, 1944–1950 (2002).
7. Cysique, L. A. J., Maruff, P. & Brew, B. J. The neuropsychological profile of symptomatic AIDS and ADC patients in the pre-HAART era: a meta-analysis. *J. Int. Neuropsychol. Soc. JINS* **12**, 368–382 (2006).
8. Woods, S. P., Moore, D. J., Weber, E. & Grant, I. Cognitive neuropsychology of HIV-associated neurocognitive disorders. *Neuropsychol. Rev.* **19**, 152–168 (2009).
9. Skinner, S., Adewale, A. J., DeBlock, L., Gill, M. J. & Power, C. Neurocognitive screening tools in HIV/AIDS: comparative performance among patients exposed to antiretroviral therapy. *HIV Med.* **10**, 246–252 (2009).
10. Power, C., Selnes, O. A., Grim, J. A. & McArthur, J. C. HIV Dementia Scale: a rapid screening test. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirology Off. Publ. Int. Retrovirology Assoc.* **8**, 273–278 (1995).
11. Bottiggi, K. A. *et al.* The HIV Dementia Scale: predictive power in mild dementia and HAART. *J. Neurol. Sci.* **260**, 11–15 (2007).
12. Sacktor, N. C. *et al.* The International HIV Dementia Scale: a new rapid screening test for HIV dementia. *AIDS Lond. Engl.* **19**, 1367–1374 (2005).
13. Tate DF. Tate DF. Neuroimaging among HIV-infected patients: current knowledge and future directions. In: Paul RH, editor. HIV and the brain. New York: Humana Press; 2009. pp. 75–107. *Humana Press* (2009).
14. Stout, J. C. *et al.* Progressive cerebral volume loss in human immunodeficiency virus infection: a longitudinal volumetric magnetic resonance imaging study. HIV Neurobehavioral Research Center Group. *Arch. Neurol.* **55**, 161–168 (1998).
15. Patel, S. H. *et al.* Correlation between percentage of brain parenchymal volume and neurocognitive performance in HIV-infected patients. *AJNR Am. J. Neuroradiol.* **23**, 543–549 (2002).



16. Chiang, M.-C. *et al.* 3D pattern of brain atrophy in HIV/AIDS visualized using tensor-based morphometry. *NeuroImage* **34**, 44–60 (2007).
17. Samuelsson, K. *et al.* The nervous system in early HIV infection: a prospective study through 7 years. *Eur. J. Neurol.* **13**, 283–291 (2006).
18. Dal Pan, G. J. *et al.* Patterns of cerebral atrophy in HIV-1-infected individuals: results of a quantitative MRI analysis. *Neurology* **42**, 2125–2130 (1992).
19. McArthur, J. C. *et al.* Incidental white matter hyperintensities on magnetic resonance imaging in HIV-1 infection. Multicenter AIDS Cohort Study. *J. Acquir. Immune Defic. Syndr.* **3**, 252–259 (1990).
20. Paul, R., Cohen, R., Navia, B. & Tashima, K. Relationships between cognition and structural neuroimaging findings in adults with human immunodeficiency virus type-1. *Neurosci. Biobehav. Rev.* **26**, 353–359 (2002).
21. Archibald, S. L. *et al.* Correlation of in vivo neuroimaging abnormalities with post-mortem human immunodeficiency virus encephalitis and dendritic loss. *Arch. Neurol.* **61**, 369–376 (2004).
22. Brew BJ. AIDS dementia complex. *Handbook of clinical neurology*. Amsterdam, The Netherlands: Elsevier; 2007. pp. 79–91.
23. Chang, L., Ernst, T., Leonido-Yee, M., Walot, I. & Singer, E. Cerebral metabolite abnormalities correlate with clinical severity of HIV-1 cognitive motor complex. *Neurology* **52**, 100–108 (1999).
24. Mohamed, M. A. *et al.* Factor analysis of proton MR spectroscopic imaging data in HIV infection: metabolite-derived factors help identify infection and dementia. *Radiology* **254**, 577–586 (2010).
25. Sacktor, N. *et al.* A multicenter study of two magnetic resonance spectroscopy techniques in individuals with HIV dementia. *J. Magn. Reson. Imaging JMRI* **21**, 325–333 (2005).
26. Tucker, K. A. *et al.* Neuroimaging in human immunodeficiency virus infection. *J. Neuroimmunol.* **157**, 153–162 (2004).
27. Stankoff, B. *et al.* Clinical and spectroscopic improvement in HIV-associated cognitive impairment. *Neurology* **56**, 112–115 (2001).
28. Chang, L. *et al.* Highly active antiretroviral therapy reverses brain metabolite abnormalities in mild HIV dementia. *Neurology* **53**, 782–789 (1999).
29. Ernst, T., Itti, E., Itti, L. & Chang, L. Changes in cerebral metabolism are detected prior to perfusion changes in early HIV-CMC: A coregistered (<sup>1</sup>H)MRS and SPECT study. *J. Magn. Reson. Imaging JMRI* **12**, 859–865 (2000).
30. Paul, R. H. *et al.* Relative sensitivity of magnetic resonance spectroscopy and quantitative magnetic resonance imaging to cognitive function among nondemented individuals infected with HIV. *J. Int. Neuropsychol. Soc. JINS* **14**, 725–733 (2008).
31. Pomara, N., Crandall, D. T., Choi, S. J., Johnson, G. & Lim, K. O. White matter abnormalities in HIV-1 infection: a diffusion tensor imaging study. *Psychiatry Res.* **106**, 15–24 (2001).

32. Ragin, A. B. *et al.* Diffusion tensor imaging of subcortical brain injury in patients infected with human immunodeficiency virus. *J. Neurovirol.* **11**, 292–298 (2005).
33. Gongvatana, A. *et al.* White matter tract injury and cognitive impairment in human immunodeficiency virus-infected individuals. *J. Neurovirol.* **15**, 187–195 (2009).
34. Chen, Y. *et al.* White matter abnormalities revealed by diffusion tensor imaging in non-demented and demented HIV+ patients. *NeuroImage* **47**, 1154–1162 (2009).
35. Tracey, I. *et al.* Increased cerebral blood volume in HIV-positive patients detected by functional MRI. *Neurology* **50**, 1821–1826 (1998).
36. Ernst, T. *et al.* Declined neural efficiency in cognitively stable human immunodeficiency virus patients. *Ann. Neurol.* **65**, 316–325 (2009).
37. Ernst, T., Chang, L. & Arnold, S. Increased glial metabolites predict increased working memory network activation in HIV brain injury. *NeuroImage* **19**, 1686–1693 (2003).
38. Chang, L., Ernst, T., Leonido-Yee, M. & Speck, O. Perfusion MRI detects rCBF abnormalities in early stages of HIV-cognitive motor complex. *Neurology* **54**, 389–396 (2000).
39. Ances, B. M. *et al.* Resting cerebral blood flow: a potential biomarker of the effects of HIV in the brain. *Neurology* **73**, 702–708 (2009).
40. Dousset, V. *et al.* Magnetization transfer study of HIV encephalitis and progressive multifocal leukoencephalopathy. Groupe d'Epidémiologie Clinique du SIDA en Aquitaine. *AJNR Am. J. Neuroradiol.* **18**, 895–901 (1997).
41. Wu, Y. *et al.* Whole brain and localized magnetization transfer measurements are associated with cognitive impairment in patients infected with human immunodeficiency virus. *AJNR Am. J. Neuroradiol.* **29**, 140–145 (2008).
42. Sacktor, N. *et al.* A comparison of cerebral SPECT abnormalities in HIV-positive homosexual men with and without cognitive impairment. *Arch. Neurol.* **52**, 1170–1173 (1995).
43. Christensson, B., Ljungberg, B., Ryding, E., Svenson, G. & Rosén, I. SPECT with 99mTc-HMPAO in subjects with HIV infection: cognitive dysfunction correlates with high uptake. *Scand. J. Infect. Dis.* **31**, 349–354 (1999).
44. von Giesen, H. J. *et al.* Potential time course of human immunodeficiency virus type 1-associated minor motor deficits: electrophysiologic and positron emission tomography findings. *Arch. Neurol.* **57**, 1601–1607 (2000).
45. Ellis, R. J. *et al.* Human immunodeficiency virus-1 RNA levels in cerebrospinal fluid exhibit a set point in clinically stable patients not receiving antiretroviral therapy. *J. Infect. Dis.* **187**, 1818–1821 (2003).
46. Cinque, P. *et al.* Cerebrospinal fluid HIV-1 RNA levels: correlation with HIV encephalitis. *AIDS Lond. Engl.* **12**, 389–394 (1998).
47. Hagberg, L. *et al.* Cerebrospinal fluid neopterin: an informative biomarker of central nervous system immune activation in HIV-1 infection. *AIDS Res. Ther.* **7**, 15 (2010).

48. Enting, R. H. *et al.* Cerebrospinal fluid beta2-microglobulin, monocyte chemo-tactic protein-1, and soluble tumour necrosis factor alpha receptors before and after treatment with lamivudine plus zidovudine or stavudine. *J. Neuroimmunol.* **102**, 216–221 (2000).
49. Cinque, P. *et al.* Elevated cerebrospinal fluid levels of monocyte chemotactic protein-1 correlate with HIV-1 encephalitis and local viral replication. *AIDS Lond. Engl.* **12**, 1327–1332 (1998).
50. Kelder, W., McArthur, J. C., Nance-Sproson, T., McClernon, D. & Griffin, D. E. Beta-chemokines MCP-1 and RANTES are selectively increased in cerebrospinal fluid of patients with human immunodeficiency virus-associated dementia. *Ann. Neurol.* **44**, 831–835 (1998).
51. Abdulle, S. *et al.* CSF neurofilament protein (NFL) -- a marker of active HIV-related neurodegeneration. *J. Neurol.* **254**, 1026–1032 (2007).
52. Staprans, S. *et al.* Time course of cerebrospinal fluid responses to antiretroviral therapy: evidence for variable compartmentalization of infection. *AIDS Lond. Engl.* **13**, 1051–1061 (1999).
53. Ellis, R. J. *et al.* Cerebrospinal fluid HIV RNA originates from both local CNS and systemic sources. *Neurology* **54**, 927–936 (2000).
54. Cinque, P. *et al.* Effect of genotypic resistance on the virological response to highly active antiretroviral therapy in cerebrospinal fluid. *AIDS Res. Hum. Retroviruses* **17**, 377–383 (2001).
55. Eggers, C., Hertogs, K., Stürenburg, H.-J., van Lunzen, J. & Stellbrink, H.-J. De-layed central nervous system virus suppression during highly active antiretroviral therapy is associated with HIV encephalopathy, but not with viral drug resistance or poor central nervous system drug penetration. *AIDS Lond. Engl.* **17**, 1897–1906 (2003).
56. Mellgren, A. *et al.* Antiretroviral treatment reduces increased CSF neurofilament protein (NFL) in HIV-1 infection. *Neurology* **69**, 1536–1541 (2007).
57. Sevigny, J. J. *et al.* Evaluation of HIV RNA and markers of immune activation as predictors of HIV-associated dementia. *Neurology* **63**, 2084–2090 (2004).
58. Cysique, L. A. *et al.* Undetectable cerebrospinal fluid HIV RNA and beta-2 mi-croglobulin do not indicate inactive AIDS dementia complex in highly active antiretroviral therapy-treated patients. *J. Acquir. Immune Defic. Syndr.* **1999** **39**, 426–429 (2005).
59. Spudich, S., Lollo, N., Liegler, T., Deeks, S. G. & Price, R. W. Treatment benefit on cerebrospinal fluid HIV-1 levels in the setting of systemic virological suppression and failure. *J. Infect. Dis.* **194**, 1686–1696 (2006).
60. Yilmaz, A., Svennerholm, B., Hagberg, L. & Gisslén, M. Cerebrospinal fluid viral loads reach less than 2 copies/ml in HIV-1-infected patients with effective an-tiretroviral therapy. *Antivir. Ther.* **11**, 833–837 (2006).
61. Probasco, J. C. *et al.* Cerebrospinal fluid in HIV-1 systemic viral controllers: ab-

- sence of HIV-1 RNA and intrathecal inflammation. *AIDS Lond. Engl.* **24**, 1001–1005 (2010).
62. Edén, A. *et al.* HIV-1 viral escape in cerebrospinal fluid of subjects on suppressive antiretroviral treatment. *J. Infect. Dis.* **202**, 1819–1825 (2010).
  63. Canestri, A. *et al.* Discordance between cerebral spinal fluid and plasma HIV replication in patients with neurological symptoms who are receiving suppressive antiretroviral therapy. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **50**, 773–778 (2010).
  64. Gisolf, E. H. *et al.* Increasing cerebrospinal fluid chemokine concentrations despite undetectable cerebrospinal fluid HIV RNA in HIV-1-infected patients receiving antiretroviral therapy. *J. Acquir. Immune Defic. Syndr.* **1999** **25**, 426–433 (2000).
  65. Edén, A. *et al.* Immune activation of the central nervous system is still present after >4 years of effective highly active antiretroviral therapy. *J. Infect. Dis.* **196**, 1779–1783 (2007).
  66. Conde, J. R. & Streit, W. J. Microglia in the aging brain. *J. Neuropathol. Exp. Neurol.* **65**, 199–203 (2006).
  67. González-Scarano, F. & Baltuch, G. Microglia as mediators of inflammatory and degenerative diseases. *Annu. Rev. Neurosci.* **22**, 219–240 (1999).
  68. Gisslén, M. *et al.* Amyloid and tau cerebrospinal fluid biomarkers in HIV infection. *BMC Neurol.* **9**, 63 (2009).
  69. Wilkinson, J., Radkowski, M. & Laskus, T. Hepatitis C virus neuroinvasion: identification of infected cells. *J. Virol.* **83**, 1312–1319 (2009).
  70. Wright, E. J. *et al.* Cardiovascular risk factors associated with lower baseline cognitive performance in HIV-positive persons. *Neurology* **75**, 864–873 (2010).
  71. Robertson, K. R. *et al.* Neurocognitive effects of treatment interruption in stable HIV-positive patients in an observational cohort. *Neurology* **74**, 1260–1266 (2010).
  72. Cinque, P. *et al.* The urokinase receptor is overexpressed in the AIDS dementia complex and other neurological manifestations. *Ann. Neurol.* **55**, 687–694 (2004).
  73. Laspiur, J. P. *et al.* CSF proteomic fingerprints for HIV-associated cognitive impairment. *J. Neuroimmunol.* **192**, 157–170 (2007).
  74. Wikoff, W. R., Pendyala, G., Siuzdak, G. & Fox, H. S. Metabolomic analysis of the cerebrospinal fluid reveals changes in phospholipase expression in the CNS of SIV-infected macaques. *J. Clin. Invest.* **118**, 2661–2669 (2008).
  75. Budka, H. Neuropathology of human immunodeficiency virus infection. *Brain Pathol. Zurich Switz.* **1**, 163–175 (1991).
  76. Bell, J. E. An update on the neuropathology of HIV in the HAART era. *Histopathology* **45**, 549–559 (2004).
  77. Wiley, C. A. *et al.* Distribution of brain HIV load in AIDS. *Brain Pathol. Zurich Switz.* **8**, 277–284 (1998).
  78. Everall, I. P. *et al.* Cortical synaptic density is reduced in mild to moderate human

- immunodeficiency virus neurocognitive disorder. HNRC Group. HIV Neurobehavioral Research Center. *Brain Pathol. Zurich Switz.* **9**, 209–217 (1999).
79. Moore, D. J. *et al.* Cortical and subcortical neurodegeneration is associated with HIV neurocognitive impairment. *AIDS Lond. Engl.* **20**, 879–887 (2006).
  80. Cherner, M. *et al.* Neurocognitive dysfunction predicts postmortem findings of HIV encephalitis. *Neurology* **59**, 1563–1567 (2002).
  81. Johnson, R. T., Glass, J. D., McArthur, J. C. & Chesebro, B. W. Quantitation of human immunodeficiency virus in brains of demented and nondemented patients with acquired immunodeficiency syndrome. *Ann. Neurol.* **39**, 392–395 (1996).
  82. Ances, B. M. & Ellis, R. J. Dementia and neurocognitive disorders due to HIV-1 infection. *Semin. Neurol.* **27**, 86–92 (2007).
  83. Brew, B. J., Rosenblum, M., Cronin, K. & Price, R. W. AIDS dementia complex and HIV-1 brain infection: clinical-virological correlations. *Ann. Neurol.* **38**, 563–570 (1995).
  84. Overall, I. *et al.* Cliniconeuropathologic correlates of human immunodeficiency virus in the era of antiretroviral therapy. *J. Neurovirol.* **15**, 360–370 (2009).
  85. Kumar, A. M., Borodowsky, I., Fernandez, B., Gonzalez, L. & Kumar, M. Human immunodeficiency virus type 1 RNA Levels in different regions of human brain: quantification using real-time reverse transcriptase-polymerase chain reaction. *J. Neurovirol.* **13**, 210–224 (2007).
  86. Glass, J. D., Fedor, H., Wesselingh, S. L. & McArthur, J. C. Immunocytochemical quantitation of human immunodeficiency virus in the brain: correlations with dementia. *Ann. Neurol.* **38**, 755–762 (1995).
  87. Anthony, I. C., Ramage, S. N., Carnie, F. W., Simmonds, P. & Bell, J. E. Influence of HAART on HIV-related CNS disease and neuroinflammation. *J. Neuropathol. Exp. Neurol.* **64**, 529–536 (2005).
  88. Langford, D. *et al.* Relationship of antiretroviral treatment to postmortem brain tissue viral load in human immunodeficiency virus-infected patients. *J. Neurovirol.* **12**, 100–107 (2006).
  89. Jellinger, K. A. *et al.* Neuropathology and general autopsy findings in AIDS during the last 15 years. *Acta Neuropathol. (Berl.)* **100**, 213–220 (2000).
  90. Vago, L. *et al.* Pathological findings in the central nervous system of AIDS patients on assumed antiretroviral therapeutic regimens: retrospective study of 1597 autopsies. *AIDS Lond. Engl.* **16**, 1925–1928 (2002).
  91. Gray, F., Chrétien, F., Vallat-Decouvelaere, A. V. & Scaravilli, F. The changing pattern of HIV neuropathology in the HAART era. *J. Neuropathol. Exp. Neurol.* **62**, 429–440 (2003).
  92. Masliah, E., DeTeresa, R. M., Mallory, M. E. & Hansen, L. A. Changes in pathological findings at autopsy in AIDS cases for the last 15 years. *AIDS Lond. Engl.* **14**, 69–74 (2000).

93. Davis, L. E. *et al.* Early viral brain invasion in iatrogenic human immunodeficiency virus infection. *Neurology* **42**, 1736–1739 (1992).
94. Schacker, T., Collier, A. C., Hughes, J., Shea, T. & Corey, L. Clinical and epidemiologic features of primary HIV infection. *Ann. Intern. Med.* **125**, 257–264 (1996).
95. Perry, V. H. & Gordon, S. Modulation of CD<sub>4</sub> antigen on macrophages and microglia in rat brain. *J. Exp. Med.* **166**, 1138–1143 (1987).
96. Gisslén, M., Fuchs, D., Svennerholm, B. & Hagberg, L. Cerebrospinal fluid viral load, intrathecal immunoactivation, and cerebrospinal fluid monocytic cell count in HIV-1 infection. *J. Acquir. Immune Defic. Syndr.* **1999** **21**, 271–276 (1999).
97. Price, R. W. *et al.* The brain in AIDS: central nervous system HIV-1 infection and AIDS dementia complex. *Science* **239**, 586–592 (1988).
98. Kim, W.-K., Alvarez, X. & Williams, K. The role of monocytes and perivascular macrophages in HIV and SIV neuropathogenesis: information from non-human primate models. *Neurotox. Res.* **8**, 107–115 (2005).
99. González-Scarano, F. & Martín-García, J. The neuropathogenesis of AIDS. *Nat. Rev. Immunol.* **5**, 69–81 (2005).
100. Churchill, M. J. *et al.* Extensive astrocyte infection is prominent in human immunodeficiency virus-associated dementia. *Ann. Neurol.* **66**, 253–258 (2009).
101. Gorry, P. R. *et al.* Astrocyte infection by HIV-1: mechanisms of restricted virus replication, and role in the pathogenesis of HIV-1-associated dementia. *Curr. HIV Res.* **1**, 463–473 (2003).
102. Minagar, A. *et al.* The role of macrophage/microglia and astrocytes in the pathogenesis of three neurologic disorders: HIV-associated dementia, Alzheimer disease, and multiple sclerosis. *J. Neurol. Sci.* **202**, 13–23 (2002).
103. Power, C. *et al.* Cerebral white matter changes in acquired immunodeficiency syndrome dementia: alterations of the blood-brain barrier. *Ann. Neurol.* **34**, 339–350 (1993).
104. McArthur, J. C. *et al.* The diagnostic utility of elevation in cerebrospinal fluid beta 2-microglobulin in HIV-1 dementia. Multicenter AIDS Cohort Study. *Neurology* **42**, 1707–1712 (1992).
105. Andersson, L. M., Hagberg, L., Fuchs, D., Svennerholm, B. & Gisslén, M. Increased blood-brain barrier permeability in neuro-asymptomatic HIV-1-infected individuals--correlation with cerebrospinal fluid HIV-1 RNA and neopterin levels. *J. Neurovirol.* **7**, 542–547 (2001).
106. Kaul, M. & Lipton, S. A. Mechanisms of neuroimmunity and neurodegeneration associated with HIV-1 infection and AIDS. *J. Neuroimmune Pharmacol. Off. J. Soc. Neuroimmune Pharmacol.* **1**, 138–151 (2006).
107. Gisslén, M. *et al.* Elevated cerebrospinal fluid neurofilament light protein concentrations predict the development of AIDS dementia complex. *J. Infect. Dis.* **195**, 1774–1778 (2007).
108. Green, D. A. *et al.* Brain deposition of beta-amyloid is a common pathologic feature in HIV positive patients. *AIDS Lond. Engl.* **19**, 407–411 (2005).

109. Clifford, D. B. *et al.* CSF biomarkers of Alzheimer disease in HIV-associated neurologic disease. *Neurology* **73**, 1982–1987 (2009).
110. Price, R. W. & Spudich, S. Antiretroviral therapy and central nervous system HIV type 1 infection. *J. Infect. Dis.* **197 Suppl 3**, S294–306 (2008).
111. Harrington, P. R. *et al.* Cross-sectional characterization of HIV-1 env compartmentalization in cerebrospinal fluid over the full disease course. *AIDS Lond. Engl.* **23**, 907–915 (2009).
112. Schnell, G., Spudich, S., Harrington, P., Price, R. W. & Swanstrom, R. Compartmentalized human immunodeficiency virus type 1 originates from long-lived cells in some subjects with HIV-1-associated dementia. *PLoS Pathog.* **5**, e1000395 (2009).
113. Dunfee, R. L. *et al.* The HIV Env variant N283 enhances macrophage tropism and is associated with brain infection and dementia. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 15160–15165 (2006).
114. Valcour, V. *et al.* Lowest ever CD4 lymphocyte count (CD4 nadir) as a predictor of current cognitive and neurological status in human immunodeficiency virus type 1 infection--The Hawaii Aging with HIV Cohort. *J. Neurovirol.* **12**, 387–391 (2006).
115. Valcour, V. *et al.* Age, apolipoprotein E4, and the risk of HIV dementia: the Hawaii Aging with HIV Cohort. *J. Neuroimmunol.* **157**, 197–202 (2004).
116. Singh, K. K. *et al.* CCR2 polymorphisms affect neuropsychological impairment in HIV-1-infected adults. *J. Neuroimmunol.* **157**, 185–192 (2004).
117. Mishra, M., Vetrivel, S., Siddappa, N. B., Ranga, U. & Seth, P. Clade-specific differences in neurotoxicity of human immunodeficiency virus-1 B and C Tat of human neurons: significance of dicysteine C30C31 motif. *Ann. Neurol.* **63**, 366–376 (2008).
118. Sacktor, N. *et al.* HIV subtype D is associated with dementia, compared with subtype A, in immunosuppressed individuals at risk of cognitive impairment in Kampala, Uganda. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **49**, 780–786 (2009).
119. Boivin, M. J. *et al.* HIV-subtype A is associated with poorer neuropsychological performance compared with subtype D in antiretroviral therapy-naive Ugandan children. *AIDS Lond. Engl.* **24**, 1163–1170 (2010).
120. Sidtis, J. J. *et al.* Zidovudine treatment of the AIDS dementia complex: results of a placebo-controlled trial. AIDS Clinical Trials Group. *Ann. Neurol.* **33**, 343–349 (1993).
121. Portegies, P. *et al.* Declining incidence of AIDS dementia complex after introduction of zidovudine treatment. *BMJ* **299**, 819–821 (1989).
122. Tozzi, V. *et al.* Positive and sustained effects of highly active antiretroviral therapy on HIV-1-associated neurocognitive impairment. *AIDS Lond. Engl.* **13**, 1889–1897 (1999).
123. Cysique, L. A. J., Maruff, P. & Brew, B. J. Variable benefit in neuropsychologi-

- cal function in HIV-infected HAART-treated patients. *Neurology* **66**, 1447–1450 (2006).
124. Tozzi, V. *et al.* Persistence of neuropsychologic deficits despite long-term highly active antiretroviral therapy in patients with HIV-related neurocognitive impairment: prevalence and risk factors. *J. Acquir. Immune Defic. Syndr.* **1999** **45**, 174–182 (2007).
  125. Robertson, K. R. *et al.* Highly active antiretroviral therapy improves neurocognitive functioning. *J. Acquir. Immune Defic. Syndr.* **1999** **36**, 562–566 (2004).
  126. Marra, C. M. *et al.* Changes in CSF and plasma HIV-1 RNA and cognition after starting potent antiretroviral therapy. *Neurology* **60**, 1388–1390 (2003).
  127. McCutchan, J. A. *et al.* HIV suppression by HAART preserves cognitive function in advanced, immune-reconstituted AIDS patients. *AIDS Lond. Engl.* **21**, 1109–1117 (2007).
  128. Cysique, L. A. *et al.* Dynamics of cognitive change in impaired HIV-positive patients initiating antiretroviral therapy. *Neurology* **73**, 342–348 (2009).
  129. Letendre, S. L. *et al.* Enhancing antiretroviral therapy for human immunodeficiency virus cognitive disorders. *Ann. Neurol.* **56**, 416–423 (2004).
  130. Letendre, S. *et al.* Validation of the CNS Penetration-Effectiveness rank for quantifying antiretroviral penetration into the central nervous system. *Arch. Neurol.* **65**, 65–70 (2008).
  131. Letendre S, Ellis R, Deutsch R, Clifford D, Marra C, McCutchan A, *et al.* Correlates of time-to-loss-of-viral-response in CSF and plasma in the CHARTER cohort. In: CROI, paper #430. San Francisco; 2010.
  132. Letendre, S. L., Ellis, R. J., Ances, B. M. & McCutchan, J. A. Neurologic complications of HIV disease and their treatment. *Top. HIV Med. Publ. Int. AIDS Soc. USA* **18**, 45–55 (2010).
  133. Tozzi, V. *et al.* Changes in cognition during antiretroviral therapy: comparison of 2 different ranking systems to measure antiretroviral drug efficacy on HIV-associated neurocognitive disorders. *J. Acquir. Immune Defic. Syndr.* **1999** **52**, 56–63 (2009).
  134. Smurzynski, M. *et al.* Effects of central nervous system antiretroviral penetration on cognitive functioning in the ALLRT cohort. *AIDS Lond. Engl.* **25**, 357–365 (2011).
  135. Marra, C. M. *et al.* Impact of combination antiretroviral therapy on cerebrospinal fluid HIV RNA and neurocognitive performance. *AIDS Lond. Engl.* **23**, 1359–1366 (2009).
  136. Cysique, L. A. & Brew, B. J. Neuropsychological functioning and antiretroviral treatment in HIV/AIDS: a review. *Neuropsychol. Rev.* **19**, 169–185 (2009).
  137. Schweinsburg, B. C. *et al.* Brain mitochondrial injury in human immunodeficiency virus-seropositive (HIV+) individuals taking nucleoside reverse transcriptase inhibitors. *J. Neurovirol.* **11**, 356–364 (2005).
  138. Piccinini, M. *et al.* The HIV protease inhibitors nelfinavir and saquinavir, but




- not a variety of HIV reverse transcriptase inhibitors, adversely affect human proteasome function. *Antivir. Ther.* **10**, 215–223 (2005).
139. Rihs, T. A. *et al.* Efavirenz and chronic neuropsychiatric symptoms: a cross-sectional case control study. *HIV Med.* **7**, 544–548 (2006).
140. Clifford, D. B. *et al.* Long-term impact of efavirenz on neuropsychological performance and symptoms in HIV-infected individuals (ACTG 50975). *HIV Clin. Trials* **10**, 343–355 (2009).
141. Gisslén, M., Rosengren, L., Hagberg, L., Deeks, S. G. & Price, R. W. Cerebrospinal fluid signs of neuronal damage after antiretroviral treatment interruption in HIV-1 infection. *AIDS Res. Ther.* **2**, 6 (2005).
142. Childers, M. E. *et al.* Cognitive functioning during highly active antiretroviral therapy interruption in human immunodeficiency virus type 1 infection. *J. Neurovirol.* **14**, 550–557 (2008).
143. Strategies for Management of Antiretroviral Therapy (SMART) Study Group *et al.* CD4+ count-guided interruption of antiretroviral treatment. *N. Engl. J. Med.* **355**, 2283–2296 (2006).
144. McArthur, J. C., Steiner, J., Sacktor, N. & Nath, A. Human immunodeficiency virus-associated neurocognitive disorders: Mind the gap. *Ann. Neurol.* **67**, 699–714 (2010).
145. Hardy, D. J. & Hinkin, C. H. Reaction time performance in adults with HIV/AIDS. *J. Clin. Exp. Neuropsychol.* **24**, 912–929 (2002).
146. Dawes, S. *et al.* Variable patterns of neuropsychological performance in HIV-1 infection. *J. Clin. Exp. Neuropsychol.* **30**, 613–626 (2008).
147. Levine, A. J. *et al.* Elements of attention in HIV-infected adults: evaluation of an existing model. *J. Clin. Exp. Neuropsychol.* **30**, 53–62 (2008).
148. Carey, C. L. *et al.* Prospective memory in HIV-1 infection. *J. Clin. Exp. Neuropsychol.* **28**, 536–548 (2006).
149. Robertson, K. R. *et al.* Timed Gait test: normative data for the assessment of the AIDS dementia complex. *J. Clin. Exp. Neuropsychol.* **28**, 1053–1064 (2006).
150. Gonzalez, R. *et al.* Computerized reaction time battery versus a traditional neuropsychological battery: detecting HIV-related impairments. *J. Int. Neuropsychol. Soc. JINS* **9**, 64–71 (2003).
151. Maruff, P. *et al.* Validity of the CogState brief battery: relationship to standardized tests and sensitivity to cognitive impairment in mild traumatic brain injury, schizophrenia, and AIDS dementia complex. *Arch. Clin. Neuropsychol. Off. J. Natl. Acad. Neuropsychol.* **24**, 165–178 (2009).
152. Cinque, P., Brew, B. J., Gisslén, M., Hagberg, L. & Price, R. W. Cerebrospinal fluid markers in central nervous system HIV infection and AIDS dementia complex. *Handb. Clin. Neurol.* **85**, 261–300 (2007).
153. Jayadev, S. & Garden, G. A. Host and viral factors influencing the pathogenesis of HIV-associated neurocognitive disorders. *J. Neuroimmune Pharmacol. Off. J. Soc. NeuroImmune Pharmacol.* **4**, 175–189 (2009).

154. Tozzi, V. *et al.* Prevalence and risk factors for human immunodeficiency virus-associated neurocognitive impairment, 1996 to 2002: results from an urban observational cohort. *J. Neurovirol.* **11**, 265–273 (2005).
155. Parsons, T. D. *et al.* Neurocognitive functioning and HAART in HIV and hepatitis C virus co-infection. *AIDS Lond. Engl.* **20**, 1591–1595 (2006).
156. Hinkin, C. H., Castellon, S. A., Levine, A. J., Barclay, T. R. & Singer, E. J. Neurocognition in individuals co-infected with HIV and hepatitis C. *J. Addict. Dis.* **27**, 11–17 (2008).
157. Kirk, J. B. & Goetz, M. B. Human immunodeficiency virus in an aging population, a complication of success. *J. Am. Geriatr. Soc.* **57**, 2129–2138 (2009).
158. Valcour, V. *et al.* Higher frequency of dementia in older HIV-1 individuals: the Hawaii Aging with HIV-1 Cohort. *Neurology* **63**, 822–827 (2004).
159. Rippeth, J. D. *et al.* Methamphetamine dependence increases risk of neuropsychological impairment in HIV infected persons. *J. Int. Neuropsychol. Soc. JINS* **10**, 1–14 (2004).
160. Ancuta, P. *et al.* Microbial translocation is associated with increased monocyte activation and dementia in AIDS patients. *PLoS One* **3**, e2516 (2008).
161. Valcour, V. G. *et al.* Insulin resistance is associated with cognition among HIV-1-infected patients: the Hawaii Aging With HIV cohort. *J. Acquir. Immune Defic. Syndr.* **1999** **43**, 405–410 (2006).
162. Foley, J. *et al.* Neurocognitive functioning in HIV-1 infection: effects of cerebrovascular risk factors and age. *Clin. Neuropsychol.* **24**, 265–285 (2010).
163. McArthur, J. C. *et al.* Dementia in AIDS patients: incidence and risk factors. Multicenter AIDS Cohort Study. *Neurology* **43**, 2245–2252 (1993).
164. Carter, S. L., Rourke, S. B., Murji, S., Shore, D. & Rourke, B. P. Cognitive complaints, depression, medical symptoms, and their association with neuropsychological functioning in HIV infection: a structural equation model analysis. *Neuropsychology* **17**, 410–419 (2003).
165. Wachtman, L. M. *et al.* Platelet decline: an avenue for investigation into the pathogenesis of human immunodeficiency virus -associated dementia. *Arch. Neurol.* **64**, 1264–1272 (2007).
166. Kumar, A. M. *et al.* Human immunodeficiency virus type 1 in the central nervous system leads to decreased dopamine in different regions of postmortem human brains. *J. Neurovirol.* **15**, 257–274 (2009).
167. Angrist, B. *et al.* Central nervous system stimulants as symptomatic treatments for AIDS-related neuropsychiatric impairment. *J. Clin. Psychopharmacol.* **12**, 268–272 (1992).
168. Hinkin, C. H. *et al.* Methylphenidate improves HIV-1-associated cognitive slowing. *J. Neuropsychiatry Clin. Neurosci.* **13**, 248–254 (2001).
169. Sacktor, N. *et al.* Transdermal selegiline in HIV-associated cognitive impairment: pilot, placebo-controlled study. *Neurology* **54**, 233–235 (2000).
170. A randomized, double-blind, placebo-controlled trial of deprenyl and thioctic

- acid in human immunodeficiency virus-associated cognitive impairment. Dana Consortium on the Therapy of HIV Dementia and Related Cognitive Disorders. *Neurology* **50**, 645–651 (1998).
171. Schifitto, G. *et al.* Selegiline and oxidative stress in HIV-associated cognitive impairment. *Neurology* **73**, 1975–1981 (2009).
  172. Evans, S. R. *et al.* Selegiline transdermal system (STS) for HIV-associated cognitive impairment: open-label report of ACTG 5090. *HIV Clin. Trials* **8**, 437–446 (2007).
  173. Dou, H. *et al.* Neuroprotective activities of sodium valproate in a murine model of human immunodeficiency virus-1 encephalitis. *J. Neurosci. Off. J. Soc. Neurosci.* **23**, 9162–9170 (2003).
  174. Dragunow, M. *et al.* Valproic acid induces caspase 3-mediated apoptosis in microglial cells. *Neuroscience* **140**, 1149–1156 (2006).
  175. Robinson, B., Turchan, J., Anderson, C., Chauhan, A. & Nath, A. Modulation of human immunodeficiency virus infection by anticonvulsant drugs. *J. Neurovirol.* **12**, 1–4 (2006).
  176. Schifitto, G. *et al.* Valproic acid adjunctive therapy for HIV-associated cognitive impairment: a first report. *Neurology* **66**, 919–921 (2006).
  177. Cysique, L. A., Maruff, P. & Brew, B. J. Valproic acid is associated with cognitive decline in HIV-infected individuals: a clinical observational study. *BMC Neurol.* **6**, 42 (2006).
  178. Schifitto, G. *et al.* Randomized trial of the platelet-activating factor antagonist lexipafant in HIV-associated cognitive impairment. Neurological AIDS Research Consortium. *Neurology* **53**, 391–396 (1999).
  179. Navia, B. A. *et al.* A phase I/II trial of nimodipine for HIV-related neurologic complications. *Neurology* **51**, 221–228 (1998).
  180. Anderson, E. R., Gendelman, H. E. & Xiong, H. Memantine protects hippocampal neuronal function in murine human immunodeficiency virus type 1 encephalitis. *J. Neurosci. Off. J. Soc. Neurosci.* **24**, 7194–7198 (2004).
  181. Nath, A. *et al.* Synergistic neurotoxicity by human immunodeficiency virus proteins Tat and gp120: protection by memantine. *Ann. Neurol.* **47**, 186–194 (2000).
  182. Zhao, Y. *et al.* Memantine for AIDS dementia complex: open-label report of ACTG 301. *HIV Clin. Trials* **11**, 59–67 (2010).
  183. Schifitto, G. *et al.* Memantine and HIV-associated cognitive impairment: a neuropsychological and proton magnetic resonance spectroscopy study. *AIDS Lond. Engl.* **21**, 1877–1886 (2007).
  184. Zink, M. C. *et al.* Neuroprotective and anti-human immunodeficiency virus activity of minocycline. *JAMA* **293**, 2003–2011 (2005).
  185. Si, Q. *et al.* A novel action of minocycline: inhibition of human immunodeficiency virus type 1 infection in microglia. *J. Neurovirol.* **10**, 284–292 (2004).
  186. Kraus, R. L. *et al.* Antioxidant properties of minocycline: neuroprotection in an

- oxidative stress assay and direct radical-scavenging activity. *J. Neurochem.* **94**, 819–827 (2005).
187. Szeto, G. L. *et al.* Minocycline attenuates HIV infection and reactivation by suppressing cellular activation in human CD4+ T cells. *J. Infect. Dis.* **201**, 1132–1140 (2010).
188. Everall, I. P. *et al.* Lithium ameliorates HIV-gp120-mediated neurotoxicity. *Mol. Cell. Neurosci.* **21**, 493–501 (2002).
189. Letendre, S. L. *et al.* Lithium improves HIV-associated neurocognitive impairment. *AIDS Lond. Engl.* **20**, 1885–1888 (2006).
190. Schifitto, G. *et al.* Lithium therapy for human immunodeficiency virus type 1-associated neurocognitive impairment. *J. Neurovirol.* **15**, 176–186 (2009).
191. Clifford, D. B. *et al.* A randomized clinical trial of CPI-1189 for HIV-associated cognitive-motor impairment. *Neurology* **59**, 1568–1573 (2002).
192. Allard, J. P. *et al.* Effects of vitamin E and C supplementation on oxidative stress and viral load in HIV-infected subjects. *AIDS Lond. Engl.* **12**, 1653–1659 (1998).
193. Rrapo, E. *et al.* Green Tea-EGCG reduces GFAP associated neuronal loss in HIV-1 Tat transgenic mice. *Am. J. Transl. Res.* **1**, 72–79 (2009).
194. Nance, C. L., Siwak, E. B. & Shearer, W. T. Preclinical development of the green tea catechin, epigallocatechin gallate, as an HIV-1 therapy. *J. Allergy Clin. Immunol.* **123**, 459–465 (2009).
195. Giunta, B. *et al.* EGCG mitigates neurotoxicity mediated by HIV-1 proteins gp120 and Tat in the presence of IFN-gamma: role of JAK/STAT1 signaling and implications for HIV-associated dementia. *Brain Res.* **1123**, 216–225 (2006).
196. Safety and tolerability of the antioxidant OPC-14117 in HIV-associated cognitive impairment. The Dana Consortium on the Therapy of HIV Dementia and Related Cognitive Disorders. *Neurology* **49**, 142–146 (1997).
197. Letendre, S. L. *et al.* The role of cohort studies in drug development: clinical evidence of antiviral activity of serotonin reuptake inhibitors and HMG-CoA reductase inhibitors in the central nervous system. *J. Neuroimmune Pharmacol. Off. J. Soc. NeuroImmune Pharmacol.* **2**, 120–127 (2007).
198. Wong, H. L., Chattopadhyay, N., Wu, X. Y. & Bendayan, R. Nanotechnology applications for improved delivery of antiretroviral drugs to the brain. *Adv. Drug Deliv. Rev.* **62**, 503–517 (2010).
199. Mahajan, S. D. *et al.* Enhancing the delivery of anti retroviral drug 'Saquinavir' across the blood brain barrier using nanoparticles. *Curr. HIV Res.* **8**, 396–404 (2010).



# Multivariate normative comparison, a novel method for more reliably detecting cognitive impairment in HIV infection

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## Abstract

### Objective

The objective of this study is to assess whether multivariate normative comparison (MNC) improves detection of HIV-associated neurocognitive disorder (HAND) as compared with Frascati and Gisslén criteria.

### Methods

One hundred and three HIV-1-infected men with suppressed viremia on combination antiretroviral therapy (cART) for at least 12 months and 74 HIV-uninfected male controls (comparable regarding age, ethnicity, sexual orientation, premorbid intelligence and educational level), aged  $\geq 45$  years, underwent neuropsychological assessment covering six cognitive domains (fluency, attention, information processing speed, executive function, memory, and motor function). Frascati and Gisslén criteria were applied to detect HAND. Next, MNC was performed to compare the cognitive scores of each HIV-positive individual against the cognitive scores of the control group.

### Results

HIV-infected men showed significantly worse performance on the cognitive domains of attention, information processing speed and executive function compared with HIV-uninfected controls. HAND by Frascati criteria was highly prevalent in HIV-infected (48%, 95% confidence interval (CI) 38-58) but nearly equally so in HIV-uninfected men (36%, 95% CI 26-48), confirming the low specificity of this method. Applying Gisslén criteria, HAND prevalence was reduced to 5% (95% CI 1-9) in HIV-infected men and to 1% (95% CI 1-3) among HIV-uninfected controls, indicating better specificity but reduced sensitivity. MNC identified cognitive impairment in 17% (95% CI 10-24) of HIV-infected men and in 5% (95% CI 0-10) of the control group ( $P=0.02$ , one-tailed), showing an optimal balance between sensitivity and specificity.

### Conclusions

Prevalence of cognitive impairment in HIV-infected men with suppressed viremia on cART estimated by MNC was much higher than that estimated by Gisslén criteria, while the false positive rate was greatly reduced compared with the Frascati criteria.

### Video abstract

[http://download.lww.com/wolterskluwer\\_vitalstream\\_com/PermaLink/QAD/A/QAD\\_2015\\_01\\_05\\_SCHOUTEN\\_AIDS-D-14-00819\\_SDC2.mov](http://download.lww.com/wolterskluwer_vitalstream_com/PermaLink/QAD/A/QAD_2015_01_05_SCHOUTEN_AIDS-D-14-00819_SDC2.mov)

## Introduction

The introduction of combination antiretroviral therapy (cART) has resulted in a dramatic decline in AIDS-associated mortality and morbidity, including AIDS dementia complex, which has largely disappeared from clinical practice<sup>1–3</sup>. Nonetheless, HIV-infected individuals on cART are increasingly reported to experience a broad range of non-AIDS related comorbidities, including cardiovascular, chronic liver and kidney disease, diabetes mellitus and cognitive impairment (CI).<sup>4,5</sup> In the past few years, a high but varying prevalence of milder forms of CI has been reported among HIV-infected individuals ranging from 15 to 69%, including those with systemically well controlled infection.<sup>6–11</sup>

To classify this broadening clinical spectrum of HIV-associated neurocognitive disorders (HAND), a set of diagnostic criteria, commonly referred to as the Frascati criteria, has been developed (**Table 6.1**).<sup>12</sup> The Frascati criteria have a low threshold for detecting milder forms of HAND and may overestimate HAND prevalence.<sup>13</sup> Thus, applying the Frascati criteria probably results in high false-positive rates. Consequently, the exact prevalence of HAND remains heavily debated.<sup>13</sup> Gisslén et al.<sup>13</sup> proposed modified criteria in an attempt to increase specificity (**Table 6.1**). A statistical method specifically designed to control false-positive rate while retaining sensitivity is multivariate normative comparison (MNC), a novel and potentially more accurate technique for evaluating CI.<sup>14</sup>

The purpose of this study was to determine the prevalence of HIV-associated CI among HIV-infected men with suppressed viremia on cART compared with highly comparable uninfected male controls aged  $\geq 45$  years, and to assess whether MNC improves the detection of HAND as compared with Frascati and Gisslén criteria.

## Methods

### Study design and participants

The AGE<sub>h</sub>IV Cohort Study is a prospective comparative cohort study investigating prevalence, incidence and risk factors of ageing-associated

**Table 6.1: Overview of the Frascati and Gisslén criteria**

HAND subcategory	Definition of abnormal test result	Definition of abnormal cognitive domain	Affected cognitive domains	Interference with daily functioning
<b>Frascati criteria</b>				
Asymptomatic neurocognitive impairment (ANI)	-1 SD	≥1 test result abnormal	≥2	No interference
Mild neurocognitive disorder (MND)	-1 SD	≥1 test result abnormal	≥2	Mild interference
HIV-associated dementia (HAD)	-2 SD	≥1 test result abnormal	≥2	Marked interference
<b>Gisslén criteria</b>				
Asymptomatic neurocognitive impairment (ANI)	-1.5 SD	Mean domain performance abnormal	≥2	No interference
Mild neurocognitive disorder (MND)	-1.5 SD	Mean domain performance abnormal	≥2	Mild interference
HIV-associated dementia (HAD)	-2 SD	Mean domain performance abnormal	≥2	Marked interference

Abbreviations: HAND=HIV-associated neurocognitive disorder, SD=standard deviation.



comorbidities and organ dysfunction among HIV-1-infected individuals and highly comparable HIV-uninfected controls. Inclusion criteria are age of at least 45 years and laboratory-confirmed presence or absence of HIV-infection.

HIV-infected participants were recruited at the HIV outpatient clinic of the Academic Medical Center in Amsterdam, The Netherlands, and HIV-uninfected controls from the ongoing Amsterdam Cohort Studies on HIV/AIDS and among persons attending the sexual health clinic of the Amsterdam Public Health Service (details concerning AGE<sub>h</sub>IV Cohort Study have been described in a previous publication).<sup>15</sup>

All eligible participants from the main AGE<sub>h</sub>IV Cohort were consecutively invited to participate in a nested cognitive substudy, which began enrolment in December 2011. Additional eligibility criteria for the substudy were male sex (as the availability of Dutch-speaking women in the main AGE<sub>h</sub>IV Cohort was very limited), and for the HIV-infected group, sustained suppression of HIV viremia on antiretroviral treatment (plasma HIV RNA <40 copies/mL) for at least 12 months; the presence of so-called viral 'blips' (transient low-level viremia) was not an exclusion criterion.

Exclusion criteria for the substudy were a history of severe neurological disorder (e.g. stroke, seizure disorders, multiple sclerosis, dementia (including previous or current diagnosis of HIV-associated dementia (HAD)), history of traumatic brain injury with loss of consciousness more than 30 minutes, current/past (HIV-associated) central nervous system infection or tumour, current severe psychiatric disorder (e.g. psychosis, major depression), current intravenous drug use, daily use of illicit drugs (with the exception of daily cannabis use), current excessive alcohol consumption (>48 units of alcohol/week), insufficient command of the Dutch language, and mental retardation. With respect to major depression as one of the exclusion criteria, depressive symptoms were assessed in the main AGE<sub>h</sub>IV Cohort Study by the 9-item Patient Health Questionnaire (PHQ-9). Participants with a PHQ-9 score of at least 15 (indicative of severe depressive symptoms and potentially of major depression) were excluded from participation in the substudy.<sup>16</sup>

#### Standard protocol approval, registration and patient consent

The protocol of the AGE<sub>n</sub>IV Cohort Study (including the above-mentioned substudy) was approved by the local ethics committee and has been registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) (identifier: NCT01466582). Written informed consent was obtained from all participants, both for the main study and substudy separately.

#### Study procedures

Neuropsychological assessment (NPA) was performed by trained neuropsychologists and covered six cognitive domains commonly affected by HAND, including fluency, attention, information processing speed, executive function, memory, and motor function (details concerning neuropsychological test battery are provided in **Supplementary 6.1**). Depressive symptoms were assessed using the Beck Depression Inventory (BDI)<sup>17</sup> and subjective cognitive complaints with the Cognitive Failure Questionnaire (CFQ).<sup>18</sup> Everyday functioning was assessed using the Instrumental Activities of Daily Living (IADL) questionnaire<sup>19</sup> and premorbid intelligence was estimated by the Dutch Adult Reading Test (DART).<sup>20</sup>

#### Classification of cognitive impairment according to Frascati criteria, Gisslén criteria and multivariate normative comparison

First, Frascati criteria were applied to diagnose HAND.<sup>12</sup> According to the Frascati criteria, participants are classified as having asymptomatic neurocognitive impairment (ANI) if at least one test per cognitive domain is at least 1 standard deviation (SD) below the normative mean for at least two cognitive domains, in the absence of interference with everyday functioning. Participants are classified as having mild neurocognitive disorder (MND) if they satisfy these same criteria, and if they report mild functional impairment. Participants are classified as having HAD if at least one test per cognitive domain is at least 2 SD below the normative mean for at least two cognitive domains, and if there is a marked functional impairment in daily life. CI should not be explained by opportunistic central nervous system disease, systemic illness, psychi-

## Supplementary 6.1: Overview of the administered neuropsychological test battery

	Tests administered	Test scores
Verbal fluency	Category Fluency <sup>1</sup>	Total number of animals in 1 minute Total number of occupations in 1 minute
	Letter Fluency <sup>2</sup>	Total number of words, 1 minute for each of 3 letters
Executive function	Trail Making Test-B <sup>3</sup>	Total time to complete
	Wisconsin CST <sup>4</sup>	Percentage of perseverative errors
	Stroop color-word test <sup>5</sup>	Interference condition: Time to complete
Information processing speed	Trail Making Test-A <sup>3</sup>	Time to complete
	Digit Symbol <sup>6</sup>	Total correct symbols
	Symbol Search <sup>6</sup>	Total correct symbols
Attention	PASAT 3.2 <sup>7</sup>	Total correct summations
	PASAT 2.8 <sup>7</sup>	Total correct summations
Memory	Letter-number sequencing <sup>6</sup>	Total correct sequences
	Rey AVLT-learning <sup>8</sup>	Total recalled words trails 1-5
	Rey AVLT-recall <sup>8</sup>	Total words recalled
	VR learning <sup>9</sup>	Total score
Motor function	VR recall <sup>9</sup>	Total score
	Grooved pegboard <sup>10</sup>	Dominant hand: Time to complete
		Non-dominant hand: Time to complete
	Finger tapping <sup>10</sup>	Dominant hand: Median number of taps Non-dominant hand: Median number of taps

Abbreviations: CST=card sorting test, PASAT=paced auditory serial addition task, AVLT=adult verbal learning test, VR=visual reproduction.

### References

- 1 Luteijn F, Barelids D. Groningen Intelligence Test 2 (GIT2): Manual. Amsterdam: Harcourt Test Publishers; 2004.
- 2 Benton, A. L., Hamsher, K., Sivan A b. Multilingual Aphasia Examination. Iowa city, Iowa, USA: AJA associates; 1983.
- 3 Reitan RM. Trail Making Test. Manual for administration and scoring. Tuscon, Arizona: Reitan Neuropsychological Laboratory; 1992.
- 4 Heaton, R. K., Chelune, G. J., Talley, J. L., Kay, G. G., Curtiss G. Wisconsin card sorting test manual revised and expanded. Lutz, USA: PAR;
- 5 Stroop JR. Studies of interference in serial verbal reactions. *J Exp pschology*. 1935;18:643–662.
- 6 Wechsler D. Wechsler Adult Intelligence Scale -Third Edition, Dutch language version (WAIS-III NL): Test and scoring manual. Uterwijk JM, editor. Lisse: Swets Test Publishers; 2004.
- 7 Gronwall D. Paced auditory serial-addition task: a measure of recovery form concussion. *Percept Mot Skills*. 1977;44(2):367–373.
- 8 Rey A. L'examen clinique en psychologie. Paris, France: Presses Universitaires de France; 1964.
- 9 Wechsler D. Wechsler Memory Scale - Fourth Edition (WMS-IV): Test and scoring manual. Pearson; 2009.
- 10 Strauss, E., Sherman, E. M. S., Spreen A. A compendium of neuropsychological tests. New York: Oxford University Press Inc; 2006.

atric illness, substance use disorders, or medications with central nervous system effects.

Second, modified Frascati criteria for HAND as proposed by Gisslén et al.<sup>13</sup> (from now on referred to as the Gisslén criteria) were applied. According to the Gisslén criteria, the definition of abnormal cognitive performance of ANI and MND should be modified by changing the cut-off, of preferably the mean domain performance, to below 1.5 SD to lower the false-positive rate. Also, concerning the criteria for HAD, the averaged domain performance should be at least 2 SD below the normative mean. All other criteria for HAND remained the same. See **Table 6.1** for an overview of the Frascati and Gisslén criteria.

We used the CFQ-score as a surrogate for interference with everyday functioning to distinguish between ANI and MND, in which a cut-off score of 42 or higher (reflecting the 5% highest scores of the controls) was used to indicate a significant degree of subjective cognitive complaints. We used the IADL scale to assess functional impairment in daily life.

Finally, MNC was applied to diagnose CI. MNC is a statistical method that may be seen as a multivariate version of Student's t test for one sample. It can be used to statistically compare multiple cognitive scores of each single study participant against the distributions of the same scores of a control sample, taking the covariance between all test scores into account.

The MNC method is able to control family-wise error (the probability of making one or more false discoveries) by performing only one comparison in a multivariate manner. A complete cognitive profile is therefore compared in a single instance with the control sample, rather than comparing each test result separately to its norm. Thus, MNC was applied to compare the complete cognitive profile of each HIV-infected participant with the cognitive profile of the HIV-uninfected control group as a whole. The test statistic is Hotelling's  $T^2$ . The false-positive rate, that is erroneously concluding that an individual deviates from the control sample while this is not the case, is limited by the level of significance (alpha). In the present study, alpha was set at 5% one-tailed. Consequently, the MNC has a specificity of at least 95%.<sup>14</sup>

### Statistical analysis

Normality of distribution and heterogeneity of variance were checked using the Kolmogorov-Smirnov test and Levene's test. Group comparisons were performed using  $\chi^2$ , Fisher's exact or Mann-Whitney U test as appropriate.

Neuropsychological test scores were converted to age and education corrected scores (z-scores) using normative standards, except for two tests for which demographically corrected norms were not available (PASAT and finger tapping). Therefore, we used the data of the controls to calculate age and education-corrected z-scores for these two tests. We performed a multivariate analysis of variance (MANOVA) for each cognitive domain to compare cognitive test scores of the HIV-infected and uninfected groups. Missing neuropsychological test scores (1.3%, due to colour blindness, severe hand injuries, and hearing difficulty) were imputed by the average of participants with similar HIV status, age and educational level.

MNC analyses were performed using R statistical software (<http://purl.oclc.org/NET/RGRASMAN/MNC>), while for remaining analyses, SPSS (version 20.0, IBM) was used. Inter-agreement percentages between the three classification methods were calculated.

## Results

### Participants characteristics

One-hundred and three HIV-infected and 74 HIV-uninfected participants were consecutively enrolled into the substudy between December 2011 and August 2013.

Demographic and HIV-related characteristics are summarized in **Table 6.2**. Both groups were highly comparable, with a median age of 54 years in both groups, and the majority being MSM.

HIV-infected participants were known to be infected and treated with antiretroviral medication for a prolonged period of time, and 35% had previously been diagnosed with AIDS. The majority had experienced substantial immune recovery on treatment, with a median nadir CD4+

**Table 6.2: Baseline demographic and HIV-related characteristics**

	(a) HIV- uninfected (n=74)	(b) HIV- infected (n=103)	(c) HIV-infected without cognitive impairment by MNC (n=86)	(d) HIV-infected with cognitive impairment by MNC (n=17)	P value
Age (years)	54 (49-61)	54 (49-62)	54 (49-62)	56 (52-63)	$P_{a,b}=0.94^e$ $P_{c,d}=0.26^e$
MSM (%) <sup>a</sup>	90%	93%	93%	94%	$P_{a,b}=0.48^f$ $P_{c,d}=0.87^f$
Hepatitis C RNA positive (%)	0%	1%	1%	0%	$P_{a,b}=1.00^g$ $P_{c,d}=1.00^g$
Hepatitis B antigen and/or hepatitis B DNA positive (%)	0%	2%	1%	6%	$P_{a,b}=0.51^g$ $P_{c,d}=0.30^g$
Duration of known HIV seropositivity (years)	-	13.5 (7.4-17.1)	13.4 (7.9-17.5)	13.5 (5.9-15.6)	- $P_{c,d}=0.48^e$
CD4+ T-cell count at enrolment (cells/mm <sup>3</sup> )	-	625 (475-800)	635 (490-800)	593 (430-737)	- $P_{c,d}=0.65^e$
Nadir CD4+ T-cell count (cells/mm <sup>3</sup> )	-	170 (60-250)	185 (100-260)	50 (10-110)	- $P_{c,d}=0.001^e$
Duration of undetectable plasma viral load (years) <sup>b</sup>	-	8.3 (3.5-11.2)	8.2 (3.5-11.1)	9.5 (3.5-13.8)	- $P_{c,d}=0.53^e$
Duration since start of first ART (years)	-	11.6 (4.9-14.9)	11.4 (4.9-14.9)	13.1 (4.4-15.4)	- $P_{c,d}=0.70^e$
Naive at start of cART (%) <sup>c</sup>	-	80%	81%	71%	- $P_{c,d}=0.31^f$
Prior clinical AIDS (%) <sup>d</sup>	-	35%	34%	41%	- $P_{c,d}=0.56^f$

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

(a) HIV-uninfected studygroup, (b) HIV-infected studygroup, (c) HIV-infected studygroup without cognitive impairment as diagnosed using MNC, (d) HIV-infected studygroup with cognitive impairment as diagnosed using MNC. Abbreviation: MNC=multivariate normative comparison.

<sup>a</sup> The term 'MSM' applied to male participants who stated in the questionnaire to feel mostly or exclusively sexually attracted to men. Two of the 74 HIV-uninfected controls in this study did not complete this questionnaire; all HIV-infected participants completed the questionnaire.

<sup>b</sup> Duration of undetectable plasma viral load was defined as: number of years since last plasma viral load >200 copies/mL.

<sup>c</sup> The term 'cART' (combination antiretroviral therapy) was used for a combination of  $\geq 3$  antiretroviral drugs, other than ritonavir used as a pharmacological booster.

<sup>d</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>e</sup> Mann-Whitney U test

<sup>f</sup>  $\chi^2$  test

<sup>g</sup> Fisher's exact test

T-cell count of 170 cells/mm<sup>3</sup> and current median CD4+ T-cell count of 625 cells/mm<sup>3</sup>.

Factors related to cognition and behaviour are presented in **Table 6.3**. Both groups were comparable regarding educational level, number of depressive symptoms, and use of psychotropic medication. Ecstasy use was more prevalent among HIV-uninfected controls (12% vs. 2%,  $P=0.008$ ), whereas cannabis, cocaine, and alcohol use were comparable between the two groups.

#### Neuropsychological test results

HIV-infected individuals as a group performed worse compared with HIV-uninfected controls on the majority of cognitive tests. Statistically significant (one-tailed) small group differences were found for the cognitive domains: attention ( $P=0.03$ ) and executive function ( $P=0.02$ ). A trend was found for the cognitive domain information processing speed ( $P=0.05$ ). No significant group difference was found for the cognitive domains of fluency ( $P=0.41$ ), memory ( $P=0.46$ ) and motor function ( $P=0.13$ ). See **Supplementary 6.2** for details.

#### Cognitive impairment by Frascati criteria, Gisslén criteria and multivariate normative comparison

Applying Frascati criteria, HAND was present in not only 49 of 103 HIV-infected men (48%, 95% confidence interval (CI) 38-58) but also in 27 of 74 HIV-uninfected men (36%, 95% CI 26-48,  $P=0.09$ , one-tailed; **Table 6.4**). Applying Gisslén criteria, HAND was present in 5 of 103 HIV-infected men (5%, 95% CI 1-9) and 1 of 74 HIV-uninfected men (1%, 95% CI 1-3,  $P=0.20$ , one-tailed).

Using MNC, CI was detected in 17 HIV-infected men (17%, 95% CI 10-24). To verify the specificity of the MNC criterion, which was assumed to be at least 95%, we compared the scores of each individual HIV-uninfected control with the scores of the remaining control group ( $n=73$ ), using MNC. Four (5%, 95% CI 0-10) controls showed test results significantly deviating in a negative sense, supporting the assumption of 95% specificity.

**Table 6.3: Baseline characteristics related to cognition and behaviour**

	(a) HIV- uninfected (n=74)	(b) HIV- infected (n=103)	(c) HIV-infected without cognitive impairment by MNC (n=86)	(d) HIV-infected with cognitive impairment by MNC (n=17)	P value
Dutch as native language (%)	95%	91%	93%	82%	$P_{a,b}=0.56^j$ $P_{c,d}=0.17^j$
Education (ISCED level) <sup>a</sup>	6 (5-6)	6 (5-6)	5 (5-6)	6 (5-7)	$P_{a,b}=0.50^k$ $P_{c,d}=0.24^k$
Premorbid intelligence (IQ) <sup>b</sup>	103 (96-112)	101 (95-111)	102 (95-111)	99 (93-120)	$P_{a,b}=0.48^k$ $P_{c,d}=0.93^k$
Subjective cognitive complaints (%) <sup>c</sup>	5%	13%	10%	24%	$P_{a,b}=0.13^j$ $P_{c,d}=0.22^j$
Mild to moderate depressive symptoms (%) <sup>d</sup>	4%	6%	6%	12%	$P_{a,b}=0.74^j$ $P_{c,d}=0.33^j$
Severe depressive symptoms (%) <sup>e</sup>	0	0	0	0	–
Using psychotropic medication (%) <sup>f</sup>	14%	16%	15%	18%	$P_{a,b}=0.71^l$ $P_{c,d}=0.79^l$
Level of daily functioning (IADL score) <sup>g</sup>	0	0	0	0	–
Weekly to monthly use of ecstasy (%) <sup>h</sup>	13%	2%	2%	0%	$P_{a,b}=0.008^j$ $P_{c,d}=1.00^j$
Weekly to monthly use of cocaine (%) <sup>h</sup>	4%	4%	5%	0%	$P_{a,b}=1.00^j$ $P_{c,d}=1.00^j$
Daily to monthly use of cannabis (%) <sup>h</sup>	15%	16%	9%	47%	$P_{a,b}=0.96^l$ $P_{c,d}=0.001^l$
Past intravenous drug use (%) <sup>i</sup>	0	0	0	0	–
Alcohol intake (units per week)	5 (3-12)	6 (2-14)	6 (2-14)	4 (0-11)	$P_{a,b}=0.89^k$ $P_{c,d}=0.18^k$

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

(a) HIV-uninfected studygroup, (b) HIV-infected studygroup, (c) HIV-infected studygroup without cognitive impairment as diagnosed using MNC, (d) HIV-infected studygroup with cognitive impairment as diagnosed using MNC.

Abbreviation: MNC=multivariate normative comparison.

<sup>a</sup> Educational level was defined using the International Standard Classification of Education (ISCED) 2011.

<sup>b</sup> Premorbid intelligence quotient (IQ) was estimated using the Dutch Adult Reading Test (DART). One of 74 HIV-uninfected controls and six of 103 HIV-infected individuals were unable to complete this test due to dyslexia.

<sup>c</sup> Subjective cognitive complaints were assessed using Cognitive Failure Questionnaire (CFQ). A cut-off of 42 or higher was used to indicate significant amount of subjective complaints, percentages scoring above this cut-off is shown.

<sup>d</sup> A Beck Depression Inventory score >13 and <29 reflects presence of mild to moderate depressive symptoms, percentages scoring >13 and <29 are shown. One of 103 HIV-infected individuals did not complete this test.

<sup>e</sup> A Beck Depression Inventory score ≥29 reflects severe depressive symptoms. None of the participants had a score ≥29. One of 103 HIV-infected individuals did not complete this test.

<sup>f</sup> Psychotropic medication included: antidepressants, benzodiazepines, methylphenidate.

<sup>g</sup> Level of day-to-day functioning was defined using the Independent Activities of Daily Living (IADL) questionnaire.

<sup>h</sup> Recreational drug use was assessed by a questionnaire; two of 74 HIV-uninfected controls did not complete this section of the questionnaire.

<sup>i</sup> Past intravenous drug use was assessed by a questionnaire; three of 74 HIV-uninfected controls did not complete this section of the questionnaire.

<sup>j</sup> Fisher's exact test

<sup>k</sup> Mann-Whitney U test

<sup>l</sup>  $\chi^2$  test



## Supplementary 6.2: Overview of mean test performance per study-group

		HIV- (n=74)	HIV+ (n=103)	P value <sup>a</sup>
<b>Fluency</b>	Animals	-0.01 (1.13)	-0.04 (1.07)	0.41
	Occupation	0.37 (1.08)	0.26 (1.10)	
	Letter	-0.11 (0.95)	-0.24 (0.96)	
<b>Executive function</b>	Trail Making Test-B	0.37 (1.06)	-0.04 (1.16)	0.02
	Wisconsin CST	0.04 (0.55)	0.04 (0.69)	
	Stroop color-word test	0.02 (0.90)	-0.29 (0.87)	
<b>Information processing speed</b>	Trail Making Test-A	0.46 (0.85)	0.21 (1.00)	0.05
	WAIS-III Digit Symbol	0.49 (1.03)	0.18 (1.00)	
	WAIS-III Symbol Search	0.60 (0.97)	0.52 (1.07)	
<b>Attention</b>	PASAT 3.2	0.00 (0.99)	-0.43 (1.28)	0.03
	PASAT 2.8	0.00 (0.99)	-0.43 (1.21)	
	Letter-number sequencing	0.72 (1.12)	0.67 (1.05)	
<b>Memory</b>	Rey AVLT learning	-0.18 (1.05)	-0.15 (1.07)	0.46
	Rey AVLT recall	-0.07 (1.14)	0.03 (1.10)	
	WMS-IV VR learning	0.29 (0.81)	0.24 (0.82)	
	WMS-IV VR recall	0.85 (0.96)	0.77 (1.00)	
<b>Motor function</b>	GP dominant hand	0.00 (0.86)	-0.09 (1.11)	0.13
	GP non-dominant hand	0.07 (0.96)	-0.31 (1.46)	
	FT dominant hand	0.00 (0.99)	0.04 (1.14)	
	FT non-dominant hand	0.00 (0.99)	0.02 (1.18)	

All data presented as mean z-score (standard deviation).

<sup>a</sup> Multivariate analysis of variance, one-tailed.

Abbreviations: CST=card sorting test, PASAT=paced auditory serial addition task, AVLT=adult verbal learning test, VR=visual reproduction, GP=grooved pegboard, FT=finger tapping.

### References of normative data

- Schmand B, Houx P, Inge de Koning, et al. Normen van psychologische tests voor gebruik in de klinische neuropsychologie. Utrecht, Ned Inst van Psychol Sect Neuropsychol. 2012.
- Heaton, R. K., Chelune, G. J., Talley, J. L., Kay, G. G., Curtiss G. Wisconsin card sorting test manual revised and expanded. Lutz, USA.
- Wechsler D. Wechsler Adult Intelligence Scale -Third Edition, Dutch language version (WAIS-III NL): Test and scoring manual. Uterwijk JM, editor. Lisse: Swets Test Publishers; 2004.
- Wechsler D. Wechsler Memory Scale - Fourth Edition (WMS-IV): Test and scoring manual. Pearson; 2009.
- Strauss, E., Sherman, E. M. S., Spreen A. A compendium of neuropsychological tests. New York: Oxford University Press Inc; 2006.

**Table 6.4: Diagnosis of cognitive impairment applying Frascati criteria, Gisslén criteria, and multivariate normative comparison method in 74 HIV-uninfected controls and 103 HIV-infected patients**

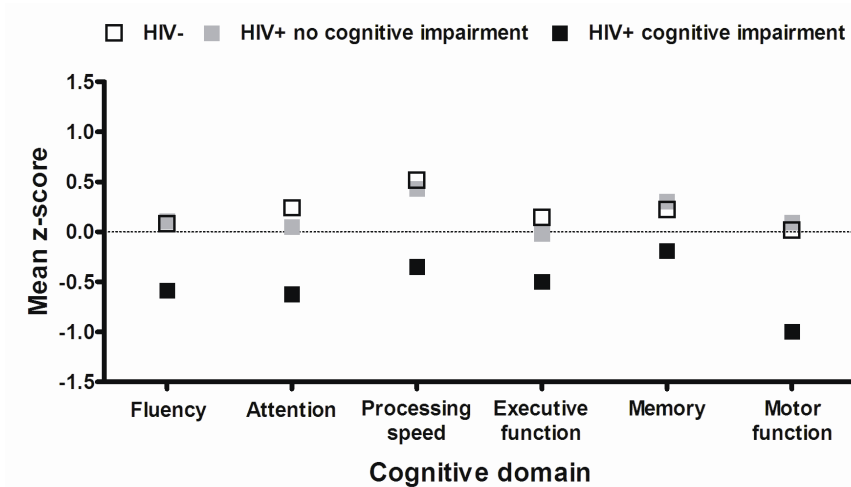
	Cognitive impairment								
	Frascati criteria			Gisslén criteria			MNC		
	HIV-	HIV+	P value <sup>a</sup>	HIV-	HIV+	P value <sup>a</sup>	HIV-	HIV+	
HAND diagnoses (no, %, 95% CI)	27 36% (26-48)	49 48% (38-58)	0.09	1 1% (1-3)	5 5% (1-9)	0.20	4 <sup>b</sup> 5% <sup>b</sup> (0-10)	17 17% (10-24)	P value <sup>a</sup> 0.02
Asymptomatic neurocognitive impairment (ANI) (no, %)	25 (34%)	41 (40%)	-	1 (1%)	4 (4%)	-			No subclassification
Mild neurocognitive disorder (MND) (no, %)	2 (3%)	8 (8%)	-	0 (0%)	1 (1%)	-			

Abbreviations: HAND=HIV-associated neurocognitive disorder, CI=confidence interval.

Of note, none of the participants with HAND as defined by Frascati or Gisslén criteria met the criteria for HAD as none of these participants reported interference with daily functioning as assessed using the IADL scale.

<sup>a</sup>  $\chi^2$  test with continuity correction, one-tailed.

<sup>b</sup> The false-positive rate (alpha) was set at 5% one-tailed, thus the specificity was at least 95%. Comparing the scores of each individual HIV-uninfected control with the distribution of test scores of the remaining HIV-uninfected control group (n=73), four (5%) of the HIV-uninfected controls showed strongly negatively deviated test results. This confirms the assumed specificity of 95%.



**Figure 6.1:** Profile of neuropsychological test performances of HIV-uninfected controls, and HIV-infected participants with and without cognitive impairment as diagnosed using multivariate normative comparison

**Figure 6.1** depicts the profile of cognitive scores of the controls and HIV-infected participants with and without CI as identified by MNC. Comparing HIV-infected participants with and without CI according to MNC, those with CI have lower median nadir CD<sub>4</sub><sup>+</sup> T-cell count ( $P=0.001$ ) and reported more often cannabis use ( $P=0.001$ ) (**Tables 6.2 and 6.3**).

Agreement between the three classification methods Frascati vs. Gisslén criteria showed an agreement of 60%, Frascati criteria vs. MNC 64%, and Gisslén criteria vs. MNC 90%.

## Discussion

### Key results

HIV-infected individuals as a group performed worse compared with HIV-uninfected controls on all cognitive domains assessed. Differences were small but significant on the cognitive domains attention, executive function, and information processing speed.

CI as defined by Frascati criteria was highly prevalent in HIV-infected but nearly equally so in HIV-uninfected men. Although prevalence of HAND was markedly reduced when applying Gisslén criteria, it remained nearly as prevalent among HIV-uninfected controls. Applying MNC, a prevalence of 17% of HAND was found in HIV-infected men with suppressed viremia on cART.

HIV-infected individuals with CI, as classified using MNC, had lower nadir CD4+ T-cell counts and reported more often cannabis use.

### Interpretation, limitations, and conclusion

Earlier studies investigating HAND as defined by Frascati criteria reported prevalences ranging from 25 to 74%.<sup>6,7,9-11,21-23</sup> This broad range was narrowed to 19-38% when Frascati criteria were applied more conservatively, for example using averaged domain scores instead of individual tests scores.<sup>7,11,22-24</sup> Two studies reported higher HAND prevalences of 59 and 74%<sup>10,25</sup> than the prevalence we found (48%). These studies included larger proportions of hepatitis C virus (HCV) co-infected participants than our HIV-infected group and HCV co-infection is assumed to worsen cognitive status.<sup>26</sup> In addition, these studies included participants with prior neurological diseases known to affect cognition, whereas such comorbidity was an exclusion criterion in our study. One study reported lower prevalence of HAND, that is 25%, which might be explained by the absence of comorbidity in their HIV-infected sample.<sup>21</sup> Two studies reported comparable HAND prevalence to what we observed, that is 37 and 49%<sup>27,28</sup>, even though their participants were slightly younger than in our study, and one of these studies had a larger proportion of participants with HCV co-infection. Most of the above-mentioned studies did not include an HIV-uninfected control group, ex-

cept for four studies.<sup>21,24,27,29</sup> Only two studies reported prevalence of CI among controls as classified by Frascati, both of 13%, which is lower than our finding of 37%. This difference may be explained by the fact that our HIV-infected and HIV-uninfected groups had similar lifestyle and risk factor profiles.

The Frascati criteria are heavily debated, as they result in high false-positive rates and are likely to overestimate HAND prevalence. We found HAND by Frascati criteria to be highly prevalent in HIV-infected participants, but nearly equally so in uninfected controls, indeed confirming low specificity of this method. The Frascati criteria suffer two major shortcomings. Firstly, a cut-off of 1 SD below the normative mean is used for the diagnosis of HAND subcategories ANI and MND. Given normally distributed test scores, 16% of the normal population will perform 1 SD below the mean on a given test.

The second shortcoming of the Frascati criteria is that an abnormal score on a single test is sufficient to classify the performance of an individual in a particular cognitive domain as abnormal. However, multiple tests are typically performed during an NPA. This increases the chance of erroneously drawing the conclusion that a result is abnormal. This is the so-called family-wise error.<sup>30</sup>

Another issue concerning the Frascati criteria is that they do not dictate how to handle multiple testing within one cognitive domain. As a result, various interpretations of the Frascati criteria have been used by different studies.

To overcome the first shortcoming of the Frascati criteria, Gisslén et al.<sup>13</sup> proposed to modify the definition of ANI and MND by changing the cut-off for abnormality to 1.5 SD below the population mean.<sup>13,31</sup> In addition, two alternative solutions have been proposed to overcome the second shortcoming of the Frascati criteria: first, to use averaged cognitive domain scores instead of individual cognitive tests and second, to limit assessment to three to five cognitive domains, in order to limit the number of comparisons.<sup>31</sup> Although the number of comparisons is reduced by these solutions, multiple comparisons are still performed. MNC addresses the shortcomings of the Frascati criteria more adequately by performing only one comparison per individual, and comparing the complete cognitive profile of one individual with the complete cognitive

profile of the control group in one instance. It reduces the false-positive rate, while enabling comprehensive neuropsychological examination. Furthermore, MNC is able to detect deviations in patterns from the norm.<sup>14</sup> This is highly relevant as HAND is mild, with subtle abnormalities across a broad range of cognitive domains.<sup>32</sup> In addition, there is a large variability of cognitive performance in the normal population, and therefore, small deviations from the norm cannot be easily detected.<sup>33</sup> Moreover, variability in performance of a person across multiple tasks may be a more sensitive predictor for impaired daily functioning than his average cognitive performance.<sup>34</sup> MNC has proven to be successful in detection of subtle CI in Parkinson's disease<sup>14,35</sup>, and it was applied to detect deviant profiles of white matter lesion load across brain regions.<sup>36</sup>

When we applied the Gisslén criteria, the false-positive rate was greatly reduced but so was HAND prevalence, indicating reduced sensitivity. Applying MNC with its inherently low false-positive rate, we found CI in 17% of HIV-infected participants. Specificity was indeed at least 95% in the present study.

Comparing HIV-infected participants with and without CI as diagnosed by MNC, cannabis use was more common and the nadir CD4+ T-cell count was lower among HIV-infected individuals with CI. Nadir CD4+ T-cell count has been associated with HAND<sup>9,11,23,37</sup>, which suggests that cognitive CI might be a residual effect of prior periods of severe immune suppression. Cannabis use is common among HIV-infected individuals for therapeutic and recreational purposes. More cognitive deficits among HIV-infected individuals with chronic cannabis use than nonusers have been reported before.<sup>38</sup> In addition, effects of chronic cannabis use on brain metabolites of neuronal dysfunction and glial activation in HIV-infected individuals have been found.<sup>39</sup>

There are some limitations of the current study. First, we used the CFQ to measure subjective cognitive complaints. It can be debated whether this tool is appropriate to estimate functional impairment. However, similar surrogates of functional decline were used in earlier studies.<sup>6,10</sup> These studies measured subjective cognitive complaints in a more qualitative way, whereas we used a standardized, multi-item questionnaire. Second, we may have missed some cases of HAD as we assessed activities of daily living by a self-report only, without collecting

additional information from an informant. It is conceivable that ANI or MND cases who scored  $\geq 2$  SD or worse below the mean on cognitive tests may have had a lack of insight in their functional impairment. Third, ecstasy use was more often reported by the HIV-uninfected participants, which may affect cognitive performances, particularly verbal memory.<sup>40</sup> Except for this difference, the HIV-uninfected control group is very similar to our group of HIV-infected individuals. Despite this higher ecstasy use among controls, the classification accuracy in our study is probably greater than when we had compared our patients with the general population adjusting only for effects of age and education. Fourth, our groups consist exclusively of male participants. Additional studies are needed to determine whether these results can equally be applied to women. Finally, this cohort is relatively small, and we were therefore unable to make broad generalizations.

In conclusion, the MNC method, unique by its multivariate nature facilitating profile analysis, is a powerful tool with a high specificity to detect HIV-associated CI, which is characterized by multiple subtle deficits across a broad range of cognitive domains.

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## Authors' contributions

TS contributed to data collection, data analysis and interpretation, and writing of the manuscript.

JS contributed to data collection, data analysis and interpretation, and was responsible for producing and submitting the final manuscript.

GG contributed to data interpretation, and writing of the manuscript.

FW contributed to the study design, data analysis and interpretation, and writing of the manuscript.

IS contributed to the study design, data collection, data interpretation and writing of the manuscript.

MP contributed to the study design, data interpretation and writing of the manuscript.

PP contributed to study design, data interpretation and writing of the manuscript.

MC contributed to data interpretation, and writing of the manuscript.

PR conceived the main cohort study and the substudy, contributed to both study designs, to data interpretation and writing of the manuscript.

CM conceived the substudy, contributed to its design, to data interpretation and writing of the manuscript.

BS contributed to study design, supervised data analysis and interpretation, and supervised and contributed to writing of all drafts of the manuscript.




## References

1. Palella, F. J. *et al.* Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. *N. Engl. J. Med.* **338**, 853–860 (1998).
2. Wada, N. *et al.* Cause-specific life expectancies after 35 years of age for human immunodeficiency syndrome-infected and human immunodeficiency syndrome-negative individuals followed simultaneously in long-term cohort studies, 1984–2008. *Am. J. Epidemiol.* **177**, 116–125 (2013).
3. Weber, R. *et al.* Decreasing mortality and changing patterns of causes of death in the Swiss HIV Cohort Study. *HIV Med.* **14**, 195–207 (2013).
4. Hasse, B. *et al.* Morbidity and aging in HIV-infected persons: the Swiss HIV cohort study. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **53**, 1130–1139 (2011).
5. Guaraldi, G. *et al.* Premature age-related comorbidities among HIV-infected persons compared with the general population. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **53**, 1120–1126 (2011).
6. Cysique, L. A. & Brew, B. J. Prevalence of non-confounded HIV-associated neurocognitive impairment in the context of plasma HIV RNA suppression. *J. Neurovirol.* **17**, 176–183 (2011).
7. Winston, A. *et al.* Neurocognitive function in HIV infected patients on antiretroviral therapy. *PLoS One* **8**, e61949 (2013).
8. Schouten, J., Cinque, P., Gisslen, M., Reiss, P. & Portegies, P. HIV-1 infection and cognitive impairment in the cART era: a review. *AIDS Lond. Engl.* **25**, 561–575 (2011).
9. Robertson, K. R. *et al.* The prevalence and incidence of neurocognitive impairment in the HAART era. *AIDS Lond. Engl.* **21**, 1915–1921 (2007).
10. Simioni, S. *et al.* Cognitive dysfunction in HIV patients despite long-standing suppression of viremia. *AIDS Lond. Engl.* **24**, 1243–1250 (2010).
11. Heaton, R. K. *et al.* HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER Study. *Neurology* **75**, 2087–2096 (2010).
12. Antinori, A. *et al.* Updated research nosology for HIV-associated neurocognitive disorders. *Neurology* **69**, 1789–1799 (2007).
13. Gisslén, M., Price, R. W. & Nilsson, S. The definition of HIV-associated neurocognitive disorders: are we overestimating the real prevalence? *BMC Infect. Dis.* **11**, 356 (2011).
14. Huizenga, H. M., Smeding, H., Grasman, R. P. P. & Schmand, B. Multivariate normative comparisons. *Neuropsychologia* **45**, 2534–2542 (2007).
15. Schouten, J. *et al.* Cross-sectional comparison of the prevalence of age-associated comorbidities and their risk factors between HIV-infected and uninfected individuals: the AGEHIV cohort study. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **59**, 1787–1797 (2014).
16. Beck, A. T., Steer, R. A., Ball, R. & Ranieri, W. Comparison of Beck Depression

- Inventories -IA and -II in psychiatric outpatients. *J. Pers. Assess.* **67**, 588–597 (1996).
17. Beck, A. T., Ward, C. H., Mendelson, M., Mock, J. & Erbaugh, J. An inventory for measuring depression. *Arch. Gen. Psychiatry* **4**, 561–571 (1961).
  18. Broadbent, D. E., Cooper, P. F., FitzGerald, P. & Parkes, K. R. The Cognitive Failures Questionnaire (CFQ) and its correlates. *Br. J. Clin. Psychol. Br. Psychol. Soc.* **21 (Pt 1)**, 1–16 (1982).
  19. Lawton, M. P. & Brody, E. M. Assessment of older people: self-maintaining and instrumental activities of daily living. *The Gerontologist* **9**, 179–186 (1969).
  20. Schmand B, Lindeboom J, van Harskamp F. Schmand B, Lindeboom J, van Harskamp F. Dutch adult reading test. Lisse: Swets en Zeitlinger 1992. (1992).
  21. Pedersen, K. K. *et al.* Persisting inflammation and chronic immune activation but intact cognitive function in HIV-infected patients after long-term treatment with combination antiretroviral therapy. *J. Acquir. Immune Defic. Syndr.* **1999** **63**, 272–279 (2013).
  22. Morgan, E. E. *et al.* Lower cognitive reserve among individuals with syndromic HIV-associated neurocognitive disorders (HAND). *AIDS Behav.* **16**, 2279–2285 (2012).
  23. Garvey, L., Surendrakumar, V. & Winston, A. Low rates of neurocognitive impairment are observed in neuro-asymptomatic HIV-infected subjects on effective antiretroviral therapy. *HIV Clin. Trials* **12**, 333–338 (2011).
  24. Cysique, L. A. *et al.* HIV, vascular and aging injuries in the brain of clinically stable HIV-infected adults: a (1)H MRS study. *PLoS One* **8**, e61738 (2013).
  25. Bonnet, F. *et al.* Cognitive disorders in HIV-infected patients: are they HIV-related? *AIDS Lond. Engl.* **27**, 391–400 (2013).
  26. Devlin, K. N. *et al.* Neurocognitive effects of HIV, hepatitis C, and substance use history. *J. Int. Neuropsychol. Soc. JINS* **18**, 68–78 (2012).
  27. Cattie, J. E. *et al.* Planning deficits in HIV-associated neurocognitive disorders: component processes, cognitive correlates, and implications for everyday functioning. *J. Clin. Exp. Neuropsychol.* **34**, 906–918 (2012).
  28. Pumpradit, W. *et al.* Neurocognitive impairment and psychiatric comorbidity in well-controlled human immunodeficiency virus-infected Thais from the 2NN Cohort Study. *J. Neurovirol.* **16**, 76–82 (2010).
  29. Chiao, S. *et al.* Deficits in self-awareness impact the diagnosis of asymptomatic neurocognitive impairment in HIV. *AIDS Res. Hum. Retroviruses* **29**, 949–956 (2013).
  30. Sainani, K. L. The problem of multiple testing. *PM R* **1**, 1098–1103 (2009).
  31. Meyer, A.-C. L., Boscardin, W. J., Kwasa, J. K. & Price, R. W. Is it time to rethink how neuropsychological tests are used to diagnose mild forms of HIV-associated neurocognitive disorders? Impact of false-positive rates on prevalence and power. *Neuroepidemiology* **41**, 208–216 (2013).

32. Woods, S. P., Moore, D. J., Weber, E. & Grant, I. Cognitive neuropsychology of HIV-associated neurocognitive disorders. *Neuropsychol. Rev.* **19**, 152–168 (2009).
33. Schretlen, D. J., Munro, C. A., Anthony, J. C. & Pearlson, G. D. Examining the range of normal intraindividual variability in neuropsychological test performance. *J. Int. Neuropsychol. Soc. JINS* **9**, 864–870 (2003).
34. Morgan, E. E., Woods, S. P., Grant, I. & HIV Neurobehavioral Research Program (HNRP) Group. Intra-individual neurocognitive variability confers risk of dependence in activities of daily living among HIV-seropositive individuals without HIV-associated neurocognitive disorders. *Arch. Clin. Neuropsychol. Off. J. Natl. Acad. Neuropsychol.* **27**, 293–303 (2012).
35. Castelli, L. *et al.* Neuropsychological changes 1-year after subthalamic DBS in PD patients: A prospective controlled study. *Parkinsonism Relat. Disord.* **16**, 115–118 (2010).
36. González-Redondo, R. *et al.* The impact of silent vascular brain burden in cognitive impairment in Parkinson's disease. *Eur. J. Neurol.* **19**, 1100–1107 (2012).
37. Chan, L. G., Kandiah, N. & Chua, A. HIV-associated neurocognitive disorders (HAND) in a South Asian population - contextual application of the 2007 criteria. *BMJ Open* **2**, e000662 (2012).
38. Cristiani, S. A., Pukay-Martin, N. D. & Bornstein, R. A. Marijuana use and cognitive function in HIV-infected people. *J. Neuropsychiatry Clin. Neurosci.* **16**, 330–335 (2004).
39. Chang, L., Cloak, C., Yakupov, R. & Ernst, T. Combined and independent effects of chronic marijuana use and HIV on brain metabolites. *J. Neuroimmune Pharmacol. Off. J. Soc. NeuroImmune Pharmacol.* **1**, 65–76 (2006).
40. van Holst, R. J. & Schilt, T. Drug-related decrease in neuropsychological functions of abstinent drug users. *Curr. Drug Abuse Rev.* **4**, 42–56 (2011).





# Determinants of reduced cognitive performance in HIV-infected middle-aged men on combination antiretroviral therapy

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## Abstract

### Objective

The spectrum of risk factors for HIV-associated cognitive impairment (CI) is likely very broad and includes not only HIV/antiretroviral therapy-specific factors but also other comorbid conditions. The purpose of this current study was to explore possible determinants for decreased cognitive performance.

### Methods

Neuropsychological assessment was performed on 103 HIV-1-infected men with suppressed viremia on combination antiretroviral therapy for at least 12 months and 74 HIV-uninfected highly similar male controls, all aged at least 45 years. CI and cognitive performance were determined by multivariate normative comparison (MNC). Determinants of decreased cognitive performance and CI were investigated by linear and logistic regression analysis, respectively.

### Results

CI as diagnosed by MNC was found in 17% of HIV-infected men. Determinants for decreased cognitive performance by MNC as a continuous variable included cannabis use, history of prior cardiovascular disease, impaired renal function, diabetes mellitus type 2, having an above-normal waist-to-hip ratio, presence of depressive symptoms, and lower nadir CD<sub>4</sub><sup>+</sup> T-cell count. Determinants for CI, as dichotomized by MNC, included cannabis use, prior cardiovascular disease, impaired renal function, and diabetes mellitus type 2.

### Conclusions

Decreased cognitive performance probably results from a multifactorial process, including not only HIV-associated factors, such as having experienced more severe immune deficiency, but also cardiovascular/metabolic factors, cannabis use, and depressive symptoms.

## Introduction

With the introduction of combination antiretroviral therapy (cART), AIDS-associated mortality and morbidity have markedly diminished and HIV encephalopathy, previously known as AIDS dementia complex, has largely disappeared.<sup>1-3</sup> In the past few years, however, a high prevalence (15-69%) of milder forms of cognitive impairment (CI) has been reported among HIV-infected individuals, including those with systemically well controlled HIV infection.<sup>4-9</sup>

To classify this broad clinical spectrum of HIV-associated neurocognitive disorders, a set of diagnostic criteria, commonly referred to as Frascati criteria, was developed.<sup>10</sup> These criteria, however, appear over-sensitive, resulting in not only high prevalence estimates but also high false-positive rates. We recently reported multivariate normative comparison (MNC), a technique which controls the false-positive rate while retaining sensitivity, to be a more accurate method of detecting CI in the HIV-infected population.<sup>11</sup>

In this previous report, we found CI by MNC to be present in 17% of 103 HIV-infected men and in 5% of 74 HIV-uninfected controls participating in the AGE<sub>HIV</sub> Cohort Study ( $P=0.02$ , one-tailed). Applying Frascati criteria to the same study population, CI was highly prevalent in HIV-infected participants (48%), but nearly equally so in HIV-uninfected controls (36%,  $P=0.09$ , one-tailed), indicating a high-false positive rate.<sup>11</sup>

In the pre-cART era, HIV-specific factors such as HIV viral load and CD<sub>4</sub><sup>+</sup> T-cell count were most strongly associated with CI.<sup>12</sup> In cART-treated (and ageing) individuals, however, the relative contribution of other risk factors towards CI, including cardiovascular, metabolic, and other comorbid conditions, is likely to gain relative importance besides HIV/ART-specific factors such as persistent immune activation and inflammation.<sup>13</sup> The relative contribution of each of such factors to the pathogenesis of CI remains to be further elucidated.

The purpose of this current study was to explore possible determinants for decreased cognitive performance as determined by MNC in the same above-mentioned AGE<sub>HIV</sub> Cohort Study population. Within this study, which investigates age-associated comorbidity among middle-aged individuals with and without HIV infection, a nested substudy

was established focusing on cognitive functioning. We performed cross-sectional analyses on these 103 HIV-1-infected and 74 HIV-uninfected substudy participants, exploring a broad range of possible determinants for decreased cognitive performance including HIV/ART-related factors, inflammatory markers, use of illicit drugs and/or alcohol, psychiatric conditions, and metabolic and cardiovascular risk factors.

## Methods

### Study design and participants

The AGE<sub>h</sub>IV Cohort Study is a prospective cohort study investigating prevalence, incidence, and risk factors of ageing-associated comorbidities and organ dysfunction among HIV-1-infected individuals and highly comparable HIV-uninfected controls, aged at least 45, in Amsterdam, The Netherlands, the details of which have been previously described.<sup>14</sup> At baseline, and every two years thereafter, participants undergo extensive screening for age-associated comorbidity and organ dysfunction.

All eligible participants from the main AGE<sub>h</sub>IV Cohort were consecutively invited to participate in a nested cognitive substudy, which began enrolment in December 2011.<sup>11</sup> Additional eligibility criteria for the substudy were male sex (as the availability of native Dutch-speaking women in the main AGE<sub>h</sub>IV Cohort was limited), and for the HIV-infected group, sustained suppression of HIV viremia on antiretroviral treatment (plasma HIV RNA <40 copies/mL) for at least 12 months; the presence of so-called viral 'blips' (transient low-level viremia between 40 and 200 copies/mL) was not an exclusion criterion.

Exclusion criteria for the substudy were a history of severe neurological disorder (e.g. stroke, seizure disorders, multiple sclerosis, dementia (including previous or current diagnosis of HIV-associated dementia)), history of traumatic brain injury with loss of consciousness for more than 30 minutes, current/past (HIV-associated) central nervous system infection or tumour, current severe psychiatric disorder (e.g. psychosis and major depression), current intravenous drug use, daily use of illicit drugs (with the exception of daily cannabis use), current excessive alco-



hol consumption (>48 units of alcohol/week), insufficient command of the Dutch language, and mental retardation.

Individuals with a previous or current diagnosis of HIV-associated dementia were excluded from participation as they most likely already underwent interventions (e.g. adaptation of their antiretroviral treatment), biasing the results of our study.

With respect to major depression as one of the exclusion criteria, depressive symptoms were assessed in the main AGE<sub>h</sub>IV Cohort Study by the 9-item Patient Health Questionnaire (PHQ-9). Participants with a PHQ-9 score of at least 15 (indicative of severe depressive symptoms and high risk of major depression) were excluded from the participation in the substudy.<sup>15</sup>

The inclusion/exclusion criteria with regard to illicit drug use (allowing weekly to monthly use of cocaine or ecstasy, as well as daily cannabis use) were implemented to minimize selection bias and were based on illicit drug use prevalence data previously obtained from the main AGE<sub>h</sub>IV Cohort. These showed daily cannabis use and weekly to monthly cocaine or ecstasy use to be fairly common among both HIV-infected participants attending the HIV outpatient department and HIV-uninfected controls.<sup>14</sup>

#### Standard protocol approvals, registrations, and patient consents

The protocol of the AGE<sub>h</sub>IV Cohort Study, including the above-mentioned substudy, was approved by the local ethics committee and has been registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) (identifier: NCT01466582). Written informed consent was obtained from all participants, separately for the main cohort study and nested substudy.

#### Neuropsychological assessment

As part of the substudy, neuropsychological assessment was performed by trained neuropsychologists and covered six cognitive domains commonly affected by HIV-associated CI, including fluency, attention, information processing speed, executive function, memory, and motor function (details are provided in a previous publication).<sup>11</sup> Depressive symptoms were assessed using the Beck Depression

Inventory<sup>16</sup>, and subjective cognitive complaints with the Cognitive Failures Questionnaire.<sup>17</sup> Everyday functioning was assessed using the Instrumental Activities of Daily Living<sup>18</sup> questionnaire and premorbid intelligence was estimated by the Dutch Adult Reading Test.<sup>19</sup> Use of psychotropic medication was assessed and included antidepressants, benzodiazepines, and methylphenidate.

### Definitions

All definitions of investigated variables are provided as footnotes in **Tables 7.1 and 7.2**.

Cognitive impairment diagnosis by multivariate normative comparison  
MNC is a statistical method that may be seen as a multivariate version of Student's t test for one sample.<sup>11,20</sup> MNC is able to control the family-wise error (the probability of falsely diagnosing individuals as cognitively abnormal) by performing a single multivariate comparison of the complete cognitive profile of a particular patient to the distribution of all the cognitive profiles of the control sample, rather than comparing each test result separately to the reference population.

MNC thus compares the complete cognitive profile of each HIV-infected participant with the cognitive profile of the HIV-uninfected control group as a whole. The test statistic is Hotelling's  $T^2$ . The false positive rate, that is erroneously concluding that an individual deviates from the control sample while this is not the case, is limited by the level of significance ( $\alpha$ ). In the present study,  $\alpha$  was set at 5% one tailed, resulting in a specificity of at least 95%, as confirmed in our previous publication.<sup>11</sup> In that previous report, the false-positive rate for CI was shown to be much higher when applying Frascati criteria, and was greatly reduced by applying MNC, indicating MNC to be a very powerful and more accurate tool for detecting CI.

Multivariate normative comparison: cognitive impairment as a dichotomous measure and cognitive performance as a continuous measure

Applying MNC as described in the previous subsection provides a dichotomous result (CI vs. no CI). As the number of cognitively impaired participants in our cohort, as diagnosed by MNC, was relatively small, statistical power to investigate determinants was limited.

MNC, however, also provides a continuous measure: the Hotelling's  $T^2$  statistic. The Hotelling's  $T^2$  statistic reflects the degree of cognitive deviation of each HIV-infected participant compared with the HIV-uninfected control group as a whole.

The direction of the deviation (better or worse cognitive performance compared with the control population) was determined using the sum of all z-scores of the participant (being a positive or negative score). Hotelling's  $T^2$  statistics were then transformed to a normal distribution by subtracting the lowest absolute Hotelling's  $T^2$  statistic from all absolute Hotelling's  $T^2$  statistics. This way the bimodal curve of the Hotelling's  $T^2$  statistic was transformed to a curve with a single peak, approaching a normal distribution (as confirmed by skewness and kurtosis tests).

This continuous measure enabled us to perform more robust statistical analyses (linear instead of logistic regression) and increased statistical power. We therefore used this variable as the main outcome measure in the regression analyses.

### Statistical analysis

Group comparisons were performed using the nonparametric test for trend,  $\chi^2$ , Fisher's exact, or Wilcoxon rank-sum test as appropriate.

Determinants for decreased cognitive performance were analysed by linear regression using the Hotelling's  $T^2$  statistic from the MNC analysis as a continuous variable as outcome measure. As a sensitivity analysis, determinants for CI as dichotomized by MNC, were analysed by logistic regression. All regression analyses were restricted to the HIV-infected studygroup.

Plausible determinants of cognitive performance were analysed using a forward stepwise model selection with P less than 0.05 as entry and

P more than 0.1 as exit criterion, exploring the following categories of variables:

1. demographic factors (age, premorbid IQ, educational level, Dutch as native language)
2. co-infections (chronic hepatitis B/C virus co-infections)
3. factors related to psychiatric comorbidity (depressive symptoms, psychotropic medication use)
4. use of illicit drugs (cannabis/cocaine/ecstasy) and/or alcohol
5. cardiovascular and metabolic factors (hypertension, smoking, diabetes mellitus type 2, BMI, waist-to-hip ratio, cardiovascular disease, levels of total/HDL/LDL cholesterol, triglycerides, and lipoprotein(a), physical activity, positive family history for myocardial infarction/hypertension/hypercholesterolemia, renal function)
6. markers of inflammation, monocyte activation, and coagulation (high-sensitivity C-reactive protein (hsCRP), soluble CD14 (sCD14), soluble CD163 (sCD163), D-dimer)
7. HIV/ART-related factors (time since HIV diagnosis, HIV diagnosis prior to 1996, having been treated with mono or dual nucleoside-analogue reverse transcriptase inhibitors prior to starting cART, duration of ART use, duration/degree of immune deficiency, prior AIDS diagnosis, central nervous system penetration effectiveness score of the currently used cART regimen, current/ prior/duration of/use of individual (classes) of antiretroviral agents)

MNC analyses were performed using R statistical software (<http://purl.oclc.org/NET/RGRASMAN/MNC>); for remaining analyses, STATA (version 10.1; StataCorp, College Station, Texas, USA) was used.

## Results

### Participants' characteristics

One hundred and three HIV-infected and 74 HIV-uninfected men were consecutively enrolled into the substudy between December 2011 and August 2013. Demographic and HIV-related characteristics are shown in **Table 7.1**. Both the groups were highly comparable, with a median age of 54 in both the groups, the majority of whom were MSM.

HIV-infected men were known to be infected and treated with antiretroviral medication for a prolonged period of time, and 35% had previously been diagnosed with AIDS. The majority had experienced substantial immune recovery on cART, with a median nadir CD4+ T-cell count of 170 cells/mm<sup>3</sup>, current median CD4+ T-cell count of 625 cells/mm<sup>3</sup>, and undetectable plasma viral load for a median of 8 years.

Factors related to cognition, behaviour, comorbidity, and inflammation are presented in **Table 7.2**. Both groups were comparable regarding native language, educational level, premorbid intelligence, depressive symptoms, and use of psychotropic medication. Smoking was more prevalent among HIV-positives (30% vs. 19% currently smoking,  $P=0.048$ ) and ecstasy use was more prevalent among HIV-uninfected controls (13% vs. 2%,  $P=0.008$ ), whereas cannabis, cocaine, and alcohol use were comparable between the two groups. Among HIV-positives, BMI was significantly lower (24.1 (interquartile range (IQR) 22.2-26.0) vs. 25.4 (IQR 23.7-27.5) kg/m<sup>2</sup>,  $P=0.003$ ) and waist-to-hip ratio significantly higher (0.96 (IQR 0.92-1.01) vs. 0.93 (IQR 0.89-0.99),  $P=0.02$ ). Total, HDL, and LDL cholesterol, lipoprotein(a), and triglyceride levels were comparable between the two groups, as was use of lipidlowering medication, physical activity, family history for metabolic/cardiovascular disease, history of cardiovascular disease, diabetes mellitus type 2, hypertension, and estimated glomerular filtration rate. Increased urinary albumin-to-creatinine ratio ( $\geq 3$  mg/mmol) was significantly more prevalent among HIV-positives (19.2% vs. 5.8%,  $P=0.01$ ). Levels of hsCRP and sCD14 were significantly higher among HIV-positives (1.5 (IQR 0.7-3.3) vs. 1.1 (IQR 0.6-2.1) mg/L,  $P=0.02$ , and 1548 (IQR 1318-2025) vs. 1207 (IQR 995-1558) ng/mL,  $P<0.001$ , respectively). hsCRP levels above 10 mg/L were also

**Table 7.1: Baseline demographic and HIV-related characteristics**

	HIV-uninfected (n=74)	HIV-infected (n=103)	P value
Age (years)	54 (49-61)	54 (49-62)	0.94 <sup>*</sup>
MSM (%) <sup>a</sup>	90%	93%	0.48 <sup>†</sup>
Dutch origin (%)	89%	86%	0.63 <sup>†</sup>
Hepatitis C virus RNA positive (%)	0%	1%	1.00 <sup>‡</sup>
Hepatitis B virus antigen and/or hepatitis B virus DNA positive (%)	0%	2%	0.51 <sup>‡</sup>
Time since HIV diagnosis (years)	-	13.5 (7.4-17.1)	-
Diagnosed with HIV prior to 1996 (%)	-	35%	-
CD4+ T-cell count at enrolment (cells/mm <sup>3</sup> )	-	625 (475-800)	-
Nadir CD4+ T-cell count (cells/mm <sup>3</sup> )	-	170 (60-250)	-
Known duration of CD4 <350 cells/mm <sup>3</sup> (months)	-	15.4 (4.2-45.2)	-
Duration of plasma viral load ≤200 copies/mL (years) <sup>b</sup>	-	8.3 (3.5-11.2)	-
Time since ART was first initiated (years)	-	11.6 (4.9-14.9)	-
Naive at start of cART (%) <sup>c</sup>	-	80%	-
Prior clinical AIDS (%) <sup>d</sup>	-	35%	-
Use of efavirenz			
Prior use	-	47%	-
Current use	-	21%	-
Central nervous system penetration effectiveness score of current cART regimen <sup>e</sup>	-	7 (7-8)	-

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

<sup>a</sup> The term 'MSM' applied to male participants who stated in the questionnaire to feel mostly or exclusively sexually attracted to men.

<sup>b</sup> Duration of undetectable plasma viral load was defined as: number of years since last plasma viral load >200 copies/mL.

<sup>c</sup> The term 'cART' was used for a combination of ≥3 antiretroviral drugs, other than ritonavir used as a pharmacologic booster.

<sup>d</sup> The term 'prior AIDS' was used in case of a previous AIDS-defining condition according to the US Centers for Disease Control and Prevention (CDC) classification.

<sup>e</sup> Central nervous system penetration effectiveness (CPE) score of the cART regimen of each HIV-infected participant was calculated using the algorithm as proposed by Letendre et al. in 2010.<sup>53</sup>

\* Wilcoxon rank-sum test

<sup>†</sup>  $\chi^2$  test

<sup>‡</sup> Fisher's exact test

significantly more prevalent among HIV-positives (10% vs. 0%,  $P=0.005$ ). D-dimer and sCD163 levels were comparable between the two study-groups.

Cognitive impairment as diagnosed by multivariate normative comparison

As previously reported, using MNC, CI was detected in 17 (17%) HIV-infected men. Transformed Hotelling's  $T^2$  statistics of the HIV-infected men ranged between -2.39 and 1.90, with a median of -0.15 (IQR -0.87 to +0.48).

Determinants of decreased cognitive performance by multivariate normative comparison in HIV-infected cohort participants

Linear regression analysis showed cannabis use, history of prior cardiovascular disease (borderline), impaired renal function (borderline), diabetes mellitus type 2, having an above-normal waist-to-hip ratio (borderline), presence of depressive symptoms (borderline), and lower nadir CD4+ T-cell count to be independently associated with poorer cognitive performance (**Table 7.3, Model 1**).

Determinants of cognitive impairment as dichotomized by multivariate normative comparison in HIV-1-infected cohort participants (sensitivity analysis)

Logistic regression analysis showed cannabis use, history of prior cardiovascular disease, impaired renal function, and diabetes mellitus type 2 (borderline) to be independently associated with CI (**Table 7.3, Model 2**).

**Table 7.2: Baseline characteristics related to cognition, behaviour, comorbidity, and inflammation**

	HIV-uninfected (n=74)	HIV-infected (n=103)	P value
Dutch as native language (%)	95%	91%	0.56 <sup>*</sup>
Education (ISCED level) <sup>a</sup>	6 (5-6)	6 (5-6)	0.50 <sup>†</sup>
Premorbid intelligence (IQ) <sup>b</sup>	103 (96-112)	101 (95-111)	0.48 <sup>†</sup>
Subjective cognitive complaints (%) <sup>c</sup>	5%	13%	0.13 <sup>*</sup>
Mild-to-moderate depressive symptoms (%) <sup>d</sup>	4%	6%	0.74 <sup>*</sup>
Severe depressive symptoms (%) <sup>e</sup>	0	0	-
Use of psychotropic medication (%) <sup>f</sup>	14%	16%	0.71 <sup>§</sup>
Level of daily functioning (IADL score) <sup>g</sup>	0	0	-
Weekly to monthly use of ecstasy (%) <sup>h</sup>	13%	2%	0.008 <sup>*</sup>
Weekly to monthly use of cocaine (%) <sup>h</sup>	4%	4%	1.00 <sup>*</sup>
Daily to monthly use of cannabis (%) <sup>h</sup>	15%	16%	0.96 <sup>§</sup>
Past intravenous drug use (%) <sup>h</sup>	0	0	-
Use of methamphetamine (%) <sup>i</sup>	1%	0	1.00 <sup>*</sup>
Alcohol intake (units/week)	5 (3-12)	6 (2-14)	0.89 <sup>‡</sup>
Smoking status			
Never smoked	36%	24%	0.048 <sup>†</sup>
Ever smoked	44%	46%	
Currently smoking	19%	30%	
Packyears of smoking	2.3 (0.0-14.0)	9.9 (0.2-31.6)	0.005 <sup>‡</sup>
BMI (kg/m <sup>2</sup> )	25.4 (23.7-27.5)	24.1 (22.2-26.0)	0.003 <sup>‡</sup>
BMI categories			
<20 (kg/m <sup>2</sup> )	1.4%	9.7%	0.002 <sup>†</sup>
20 to <25 (kg/m <sup>2</sup> )	44.4%	55.3%	
25 to <30 (kg/m <sup>2</sup> )	38.9%	30.1%	
≥30 (kg/m <sup>2</sup> )	15.3%	4.9%	
Waist-to-hip ratio	0.93 (0.89-0.99)	0.96 (0.92-1.01)	0.02 <sup>‡</sup>
Waist-to-hip ratio higher than normal (%) <sup>j</sup>	70%	85%	0.02 <sup>§</sup>
HDL cholesterol (mmol/L)	1.32 (1.01-1.58)	1.27 (1.02-1.52)	0.60 <sup>‡</sup>
LDL cholesterol (mmol/L)	3.35 (2.80-3.84)	3.25 (2.39-3.79)	0.26 <sup>‡</sup>
Total cholesterol (mmol/L)	5.39 (5.07-6.15)	5.40 (4.57-6.22)	0.37 <sup>‡</sup>
Lipoprotein (a) (mg/L)	86 (46-205)	87 (43-324)	0.57 <sup>‡</sup>
Triglycerides (mmol/L)	1.61 (1.09-2.43)	1.85 (1.20-2.81)	0.29 <sup>‡</sup>
Use of lipidlowering medication (%)	11%	12%	0.91 <sup>§</sup>
Statins (%)	11%	11%	0.90 <sup>§</sup>
Fibrates (%)	0%	2%	0.51 <sup>*</sup>
Physical activity (%) <sup>k</sup>	46%	47%	0.87 <sup>§</sup>
Positive family history for myocardial infarction, hypertension, diabetes mellitus type 2, or hypercholesterolemia (%) <sup>l</sup>	66%	64%	0.74 <sup>§</sup>
Diabetes mellitus type 2 (%) <sup>m</sup>	4%	6%	0.74 <sup>*</sup>
Hypertension (%) <sup>n</sup>	38%	39%	0.86 <sup>§</sup>
Renal function class, by albumin-to-creatinine ratio (ACR)			
Normal (<3 mg/mmol)	94.2%	80.9%	0.01 <sup>†</sup>
Moderately impaired (3-30 mg/mmol)	5.8%	19.2%	
Severely impaired (>30 mg/mmol)	0%	0%	
Renal function class, by estimated glomerular filtration rate (eGFR)			
Normal (≥90 mL/min)	54.9%	50.5%	0.76 <sup>†</sup>
Mildly impaired (60-90 mL/min)	40.9%	49.5%	
Moderately impaired (30-60 mL/min)	4.2%	0%	
Severely impaired (15-30 mL/min)	0%	0%	
Renal failure (<15 mL/min)	0%	0%	



**Table 7.2: Baseline characteristics related to cognition, behaviour, comorbidity, and inflammation (continued)**

	HIV-uninfected (n=74)	HIV-infected (n=103)	P value
Cardiovascular disease (%) <sup>o</sup>	6%	8%	0.76 <sup>*</sup>
Angina pectoris (%)	1%	5%	0.40 <sup>*</sup>
Myocardial infarction (%)	3%	2%	1.00 <sup>*</sup>
Peripheral arterial disease (%)	1%	3%	0.65 <sup>*</sup>
hsCRP (mg/L)	1.1 (0.6-2.1)	1.5 (0.7-3.3)	0.02 <sup>‡</sup>
hsCRP >10mg/L (%)	0%	10%	0.005 <sup>*</sup>
D-dimer (mg/L)	0.27 (0.20-0.40)	0.21 (0.20-0.33)	0.06 <sup>‡</sup>
D-dimer >0.5 mg/L (%)	15%	10%	0.27 <sup>§</sup>
sCD14 (ng/mL)	1207 (995-1558)	1548 (1318-2025)	<0.001 <sup>‡</sup>
sCD163 (ng/mL)	242 (186-343)	273 (205-417)	0.12 <sup>‡</sup>

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

*a* Educational level was defined using the International Standard Classification of Education (ISCED) 2011.

*b* Premorbid intelligence quotient (IQ) was estimated using the Dutch Adult Reading Test (DART). One of in total 74 HIV-uninfected controls and six of in total 103 HIV-infected individuals were unable to complete this test because of dyslexia.

*c* Subjective cognitive complaints were assessed using Cognitive Failure Questionnaire (CFQ). A cut-off of 42 or higher was used to indicate significant amount of subjective complaints, percentages scoring above this cut-off is shown.

Among HIV-infected participants with CI as diagnosed by MNC (n=17, 17%), four participants (24%) fulfilled the criteria for experiencing subjective cognitive complaints and 13 participants did not (76%).

*d* A Beck Depression Inventory score >13 and <29 reflects presence of mild-to-moderate depressive symptoms, percentages scoring >13 and <29 are shown. One of 103 HIV-infected individuals did not complete this test.

*e* A Beck Depression Inventory score ≥29 reflects severe depressive symptoms. None of the participants had a score ≥29. One of 103 HIV-infected individuals did not complete this test.

*f* Psychotropic medication included antidepressants, benzodiazepines, and methylphenidate.

*g* Level of day-to-day functioning was defined using the Independent Activities of Daily Living (IADL) questionnaire.

*h* Participants were asked by questionnaire about any illicit drug use (type and frequency) during the 6 months prior to completing the questionnaire. No data was available on illicit drug use prior to those 6 months, with the exception of intravenous drug use (participants were asked about any intravenous drug use in their past).

*i* Participants were asked by questionnaire about any illicit drug use (type and frequency) during the six months prior to completing the questionnaire. Use of methamphetamine was reported by only a single HIV-infected participant. This participant reported to have used methamphetamine sporadically (less than once a month) during the six months prior to completing the questionnaire. This participant was not diagnosed with CI by MNC.

*j* The waist-to-hip ratio was considered higher than normal if it was ≥0.9.

*k* Physical activity was defined following the Dutch guidelines for healthy physical activity ('Combinorm'): at least 5 days/week at least 30 min of moderate physical activity or at least twice per week at least 20 min of heavy physical activity.<sup>54</sup>

*l* 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.

*m* Diabetes mellitus type 2 was considered present if HbA<sub>1c</sub> (IFCC) ≥48 mmol/mol and/or elevated blood glucose (nonfasting ≥11.1 mmol/L or fasting ≥7.0 mmol/L), or if on antidiabetic medication.<sup>55</sup>

*n* Hypertension was considered present if DBP ≥90 mmHg and/or SBP ≥140 mmHg in all three measurements (Omron 705IT) with a 1-minute interval, or if on antihypertensive medication.<sup>56</sup>

*o* The variable past cardiovascular disease included angina pectoris, myocardial infarction, and peripheral arterial disease diagnoses, each being reported by the participants in a questionnaire. All self-reported diagnoses were then validated using hospital records for HIV-positives, and general practitioners' records for HIV-negatives, provided the latter had consented to contact their general practitioner. One HIV-uninfected participant did not provide consent to contact his general practitioner, resulting in one unconfirmed cardiovascular event. Further detail concerning diagnosis and validation of cardiovascular diseases has been previously reported.<sup>14</sup>

\* Fisher's exact test

‡ Nonparametric test for trend

‡ Wilcoxon rank-sum test

§  $\chi^2$  test

**Table 7-3: Determinants for cognitive performance/impairment as determined by multivariate normative comparison**

	Model 1		Model 2	
	$\beta$ coefficient 95% CI	P value	Odds ratio 95% CI	P value
Daily to monthly use of cannabis (y/n)	-0.77 -1.25, -0.30	0.002	27.76 4.61, 167.18	<0.001
Past cardiovascular disease (y/n) <sup>a</sup>	-0.64 -1.32, 0.04	0.066	17.71 1.81, 173.42	0.014
Impaired renal function (y/n) <sup>b</sup>	-0.36 -0.79, 0.07	0.096	8.76 1.47, 52.36	0.017
Diabetes mellitus type 2 (y/n) <sup>c</sup>	-0.73 -1.40, -0.05	0.036	5.71 0.73, 44.59	0.097
Having an above-normal waist-to-hip ratio (y/n) <sup>d</sup>	-0.46 -0.93, 0.01	0.055	-	-
Presence of depressive symptoms (y/n) <sup>e</sup>	-0.69 -1.42, 0.03	0.061	-	-
Nadir CD4+ T-cell count (per 50 cells/mm <sup>3</sup> decrease)	-0.09 -0.02, -0.15	0.008	-	-

Model 1 uses cognitive performance (as a continuous variable) as determined by MNC as outcome measure. Linear regression was performed to identify determinants for decreased cognitive performance.

Model 2 uses cognitive impairment (as a dichotomous variable) as determined by MNC as outcome measure. Logistic regression was performed to identify determinants for cognitive impairment.

Both models were restricted to the HIV-infected study group.

Abbreviations: BDI=Beck depression inventory, CI=confidence interval, MNC=multivariable normative comparison.

<sup>a</sup> The variable past cardiovascular disease included angina pectoris, myocardial infarction, and peripheral arterial disease.

<sup>b</sup> Impaired renal function as determined by albumin-to-creatinine ratio in urine of  $\geq 3$  mg/mmol.

<sup>c</sup> Diabetes mellitus type 2 was considered present if HbA1c (IFCC)  $\geq 48$  mmol/mol and/or elevated blood glucose (nonfasting  $\geq 11.1$  mmol/L or fasting  $\geq 7.0$  mmol/L), or if on antidiabetic medication.

<sup>d</sup> The waist-to-hip ratio was considered higher than normal if it was  $\geq 0.9$ .

<sup>e</sup> Depressive symptoms were considered present with a BDI score  $> 13$ .

## Discussion

### Key results

Determinants for decreased cognitive performance by MNC, when used as a continuous variable, included cannabis use, history of prior cardiovascular disease, impaired renal function, diabetes mellitus type 2, having an above-normal waist-to-hip ratio, presence of depressive symptoms, and lower nadir CD4+ T-cell count. The first four determinants were also observed in a sensitivity analysis for which CI was dichotomized as being present or absent by MNC. The latter three variables were not significant determinants in this analysis.

### Interpretation, limitations, and conclusion

To appreciate these findings, some aspects of the current report need to be addressed further. Strong features of the AGE<sub>HIV</sub> Cohort Study and its nested substudy are the large similarity between the HIV-infected and the HIV-uninfected studygroups, as well as the high level of detail by which all participants have been characterized. In addition, extensive clinical and biochemical data were obtained allowing for detailed assessment of relationships and adjustment for confounding.

Our results being those of cross-sectional analyses, we are merely able to demonstrate associations rather than causality. 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 exploring the effect of each factor towards cognitive (dys)function, and incorporating adjustment for those factors with a significant effect. Nonetheless, differences in remaining unmeasured confounders potentially influencing our results cannot be excluded.

In addition, some unique characteristics of this cohort (participants being mostly white middle-aged MSM with sustained viral suppression, with a low prevalence of chronic hepatitis B and C) may limit generalization of the results to other populations. Additional studies are needed to determine whether our findings apply equally to other populations with different characteristics.

When analysing determinants of cognitive impairment/performance by MNC, we found cannabis use to be strongly associated with cognitive dysfunction. Both in the general population and among HIV-positives, cannabis use has been associated with decreased cognitive function.<sup>21,22</sup> In the context of HIV infection, cannabis use is common, not only for recreational but also for medicinal use (treating neuropathic pain, anorexia, nausea, or mood disturbances).<sup>23,24</sup> In addition to direct effects of cannabis on cognition, the observed association could also be partly explained by some of the above-mentioned conditions for which medicinal use of cannabis is indicated, which themselves may be associated with effects on cognition. The underlying reason for cannabis use (medicinal vs. recreational) unfortunately was not captured as part of data collection, and we were therefore unable to explore this hypothesis further.

We also found multiple metabolic/cardiovascular factors to be associated with CI as well as decreased cognitive performance.

Both in the general population and among HIV-positives, hypercholesterolemia, diabetes mellitus type 2, and central obesity have been associated with decreased cognitive function.<sup>25-35</sup>

We also found (prior) cardiovascular disease (i.e. angina pectoris, myocardial infarction, or peripheral arterial disease), to be associated with cognitive impairment/performance. In both the general and HIV-infected population, prior cardiovascular disease and subclinical atherosclerotic disease have been associated with cognitive decline.<sup>28,31,36-38</sup>

In addition, we found albuminuria to be associated with cognitive dysfunction, which is in line with other studies, both in the general and the HIV-infected population.<sup>38-40</sup>

Interpreting these results, cardiovascular/metabolic factors may substantially contribute to poorer cognitive performance among HIV-infected individuals. Cerebral damage resulting from (micro)vascular disease may therefore importantly contribute towards HIV-associated CI. Several neuroimaging studies among HIV-infected individuals have also demonstrated cardiovascular/metabolic factors to be associated with cerebral damage, thereby supporting this hypothesis.<sup>41-43</sup>

Evidence of renal impairment and past cardiovascular disease (each of which are associated with cognitive dysfunction in our analyses) are

likely manifestations of (micro)vascular organ damage in many cases, and may (partly) share pathophysiological mechanisms with cerebral damage.

Presence of depressive symptoms was identified as an additional risk factor for decreased cognitive performance (but not for CI). In the general population, depression has been associated with cognitive deficits.<sup>44</sup> Among HIV-infected individuals, depressive symptoms have also been associated with decreased cognitive function,<sup>45</sup> although one study did not report an association between cognitive function and depressive symptoms.<sup>46</sup>

We also found severity of prior immune deficiency, as reflected in a lower nadir CD4+ T-cell count, to be associated with decreased cognitive performance, which is also consistent with the earlier findings.<sup>12,47-49</sup> Although HIV infection is known to cause immune deficiency by depleting CD4+ T-cells, it is also associated with activation of the immune system and inflammation. This is partly driven by depletion of CD4+ T-cells within the intestinal mucosa resulting in increased permeability and translocation of microbial products across the mucosa. This results in stimulation of both the innate and adaptive immune systems that persists, albeit at a reduced level, among cART-treated HIV-infected patients with suppressed viremia.<sup>50,51</sup>

Atherosclerosis and cardiovascular disease are also closely related to immune activation and inflammation and have been shown to be highly prevalent among HIV-infected individuals, as is the case for many cardiovascular/metabolic risk factors (such as dyslipidemia, smoking, and central obesity).<sup>52</sup> Immune activation and inflammation may therefore contribute to CI in a direct manner, but also indirectly, by the association with vascular damage and cerebral small vessel disease.

Three factors were identified as risk factors for decreased cognitive performance, but not for CI as a dichotomous outcome: having an above-normal waist-to-hip ratio, presence of depressive symptoms, and a lower nadir CD4+ T-cell count. This discrepancy might very well be explained by reduced statistical power when using CI as a dichotomous outcome measure instead of cognitive performance as a continuous outcome measure.

In conclusion, our results indicate that reduced cognitive perfor-

mance in HIV-infected men with sustained suppressed viremia on cART is likely the result of a multifactorial process, in which not only HIV-associated factors, such as having experienced more severe immune deficiency, but also cardiovascular/metabolic factors, cannabis use, and depressive symptoms are key contributors. These are likely to gain increased importance as the population of people living with HIV continues to age.

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## Authors' contributions

JS contributed to data collection, data analysis and interpretation, writing of all drafts of the manuscript, and was responsible for producing and submitting the final article.

TS contributed to data collection, data analysis, and writing of the manuscript.

FW contributed to the study design, data analysis and interpretation, and writing of the article.

NK contributed to data collection, data interpretation, and writing of the article.

MC contributed to data analysis and interpretation, and writing of the article.

GG contributed to data analysis and interpretation, and contributed to writing of all drafts of the article.

BS contributed to the study design, data analysis and interpretation, and contributed to writing of all drafts of the article.

IS contributed to the study design, data collection, data interpretation, and writing of the article.

MP contributed to the study design, data interpretation, and writing of the article.

CM conceived the nested cognitive substudy, contributed to its design, to data interpretation, and writing of the article.

PP contributed to the study design, data interpretation, and writing of the article.

PR conceived the main cohort study and the nested cognitive substudy, contributed to both study designs, to data interpretation, and writing of all drafts of the article.

## References


1. Palella, F. J. *et al.* Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. *N. Engl. J. Med.* **338**, 853–860 (1998).
2. Wada, N. *et al.* Cause-specific life expectancies after 35 years of age for human immunodeficiency syndrome-infected and human immunodeficiency syndrome-negative individuals followed simultaneously in long-term cohort studies, 1984–2008. *Am. J. Epidemiol.* **177**, 116–125 (2013).
3. Weber, R. *et al.* Decreasing mortality and changing patterns of causes of death in the Swiss HIV Cohort Study. *HIV Med.* **14**, 195–207 (2013).
4. Cysique, L. A. & Brew, B. J. Prevalence of non-confounded HIV-associated neurocognitive impairment in the context of plasma HIV RNA suppression. *J. Neurovirol.* **17**, 176–183 (2011).
5. Winston, A. *et al.* Neurocognitive function in HIV infected patients on antiretroviral therapy. *PLoS One* **8**, e61949 (2013).
6. Schouten, J., Cinque, P., Gisslen, M., Reiss, P. & Portegies, P. HIV-1 infection and cognitive impairment in the cART era: a review. *AIDS Lond. Engl.* **25**, 561–575 (2011).
7. Robertson, K. R. *et al.* The prevalence and incidence of neurocognitive impairment in the HAART era. *AIDS Lond. Engl.* **21**, 1915–1921 (2007).

8. Simioni, S. *et al.* Cognitive dysfunction in HIV patients despite long-standing suppression of viremia. *AIDS Lond. Engl.* **24**, 1243–1250 (2010).
9. Heaton, R. K. *et al.* HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER Study. *Neurology* **75**, 2087–2096 (2010).
10. Antinori, A. *et al.* Updated research nosology for HIV-associated neurocognitive disorders. *Neurology* **69**, 1789–1799 (2007).
11. Su, T. *et al.* Multivariate normative comparison, a novel method for more reliably detecting cognitive impairment in HIV infection. *AIDS Lond. Engl.* **29**, 547–557 (2015).
12. Heaton, R. K. *et al.* HIV-associated neurocognitive disorders before and during the era of combination antiretroviral therapy: differences in rates, nature, and predictors. *J. Neurovirol.* **17**, 3–16 (2011).
13. Clifford, D. B. & Ances, B. M. HIV-associated neurocognitive disorder. *Lancet Infect. Dis.* **13**, 976–986 (2013).
14. Schouten, J. *et al.* Cross-sectional comparison of the prevalence of age-associated comorbidities and their risk factors between HIV-infected and uninfected individuals: the AGEHIV cohort study. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **59**, 1787–1797 (2014).
15. Beck, A. T., Steer, R. A., Ball, R. & Ranieri, W. Comparison of Beck Depression Inventories -IA and -II in psychiatric outpatients. *J. Pers. Assess.* **67**, 588–597 (1996).
16. Beck, A. T., Ward, C. H., Mendelson, M., Mock, J. & Erbaugh, J. An inventory for measuring depression. *Arch. Gen. Psychiatry* **4**, 561–571 (1961).
17. Broadbent, D. E., Cooper, P. F., FitzGerald, P. & Parkes, K. R. The Cognitive Failures Questionnaire (CFQ) and its correlates. *Br. J. Clin. Psychol. Br. Psychol. Soc.* **21 (Pt 1)**, 1–16 (1982).
18. Lawton, M. P. & Brody, E. M. Assessment of older people: self-maintaining and instrumental activities of daily living. *The Gerontologist* **9**, 179–186 (1969).
19. Schmand B, Lindeboom J, van Harskamp F. Schmand B, Lindeboom J, van Harskamp F. Dutch adult reading test. Lisse: Swets en Zeitlinger 1992. (1992).
20. Huizenga, H. M., Smeding, H., Grasman, R. P. P. & Schmand, B. Multivariate normative comparisons. *Neuropsychologia* **45**, 2534–2542 (2007).
21. Thames, A. D., Arbid, N. & Sayegh, P. Cannabis use and neurocognitive functioning in a non-clinical sample of users. *Addict. Behav.* **39**, 994–999 (2014).
22. Cristiani, S. A., Pukay-Martin, N. D. & Bornstein, R. A. Marijuana use and cognitive function in HIV-infected people. *J. Neuropsychiatry Clin. Neurosci.* **16**, 330–335 (2004).
23. Lutge, E. E., Gray, A. & Siegfried, N. The medical use of cannabis for reducing morbidity and mortality in patients with HIV/AIDS. *Cochrane Database Syst. Rev.* CD005175 (2013).
24. Hazekamp, A. & Heerdink, E. R. The prevalence and incidence of medicinal



- cannabis on prescription in The Netherlands. *Eur. J. Clin. Pharmacol.* **69**, 1575–1580 (2013).
25. Lorius, N. *et al.* Vascular disease and risk factors are associated with cognitive decline in the alzheimer disease spectrum. *Alzheimer Dis. Assoc. Disord.* **29**, 18–25 (2015).
  26. Raffaitin, C. *et al.* Metabolic syndrome and cognitive decline in French elders: the Three-City Study. *Neurology* **76**, 518–525 (2011).
  27. Reijmer, Y. D. *et al.* Development of vascular risk factors over 15 years in relation to cognition: the Hoorn Study. *J. Am. Geriatr. Soc.* **60**, 1426–1433 (2012).
  28. Wright, E. J. *et al.* Cardiovascular risk factors associated with lower baseline cognitive performance in HIV-positive persons. *Neurology* **75**, 864–873 (2010).
  29. Bordier, L., Doucet, J., Boudet, J. & Bauduceau, B. Update on cognitive decline and dementia in elderly patients with diabetes. *Diabetes Metab.* **40**, 331–337 (2014).
  30. Ganguli, M., Fu, B., Snitz, B. E., Hughes, T. F. & Chang, C.-C. H. Mild cognitive impairment: incidence and vascular risk factors in a population-based cohort. *Neurology* **80**, 2112–2120 (2013).
  31. Fabbiani, M. *et al.* Cardiovascular risk factors and carotid intima-media thickness are associated with lower cognitive performance in HIV-infected patients. *HIV Med.* **14**, 136–144 (2013).
  32. Valcour, V. G. *et al.* Diabetes, insulin resistance, and dementia among HIV-1-infected patients. *J. Acquir. Immune Defic. Syndr.* **38**, 31–36 (2005).
  33. Sanz, C. M. *et al.* Relationship between markers of insulin resistance, markers of adiposity, HbA1c, and cognitive functions in a middle-aged population-based sample: the MONA LISA study. *Diabetes Care* **36**, 1512–1521 (2013).
  34. Crichton, G. E. *et al.* Metabolic syndrome, cognitive performance, and dementia. *J. Alzheimers Dis. JAD* **30 Suppl 2**, S77–87 (2012).
  35. McCutchan, J. A. *et al.* Role of obesity, metabolic variables, and diabetes in HIV-associated neurocognitive disorder. *Neurology* **78**, 485–492 (2012).
  36. Weinstein, G., Goldbourt, U. & Tanne, D. Angina pectoris severity among coronary heart disease patients is associated with subsequent cognitive impairment. *Alzheimer Dis. Assoc. Disord.* **29**, 6–11 (2015).
  37. Reis, J. P. *et al.* Subclinical atherosclerotic calcification and cognitive functioning in middle-aged adults: the CARDIA study. *Atherosclerosis* **231**, 72–77 (2013).
  38. Becker, J. T. *et al.* Vascular risk factors, HIV serostatus, and cognitive dysfunction in gay and bisexual men. *Neurology* **73**, 1292–1299 (2009).
  39. Anand, S., Johansen, K. L. & Kurella Tamura, M. Aging and chronic kidney disease: the impact on physical function and cognition. *J. Gerontol. A. Biol. Sci. Med. Sci.* **69**, 315–322 (2014).
  40. Kalayjian, R. C. *et al.* Proteinuria is associated with neurocognitive impairment in antiretroviral therapy treated HIV-infected individuals. *J. Acquir. Immune Defic. Syndr.* **67**, 30–35 (2014).

41. Cysique, L. A. *et al.* HIV, vascular and aging injuries in the brain of clinically stable HIV-infected adults: a (1)H MRS study. *PLoS One* **8**, e61738 (2013).
42. Nakamoto, B. K. *et al.* Cerebrovascular risk factors and brain microstructural abnormalities on diffusion tensor images in HIV-infected individuals. *J. Neurovirol.* **18**, 303–312 (2012).
43. McMurtray, A., Nakamoto, B., Shikuma, C. & Valcour, V. Small-vessel vascular disease in human immunodeficiency virus infection: the Hawaii aging with HIV cohort study. *Cerebrovasc. Dis. Basel Switz.* **24**, 236–241 (2007).
44. McIntyre, R. S. *et al.* Cognitive deficits and functional outcomes in major depressive disorder: determinants, substrates, and treatment interventions. *Depress. Anxiety* **30**, 515–527 (2013).
45. Malaspina, L. *et al.* Successful cognitive aging in persons living with HIV infection. *J. Neurovirol.* **17**, 110–119 (2011).
46. Cysique, L. A. *et al.* Incident major depression does not affect neuropsychological functioning in HIV-infected men. *J. Int. Neuropsychol. Soc. JINS* **13**, 1–11 (2007).
47. Ellis, R. J. *et al.* CD4 nadir is a predictor of HIV neurocognitive impairment in the era of combination antiretroviral therapy. *AIDS Lond. Engl.* **25**, 1747–1751 (2011).
48. Garvey, L., Surendrakumar, V. & Winston, A. Low rates of neurocognitive impairment are observed in neuro-asymptomatic HIV-infected subjects on effective antiretroviral therapy. *HIV Clin. Trials* **12**, 333–338 (2011).
49. McCombe, J. A., Vivithanaporn, P., Gill, M. J. & Power, C. Predictors of symptomatic HIV-associated neurocognitive disorders in universal health care. *HIV Med.* **14**, 99–107 (2013).
50. Brenchley, J. M. *et al.* Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat. Med.* **12**, 1365–1371 (2006).
51. Klatt, N. R., Chomont, N., Douek, D. C. & Deeks, S. G. Immune activation and HIV persistence: implications for curative approaches to HIV infection. *Immunol. Rev.* **254**, 326–342 (2013).
52. Zanni, M. V., Schouten, J., Grinspoon, S. K. & Reiss, P. Risk of coronary heart disease in patients with HIV infection. *Nat. Rev. Cardiol.* **11**, 728–741 (2014).
53. Letendre SL, Fitzsimons CA, Ellis RJ, Clifford D, Collier AC, Gelman B, et al. Correlates of CSF viral loads in 1221 volunteers of the CHARTER Cohort. 17th Conference on Retroviruses and Opportunistic Infections, 16–19 February 2010, San Francisco, California; abstract 172. (2010).
54. Hildebrandt VH, Ooijendijk WTM, Hopman-Rock M. (Eds) *Trendrapport Bewegingen en Gezondheid 2004–2005*. Leiden: TNO, 2007. (2007).
55. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* **35 Suppl 1**, S64–71 (2012).
56. Pickering, T. G. *et al.* Recommendations for blood pressure measurement in humans: an AHA scientific statement from the Council on High Blood Pressure Research Professional and Public Education Subcommittee. *J. Clin. Hypertens. Greenwich Conn* **7**, 102–109 (2005).



# Diagnostic characteristics of four cognitive screening instruments for detecting HIV-associated cognitive impairment

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## Abstract

### Objective

To assess the performance of four cognitive screening instruments (Mini Mental State Examination (MMSE), HIV Dementia Scale (HDS), Montreal Cognitive Assessment (MoCA), and the questionnaire as proposed by Simioni et al. (Simioni questionnaire)) for detecting HIV-associated cognitive impairment (CI).

### Methods

One hundred and three HIV-1-infected men with suppressed viremia on combination antiretroviral therapy (cART) for  $\geq 12$  months, and 74 highly similar HIV-uninfected men, all aged  $\geq 45$ , underwent neuropsychological assessment (NPA) as well as the above four cognitive screening instruments.

CI was diagnosed using Frascati criteria as well as using multivariate normative comparison (MNC), a more accurate method for diagnosing CI.

Scores of each cognitive screening instrument were compared between the two study-groups. In the HIV-infected group, sensitivity and specificity, area under the curve by receiver operator characteristics (ROC) analyses, and optimal cut-off point of each screening instrument were assessed, using CI by Frascati criteria or by MNC as the gold standard.

### Results

All cognitive screening tools showed comparable scores and abnormality rates among HIV-infected and HIV-uninfected participants.

Each cognitive screening instrument showed low sensitivity and moderate specificity.

By ROC analyses, MMSE, HDS, and MoCA, irrespective of the gold standard used, showed at best moderate accuracy for identifying CI.

### Conclusions

All cognitive screening instruments, irrespective of which of both gold standards was used, showed at best moderate accuracy for identifying cognitive impairment. Cognitive deficits in the context of HIV are subtle, and no cognitive screening instrument so far seems optimal for use in clinical practice.

## Introduction

With the introduction of combination antiretroviral therapy (cART), AIDS-associated mortality and morbidity have markedly diminished. Severe HIV encephalopathy or HIV-associated dementia (HAD, previously also known as AIDS dementia complex), have largely disappeared.<sup>1-3</sup> In the past few years however, a high but varying prevalence of milder forms of cognitive impairment (CI), ranging from 15-69%, has been reported among HIV-infected people, including those with systemically well-controlled infection.<sup>4-9</sup>

To classify this broadening clinical spectrum of HIV-associated neurocognitive disorders (HAND), a set of research criteria, commonly referred to as the Frascati criteria, have been developed.<sup>10</sup> These criteria are heavily debated, as they are probably oversensitive, resulting in unlikely high prevalence estimates and high false-positive rates. In view of these limitations and a lack of clinical application for the Frascati criteria, we have recently shown multivariate normative comparison (MNC) to be a more accurate method of detecting CI.<sup>11</sup>

Irrespective of the method of classification, comprehensive neuropsychological assessment (NPA) is the recommended method for establishing the diagnosis of HIV-associated CI, which is a time-consuming examination. The availability of a short and accurate cognitive screening tool to identify those who are most likely to actually have HIV-associated CI and can subsequently be referred for NPA, would therefore be of great importance.

Several cognitive screening instruments have been proposed, including the Mini Mental State Examination (MMSE), the HIV Dementia Scale (HDS), the Montreal Cognitive Assessment (MoCA), and the 3-item questionnaire as published by Simioni et al. (from now on referred to as the Simioni questionnaire).<sup>12</sup> The latter, which assesses cognitive complaints in daily life, is proposed by the European AIDS Clinical Society in their most recent HIV treatment guidelines for identifying those most likely of having HIV-associated CI and who should be referred for NPA.<sup>13</sup>

The usefulness of each of these screening instruments for detecting especially milder forms of HIV-associated CI remains to be clarified. Therefore, the purpose of this study was to assess diagnostic character-

istics of four cognitive screening instruments (MMSE, HDS, MoCA, and the Simioni questionnaire), comparing scores and rates with abnormal scores between HIV-infected and highly comparable HIV-uninfected participants, and assessing sensitivity and specificity, area under the curve by receiver operator characteristics analyses, and optimal cut-off point of each screening instrument, using an abnormal NPA (CI as diagnosed by Frascati criteria (to enable comparison with previous publications) or CI as diagnosed by MNC) as two possible gold standards.

## Methods

### Study design and participants

The AGE<sub>h</sub>IV Cohort Study is a prospective cohort study investigating prevalence, incidence and risk factors of ageing-associated comorbidities and organ dysfunction among HIV-1-infected individuals and highly comparable HIV-uninfected controls, aged  $\geq 45$ , in Amsterdam, The Netherlands, the details of which have previously been described.<sup>14</sup> At baseline, and every two years thereafter, participants undergo extensive screening for age-associated comorbidity and organ dysfunction.

All eligible participants from the main AGE<sub>h</sub>IV Cohort were consecutively invited to participate in a nested cognitive substudy, which began enrolment in December 2011.<sup>11</sup> Additional eligibility criteria for the substudy were male sex (as the availability of Dutch-speaking women in the main AGE<sub>h</sub>IV Cohort was limited), and for the HIV-infected group, sustained suppression of HIV viremia on antiretroviral treatment (plasma HIV RNA  $< 40$  copies/mL) for at least 12 months; the presence of so-called viral 'blips' (transient low-level viremia) was not an exclusion criterion.

Exclusion criteria for the substudy were a history of severe neurological disorder (e.g. stroke, seizure disorders, multiple sclerosis, dementia (including previous or current diagnosis of HAD)), history of traumatic brain injury with loss of consciousness for more than 30 minutes, current/past (HIV-associated) central nervous system infection or tumour, current severe psychiatric disorder (e.g. psychosis, major depression), current intravenous drug use, daily use of illicit drugs (with the exception

of daily cannabis use), current excessive alcohol consumption (>48 units of alcohol/week), insufficient command of the Dutch language and mental retardation. With respect to major depression as one of the exclusion criteria, depressive symptoms were assessed in the main AGE<sub>h</sub>IV Cohort Study by the 9-item Patient Health Questionnaire (PHQ-9). Participants with a PHQ-9 score of at least 15 (indicative of severe depressive symptoms and potentially of major depression) were excluded from participation in the substudy.<sup>15</sup>

#### Standard protocol approval, registration, and patient consent

The protocol of the AGE<sub>h</sub>IV Cohort Study (including the above-mentioned substudy) was approved by the local ethics committee and has been registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) (identifier: NCT01466582). Written informed consent was obtained from all participants, both for the main study and substudy.

#### Neuropsychological assessment (NPA)

As part of the substudy, NPA was performed by trained neuropsychologists and covered 6 cognitive domains commonly affected by HIV-associated CI, including fluency, attention, information processing speed, executive function, memory, and motor function (details are provided in a previous publication).<sup>11</sup> Depressive symptoms were assessed using the Beck Depression Inventory (BDI)<sup>16</sup>, and subjective cognitive complaints with the Cognitive Failures Questionnaire (CFQ)<sup>17</sup>. Everyday functioning was assessed using the Instrumental Activities of Daily Living (IADL)<sup>18</sup> questionnaire and pre-morbid intelligence was estimated by the Dutch Adult Reading Test (DART)<sup>19</sup>. Use of psychotropic medication was assessed and included use of antidepressants, benzodiazepines, and methylphenidate.

#### Diagnosis of CI according to Frascati criteria and MNC

As reported in detail in a previous publication, Frascati criteria as well as MNC were applied to diagnose CI.<sup>11</sup> Frascati criteria were applied as pub-

lished by Antinori et al.<sup>11,20</sup> MNC is a statistical method that may be seen as a multivariate version of Student's t test for one sample.<sup>11,21</sup> MNC is able to control the family-wise error (the probability of falsely diagnosing individuals as cognitively abnormal) by performing a single multivariate comparison of the complete cognitive profile of a particular patient to the distribution of all the cognitive profiles of the control sample, rather than comparing each test result separately to the reference population. MNC thus compares the complete cognitive profile of each HIV-infected participant with the cognitive profile of the HIV-uninfected control group as a whole. The test statistic is Hotelling's  $T^2$ . The false positive rate, i.e. erroneously concluding that an individual deviates from the control sample while this is not the case, is limited by the level of significance ( $\alpha$ ). In the present study  $\alpha$  was set at 5% one-tailed, resulting in a specificity of at least 95%, as confirmed in our previous publication.<sup>11</sup> In this previous report, the false-positive rate for CI was shown to be high when applying Frascati criteria, and was greatly reduced by applying MNC, indicating MNC to be a very powerful and more accurate tool for detecting CI.

#### Cognitive screening instruments

MMSE, HDS, Simioni questionnaire, and MoCA were applied to each sub-study participant. The following (classical) cut-offs were used to define abnormal scores: MMSE  $\leq 24/30$ , MoCA  $\leq 25/30$ , and HDS  $\leq 10/16$ . The more recently proposed HDS cut-off of  $\leq 14/16$  as proposed by Simioni et al. was used as well.<sup>12</sup>

The Simioni questionnaire was considered abnormal when at least one of the following three questions was answered with "yes, definitely": 1) "Do you experience frequent memory loss (e.g. do you forget the occurrence of special events even the more recent ones, appointments, etc.)?", 2) "Do you feel that you are slower when reasoning, planning activities, or solving problems?", 3) "Do you have difficulties paying attention (e.g. to a conversation, a book, or a movie)?" (each question to be answered with "never", "hardly ever", or "yes, definitely").<sup>12</sup>



### Statistical analysis

Group comparisons were performed using the non-parametric test for trend,  $\chi^2$ , Fisher's exact, or Wilcoxon rank-sum test as appropriate.

Predictive validity of each of the four cognitive screening instruments (MMSE, HDS, MoCA, and Simioni questionnaire) was assessed using either of the two different gold standards, i.e. CI diagnosis by Frascati criteria or by MNC, respectively. These analyses were restricted to the HIV-infected studygroup. A nonparametric receiver operator characteristic (ROC) analysis was performed to examine the ability of the MMSE, HDS, and MoCA to detect CI using the presence of CI (as diagnosed by Frascati criteria or by MNC, respectively) as the outcome. Furthermore, Youden index was calculated, in search of an optimal cut-off point for MMSE, HDS, and MoCA, using CI as diagnosed by Frascati criteria and CI by MNC as the gold standards.

MNC analyses were performed using *R* statistical software (<http://purl.oclc.org/NET/RGRASMAN/MNC>); for remaining analyses STATA (version 10.1, StataCorp, Texas, USA) was used.

## Results

### Participants characteristics

One hundred and three HIV-infected and 74 HIV-uninfected participants were consecutively enrolled into the substudy between December 2011 and March 2014. Demographic and HIV-related characteristics, as well as factors related to behaviour and cognition, are shown in **Table 8.1**. Both groups were highly comparable, with a median age of 54 years in both groups, and the majority being men who have sex with men (MSM). Both groups were comparable regarding native language, educational level, premorbid intelligence, subjective cognitive complaints, depressive symptoms, and use of psychotropic medication. Smoking was more prevalent among HIV-positives (30% vs. 19% currently smoking,  $P=0.048$ ) and ecstasy use was more prevalent among HIV-uninfected controls (13% vs. 2%,  $P=0.008$ ), whereas cannabis, cocaine, and alcohol use were comparable between the two groups.

**Table 8.1: Baseline demographic and HIV-related characteristics, and factors related to cognition and behaviour**

	HIV-uninfected (n=74)	HIV-infected (n=103)	P value
Age (years)	54 (49-61)	54 (49-62)	0.94 <sup>a</sup>
MSM (%) <sup>1</sup>	90%	93%	0.48 <sup>b</sup>
Hepatitis C RNA positive (%)	0%	1%	1.00 <sup>c</sup>
Hepatitis B antigen and/or hepatitis B DNA positive (%)	0%	2%	0.51 <sup>c</sup>
Dutch as native language (%)	95%	91%	0.56 <sup>c</sup>
Education (ISCED level) <sup>2</sup>	6 (5-6)	6 (5-6)	0.50 <sup>d</sup>
Premorbid intelligence (IQ) <sup>3</sup>	103 (96-112)	101 (95-111)	0.48 <sup>c</sup>
Subjective cognitive complaints (%) <sup>4</sup>	5%	13%	0.13 <sup>c</sup>
Mild to moderate depressive symptoms (%) <sup>5</sup>	4%	6%	0.74 <sup>c</sup>
Severe depressive symptoms (%) <sup>6</sup>	0	0	-
Use of psychotropic medication (%) <sup>7</sup>	14%	16%	0.71 <sup>b</sup>
Level of daily functioning (IADL score) <sup>8</sup>	0	0	-
Smoking status			
Never smoked	36%	24%	0.048 <sup>d</sup>
Ever smoked	44%	46%	
Currently smoking	19%	30%	
Packyears of smoking (packyears)	2.3 (0.0-14.0)	9.9 (0.2-31.6)	0.005 <sup>a</sup>
Weekly to monthly use of ecstasy (%)	13%	2%	0.008 <sup>c</sup>
Weekly to monthly use of cocaine (%)	4%	4%	1.00 <sup>c</sup>
Daily to monthly use of cannabis (%)	15%	16%	0.96 <sup>b</sup>
Alcohol intake (units per week)	5 (3-12)	6 (2-14)	0.89 <sup>a</sup>
Time since HIV diagnosis (years)	-	13.5 (7.4-17.1)	-
Diagnosed with HIV before 1996 (%)	-	35%	-
CD <sub>4</sub> + T-cell count at enrolment (cells/mm <sup>3</sup> )	-	625 (475-800)	-
Nadir CD <sub>4</sub> + T-cell count (cells/mm <sup>3</sup> )	-	170 (60-250)	-
Known duration of CD <sub>4</sub> <350 cells/mm <sup>3</sup> (months)	-	15.4 (4.2-45.2)	-
Duration of plasma viral load ≤200 copies/mL (years) <sup>9</sup>	-	8.3 (3.5-11.2)	-
Time since ART was first initiated (years)	-	11.6 (4.9-14.9)	-
Naive at start of cART (%) <sup>10</sup>	-	80%	-

**Table 8.1: Baseline demographic and HIV-related characteristics, and factors related to cognition and behaviour (continued)**

	HIV-uninfected (n=74)	HIV-infected (n=103)	P value
Prior clinical AIDS (%) <sup>11</sup>	-	35%	-
Use of efavirenz			
Prior use	-	47%	-
Current use	-	21%	-
Central nervous system penetration effectiveness score of current cART regimen <sup>12</sup>	-	7 (7-8)	-

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

Test type used: <sup>a</sup> Wilcoxon rank-sum test, <sup>b</sup>  $\chi^2$  test, <sup>c</sup> Fisher's exact test, <sup>d</sup> Nonparametric test for trend.

<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> Educational level was defined using the International Standard Classification of Education (ISCED) 2011.

<sup>3</sup> Premorbid intelligence quotient (IQ) was estimated using the Dutch Adult Reading Test (DART). One of in total 74 HIV-uninfected controls and six of in total 103 HIV-infected individuals were unable to complete this test due to dyslexia.

<sup>4</sup> Subjective cognitive complaints were assessed using Cognitive Failure Questionnaire (CFQ). A cut-off of  $\geq 42$  was used to indicate significant amount of subjective complaints, percentages scoring above this cut-off is shown.

<sup>5</sup> A Beck Depression Inventory score  $>13$  and  $<29$  reflects presence of mild to moderate depressive symptoms, percentages scoring  $>13$  and  $<29$  are shown. One of 103 HIV-infected individuals did not complete this test.

<sup>6</sup> A Beck Depression Inventory score  $\geq 29$  reflects severe depressive symptoms. None of the participants had a score  $\geq 29$ . One of 103 HIV-infected individuals did not complete this test.

<sup>7</sup> Psychotropic medication included: antidepressants, benzodiazepines, methylphenidate.

<sup>8</sup> Level of day-to-day functioning was defined using the Independent Activities of Daily Living (IADL) questionnaire.

<sup>9</sup> Duration of undetectable plasma viral load was defined as: number of years since last plasma viral load  $>200$  copies/mL.

<sup>10</sup> The term 'cART' was used for a combination of  $\geq 3$  antiretroviral drugs, other than ritonavir used as a pharmacologic booster.

<sup>11</sup> The term 'prior AIDS' was used in case of a previous AIDS-defining condition according to the United States Centers for Disease Control and Prevention (CDC) classification.

<sup>12</sup> Central nervous system penetration effectiveness (CPE) score of the cART regimen of each HIV-infected participant was calculated using the algorithm as proposed by Letendre et al. in 2010.<sup>40</sup>

HIV-infected participants were known to be infected and treated with antiretroviral medication for a prolonged period of time, and 35% had previously been diagnosed with AIDS. The majority had experienced substantial immune recovery on treatment, with a median nadir CD4+ T-cell count of 170 cells/mm<sup>3</sup>, current median CD4+ T-cell count of 625 cells/mm<sup>3</sup>, and undetectable plasma viral load for a median of 8 years.

Cognitive impairment by Frascati criteria and by multivariate normative comparison

Applying Frascati criteria, CI was present in 49 of 103 (48%) HIV-infected, but also in 27 of 74 (36%) HIV-uninfected men ( $P=0.14$ ).

Using MNC, CI was detected in 17 (17%) HIV-infected men. To verify the specificity of the MNC criterion, which was assumed to be at least 95%, we compared the scores of each individual HIV-uninfected control with the scores of the remaining control group ( $n=73$ ), using MNC. Four (5%,  $P=0.02$ ) uninfected controls showed test results significantly below the remainder of the group, supporting the assumption of 95% specificity.

Results of the MMSE, HDS, MoCA, and Simioni questionnaire (**Table 8.2**)

All 177 substudy participants had an available MMSE, HDS, and MoCA score. Simioni questionnaires were missing from two HIV-uninfected participants.

None of the cognitive screening instruments showed statistically significant differences between the HIV-infected and HIV-uninfected studygroups, neither in terms of abnormal scoring nor regarding median scores.

Sensitivity and specificity of the MMSE (**Table 8.3**)

Using CI by Frascati criteria, or CI by MNC as the gold standard, an MMSE score  $\leq 24/30$  showed a very low sensitivity of 2% and 6%, respectively. Specificity was 100% irrespective of the gold standard used. The single

**Table 8.2: Results of the Mini Mental State Examination, HIV Dementia Scale, Montreal Cognitive Assessment and Simioni questionnaire**

	HIV-uninfected (n=74)	HIV-infected (n=103)	P value
Mini Mental State Examination (MMSE) score	29 (28-30)	29 (28-30)	0.37 <sup>a</sup>
Mini Mental State Examination (MMSE) $\leq 24/30$	0% (n=0)	1% (n=1)	1.00 <sup>b</sup>
HIV Dementia Scale (HDS) score	14.3 (13.5-16.0)	15.0 (13.5-16.0)	0.30 <sup>a</sup>
HIV Dementia Scale (HDS) $\leq 10/16$	8% (n=6)	4% (n=4)	0.32 <sup>b</sup>
HIV Dementia Scale (HDS) $\leq 14/16$	50% (n=37)	38% (n=39)	0.11 <sup>c</sup>
Montreal Cognitive Assessment (MoCA) score	29 (27-29)	28 (27-29)	0.10 <sup>a</sup>
Montreal Cognitive Assessment (MoCA) $\leq 25/30$	11% (n=8)	13% (n=13)	0.71 <sup>c</sup>
Simioni questionnaire abnormal	31% (n=22)	30% (n=31)	0.95 <sup>c</sup>

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

Test type used: <sup>a</sup> Wilcoxon rank-sum test, <sup>b</sup> Fisher's exact test, <sup>c</sup>  $\chi^2$  test.

**Table 8.3: Sensitivity and specificity of the Mini Mental State Examination, HIV Dementia Scale, Montreal Cognitive Assessment and Simioni questionnaire**

	Gold standard= CI as diagnosed by Frascati criteria		Gold standard= CI as diagnosed by MNC	
	Sensitivity	Specificity	Sensitivity	Specificity
Mini Mental State Examination (MMSE) $\leq 24/30$	2%	100%	6%	100%
HIV Dementia Scale (HDS) $\leq 10/16$	6%	98%	6%	97%
HIV Dementia Scale (HDS) $\leq 14/16$	45%	69%	71%	69%
Montreal Cognitive Assessment (MoCA) $\leq 25/30$	20%	94%	24%	90%
Simioni questionnaire abnormal	35%	74%	41%	72%
HIV Dementia Scale (HDS) $\leq 14/16$ among participants with cognitive complaints <sup>1</sup>	41%	79%	71%	79%
HIV Dementia Scale (HDS) $\leq 14/16$ among participants without cognitive complaints <sup>1</sup>	47%	65%	70%	65%

Abbreviations: CI=cognitive impairment, MNC=multivariate normative comparison.

Analyses were restricted to the HIV-infected studygroup.

<sup>1</sup> Cognitive complaints were defined by an abnormal Simioni questionnaire.

HIV-infected participant with an MMSE score  $\leq 24/30$  was diagnosed as being cognitively impaired by Frascati criteria as well as by MNC.

**Sensitivity and specificity of the HDS (Table 8.3)**

An HDS score  $\leq 10/16$  showed a low sensitivity of 6%, using either CI by Frascati criteria, or CI by MNC as the gold standard. Specificity was 97-98%. Raising the cut-off to  $\leq 14/16$  showed a sensitivity of 45% using CI by Frascati criteria as the gold standard, and a higher sensitivity of 71% using CI by MNC as the gold standard. Irrespective of the gold standard used, specificity was low (69%).

**Sensitivity and specificity of the MoCA (Table 8.3)**

A MoCA score  $\leq 25/30$  showed a low sensitivity of around 20% and a reasonable specificity of around 90%, by both gold standards.

**Sensitivity and specificity of the Simioni questionnaire (Table 8.3)**

An abnormal Simioni questionnaire showed a low sensitivity of around 40% and low specificity of around 70%, by both gold standards.

**Sensitivity and specificity of an HDS cut-off  $\leq 14/16$  among those with cognitive complaints (as defined by an abnormal Simioni questionnaire) (Table 8.3)**

Applying an HDS cut-off  $\leq 14/16$  to HIV-infected participants with cognitive complaints, specificity was increased by 10% to 79% (using both gold standards); sensitivity remained virtually unchanged when compared to the HDS using a cut-off of  $\leq 14/16$  irrespective of cognitive complaints.

**Performance of all the above screening cognitive tools when only including diagnoses of MND, but not ANI, as the gold standard for cognitive impairment according to Frascati criteria**

When using this approach, in which participants with ANI were considered to be cognitively unimpaired, performance of all the above screening cognitive instruments remained virtually unchanged (data not shown).

**Table 8.4: Optimal cut-off points (including corresponding sensitivity and specificity) of the Mini Mental State Examination, HIV Dementia Scale, and Montreal Cognitive Assessment**

	Gold standard= CI as diagnosed by Frascati criteria			Gold standard= CI as diagnosed by MNC		
	Optimal cut-off	Sens	Spec	Optimal cut-off	Sens	Spec
Mini Mental State Examination (MMSE)	≤29	67%	54%	≤28	59%	76%
Montreal Cognitive Assessment (MoCA)	≤27	57%	74%	≤25	24%	90%
HIV Dementia Scale (HDS)	≤13.5	37%	81%	≤14	71%	69%
HIV Dementia Scale (HDS) among participants with cognitive complaints <sup>1</sup>	≤13	35%	93%	≤13.5	71%	83%

Abbreviations: CI=cognitive impairment, MNC=multivariate normative comparison, sens=sensitivity, spec=specificity.

Analyses were restricted to the HIV-infected studygroup. Optimal cut-off points were calculated using Youden index.

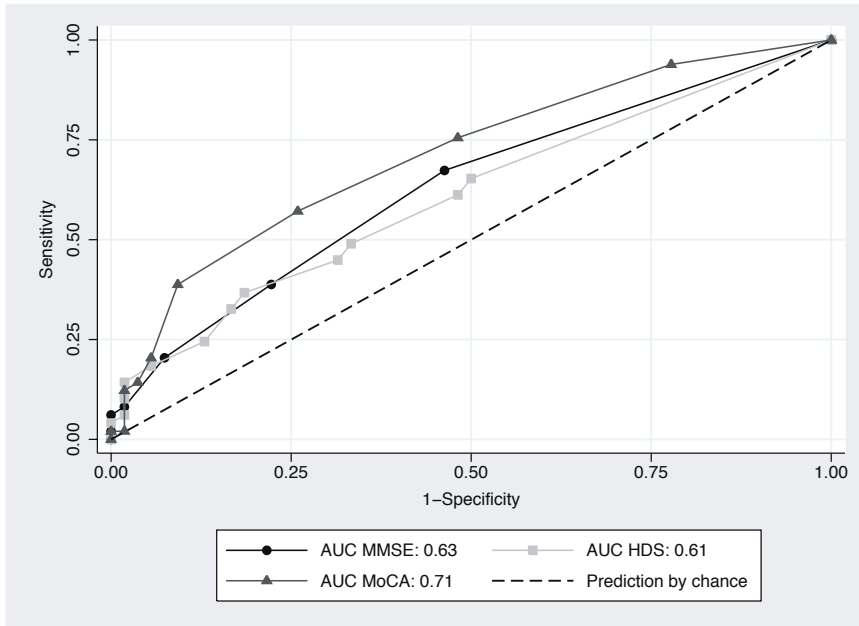
<sup>1</sup> Cognitive complaints were defined by an abnormal Simioni questionnaire.

ROC analyses of the MMSE, HDS, and MoCA

**Figure 8.1** depicts the ROC curve for MMSE, HDS, and MoCA using CI as diagnosed by Frascati criteria as the gold standard. The area under the curve (AUC) for the MMSE, HDS, and MoCA was 0.63 (95% confidence interval (CI) 0.53-0.73), 0.61 (95% CI 0.50-0.71), and 0.71 (95% CI 0.61-0.81), respectively.

**Figure 8.2** depicts the ROC curve for MMSE, HDS, and MoCA when using CI as diagnosed by MNC as the gold standard. The AUC for the MMSE, HDS, and MoCA was 0.70 (95% CI 0.55-0.84), 0.67 (95% CI 0.52-0.83), and 0.58 (95% CI 0.44-0.73), respectively.

Optimal cut-off points (including corresponding sensitivity and specificity) of each cognitive screening instrument were calculated by Youden index, and are listed in **Table 8.4**. Even when using the optimal cut-off scores, no large improvements in sensitivity or specificity were seen. Applying the HDS with a cut-off of ≤13.5 (using CI by MNC as the gold standard) among participants with cognitive complaints as defined by an abnormal Simioni questionnaire showed the best, albeit still moderate performance, with a sensitivity of 71% and a specificity of 83%.

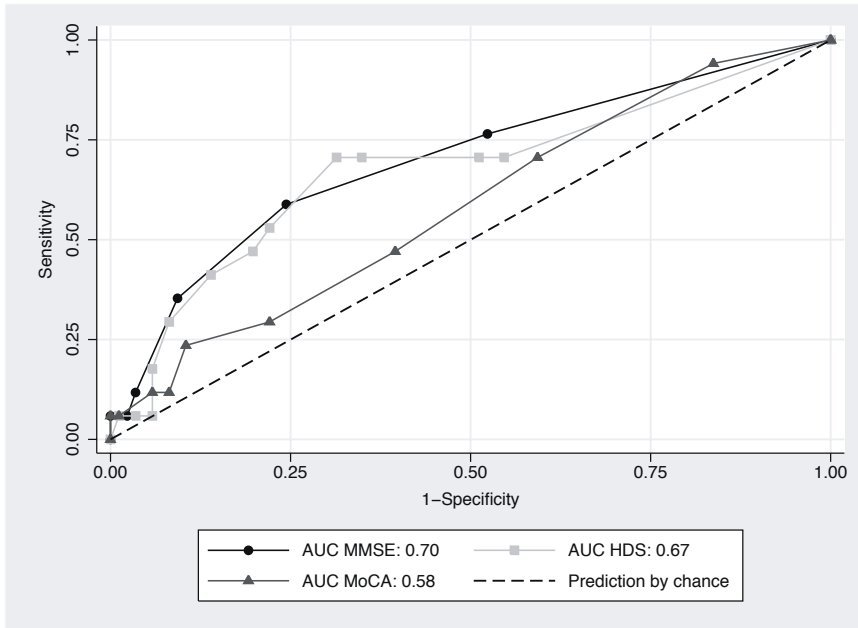


**Figure 8.1: ROC curves of the Mini Mental State Examination, HIV Dementia Scale, and Montreal Cognitive Assessment using cognitive impairment as diagnosed by Frascati criteria as the gold standard**

Abbreviations: ROC=receiver operator characteristics, AUC=area under the curve, MMSE=mini mental state examination, HDS=HIV dementia scale, MoCA=Montreal Cognitive Assessment.

Analyses were restricted to the HIV-infected studygroup.





**Figure 8.2: ROC curves of the Mini Mental State Examination, HIV Dementia Scale, and Montreal Cognitive Assessment using cognitive impairment as diagnosed by multivariate normative comparison as the gold standard**

Abbreviations: ROC=receiver operator characteristics, AUC=area under the curve, MMSE=mini mental state examination, HDS=HIV dementia scale, MoCA=Montreal Cognitive Assessment.

Analyses were restricted to the HIV-infected studygroup.

## Discussion

### Key results

All four cognitive screening tools showed comparable scores and abnormality rates among HIV-infected and HIV-uninfected study participants. When using either CI as diagnosed by Frascati criteria, or CI as diagnosed by MNC as the gold standard, having an abnormal Simioni questionnaire, or an abnormal MMSE, HDS, or MoCA score (using the classical cut-offs), each showed low sensitivity and moderate specificity.

The more recently proposed HDS cut-off of  $\leq 14/16$  showed the highest, but still moderate, sensitivity, especially when using CI by MNC as the gold standard (71%). Specificity for this cut-off was 69%. The specificity was increased further to 79% when applying this cut-off to HIV-infected participants with cognitive complaints as defined by an abnormal Simioni questionnaire. Sensitivity remained virtually unchanged.

When using CI by Frascati criteria as the gold standard, ROC analyses showed MoCA to perform slightly better compared to HDS and MMSE. When using CI as diagnosed by MNC, ROC analyses showed MMSE and HDS to perform slightly better than MoCA.

However, all cognitive screening instruments, irrespective of which of both gold standards was used, showed at best moderate accuracy for identifying CI.

Exploring different cut-offs for MMSE, HDS, and MoCA, in search of an optimal cut-off to detect HIV-associated CI, no large improvements in sensitivity or specificity were observed.

### Interpretation, limitations, and conclusion

Given that all four cognitive screening tools show comparable scores and abnormality rates among HIV-infected and uninfected study participants, concerns arise about the ability of these instruments to actually detect HIV-associated CI.

The MMSE is the most widely used cognitive screening tool for Alzheimer's disease (AD), using a cut-off of  $\leq 24$ .<sup>22,23</sup> Cortical dysfunction is a hallmark of AD, but in the context of HIV, subcortical dysfunction is more common than cortical impairment.<sup>24</sup> MMSE, not capturing ex-

ecutive function or motor skill, is therefore less sensitive to subcortical dysfunction.<sup>25</sup> Another limitation of the MMSE is the ceiling effect, especially among people with high premorbid intelligence or educational level.<sup>23</sup>

In the context of HIV, two studies have investigated the usefulness of the MMSE in detecting HIV-associated CI using full NPA as the gold standard.<sup>26,27</sup> These studies showed low sensitivity (24% and 46%) of the MMSE, which is in line with other publications (although these did not use full NPA as the gold standard).<sup>25,26,28,29</sup> In our study MMSE showed a very low sensitivity (2-6%), confirming these earlier reports, and we therefore consider the usefulness of the MMSE as a screening instrument for HIV-associated CI to be poor.

The HDS was developed specifically for the detection of HAD, using a cut-off of  $\leq 10$ .<sup>30</sup> The usefulness of the HDS in detecting especially the milder forms of HIV-associated CI is under debate, with earlier studies reporting sensitivities of 39%-55% and specificities of 75%-96%.<sup>12,25,26,29,31-34</sup> One study, additionally adjusting for age and education, managed to increase sensitivity to 71%.<sup>35</sup> In an attempt to increase sensitivity further, a cut-off of  $\leq 14$  was proposed by Simioni et al., showing a sensitivity of 83-88% and specificity of 63-67%.<sup>12</sup> Additionally, when they distinguished between participants with and without cognitive complaints, as determined by a short questionnaire they developed (the Simioni questionnaire), the positive predictive value of an HDS score of  $\leq 14$  among participants with cognitive complaints was 92% versus 82% among those without cognitive complaints.

In our study, the HDS cut-off of  $\leq 10$  showed a low sensitivity of 6% and a high specificity of 97-98%. HAD being an exclusion criterion, this possibly accounts for the lower sensitivity in our analyses compared to previous studies.

The higher HDS cut-off of  $\leq 14$  showed a sensitivity of 45% and specificity of 69% when using CI as diagnosed by Frascati criteria as the gold standard, which is lower than the sensitivity as published by Simioni et al. Prevalence of CI was also lower in our cohort compared to CI prevalence as reported by Simioni et al. This might be explained by absence of HAD cases in our cohort, a higher hepatitis C co-infection rate in the

Simioni cohort, as well as inclusion of participants with past cerebral toxoplasmosis in their cohort.

Using CI as diagnosed by MNC as the gold standard, an HDS cut-off of  $\leq 14$  showed a somewhat higher sensitivity (compared to CI by Frascati criteria as the gold standard) of 71% and a comparable specificity of 69%.

Analogous to the publication by Simioni et al. we also explored the performance of this higher HDS cut-off of  $\leq 14$  among participants with and without cognitive complaints as defined by an abnormal Simioni questionnaire. Among those with cognitive complaints, sensitivity remained virtually unchanged, whereas specificity increased by 10%, irrespective of which of both gold standards was used.

The Simioni questionnaire itself as a screening cognitive instrument in our analyses showed a low sensitivity (35-41%) and moderate specificity (72-74%) for detecting CI. The sensitivity of the Simioni questionnaire in our cohort was lower than the sensitivity as published by Simioni (57%). A recent study reported a sensitivity and specificity of the Simioni questionnaire of 78% and 32%, respectively.<sup>27</sup> The characteristics of this cohort however differed substantially from ours, with 12% HAD cases.

Altogether we consider the usefulness of the Simioni questionnaire to detect HIV-associated CI therefore to be poor.<sup>22</sup> Notably, and as described above, the Simioni questionnaire was developed solely as a scientific tool to distinguish those individuals with and without cognitive complaints, and as a complementary tool to the HDS, and not as a cognitive screening tool by itself. This is in line with the most recent European AIDS Clinical Society guidelines which suggest for clinicians to focus on patients reporting complaints of CI rather than rely too much on the Simioni questionnaire as a screening tool.<sup>13</sup>

The HDS using the higher cut-off of  $\leq 14$  in combination with the Simioni questionnaire to determine subjective cognitive complaints in our hands performed modestly better and seemed to be the most appropriate cognitive screening tool.

The MoCA has been designed as a screening instrument for mild cognitive impairment, using a cut-off of  $\leq 25$ .<sup>36</sup> A few studies have examined its usefulness for detecting HIV-associated CI, reporting a sensitivity of 53-63% and specificity of 71-73%.<sup>25,26,29,34,37,38</sup> A more recent publication by Milanini et al., investigating HIV-infected individuals aged above 60,

reported a higher sensitivity of 72%.<sup>39</sup> In our study, MoCA showed a low sensitivity of 20-24% and a specificity of 90-94%, the sensitivity being substantially lower in our analyses compared to those previously published. The studies by Ku and Overton however concern HIV-infected populations with higher hepatitis C co-infection rates of 3.7% and 8.0%, respectively, compared to 1% in our HIV-infected cohort. Furthermore, substance abuse and depressive symptoms, both factors known to be associated with CI, were not reported in both papers.<sup>37,38</sup> The study by Milanini et al. concerns an older HIV-infected population compared to our cohort, and information concerning substance abuse, presence of depressive symptoms, and hepatitis C co-infection is not included in the paper, each of which may influence rates of CI and performance of MoCA.<sup>39</sup> Joska et al. also investigated the usefulness of the MoCA in detecting HIV-associated CI but using a different cut-off of  $\leq 26$ ; they reported a sensitivity and specificity of 89% and 23%, respectively.<sup>27</sup> Their cohort substantially differs from ours with HAD being present in 12% of participants.

Our participants were all male and predominantly of Caucasian descent, and future studies will be needed to determine whether our findings apply equally to women and populations with other ethnic backgrounds.

In conclusion, all investigated cognitive screening instruments performed poorly for detecting HIV-associated CI. Cognitive deficits in the context of treated HIV infection are subtle, and none of the currently available cognitive screening instruments seems sufficiently adequate for use in clinical practice.

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## Authors' contributions

JS contributed to data collection, data analysis and interpretation, writing of all drafts of the manuscript, and was responsible for producing and submitting the final manuscript. RZ contributed to data collection, data analysis and interpretation, and writing of the manuscript.

GG contributed to data analysis and interpretation, and contributed to writing of all drafts of the manuscript.

FW contributed to the study design, data analysis and interpretation, and writing of the manuscript.

TS contributed to data collection, data analysis, and writing of the manuscript.

KK contributed to data collection, data analysis, and writing of the manuscript.

MC contributed to data analysis and interpretation, and writing of the manuscript.

CM conceived the nested cognitive substudy, contributed to its design, to data interpretation, and writing of the manuscript.

MP contributed to the study design, data interpretation, and writing of the manuscript.

AW contributed to data interpretation, and writing of the manuscript.

PR conceived the main cohort study and the nested cognitive substudy, contributed to both study designs, to data interpretation, and writing of all drafts of the manuscript.

PP contributed to the study design, data interpretation, and writing of the manuscript.

BS contributed to the study design, data analysis and interpretation, and contributed to writing of all drafts of the manuscript.

## References

1. Palella, F. J. *et al.* Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. *N. Engl. J. Med.* **338**, 853–60 (1998).
2. Wada, N. *et al.* Cause-Specific Life Expectancies After 35 Years of Age for Human Immunodeficiency Syndrome-Infected and Human Immunodeficiency Syndrome-Negative Individuals Followed Simultaneously in Long-term Cohort Studies, 1984–2008. *Am. J. Epidemiol.* **177**, 116–125 (2013).
3. Weber, R. *et al.* Decreasing mortality and changing patterns of causes of death in the Swiss HIV Cohort Study. *HIV Med.* **14**, 195–207 (2013).
4. Cysique, L. A. & Brew, B. J. Prevalence of non-confounded HIV-associated neurocognitive impairment in the context of plasma HIV RNA suppression. *J. Neurovirol.* **17**, 176–83 (2011).
5. Winston, A. *et al.* Neurocognitive Function in HIV Infected Patients on Antiretroviral Therapy. *PLoS ONE* **8**, e61949 (2013).
6. Schouten, J., Cinque, P., Gisslen, M., Reiss, P. & Portegies, P. HIV-1 infection and cognitive impairment in the cART era: a review. *AIDS Lond. Engl.* **25**, 561–75 (2011).
7. Robertson, K. R. *et al.* The prevalence and incidence of neurocognitive impairment in the HAART era. *AIDS Lond. Engl.* **21**, 1915–21 (2007).
8. Simioni, S. *et al.* Cognitive dysfunction in HIV patients despite long-standing suppression of viremia. *AIDS Lond. Engl.* **24**, 1243–50 (2010).
9. Heaton, R. K. *et al.* HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER Study. *Neurology* **75**, 2087–96 (2010).
10. Antinori, A. *et al.* Updated research nosology for HIV-associated neurocognitive disorders. *Neurology* **69**, 1789–99 (2007).
11. Su, T. *et al.* Multivariate normative comparison, a novel method for more reliably detecting cognitive impairment in HIV infection. *AIDS Lond. Engl.* **29**, 547–557 (2015).
12. Simioni, S. *et al.* Cognitive dysfunction in HIV patients despite long-standing suppression of viremia: *AIDS* **1** (2009).
13. European AIDS Clinical Society (EACS) treatment guidelines, version 8.0, June 2016. (2016).
14. Schouten, J. *et al.* Cross-sectional comparison of the prevalence of age-associated comorbidities and their risk factors between HIV-infected and uninfected individuals: the AGEHIV cohort study. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **59**, 1787–1797 (2014).
15. Beck, A. T., Steer, R. A., Ball, R. & Ranieri, W. Comparison of Beck Depression Inventories -IA and -II in psychiatric outpatients. *J. Pers. Assess.* **67**, 588–597 (1996).

16. Beck, A., Ward, C., Mendelson, M., Mochk, J. & Erbaugh, J. Assessment of depression: The depression inventory. *Arch. Gen. Psychiatry* **4**, 561–571 (1961).
17. Broadbent, D. E., Cooper, P. F., FitzGerald, P. & Parkes, K. R. The Cognitive Failures Questionnaire (CFQ) and its correlates. *Br. J. Clin. Psychol. Br. Psychol. Soc.* **21 (Pt 1)**, 1–16 (1982).
18. Lawton, M. & Brody, E. Assessment of older people: Self-maintaining and instrumental activities of daily living. *The Gerontologist* **9**, 179–186 (1982).
19. Schmand, B., Lindeboom, J., van Harskamp, F. *Dutch Adult Reading Test*. (Swets en Zeitlinger, 1992).
20. Antinori, A. *et al.* Updated research nosology for HIV-associated neurocognitive disorders. *Neurology* **69**, 1789–1799 (2007).
21. Huizenga, H. M., Smeding, H., Grasman, R. P. P. & Schmand, B. Multivariate normative comparisons. *Neuropsychologia* **45**, 2534–2542 (2007).
22. Folstein, M. F., Folstein, S. E. & McHugh, P. R. 'Mini-mental state'. A practical method for grading the cognitive state of patients for the clinician. *J. Psychiatr. Res.* **12**, 189–198 (1975).
23. Ismail, Z., Rajji, T. K. & Shulman, K. I. Brief cognitive screening instruments: an update. *Int. J. Geriatr. Psychiatry* **25**, 111–120 (2010).
24. Woods, S. P., Moore, D. J., Weber, E. & Grant, I. Cognitive neuropsychology of HIV-associated neurocognitive disorders. *Neuropsychol. Rev.* **19**, 152–168 (2009).
25. Valcour, V., Paul, R., Chiao, S., Wendelken, L. A. & Miller, B. Screening for Cognitive Impairment in Human Immunodeficiency Virus. *Clin. Infect. Dis.* **53**, 836–842 (2011).
26. Kamminga, J., Cysique, L. A., Lu, G., Batchelor, J. & Brew, B. J. Validity of Cognitive Screens for HIV-Associated Neurocognitive Disorder: A Systematic Review and an Informed Screen Selection Guide. *Curr. HIV/AIDS Rep.* **10**, 342–355 (2013).
27. Joska, J. A. *et al.* A Comparison of Five Brief Screening Tools for HIV-Associated Neurocognitive Disorders in the USA and South Africa. *AIDS Behav.* (2016).
28. Muniyandi, K., Arutselvi, T., Venkatesan, J. & Jayaseelan, V. Study to assess the prevalence, nature and extent of cognitive impairment in people living with AIDS. *Indian J. Psychiatry* **54**, 149 (2012).
29. Zipursky, A. R. *et al.* Evaluation of brief screening tools for neurocognitive impairment in HIV/AIDS: a systematic review of the literature. *AIDS* **27**, 2385–2401 (2013).
30. Power, C., Selnes, O. A., Grim, J. A. & McArthur, J. C. HIV Dementia Scale: a rapid screening test. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirology Off. Publ. Int. Retrovirology Assoc.* **8**, 273–278 (1995).
31. Bottiggi, K. A. *et al.* The HIV Dementia Scale: predictive power in mild dementia and HAART. *J. Neurol. Sci.* **260**, 11–15 (2007).
32. Richardson, M. A. *et al.* Utility of the HIV dementia scale in assessing risk for



- significant HIV-related cognitive-motor deficits in a high-risk urban adult sample. *AIDS Care* **17**, 1013–1021 (2005).
33. Smith, C. A., van Gorp, W. G., Ryan, E. R., Ferrando, S. J. & Rabkin, J. Screening subtle HIV-related cognitive dysfunction: the clinical utility of the HIV dementia scale. *J. Acquir. Immune Defic. Syndr.* **1999** **33**, 116–118 (2003).
  34. Janssen, M. A. M., Bosch, M., Koopmans, P. P. & Kessels, R. P. C. Validity of the Montreal Cognitive Assessment and the HIV Dementia Scale in the assessment of cognitive impairment in HIV-1 infected patients. *J. Neurovirol.* (2015).
  35. Morgan, E. E. *et al.* Predictive validity of demographically adjusted normative standards for the HIV Dementia Scale. *J. Clin. Exp. Neuropsychol.* **30**, 83–90 (2008).
  36. Nasreddine, Z. S. *et al.* The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *J. Am. Geriatr. Soc.* **53**, 695–699 (2005).
  37. Ku, N. *et al.* HIV-associated neurocognitive disorder in HIV-infected Koreans: the Korean NeuroAIDS Project: HAND in HIV-infected Koreans. *HIV Med.* n/a-n/a (2014).
  38. Overton, E. T. *et al.* The Alzheimer's disease-8 and Montreal Cognitive Assessment as screening tools for neurocognitive impairment in HIV-infected persons. *J. Neurovirol.* **19**, 109–116 (2013).
  39. Milanini, B. *et al.* The Montreal Cognitive Assessment (MoCA) to screen for cognitive impairment in HIV over age 60: *JAIDS J. Acquir. Immune Defic. Syndr.* **1** (2014).
  40. Letendre, S. L., Fitzsimons, C. A., Ellis, R. J. & *et al.* Correlates of CSF viral loads in 1221 volunteers of the CHARTER Cohort. 17th Conference on Retroviruses and Opportunistic Infections, February 16–19, San Francisco, CA Abstract 172.





# Summary and discussion

## Introduction

With the introduction of combination antiretroviral therapy (cART) in 1996, HIV infection changed from an inevitably fatal condition to a chronic manageable disease. Subsequently, as AIDS-associated complications diminished and it became possible for the HIV-infected population to survive into older age, non-AIDS/ageing-associated comorbidities emerged and gained importance in this population. Several reports were published suggesting an excess prevalence of non-AIDS/ageing-associated comorbidities among people living with HIV.<sup>1,2</sup> However, it remained unclear whether non-AIDS/ageing-associated comorbidities were truly occurring more frequently, and if so, whether these were occurring at a younger age among properly-treated HIV-infected individuals compared to uninfected individuals.

To obtain more insight into these issues, we initiated the AGE<sub>HIV</sub> Cohort Study in 2010 in Amsterdam, The Netherlands, investigating the prevalence, incidence and risk factors of non-AIDS/ageing-associated comorbidities and organ dysfunction among HIV-infected individuals and highly similar HIV-uninfected controls, all 45 years of age and above.

A nested neurological substudy within the main AGE<sub>h</sub>IV Cohort Study (from now on referred to as the neurological substudy), was initiated in 2011, investigating cognitive function and additional parameters related to the central nervous system among a subset of male HIV-infected (all with suppressed viremia) and uninfected participants of the main AGE<sub>h</sub>IV Cohort Study.

This thesis is based on cross-sectional analyses of baseline data gathered through the AGE<sub>h</sub>IV main study and neurological substudy. Constructed as a diptych, this thesis focuses on two prominent and concerning non-AIDS/ageing-associated comorbidities in the context of chronic treated HIV infection: vascular complications ('heart') and cognitive impairment ('head').

## Part I Heart: vascular complications

An introduction of Part I is provided in **chapter 2**, in terms of a published review summarizing our understanding of coronary heart disease in treated HIV infection.

In **chapter 3**, the prevalence of a number of non-AIDS/ageing-associated comorbidities investigated within the AGE<sub>h</sub>IV Cohort Study was compared between the HIV-infected ( $n=540$ ) and uninfected ( $n=524$ ) studygroups at the time of enrolment into the cohort. Investigated comorbidities included: hypertension, myocardial infarction, peripheral arterial disease, angina pectoris, diabetes mellitus type 2, obstructive pulmonary disease, impaired renal function, non-AIDS malignancies, and atraumatic fractures/osteoporosis.

HIV-infected participants had a significantly higher mean number of comorbidities than uninfected controls (1.3 (standard deviation (SD) 1.14) vs. 1.0 (SD 0.95),  $P<0.001$ ) and significantly more HIV-infected participants had  $\geq 1$  comorbidity (69.4% vs. 61.8%,  $P=0.009$ ). All comorbidities were numerically more prevalent among HIV-infected participants, and hypertension, myocardial infarction, peripheral arterial disease, and impaired renal function significantly so.

The risk of comorbidity was analysed by logistic regression and was

independently associated with higher age, smoking, positive family history for cardiovascular/metabolic disease, and higher waist-to-hip ratio, but also with HIV infection (odds ratio (OR) 1.58, 95% confidence interval (CI) 1.23-2.03,  $P < 0.001$ ). In those with HIV, longer time spent with severe immunodeficiency (longer exposure to CD4+ T-cell counts  $< 200$  cells/mm<sup>3</sup>) increased the risk of a higher composite comorbidity burden. There was also a contribution (though less pronounced) from residual inflammation and immune activation (as measured by high-sensitivity C-reactive protein (hsCRP) and soluble CD14 (sCD14) plasma concentrations), and prior high-dose ritonavir use ( $\geq 400$  mg/24 hours).

Since vascular complications are the most prevalent category of non-AIDS/ageing-associated comorbidities among our HIV-infected participants, in **chapter 4** we investigated aortic stiffness as assessed by pulse wave velocity (PWV) at baseline within the AGE<sub>HIV</sub> Cohort Study.

Aortic stiffening is a degenerative process, that typically occurs with ageing and is accelerated by hypertension, metabolic changes and inflammation. Aortic stiffness, assessed by measuring aortic PWV, is independently associated with cardiovascular events and mortality in the general population, and may be seen as a preclinical vascular abnormality, with higher PWV indicating more aortic stiffness.

Among our study participants, PWV was higher in the HIV-infected ( $n=566$ ) studygroup compared to the uninfected ( $n=507$ ) control group (7.9 m/s vs. 7.7 m/s,  $P=0.004$ ). Using multivariable linear regression, we investigated whether HIV infection was independently associated with higher PWV. After adjusting for known confounders (mean arterial pressure (MAP), age and gender), we found that the association between HIV status and aortic PWV remained statistically significant (+0.20 m/s, 95% CI 0.02-0.38 m/s,  $P=0.03$ ). However, the regression coefficient of HIV-infected status was attenuated by packyears of smoking (to +0.12 m/s, 95% CI -0.06-0.29,  $P=0.18$ ) and further attenuated by use of antihypertensive drugs (to +0.09 m/s, 95% CI -0.09-0.26,  $P=0.33$ ).

Additional factors that were associated with higher PWV were: both a body mass index (BMI)  $\geq 25$  kg/m<sup>2</sup> and a BMI  $< 18.5$  kg/m<sup>2</sup>, lower HDL cholesterol levels, higher triglycerides, and higher hsCRP levels.

Finally, in HIV-infected participants, having a nadir CD4+ T-cell count  $\leq 100$  cells/mm<sup>3</sup> was independently associated with a higher PWV (+0.33

m/s, 95% CI 0.06-0.61,  $P=0.02$ ). This association was slightly attenuated by level of triglycerides (to +0.31 m/s, 95% CI 0.03-0.59,  $P=0.03$ ) and by hsCRP (to +0.28 m/s, 95% CI 0.00-0.56,  $P=0.05$ ).

#### AGE<sub>HIV</sub> Cohort characteristics

We succeeded in compiling two studygroups that were highly similar in terms of socio-demographic characteristics and lifestyle/behaviour-related factors. In both studygroups, median age was 52, and the majority were male and men who have sex with men.

On average, HIV-infected participants were known to have been infected for a prolonged period of time, and 30% had prior AIDS. Virtually all had been on cART for many years, and currently had undetectable HIV plasma viral loads. The majority had experienced immune recovery on treatment, with a median nadir CD<sub>4</sub>+ T-cell count of 180 cells/mm<sup>3</sup> and current median CD<sub>4</sub>+ T-cell count of 565 cells/mm<sup>3</sup>.

Our HIV-infected cohort may be seen as an 'intermediate' cohort; 20% of the cohort has been treated with mono/dual ART regimens before initiating cART, while the other 80% was ART-naïve when starting cART. Furthermore, the level of immune deficiency experienced by our cohort participants varies greatly, with 56%, 32%, 8%, and 3% having had nadir CD<sub>4</sub>+ T-cell counts <200, 200-350, 350-500, and ≥500 cells/mm<sup>3</sup>, respectively.

#### Non-AIDS/ageing-associated comorbidity

Our observation that many non-AIDS/ageing-associated comorbidities, especially vascular complications, are more prevalent among HIV-infected individuals is in line with earlier reports<sup>1-8</sup> and has also been confirmed by more recent publications.<sup>9-11</sup> The most robust clinical evidence has been provided by Freiberg et al., who reported a 50% increased risk of incident acute myocardial infarction among HIV-infected individuals beyond that explained by recognized vascular risk factors, even among those with well-controlled infection.<sup>8</sup>

### Risk factors for (vascular) complications

In the AGE<sub>HIV</sub> Cohort, we identified several important traditional recognized risk factors for (vascular) comorbidity/complications, including smoking, hypertension, dyslipidemia, and renal dysfunction. All were significantly more prevalent among the HIV-infected participants.

#### *Smoking*

We found a strong association between smoking and comorbidity risk in our cohort. Furthermore, the observed difference in aortic stiffness between HIV-infected and uninfected participants was largely explained by the higher prevalence of smoking among HIV-infected participants (32% vs. 25% of uninfected controls, and vs. 23% of the general population in The Netherlands).<sup>12</sup> This is a phenomenon seen in other HIV-infected cohorts as well, with smoking prevalence rates of up to 60%.<sup>10,13–16</sup> Aside from the high smoking prevalence among HIV-infected populations, and the well-known association of smoking with many comorbidities, there are also suggestions that HIV infection and smoking may exert a synergistic negative effect on non-AIDS comorbidity and mortality.<sup>14,17</sup> The latter has not been confirmed in our analyses.

#### *Hypertension*

Hypertension, a well-known risk factor of clinical vascular events, was also highly prevalent among our HIV-infected participants (45%, compared to 31% of uninfected controls, and compared to 31% of the general population in The Netherlands).<sup>12</sup> Aside from smoking, hypertension seemed the most important driver for the increased aortic stiffness among HIV-infected participants. Previously reported hypertension prevalence varies from 13% to 49% in HIV-infected populations, although the results from studies comparing the prevalence of hypertension among HIV-infected individuals are conflicting.<sup>18–22</sup> Potential risk factors for an increased risk of hypertension include common risk factors such as age, gender, and BMI<sup>19,21–24</sup>, but also immune activation and inflammation<sup>25</sup>, immune deficiency<sup>26</sup>, as well as ART-related factors.<sup>22,26–29</sup>

In analyses of determinants of hypertension within the AGE<sub>HIV</sub> Cohort, not reported as part of this thesis, our group has shown that HIV

infection was significantly associated with hypertension risk after adjustment for recognized risk factors (age, sex, ethnicity, family history of hypertension, smoking, alcohol use, physical activity, and BMI) with an OR of 1.65 (95% CI 1.25-2.19), but was attenuated after additional adjustment for waist-to-hip ratio. Among HIV-infected individuals, particularly among those with mono/dual nucleoside reverse transcriptase inhibitor therapy prior to cART, stavudine exposure was independently associated with hypertension (OR 1.54, 95% CI 1.04-2.30). This association was attenuated after additional adjustment for either waist-to-hip ratio or hip circumference. These findings suggested that changes in body composition, involving both abdominal obesity and stavudine-induced peripheral lipoatrophy, contribute to the higher prevalence of hypertension in HIV-infected individuals.<sup>30</sup>

### *Dyslipidemia*

With 14% of our HIV-infected participants using lipid-lowering therapy compared to 7% of uninfected controls, in combination with lower HDL cholesterol levels and higher triglyceride levels among HIV-positive individuals, dyslipidemia was highly prevalent in the HIV-positive group. This is in line with findings in other cohorts.<sup>7,31</sup> Although not associated with comorbidity burden in our cohort, lower HDL cholesterol levels, as well as higher triglycerides, were associated with aortic stiffness. Both HIV infection itself and cART may induce unfavourable changes in lipid profiles, including reduced HDL cholesterol and higher triglyceride levels.<sup>32-34</sup> The extent to which the dyslipidemia in our HIV-infected participants originated from HIV-related lipid changes during their pre-treatment period, or whether it is the result of the use of specific antiretroviral agents, is as yet unclear.

### *Renal dysfunction*

Renal dysfunction may be both a risk factor for, or the result of, clinical vascular disease. In our cohort, renal dysfunction was significantly more prevalent among HIV-infected participants: 4.3% compared to 2.1% of uninfected controls. Presence of renal disease did not seem to affect results when we analysed determinants of increased aortic stiffness.

While HIV itself is a well-known contributor to nephropathy, renal



dysfunction is also very prevalent in cART-treated HIV-infected cohorts, which is in line with our findings.<sup>35-38</sup> The exact mechanisms towards chronic kidney disease are yet to be elucidated, but potential factors include past damage by HIV itself, renal damage related to metabolic and vascular disease, chronic inflammation, and potential nephrotoxicity of (antiretroviral) medication.<sup>39</sup>

Aside from these traditional recognized risk factors, we also found determinants of non-AIDS/ageing-associated comorbidity/complications that are related to being HIV-infected and/or treated with ART.

#### *Changes in body composition*

An above-normal waist-to-hip ratio was highly prevalent among our HIV-infected participants: 84%, compared to 63% of uninfected controls. Both increased waist circumference (indicating abdominal fat accumulation) and decreased hip circumference (indicating peripheral lipoatrophy) contributed to this difference.

We found a significant association between abnormal waist-to-hip ratio and comorbidity in our cohort, which is in line with the existing literature.<sup>15,40</sup> As mentioned previously, we also found an abnormal waist-to-hip ratio to be a major driver of the difference in hypertension prevalence between the two studygroups. When we analysed the separate components of waist-to-hip ratio in relation to hypertension risk, we found abdominal obesity to be of importance in all HIV-infected individuals, and lipoatrophy of particular importance in those with prior exposure to the thymidine analogue stavudine.<sup>30</sup>

Even though waist-to-hip ratio was not associated with increased aortic stiffness in our cohort, abnormality in BMI (both above and below normal) was.

#### *Specific ART*

Analysis of associations between comorbidity risk and specific ART revealed a significant association with prior high-dose ( $\geq 400$  mg/24 hours) use of ritonavir (a protease inhibitor, PI) in our cohort. Other studies have also reported an association between PI's and vascular comorbidity.<sup>41</sup> Plausible mechanisms by which ritonavir may contribute to comorbidity

risk include its known dose-dependent effect on lipids<sup>41</sup>, induction of endothelial dysfunction<sup>42</sup>, and cellular accumulation of prelamin A, which may result in premature cellular senescence similar to that observed in some genetically determined premature ageing syndromes.<sup>43,44</sup>

In terms of hypertension determinants in our cohort, our group has previously reported that the effect of HIV infection is mediated by increased waist-to-hip ratio, which in turn seems partly driven by stavudine-related lipoatrophy. Lipoatrophy is highly associated with use of nucleoside reverse transcriptase inhibitors (NRTI's), and particularly thymidine analogues such as stavudine.<sup>40</sup> An important mechanism underlying this lipoatrophy is thought to be mitochondrial toxicity.<sup>40</sup> However, in contemporary cART combinations both high-dose ritonavir and stavudine are no longer used, and therefore, in our cohort, exposure to high-dose ritonavir and stavudine had occurred many years prior to when participants underwent baseline assessment for the study.

### *Inflammation*

We found most investigated inflammatory markers to be higher among the HIV-infected participants compared to uninfected controls. Moreover, in the HIV-infected study group, some of these markers were associated with both clinical comorbidity burden (hsCRP and the monocyte activation marker sCD14) and increased aortic stiffness (hsCRP).

HIV infection results in both immune activation and immune deficiency.<sup>45-47</sup> Treatment with cART lowers the levels of many immune activation and inflammatory markers, albeit not down to levels seen in individuals without HIV infection.<sup>47,48</sup> Potential explanations for persistent immune activation and inflammation include persistent HIV replication, continued microbial translocation, and co-infections (for example with cytomegalovirus, hepatitis C virus, or both).<sup>47,49,50</sup>

In the general population, vascular disease, as well as many vascular risk factors, is also strongly associated with a pro-inflammatory state, and inflammation is considered highly pro-atherogenic.<sup>51,52</sup>

In HIV infection, immune activation and inflammation caused by, or related to, the infection could synergize with metabolic and lifestyle-related vascular risk factors, thereby potentially enhancing vascular damage.

### *Immune deficiency*

In our cohort, prior immune deficiency (reflected by low nadir CD<sub>4</sub><sup>+</sup> T-cell count) was identified as a risk factor for comorbid disease as well as increased aortic stiffness. The association between nadir CD<sub>4</sub><sup>+</sup> T-cell count and aortic stiffness seemed partially mediated by hsCRP. This finding confirms those of other studies, linking prior immune deficiency to diverse complications, and suggests that past immune deficiency has a long-lasting effect and is associated with inflammation.<sup>47,53</sup>

### Arterial stiffness as a risk factor of clinical vascular events in the AGE<sub>h</sub>IV Cohort

Among HIV-infected participants of the AGE<sub>h</sub>IV Cohort, PWV was significantly higher compared with uninfected controls (7.9 m/s vs. 7.7 m/s). Published reference values for healthy adults without cardiovascular risk factors report PWV's of 8.3, 10.3, and 10.9 m/s in the age categories 50-59, 60-69, and ≥70 years, respectively.<sup>54</sup> A PWV of >12 m/s is considered abnormal and a symptom of subclinical organ damage.<sup>55</sup> Thus, compared with these numbers, the PWV values of our study participants fall within the normal range, and the increase in PWV of 0.20 m/s among HIV-positives seems marginal. Furthermore, as we could not demonstrate a significant association between PWV and clinical vascular disease, this suggests a relatively small role for aortic stiffening in the observed increased CVD risk in well-treated HIV infection. To appropriately nuance this assumption, however, some methodological issues should be mentioned. First, we analysed PWV measured at baseline and clinical events that occurred in the past, with, in many cases, a long interval between the PWV measurement and the prior occurrence of the clinical vascular event. The relationship between PWV and clinical vascular disease may therefore have been distorted by changes in lifestyle or medication following the event. Furthermore, the absolute number of past clinical events was small, limiting the statistical power in our analysis. Longitudinal analyses will therefore be of interest to compare the PWV trajectories of both studygroups over the years, and to investigate any deviation between the two trajectories and any association with incident clinical vascular events.

### Ageing

The hypothesis of HIV-infected individuals ageing in an accelerated and/or accentuated manner has been proposed in previous papers<sup>4,56,57</sup>, but without a proper definition or gold standard of biological ageing, this remains very difficult to confirm or reject. In our analyses we did find a borderline significant interaction between age and HIV infection towards comorbidity burden, suggesting a somewhat stronger age effect among HIV-infected individuals, but our cross-sectional analyses did not allow for any firm conclusions. Therefore, whether or not people infected with HIV truly age differently remains to be elucidated. Continuation of our cohort, enabling longitudinal analyses, and measurement of potential biomarkers of biological age will hopefully provide further insight into this question.

## Part II Head: cognitive impairment

**Chapter 5** provides an introduction to Part II in the form of a published review of HIV-associated cognitive impairment (CI) in treated HIV infection.

In **chapter 6**, we investigated the prevalence of HIV-associated CI among 103 HIV-infected men with suppressed viremia on cART and 74 uninfected controls, all participants of both the main AGE<sub>HIV</sub> study as well as the AGE<sub>HIV</sub> neurological substudy. Firstly, CI was diagnosed using the existing Frascati criteria<sup>58</sup>, revealing that 48% of HIV-infected but also 36% of uninfected participants were cognitively impaired, indicating a high false-positive rate and low specificity. CI was subsequently diagnosed using the Gisslén criteria (a modified version of the Frascati criteria that is intended to reduce false-positivity)<sup>59</sup>, showing 5% of HIV-infected and 1% of uninfected participants to be cognitively impaired. These results indicated better specificity, but reduced sensitivity. As an alternative method to measure CI, we next investigated multivariate normative comparison (MNC), a novel method for more reliably detecting CI.<sup>60–63</sup> MNC is a statistical technique specifically designed to control the false-positive rate, while retaining sensitivity. MNC is able to control

the family-wise error (the probability of falsely diagnosing individuals as cognitively abnormal) by performing a single multivariate comparison of the complete cognitive profile of a particular patient with the distribution of all the cognitive profiles of the control sample, rather than comparing each test result separately to the reference population. In other words, the complete cognitive profile of each HIV-infected participant can be compared with the cognitive profile of the HIV-uninfected control group as a whole. The false-positive rate is limited by the level of significance (alpha) chosen in the MNC algorithm. In the present study, alpha was set at 5% one-tailed. Consequently, MNC has a specificity of at least 95%.<sup>60,61</sup>

When applying MNC we found 17% of HIV-infected men to be cognitively impaired. Investigating the specificity of MNC, we compared the cognitive profile of each HIV-uninfected participant with the cognitive profile of the rest of the control group. Four (5%) uninfected controls showed negative deviation, confirming the assumed 95% specificity.

Finally, we analysed which cognitive domains were affected in those individuals with decreased cognitive functioning, and found that CI was characterized by multiple subtle deficits across a broad range of cognitive domains.

In **chapter 7**, determinants of decreased cognitive performance and CI as diagnosed by MNC were investigated in the AGE<sub>hIV</sub> neurological substudy. Determinants for decreased cognitive performance by MNC as a continuous variable included cannabis use, history of prior cardiovascular disease, impaired renal function, diabetes mellitus type 2, an above-normal waist-to-hip ratio, depressive symptoms, and a prior lower nadir CD4+ T-cell count. Determinants for CI, as dichotomized by MNC, included cannabis use, prior cardiovascular disease, impaired renal function, and diabetes mellitus type 2.

In **chapter 8**, we assessed the performance of four cognitive screening instruments (Mini Mental State Examination (MMSE)<sup>64</sup>, HIV Dementia Scale (HDS)<sup>65</sup>, Montreal Cognitive Assessment (MoCA)<sup>66</sup>, and the questionnaire as proposed by Simioni et al. (Simioni questionnaire)<sup>67</sup>) for detecting HIV-associated CI. Scores of each cognitive screening instrument were compared between the two studygroups of the AGE<sub>hIV</sub> neurological substudy. In the HIV-infected group, sensitivity and specificity,

area under the curve by receiver operator characteristics (ROC) analyses, and optimal cut-off point of each screening instrument were assessed, using CI by Frascati criteria or by MNC as the gold standard.

All cognitive screening tools showed comparable scores and abnormality rates among HIV-infected and HIV-uninfected participants. Each cognitive screening instrument showed low sensitivity and moderate specificity. Based on ROC analyses and irrespective of the gold standard used, accuracy of MMSE, HDS, and MoCA in identifying CI was, at best, moderate.

#### Diagnosis, prevalence, and nature of cognitive impairment

The optimal method for diagnosing HIV-associated CI has been subject to much debate in recent years. The Frascati criteria, developed by an expert panel in 2007, are probably oversensitive, resulting in an overestimation of the rate of CI.<sup>59,68</sup> This is reflected in the reported wide range of CI prevalence (15-69%), mostly based on diagnosis by the Frascati criteria, and therefore questions the utility of these criteria.<sup>59,68</sup> In our cohort, we confirmed the high false-positive rate and low specificity of the Frascati criteria. MNC seems a more elegant and accurate research method for detecting CI, as demonstrated in chapter 6. Using MNC, we found a CI prevalence among HIV-infected substudy participants of 17%. Although this number cannot be compared with a gold standard, based on clinical experience, this seems a more reasonable estimation of cognitively impaired individuals. Among those with CI, subtle deficits were found across a broad range of cognitive domains, which is in line with earlier reports.<sup>69,70</sup>

#### Determinants of cognitive dysfunction

We found a variety of risk factors and classes of risk factors to be associated with decreased cognitive performance, including substance use (cannabis), vascular/metabolic risk factors (prior cardiovascular disease, impaired renal function, diabetes mellitus type 2, and above-normal waist-to-hip ratio), psychiatric comorbidity (depressive symptoms), and immune deficiency (nadir CD4+ T-cell count). This is in line with the broad

range of risk factors that have been identified by other study groups<sup>71</sup>, and indicates that decreased cognitive performance probably results from a multifactorial process.

#### Screening instruments for HIV-associated cognitive dysfunction

Given that, even in cognitively impaired individuals, abnormalities are subtle, it is not surprising that none of the investigated screening cognitive instruments was shown to be useful in clinical practice. This is in line with recently published results from other research groups.<sup>72</sup>

#### Progression of cognitive impairment

Cognitive dysfunction thus far observed in our cohort seems to be subtle, although the rate of progression of cognitive dysfunction over time still remains to be analysed. Many patients and physicians have been worrying whether subtle cognitive deficits will progress with time, resulting in HIV-associated dementia, as was the case in the pre-cART era. To date, the most robust longitudinal data on this subject have been published by Heaton et al. from the CHARTER cohort, who found no clinically relevant progression of cognitive dysfunction over a 3-year observation period.<sup>73</sup> In combination with the subtleness of the abnormalities they described, these findings are reassuring and make progression to HIV-associated dementia unlikely. Of note though, the relatively short follow-up period of three years should caution us to draw too firm conclusions. Given the high prevalence of vascular/metabolic disease and risk factors among our increasingly ageing HIV-infected population, the concern does arise that vascular and HIV-associated damage to the brain in the longer term will increasingly interact with one another, and impact cognitive function.

## The big picture: modification of comorbidity risk profile

In the context of chronic treated HIV infection, there is a complex interplay and entanglement between/of many comorbidity risk factors. The

two comorbidities investigated in this thesis, vascular disease and cognitive impairment, share many risk factors and therefore these comorbidities are likely to be linked. Two categories of relevant risk factors consistently surface, both in the AGE<sub>r</sub>IV Cohort Study and in the published scientific literature, namely immune deficiency and metabolic/vascular factors. Avoiding, modifying, or treating these factors is therefore of great importance to minimize the risk of developing comorbidity in the long term.

### Immune deficiency

After becoming infected with HIV, the disease may be asymptomatic for a long period of time. Even though the immune system is progressively affected during this latent period, clinical symptoms (AIDS) only arise once CD<sub>4</sub><sup>+</sup> T-cell counts reach critically low levels. Since clinical symptoms are largely absent in the first latent phase of the infection, too many patients are diagnosed with HIV at a relatively late stage of the infection, when their CD<sub>4</sub> counts have already decreased to levels far below normal. For instance, of the newly diagnosed patients in The Netherlands in 2015, 52% were late-presenters ((i.e., individuals either presenting for care with a CD<sub>4</sub><sup>+</sup> T-cell count below 350 cells/mm<sup>3</sup> or presenting with an AIDS-defining event).<sup>74</sup> This is in line with observations across Europe and other parts of the world.<sup>75,76</sup>

Avoiding CD<sub>4</sub><sup>+</sup> T-cell depletion and subsequent immune deficiency is very important, in the context of not only AIDS-related complications, but also non-AIDS comorbidities, as several studies have reported associations between risk of (cardiovascular) comorbidity and level of prior immune deficiency.<sup>4,27,77,78</sup>

The START study convincingly demonstrated that initiating cART treatment early (with a CD<sub>4</sub><sup>+</sup> T-cell count >500 cells/mm<sup>3</sup>) is superior to deferral of cART therapy until the CD<sub>4</sub><sup>+</sup> T-cell count has declined to 350 cell/mm<sup>3</sup>.<sup>79</sup> This beneficial effect was evident in terms of reducing the number of incident AIDS-related and non-AIDS-related (especially non-AIDS malignancies) events. Of note, no significant difference was seen in the incidence of vascular events, although a number of important issues should be considered when interpreting these results. In particu-



lar, the study population of the START study was young, they had a low cardiovascular risk as determined by the Framingham risk assessment tool, the 'deferred therapy' group had relatively high CD<sub>4</sub><sup>+</sup> T-cell counts, and the follow-up period of three years was relatively short in the context of comorbidity incidence.<sup>79</sup> Altogether however, the beneficial effect of early diagnosis and treatment is apparent.

#### Metabolic/vascular factors

Improvement of the metabolic/vascular profile is essential in decreasing the odds for comorbidity among HIV-infected individuals. Many metabolic and vascular risk factors are highly prevalent in HIV-infected populations. In particular, smoking, hypertension, and dyslipidemia appear to be suitable key targets for modification.

The first and most obvious intervention by which to decrease the risk of a broad range of comorbidities (e.g., vascular disease, malignancy, pulmonary obstructive disease) is smoking cessation, especially since smoking might actually cause more harm in individuals living with HIV than in those without HIV.<sup>14,17</sup> Although the importance of smoking cessation is indisputable, clinical practice has shown that smoking is an unruly habit and that cessation is difficult, not only in those infected with HIV, but also in the general population.<sup>80,81</sup>

A second very important intervention to lower the risk of (vascular) comorbidity is the prevention and optimized treatment of hypertension. Apart from avoiding traditional risk factors for hypertension, in the specific context of HIV, it is important to prevent lipodystrophy by avoiding the obsolete use of stavudine. Unfortunately, this antiretroviral agent is still in use in some resource-limited settings due to cost considerations, in spite of WHO recommendations.<sup>82</sup>

In terms of primary and secondary cardiovascular disease prevention, optimal regulation of blood pressure is important. Unfortunately, this has proven to be challenging, even in a population that is as closely monitored as our HIV-infected cohort participants. A previous analysis of our cohort, which is not part of this thesis, found that of those participants not on antihypertensive medication, less than 20% were normotensive; and of those using antihypertensive treatment, more than half had not

achieved adequate hypertension control.<sup>30</sup> Among those HIV-infected participants with a prior clinical vascular event, 42% had uncontrolled hypertension (vs. 52% of uninfected controls).

Comparable suboptimal findings regarding hypertension treatment and control have been observed in other HIV-infected cohorts, as well as in the general population.<sup>34,83–87</sup>

The third key intervention is the prevention of dyslipidemia. Here too, the first step should be to manage the traditional risk factors. In addition, in the context of HIV, those antiretroviral agents most prominently associated with metabolic dysregulation and lipodystrophy should be avoided, and therefore access to recommended regimens should be ensured globally. The second step is to optimize dyslipidemia management. Once again, an analysis not part of this thesis found that, although significantly more of our HIV-positive cohort participants were using lipid-lowering medication and many of these had lipid profiles within the normal range, of those who had experienced a clinical vascular complication, 57% of the HIV-infected individuals (and 70% of uninfected controls) had an indication for lipid-lowering therapy or intensification of this therapy.

Comparable suboptimal results in treatment and control of dyslipidemia have also been observed in other HIV-infected cohorts, as well as in the general population.<sup>85,86,88–91</sup>

## The bigger picture: resilient ageing with HIV infection

Ageing in HIV-infected individuals can be seen as one of the greatest achievements in recent medical history. However, one of today's challenges is to further close the life-expectancy gap between those with and without HIV<sup>92</sup>, while at the same time enabling resilient ageing of those infected with HIV.

An important step towards achieving these goals, and in line with the above section, will be to avoid and/or successfully modify two major risk factors, namely immune deficiency and vascular/metabolic risk factors.

To avoid immune deficiency, early testing and treatment of HIV in-

fection is of the utmost importance, to reduce the risk of not only short term AIDS-related complications, but also long term non-AIDS/ageing-associated comorbidities. Another important benefit of early testing and treatment, apart from its individual benefit, is its public health benefit in terms of prevention of onward HIV transmission, which may importantly contribute to controlling the HIV epidemic.<sup>93</sup> Future research should therefore include monitoring of non-AIDS-related comorbidities in HIV-infected populations. It is also important to extend the follow-up not only of long term survivors pre-treated with mono/dual therapy, but also of populations who have started cART from naive at increasingly high CD4+ T-cell count thresholds, including those who start immediately after diagnosis as recommended by current guidelines.

As described above (under 'AGE<sub>HIV</sub> Cohort characteristics') the first two of these populations of patients are well represented in the AGE<sub>HIV</sub> Cohort Study. However, the third group, namely those who start cART immediately after diagnosis, remains underrepresented in the cohort. It may therefore be valuable to include a second wave of HIV-infected individuals who, according to the most recent guidelines, have been diagnosed and immediately treated at high CD4 counts.

Vascular disease is the number one cause of death globally in the general population and cardiovascular disease is even more prevalent among HIV-infected individuals.<sup>94</sup> Optimal primary and secondary prevention of vascular disease in HIV-infected individuals is therefore of utmost importance. While considerable efforts have been made to improve the situation in the past few decades, numerous hurdles have yet to be crossed and much progress remains to be achieved.

Improving the vascular risk profile is based on two pillars: first, medical treatment of conditions related to an increased risk of vascular disease (hypertension, dyslipidemia, renal dysfunction, diabetes, and among HIV-infected individuals: HIV replication and immune deficiency) and, second, optimizing lifestyle-related factors (smoking, diet, physical activity).

In terms of the first pillar, regular monitoring and strict treatment of vascular risk factors is very important. However, since vascular disease and risk factors remain highly prevalent, the current (mainly hospital-based) system would not appear sufficiently effective. This is because,

even though an adverse vascular risk factor profile is usually detected by a doctor, modification is largely accomplished at home and not in the hospital (i.e., medication adherence, healthy lifestyle). Thus, home-based and community-based, rather than hospital-based, interventions seem the way forward, in combination with optimal integration and application of behavioural psychology knowledge.<sup>95,96</sup> In short, healthcare providers should provide more stringent monitoring and treatment of vascular risk factors, and effective home-based strategies for patient self-management and ensuring healthier lifestyle will should be developed and implemented.

On a global scale, in many parts of the world, HIV and cardiovascular disease are becoming two increasingly intersecting and entangled epidemics. Both require long term monitoring and interventions on an individual and a societal level. Both epidemics should make optimal use of the achievements accomplished in their respective field, not only in terms of medical and scientific progress, but also in terms of the optimized provision of long term prevention and care. For example, in resource-limited regions of the world, many efforts have been made during the past decades to create an infrastructure for HIV care. Further gains in population health, including for those with HIV, could be accomplished by integrating cardiovascular and primary care within this existing infrastructure.

Cognitive impairment shares many risk factors with vascular disease, with immune deficiency and metabolic/vascular risk factors being associated with both comorbidities. As a result, similar recommendations apply to risk reduction of cognitive deficits in the context of HIV infection.

In case of cognitive complaints, patients should be referred to a neurologist, preferably one with knowledge of, and experience in, HIV infection. The differential diagnosis of cognitive impairment in treated HIV-infected individuals includes not only specific HIV-related complications such as the above-mentioned HIV-associated cognitive impairment, but also cerebral damage due to central nervous system viral escape (discordantly high viral replication in the cerebrospinal fluid compared to a lower plasma viral load). Finally, in the ageing HIV-infected population, other, more general causes of CI should also be considered.

Given the high prevalence of comorbidity and multimorbidity in

middle-aged HIV-infected individuals, complex multimorbidity and polypharmacy will likely become the norm, rather than the exception, in older-aged HIV-infected individuals. However, it is highly questionable whether, in its current form, the (Dutch) healthcare system is prepared for this situation.

Aside from the above-mentioned close monitoring and treatment of vascular risk factors, there will be a need for active screening and, if indicated adequate treatment of other comorbidities. As such, care for HIV-infected individuals should be multidisciplinary, integrating knowledge and care from different fields of expertise, including primary care medicine. It is therefore important that each HIV treating facility has access to a variety of medical specialists (e.g., neurologist, vascular specialist, cardiologist, psychiatrist) who have an affinity with HIV infection. In addition, polypharmacy will have to be actively managed to avoid drug fatigue, serious drug-drug interactions, hospitalizations, and mortality. Finally, assisted living facilities and hospices should be set up, with HIV-knowledgeable staff and appropriate means of providing optimal care.<sup>97</sup>

As we work towards achieving these goals, insights gained from research into ageing and comorbidity in the context of HIV infection will hopefully also provide additional knowledge about, and benefit to, the general population, and vice versa.

## The biggest picture: no more HIV/AIDS

Resilient ageing with HIV is an amazing achievement, but resilient ageing without HIV would even be better. With an estimated 36 million people living with HIV globally, over half of whom remain without access to treatment, it is imperative that research towards a cure, as well as an effective vaccine for HIV, continues. Only then will we be able to achieve an AIDS-free generation and ultimately a world without HIV.

## References

1. Guaraldi, G. *et al.* Premature age-related comorbidities among HIV-infected persons compared with the general population. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **53**, 1120–1126 (2011).
2. Hasse, B. *et al.* Morbidity and aging in HIV-infected persons: the Swiss HIV cohort study. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **53**, 1130–1139 (2011).
3. Hansen, A.-B. E. *et al.* Incidence of low and high-energy fractures in persons with and without HIV infection: a Danish population-based cohort study. *AIDS* **26**, 285–293 (2012).
4. Lang, S. *et al.* Increased risk of myocardial infarction in HIV-infected patients in France, relative to the general population. *AIDS Lond. Engl.* **24**, 1228–1230 (2010).
5. Obel, N. *et al.* Ischemic heart disease in HIV-infected and HIV-uninfected individuals: a population-based cohort study. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **44**, 1625–1631 (2007).
6. Triant, V. A., Brown, T. T., Lee, H. & Grinspoon, S. K. Fracture Prevalence among Human Immunodeficiency Virus (HIV)-Infected Versus Non-HIV-Infected Patients in a Large U.S. Healthcare System. *J. Clin. Endocrinol. Metab.* **93**, 3499–3504 (2008).
7. Triant, V. A., Lee, H., Hadigan, C. & Grinspoon, S. K. Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease. *J. Clin. Endocrinol. Metab.* **92**, 2506–2512 (2007).
8. Freiberg, M. S. HIV Infection and the Risk of Acute Myocardial Infarction. *JAMA Intern. Med.* **173**, 614 (2013).
9. Hanna, D. B. *et al.* Trends in cardiovascular disease mortality among persons with HIV in New York City, 2001–2012. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* (2016).
10. Hasse, B. *et al.* Strong Impact of Smoking on Multimorbidity and Cardiovascular Risk Among Human Immunodeficiency Virus-Infected Individuals in Comparison With the General Population. *Open Forum Infect. Dis.* **2**, ofv108 (2015).
11. Rasmussen, L. D. *et al.* Time trends for risk of severe age-related diseases in individuals with and without HIV infection in Denmark: a nationwide population-based cohort study. *Lancet HIV* **2**, e288–298 (2015).
12. *Nationaal Kompas Volksgezondheid; Gezondheidsmonitor GGD, CBS en RIVM 2013.* (2013).
13. Mdodo, R. *et al.* Cigarette smoking prevalence among adults with HIV compared with the general adult population in the United States: cross-sectional surveys. *Ann. Intern. Med.* **162**, 335–344 (2015).
14. Helleberg, M. *et al.* Smoking and life expectancy among HIV-infected individuals on antiretroviral therapy in Europe and North America. *AIDS Lond. Engl.* **29**, 221–229 (2015).

15. Shahmanesh, M. *et al.* The cardiovascular risk management for people living with HIV in Europe: how well are we doing? *AIDS Lond. Engl.* (2016).
16. Lifson, A. R. *et al.* Smoking-related health risks among persons with HIV in the Strategies for Management of Antiretroviral Therapy clinical trial. *Am. J. Public Health* **100**, 1896–1903 (2010).
17. Helleberg, M. *et al.* Mortality attributable to smoking among HIV-1-infected individuals: a nationwide, population-based cohort study. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **56**, 727–734 (2013).
18. Gazzaruso, C. *et al.* Hypertension among HIV patients: prevalence and relationships to insulin resistance and metabolic syndrome. *J. Hypertens.* **21**, 1377–1382 (2003).
19. Seaberg, E. C. *et al.* Association between highly active antiretroviral therapy and hypertension in a large cohort of men followed from 1984 to 2003. *AIDS Lond. Engl.* **19**, 953–960 (2005).
20. Bergersen, B. M., Sandvik, L., Dunlop, O., Birkeland, K. & Bruun, J. N. Prevalence of hypertension in HIV-positive patients on highly active retroviral therapy (HAART) compared with HAART-naïve and HIV-negative controls: results from a Norwegian study of 721 patients. *Eur. J. Clin. Microbiol. Infect. Dis. Off. Publ. Eur. Soc. Clin. Microbiol.* **22**, 731–736 (2003).
21. Jericó, C. *et al.* Hypertension in HIV-infected patients: prevalence and related factors. *Am. J. Hypertens.* **18**, 1396–1401 (2005).
22. Baekken, M., Os, I., Sandvik, L. & Oektedalen, O. Hypertension in an urban HIV-positive population compared with the general population: influence of combination antiretroviral therapy. *J. Hypertens.* **26**, 2126–2133 (2008).
23. Thiébaud, R. *et al.* Predictors of hypertension and changes of blood pressure in HIV-infected patients. *Antivir. Ther.* **10**, 811–823 (2005).
24. Willig, A. L. *et al.* Obesity is associated with race/sex disparities in diabetes and hypertension prevalence, but not cardiovascular disease, among HIV-infected adults. *AIDS Res. Hum. Retroviruses* **31**, 898–904 (2015).
25. Manner, I. W. *et al.* Markers of microbial translocation predict hypertension in HIV-infected individuals. *HIV Med.* **14**, 354–361 (2013).
26. Manner, I. W., Trøseid, M., Oektedalen, O., Baekken, M. & Os, I. Low nadir CD4 cell count predicts sustained hypertension in HIV-infected individuals. *J. Clin. Hypertens. Greenwich Conn* **15**, 101–106 (2013).
27. Lichtenstein, K. A. *et al.* Low CD4+ T cell count is a risk factor for cardiovascular disease events in the HIV outpatient study. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **51**, 435–447 (2010).
28. Crane, H. M., Van Rumpae, S. E. & Kitahata, M. M. Antiretroviral medications associated with elevated blood pressure among patients receiving highly active antiretroviral therapy. *AIDS Lond. Engl.* **20**, 1019–1026 (2006).
29. Nduka, C. U., Stranges, S., Sarki, A. M., Kimani, P. K. & Uthman, O. A. Evidence of increased blood pressure and hypertension risk among people living with

- HIV on antiretroviral therapy: a systematic review with meta-analysis. *J. Hum. Hypertens.* **30**, 355–362 (2016).
30. van Zoest, R. A. *et al.* Higher Prevalence of Hypertension in HIV-1-Infected Patients on Combination Antiretroviral Therapy Is Associated With Changes in Body Composition and Prior Stavudine Exposure. *Clin. Infect. Dis.* **63**, 205–213 (2016).
  31. Friis-Møller, N. *et al.* Cardiovascular disease risk factors in HIV patients—association with antiretroviral therapy. Results from the DAD study. *AIDS Lond. Engl.* **17**, 1179–1193 (2003).
  32. Grunfeld, C. *et al.* Lipids, lipoproteins, triglyceride clearance, and cytokines in human immunodeficiency virus infection and the acquired immunodeficiency syndrome. *J. Clin. Endocrinol. Metab.* **74**, 1045–1052 (1992).
  33. Nduka, C., Sarki, A., Uthman, O. & Stranges, S. Impact of antiretroviral therapy on serum lipoprotein levels and dyslipidemias: a systematic review and meta-analysis. *Int. J. Cardiol.* **199**, 307–318 (2015).
  34. Ballocca, F. *et al.* HIV Infection and Primary Prevention of Cardiovascular Disease: Lights and Shadows in the HAART Era. *Prog. Cardiovasc. Dis.* **58**, 565–576 (2016).
  35. Kalayjian, R. C. Renal issues in HIV infection. *Curr. HIV/AIDS Rep.* **8**, 164–171 (2011).
  36. Mocroft, A. *et al.* Deteriorating renal function and clinical outcomes in HIV-positive persons. *AIDS Lond. Engl.* **28**, 727–737 (2014).
  37. Overton, E. T., Nurutdinova, D., Freeman, J., Seyfried, W. & Mondy, K. E. Factors associated with renal dysfunction within an urban HIV-infected cohort in the era of highly active antiretroviral therapy. *HIV Med.* **10**, 343–350 (2009).
  38. Ryom, L. *et al.* Renal Impairment and Cardiovascular Disease in HIV-positive Individuals; The D:A:D Study. *J. Infect. Dis.* (2016).
  39. Rosenberg, A. Z., Naicker, S., Winkler, C. A. & Kopp, J. B. HIV-associated nephropathies: epidemiology, pathology, mechanisms and treatment. *Nat. Rev. Nephrol.* **11**, 150–160 (2015).
  40. Grinspoon, S. & Carr, A. Cardiovascular risk and body-fat abnormalities in HIV-infected adults. *N. Engl. J. Med.* **352**, 48–62 (2005).
  41. Rhew, D. C., Bernal, M., Aguilar, D., Iloeje, U. & Goetz, M. B. Association between protease inhibitor use and increased cardiovascular risk in patients infected with human immunodeficiency virus: a systematic review. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **37**, 959–972 (2003).
  42. Johnsen, S. *et al.* Carotid intimal medial thickness in human immunodeficiency virus-infected women: effects of protease inhibitor use, cardiac risk factors, and the metabolic syndrome. *J. Clin. Endocrinol. Metab.* **91**, 4916–4924 (2006).
  43. Barrowman, J., Wiley, P. A., Hudon-Miller, S. E., Hrycyna, C. A. & Michaelis, S. Human ZMPSTE24 disease mutations: residual proteolytic activity correlates with disease severity. *Hum. Mol. Genet.* **21**, 4084–4093 (2012).
  44. Perrin, S. *et al.* HIV Protease Inhibitors Do Not Cause the Accumulation of



- Prelamin A in PBMCs from Patients Receiving First Line Therapy: The ANRS EP45 'Aging' Study. *PLoS ONE* **7**, e53035 (2012).
45. So-Armah, K. A. *et al.* Do Biomarkers of Inflammation, Monocyte Activation, and Altered Coagulation Explain Excess Mortality Between HIV Infected and Uninfected People? *J. Acquir. Immune Defic. Syndr.* 1999 **72**, 206–213 (2016).
  46. Armah, K. A. *et al.* HIV Status, Burden of Comorbid Disease, and Biomarkers of Inflammation, Altered Coagulation, and Monocyte Activation. *Clin. Infect. Dis.* **55**, 126–136 (2012).
  47. Deeks, S. G., Tracy, R. & Douek, D. C. Systemic Effects of Inflammation on Health during Chronic HIV Infection. *Immunity* **39**, 633–645 (2013).
  48. Beltrán, L. M. *et al.* Reduced sTWEAK and increased sCD163 levels in HIV-infected patients: modulation by antiretroviral treatment, HIV replication and HCV co-infection. *PLoS One* **9**, e90541 (2014).
  49. Sandler, N. G. & Douek, D. C. Microbial translocation in HIV infection: causes, consequences and treatment opportunities. *Nat. Rev. Microbiol.* **10**, 655–666 (2012).
  50. Hatano, H. Immune activation and HIV persistence: considerations for novel therapeutic interventions. *Curr. Opin. HIV AIDS* **8**, 211–216 (2013).
  51. Tuttolomondo, A. *et al.* Atherosclerosis as an inflammatory disease. *Curr. Pharm. Des.* **18**, 4266–4288 (2012).
  52. Bäck, M., Weber, C. & Lutgens, E. Regulation of atherosclerotic plaque inflammation. *J. Intern. Med.* **278**, 462–482 (2015).
  53. Maia-Leite, L. H. *et al.* Aortic stiffness aging is influenced by past profound immunodeficiency in HIV-infected individuals: results from the EVAS-HIV (Evaluation of Aortic Stiffness in HIV-infected individuals). *J. Hypertens.* **34**, 1338–1346 (2016).
  54. The Reference Values for Arterial Stiffness' Collaboration. Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: 'establishing normal and reference values'. *Eur. Heart J.* **31**, 2338–2350 (2010).
  55. Mancia, G. *et al.* 2007 Guidelines for the management of arterial hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Eur. Heart J.* **28**, 1462–1536 (2007).
  56. Effros, R. B. *et al.* Aging and infectious diseases: workshop on HIV infection and aging: what is known and future research directions. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **47**, 542–553 (2008).
  57. Pathai, S., Bajillan, H., Landay, A. L. & High, K. P. Is HIV a Model of Accelerated or Accentuated Aging? *J. Gerontol. A. Biol. Sci. Med. Sci.* (2013).
  58. Antinori, A. *et al.* Updated research nosology for HIV-associated neurocognitive disorders. *Neurology* **69**, 1789–1799 (2007).
  59. Gisslén, M., Price, R. W. & Nilsson, S. The definition of HIV-associated neurocog-

- nitive disorders: are we overestimating the real prevalence? *BMC Infect. Dis.* **11**, 356 (2011).
60. Huizenga, H. M., Smeding, H., Grasman, R. P. P. & Schmand, B. Multivariate normative comparisons. *Neuropsychologia* **45**, 2534–2542 (2007).
  61. Huizenga, H. M., Agelink van Rentergem, J. A., Grasman, R. P. P., Muslimovic, D. & Schmand, B. Normative comparisons for large neuropsychological test batteries: User-friendly and sensitive solutions to minimize familywise false positives. *J. Clin. Exp. Neuropsychol.* **38**, 611–629 (2016).
  62. González-Redondo, R. *et al.* The impact of silent vascular brain burden in cognitive impairment in Parkinson's disease. *Eur. J. Neurol.* **19**, 1100–1107 (2012).
  63. Cohen, S. *et al.* Poorer cognitive performance in perinatally HIV-infected children versus healthy socioeconomically matched controls. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **60**, 1111–1119 (2015).
  64. Folstein, M. F., Folstein, S. E. & McHugh, P. R. 'Mini-mental state'. A practical method for grading the cognitive state of patients for the clinician. *J. Psychiatr. Res.* **12**, 189–198 (1975).
  65. Power, C., Selnes, O. A., Grim, J. A. & McArthur, J. C. HIV Dementia Scale: a rapid screening test. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirology Off. Publ. Int. Retrovirology Assoc.* **8**, 273–278 (1995).
  66. Nasreddine, Z. S. *et al.* The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *J. Am. Geriatr. Soc.* **53**, 695–699 (2005).
  67. Simioni, S. *et al.* Cognitive dysfunction in HIV patients despite long-standing suppression of viremia. *AIDS* **1** (2009).
  68. Meyer, A.-C. L., Boscardin, W. J., Kwasa, J. K. & Price, R. W. Is It Time to Rethink How Neuropsychological Tests Are Used to Diagnose Mild Forms of HIV-Associated Neurocognitive Disorders? Impact of False-Positive Rates on Prevalence and Power. *Neuroepidemiology* **41**, 208–216 (2013).
  69. Cysique, L. A., Maruff, P. & Brew, B. J. Prevalence and pattern of neuropsychological impairment in human immunodeficiency virus-infected/acquired immunodeficiency syndrome (HIV/AIDS) patients across pre- and post-highly active antiretroviral therapy eras: a combined study of two cohorts. *J. Neurovirol.* **10**, 350–357 (2004).
  70. Woods, S. P., Moore, D. J., Weber, E. & Grant, I. Cognitive Neuropsychology of HIV-Associated Neurocognitive Disorders. *Neuropsychol. Rev.* **19**, 152–168 (2009).
  71. Clifford, D. B. & Ances, B. M. HIV-associated neurocognitive disorder. *Lancet Infect. Dis.* **13**, 976–986 (2013).
  72. Joska, J. A. *et al.* A Comparison of Five Brief Screening Tools for HIV-Associated Neurocognitive Disorders in the USA and South Africa. *AIDS Behav.* (2016).
  73. Heaton, R. K. *et al.* Neurocognitive change in the era of HIV combination an-

- tiretroviral therapy: the longitudinal CHARTER study. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **60**, 473–480 (2015).
74. Sighem, A. van, Ebeling, S. H. & Stichting HIV Monitoring. Monitoring report 2015: monitoring of human immunodeficiencyvirus (HIV) infection in the Netherlands. (Stichting HIV Monitoring (SHM), 2015).
  75. Mocroft, A. *et al.* Risk factors and outcomes for late presentation for HIV-positive persons in Europe: results from the Collaboration of Observational HIV Epidemiological Research Europe Study (COHERE). *PLoS Med.* **10**, e1001510 (2013).
  76. Fleishman, J. A., Yehia, B. R., Moore, R. D., Gebo, K. A. & HIV Research Network. The economic burden of late entry into medical care for patients with HIV infection. *Med. Care* **48**, 1071–1079 (2010).
  77. Triant, V. A., Meigs, J. B. & Grinspoon, S. K. Association of C-reactive protein and HIV infection with acute myocardial infarction. *J. Acquir. Immune Defic. Syndr.* **1999** **51**, 268–273 (2009).
  78. Silverberg, M. J. *et al.* Immunodeficiency and risk of myocardial infarction among HIV-positive individuals with access to care. *J. Acquir. Immune Defic. Syndr.* **1999** **65**, 160–166 (2014).
  79. INSIGHT START Study Group *et al.* Initiation of Antiretroviral Therapy in Early Asymptomatic HIV Infection. *N. Engl. J. Med.* **373**, 795–807 (2015).
  80. Ledgerwood, D. M. & Yskes, R. Smoking Cessation for People Living With HIV/AIDS: A Literature Review and Synthesis. *Nicotine Tob. Res. Off. J. Soc. Res. Nicotine Tob.* (2016).
  81. Vangeli, E., Stapleton, J., Smit, E. S., Borland, R. & West, R. Predictors of attempts to stop smoking and their success in adult general population samples: a systematic review. *Addict. Abingdon Engl.* **106**, 2110–2121 (2011).
  82. *World Health Organization HIV infection treatment guidelines 2016.* (2016).
  83. Nüesch, R. *et al.* Risk of cardiovascular events and blood pressure control in hypertensive HIV-infected patients: Swiss HIV Cohort Study (SHCS). *J. Acquir. Immune Defic. Syndr.* **1999** **62**, 396–404 (2013).
  84. Redon, J., Mourad, J.-J., Schmieder, R. E., Volpe, M. & Weiss, T. W. Why in 2016 are patients with hypertension not 100% controlled? A call to action. *J. Hypertens.* **34**, 1480–1488 (2016).
  85. Reinsch, N. *et al.* Are HIV patients undertreated? Cardiovascular risk factors in HIV: results of the HIV-HEART study. *Eur. J. Prev. Cardiol.* **19**, 267–274 (2012).
  86. Balder, J. W. *et al.* Adherence to guidelines to prevent cardiovascular diseases: The LifeLines cohort study. *Neth. J. Med.* **73**, 316–323 (2015).
  87. Agyemang, C. *et al.* Hypertension control in a large multi-ethnic cohort in Amsterdam, The Netherlands: the HELIUS study. *Int. J. Cardiol.* **183**, 180–189 (2015).
  88. Myerson, M. *et al.* Prevalence, treatment, and control of dyslipidemia and hypertension in 4278 HIV outpatients. *J. Acquir. Immune Defic. Syndr.* **1999** **66**, 370–377 (2014).
  89. Gamboa, C. M. *et al.* Statin underuse and low prevalence of LDL-C control among

- U.S. adults at high risk of coronary heart disease. *Am. J. Med. Sci.* **348**, 108–114 (2014).
90. Clement, M. E. *et al.* Statin Utilization and Recommendations Among HIV- and HCV-infected Veterans: A Cohort Study. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **63**, 407–413 (2016).
  91. Mantel-Teeuwisse, A. K. *et al.* Undertreatment of hypercholesterolaemia: a population-based study. *Br. J. Clin. Pharmacol.* **55**, 389–397 (2003).
  92. Marcus, J. L. *et al.* Narrowing the gap in life expectancy between HIV-infected and HIV-uninfected individuals with access to care. *J. Acquir. Immune Defic. Syndr.* **1999** (2016).
  93. Rodger, A. J. *et al.* Sexual Activity Without Condoms and Risk of HIV Transmission in Serodifferent Couples When the HIV-Positive Partner Is Using Suppressive Antiretroviral Therapy. *JAMA* **316**, 171–181 (2016).
  94. *Fact sheet World Health Organization on cardiovascular disease.* (2016).
  95. Ariely, D. & Norton, M. I. From thinking too little to thinking too much: a continuum of decision making. *Wiley Interdiscip. Rev. Cogn. Sci.* **2**, 39–46 (2011).
  96. Schwartz, J., Riis, J., Elbel, B. & Ariely, D. Inviting Consumers To Downsize Fast-Food Portions Significantly Reduces Calorie Consumption. *Health Aff. (Millwood)* **31**, 399–407 (2012).
  97. Althoff, K. N., Smit, M., Reiss, P. & Justice, A. C. HIV and ageing: improving quantity and quality of life. *Curr. Opin. HIV AIDS* **11**, 527–536 (2016).

# Samenvatting

## “Head and heart in treated HIV infection”

### Introductie

Humaan immunodeficiëntie virus (HIV) is een retrovirus dat het immuunsysteem infecteert en beschadigt. Een essentieel aangrijpingspunt voor HIV is de CD<sub>4</sub> receptor. Deze receptor bevindt zich op bepaalde witte bloedcellen (CD<sub>4</sub>+ T-lymfocyten) waardoor deze geïnfecteerd raken en vervolgens afnemen in aantal en functie. CD<sub>4</sub>+ T-lymfocyten vervullen een centrale functie binnen het immuunsysteem. Zonder behandeling resulteert infectie met HIV dan ook in het optreden van ernstige infecties en kanker (de HIV infectie is dan symptomatisch geworden), en uiteindelijk tot de dood. Wanneer het symptomatische stadium van de HIV infectie is aangebroken spreekt men van AIDS (acquired immune deficiency syndrome). AIDS manifesteert zich pas als het zogenaamde CD<sub>4</sub> getal (hoeveelheid CD<sub>4</sub>+ T-lymfocyten in het bloed) een kritieke ondergrens is gepasseerd. Omdat er lange tijd voorbij kan gaan tussen het moment van infectie met HIV en het passeren van deze ondergrens in CD<sub>4</sub> getal, kan de HIV infectie lange tijd asymptomatisch zijn en ongemerkt verlopen. HIV wordt overgedragen via seksueel contact, bloed-bloed contact, en van moeder op kind.

De eerste manifestaties van AIDS werden begin jaren '80 beschreven. In 1983 werd duidelijk dat HIV de veroorzaker is van AIDS. In 1987 kwam het eerste antiretrovirale middel zidovudine beschikbaar. Deze nucleoside reverse-transcriptase inhibitor (NRTI) bleek HIV echter slechts tijdelijk te kunnen onderdrukken. Ditzelfde gold voor andere NRTI's die in de jaren daarna werden ontwikkeld, zelfs wanneer zij als combinaties van twee middelen werden toegepast als zogenaamde 'dual therapy'. Pas toen andere klassen antiretrovirale middelen werden ontwikkeld (protease inhibitors of PI's, en non-nucleoside reverse-transcriptase inhibitors of NNRTI's), én deze werden gecombineerd met twee NRTI's tot een zogenaamde 'triple therapie' of 'highly active antiretroviral therapy (HAART)' of 'combination antiretroviral therapy (cART)' zoals deze combinatietherapie tegenwoordig wordt genoemd, bleek langdurige onderdrukking van HIV te kunnen worden bewerkstelligd. Sinds de beschikbaarheid van cART in 1996 is HIV veranderd van een dodelijke ziekte in een chronische behandelbare aandoening.

Door de indrukwekkende daling in HIV-gerelateerde sterfte sinds de introductie van cART in 1996 konden mensen met HIV voor het eerst overleven en ouder worden. Dit heeft geresulteerd in een verouderende HIV populatie waarbij inmiddels bijna een derde van de HIV-geïnfecteerden in welvarende landen 50 jaar of ouder is. In minder welvarende landen waar cART op grotere schaal pas later beschikbaar is gekomen, is dit 10%. In Nederland is het percentage 50+'ers onder mensen met HIV gestegen van 9% in 1995 tot 42% in 2015. Wereldwijd wordt geschat dat er meer dan 3,6 miljoen HIV-patiënten ouder zijn dan 50.

Ondanks het indrukwekkende succes van cART en het terugdringen van AIDS-gerelateerde sterfte lijken ziekten (comorbiditeiten) die niet samenhangen met AIDS en in de algemene bevolking veelal geassocieerd zijn met gevorderde leeftijd vaker voor te komen onder HIV-geïnfecteerden, ook wanneer het virus met medicatie adequaat onderdrukt wordt. Voorbeelden zijn hart- en vaatziekten, hypertensie, nierfunctiestoornissen, osteoporose, diabetes mellitus, leverfunctiestoornissen, kankers die niet van oudsher geassocieerd zijn met AIDS, en cognitieve problemen.

Omdat de meeste studies geen goed vergelijkbare controlegroep

met mensen zonder HIV beschikbaar hadden, was onduidelijk of deze niet-AIDS/verouderings-gerelateerde aandoeningen werkelijk vaker vóórkwamen onder HIV-geïnfecteerden. Ook was onduidelijk of dit eventuele toegenomen risico op comorbiditeit onder mensen met HIV toe te schrijven viel aan de HIV infectie zelf (of behandeling met anti-retrovirale therapie (ART)), of aan risicofactoren die samenhangen met leefstijl zoals roken en drugsgebruik. Binnen HIV-geïnfecteerde populaties is risicogedrag namelijk frequenter voorkomend dan in de algemene bevolking.

Mogelijk zouden deze niet-AIDS/verouderings-gerelateerde comorbiditeiten ook op jongere leeftijd vóórkomen onder HIV-geïnfecteerden, hetgeen heeft geleid tot de hypothese dat mensen met HIV sneller en/of meer uitgesproken zouden verouderen dan mensen zonder HIV.

## AGE<sub>n</sub>IV Cohort Studie

Om meer inzicht te krijgen in deze vraagstukken is in 2010 de AGE<sub>n</sub>IV Cohort Studie gestart in Amsterdam.

Deze prospectieve cohortstudie vergelijkt het ontstaan, vóórkomen, en de mogelijke risicofactoren van niet-AIDS/verouderings-gerelateerde aandoeningen en orgaan dysfunctie tussen mensen met en zonder HIV, allen 45 jaar en ouder.

Werving en inclusie van HIV-geïnfecteerde deelnemers heeft plaatsgevonden via de HIV polikliniek van het Academisch Medisch Centrum in Amsterdam; ongeïnfecteerde controles zijn gerekruteerd via de polikliniek voor seksueel overdraagbare aandoeningen (SOA-poli) van de GGD Amsterdam en via de bestaande Amsterdam Cohort Studies naar HIV/AIDS. Er is bewust gekozen voor een controlegroep die een zo groot mogelijke gelijkenis heeft wat betreft risicogedrag en -profiel met de HIV-geïnfecteerde deelnemers. Door de controles te werven via de bovenstaande routes konden we mensen includeren die wel 'at risk' zijn geweest voor het oplopen van HIV infectie, maar de infectie toch niet hebben gekregen. Om een zo groot mogelijke overeenkomst tussen beide studiegroepen te bewerkstelligen, zijn bepaalde karakteristieken

(leeftijd, geslacht, etniciteit) van de HIV-geïnficeerde groep frequent gemonitord en is inclusie van de ongeïnficeerde controles hier regelmatig op aangepast.

Dit heeft geresulteerd in inclusie van 598 mensen met en 550 mensen zonder HIV, tussen 1 oktober 2010 en 30 september 2012.

Elke twee jaar ondergaan deelnemers uit beide groepen een uitgebreide screening naar niet-AIDS/verouderings-gerelateerde complicaties. Gedetailleerde informatie omtrent HIV en ART geschiedenis is verkregen via de Stichting HIV Monitoring. Deze stichting is verantwoordelijk voor het verzamelen van HIV/ART-gerelateerde gegevens van alle mensen die in zorg zijn voor HIV infectie bij een HIV behandelcentrum in Nederland.

Er is gestart met het samenstellen van een biobank door het invriezen van urine, bloed, en ontlasting. Dit maakt toekomstig onderzoek van afgenomen materialen mogelijk.

Binnen de AGE<sub>HIV</sub> Cohort Studie zoals hierboven beschreven, is in 2011 een neurologische substudie gestart gericht op het meer diepgaand onderzoeken van cognitieve stoornissen en aanvullende parameters verwant aan het centrale zenuwstelsel. Vanuit de hoofdstudie zijn 103 HIV-geïnficeerde (allemaal met onderdrukking van HIV replicatie gedurende een jaar of meer) en 74 ongeïnficeerde mannen geïnccludeerd in deze substudie. Deze substudie deelnemers ondergingen bij inclusie en twee jaar daarna vier extra procedures: uitgebreid neuropsychologisch onderzoek, beeldvorming van de hersenen door middel van diverse soorten MRI-scans, analyse van hersenvocht verkregen via een lumbaalpunctie, en beeldvorming van het netvlies.

## Proefschrift

Dit proefschrift richt zich op twee van de bovengenoemde niet-AIDS/verouderings-gerelateerde comorbiditeiten en is opgebouwd als tweeluik: vasculaire complicaties ('heart') en cognitieve stoornissen ('head'). Dit proefschrift is gebaseerd op cross-sectionele analyses van data die ver-



kregen zijn ten tijde van inclusie in zowel de AGE<sub>HIV</sub> Cohort hoofdstudie als de neurologische substudie.

## Deel I: Heart, vasculaire complicaties

**Hoofdstuk 2** is een gepubliceerd overzichtsartikel over coronaire hartziekte bij behandelde HIV infectie, en dient als een introductie voor het eerste deel van het proefschrift.

In **hoofdstuk 3** wordt beschreven dat alle onderzochte niet-AIDS/verouderings-gerelateerde comorbiditeiten binnen de AGE<sub>HIV</sub> Studie (hypertensie, myocardinfarct, angina pectoris, perifere arteriële ziekte, cerebrovasculaire ziekte, diabetes mellitus type 2, obstructieve longziekte, nierfunctiestoornissen, niet-AIDS kankers, en atraumatische fracturen/osteoporose) getalsmatig vaker voorkwamen onder deelnemers met HIV (n=540) in vergelijking met ongeïnfecteerde controles (n=524). Het verschil in vóórkomen van hypertensie, myocardinfarct, perifere arteriële ziekte, en nierfunctiestoornissen was hierbij statistisch significant. Geïnfecteerd zijn met HIV bleek onafhankelijk geassocieerd te zijn met een verhoogd risico op comorbiditeit. Onder HIV-geïnfecteerden waren doorgemaakte immuundeficiëntie (weerspiegeld door een lager laagste ooit gemeten CD<sub>4</sub> getal, of nadir CD<sub>4</sub>) en in mindere mate persisterende inflammatie en immuunactivatie, alsook gebruik van hoge dosis ritonavir ( $\geq 400$  mg/dag) in het verleden, geassocieerd met een verhoogd risico op comorbiditeit.

Omdat vasculaire ziekten de meest voorkomende categorie binnen de onderzochte comorbiditeiten bleek onder HIV-geïnfecteerde deelnemers hebben we in **hoofdstuk 4** toegenomen stijfheid van de aorta onderzocht (weerspiegeld door een toegenomen pulse wave velocity, of PWV). Toegenomen stijfheid van de aorta is een degeneratief proces dat geassocieerd is met veroudering, en versneld kan worden door hypertensie, metabole veranderingen, en inflammatie. Toegenomen PWV (als uiting van toegenomen stijfheid van de aorta) is geassocieerd met klinische cardiovasculaire ziekte en mortaliteit in de algemene bevolking en kan gezien worden als een preklinische afwijking.

Binnen de AGE<sub>HIV</sub> Studie was de mediane PWV hoger onder deelnemers met (n=566) dan deelnemers zonder (n=507) HIV. Ondanks dat geïnfecteerd zijn met HIV wel onafhankelijk geassocieerd was met dit toegenomen risico op vaatstijfheid, bleek het HIV-geassocieerde verschil in PWV tussen de twee studiegroepen grotendeels verklaard te worden door de klassieke risicofactoren roken en hypertensie, beide veel meer voorkomend onder de deelnemers met HIV in vergelijking met de ongeïnfecteerde controles. Andere factoren die geassocieerd waren met toegenomen stijfheid waren over- of ondergewicht, dyslipidemie, en inflammatie.

Onder de HIV-geïnfecteerde deelnemers was doorgemaakte immuundeficiëntie geassocieerd met toegenomen stijfheid. Deze associatie leek deels te worden gemedieerd door dyslipidemie en inflammatie.

Ondanks dat de HIV-geïnfecteerden in onze studie weliswaar een iets hogere PWV hadden, waren de mediane waarden van zowel de HIV-geïnfecteerde als de ongeïnfecteerde controles binnen de normale range. Ook vonden we geen associatie tussen PWV en klinische vasculaire ziekte. Waarschijnlijk is dus de bijdrage van toegenomen stijfheid van de aorta aan het ontstaan van klinische vasculaire ziekte relatief klein. Wel moet hierbij de kanttekening worden gemaakt dat we PWV gemeten tijdens inclusie hebben gerelateerd aan doorgemaakte klinische cardiovasculaire events in het verleden, waarbij het interval tussen het event en de meting uiteenlopend maar vaak lang was. Ook was het aantal doorgemaakte klinische events relatief klein hetgeen de statistische power limiteert. Tot slot kan er verandering van lifestyle/risicoprofiel hebben plaatsgevonden na het doorgemaakte event, wat de later gemeten PWV heeft beïnvloed en ‘gebiased’. Longitudinale analyses van de PWV bieden de mogelijkheid om het beloop van de PWV van beide studiegroepen te vervolgen en te onderzoeken of er deviatie optreedt en er associaties te vinden zullen zijn met incidente (nieuw ontstane) cardiovasculaire events.

Concluderend bleken niet-AIDS/verouderings-geassocieerde comorbiditeiten, en dan vooral vasculaire complicaties, vaker voor te komen bij HIV-geïnfecteerden.

HIV bleek een onafhankelijke risicofactor te zijn voor het hebben van comorbiditeiten. Het palet aan risicofactoren voor comorbiditeit is

zeer divers en omvat zowel HIV/ART-gerelateerde factoren (zoals door-gemaakte immuundeficiëntie en inflammatie) alsook bekende klassieke (vasculaire) risicofactoren zoals roken, hypertensie, en dyslipidemie. Deze factoren zijn ook onderling in grote mate verstrengeld. Onze bevindingen zijn in lijn met die van andere studiegroepen.

We hebben niet overtuigend aan kunnen tonen dat comorbiditeiten op jongere leeftijd vóórkomen bij HIV-geïnfekteerden. Longitudinale analyses van het AGE<sub>HIV</sub> Cohort, alsook andere cohorten, zijn echter noodzakelijk om hier in de toekomst hopelijk meer inzicht over te verschaffen.

## Deel II: Head, cognitieve stoornissen

**Hoofdstuk 5** is een gepubliceerd overzichtsartikel over cognitieve stoornissen bij behandelde HIV infectie, en dient als een introductie voor het tweede deel van het proefschrift.

In **hoofdstuk 6** hebben we het vóórkomen van cognitieve stoornissen onderzocht binnen de neurologische substudie van de AGE<sub>HIV</sub> Cohort hoofdstudie. Wanneer de bestaande (en waarschijnlijk overgevoelige) Frascati criteria worden toegepast op de studiedeelnemers, blijkt 48% van de deelnemers met HIV (n=103) en 36% van de deelnemers zonder HIV (n=74) als cognitief afwijkend geclassificeerd te worden. Dit bevestigt het vermoeden op de eerdergenoemde overgevoeligheid van de Frascati criteria. In plaats van de Frascati criteria stellen we een nieuwe en meer accurate methode voor om cognitieve stoornissen te detecteren: multivariate normative comparison (MNC). Wanneer we MNC toepassen blijkt 17% van de HIV-geïnfekteerde deelnemers geclassificeerd te worden als cognitief afwijkend. De gevonden cognitieve afwijkingen zijn subtiel en over de gehele linie van onderzochte cognitieve domeinen.

In **hoofdstuk 7** hebben we risicofactoren voor afwijkende cognitieve functie (zoals geclassificeerd met MNC) onderzocht. Hierbij hebben we de volgende risicofactoren geïdentificeerd: cannabis gebruik, door-gemaakte cardiovasculaire ziekte, nierfunctiestoornissen, diabetes melli-

tus type 2, afwijkende middel-heup ratio, aanwezigheid van depressieve symptomen, en doorgemaakte immuundeficiëntie.

In **hoofdstuk 8** hebben we vier bestaande screenende instrumenten (Mini Mental State Examination (MMSE), HIV Dementia Scale (HDS), Montreal Cognitive Assessment (MoCA), en de zogenaamde Simioni vragen) om cognitieve stoornissen te detecteren onderzocht binnen de neurologische substudie van het AGE<sub>HIV</sub> Cohort. Vanwege de matige prestaties van alle screenende instrumenten, kon geen van de onderzochte screenende instrumenten worden aanbevolen in de klinische praktijk.

Concluderend komen cognitieve stoornissen voor bij 17% van de HIV-geïnfecteerde deelnemers en lijkt multivariate normative comparison een adequatere methode te zijn voor het detecteren van cognitieve stoornissen dan de huidige Frascati criteria. De gevonden stoornissen zijn subtiel en verspreid over een brede range van cognitieve domeinen. Een groot scala aan risicofactoren is geassocieerd met cognitieve stoornissen waaronder HIV/ART-gerelateerde factoren (doorgemaakte immuundeficiëntie), vasculaire/metabole factoren, psychiatrische comorbiditeit, en cannabis gebruik. Onze bevindingen zijn conform die van andere studiegroepen.

De subtiële stoornissen kunnen niet betrouwbaar worden gedetecteerd met screenende instrumenten.

Gepubliceerde prospectieve resultaten van het CHARTER cohort tonen geen progressie van cognitieve stoornissen gedurende een driejarige observatieperiode. Dit, in combinatie met de subtiliteit van de afwijkingen, stemt enigszins gerust en maakt progressie tot HIV-dementie onwaarschijnlijk. De follow-up periode van drie jaar is echter relatief kort. En met het vele vóórkomen van vasculaire/metabole ziekte en de bijbehorende risicofactoren binnen de verouderende HIV-geïnfecteerde bevolking bestaat wel degelijk de zorg dat vasculaire en HIV-geassocieerde cerebrale schade op de lange termijn in toenemende mate zullen samengaan en het cognitief functioneren nadelig kunnen beïnvloeden.

## The big picture: optimaliseren van het risicoprofiel voor comorbiditeit

Wanneer we kijken naar het palet van risicofactoren voor niet-AIDS/verouderings-gerelateerde aandoeningen zien we dat vele van deze factoren onderling verstrengeld zijn en het ontstaan van comorbiditeit een multifactorieel proces is. Wat betreft de twee onderzochte comorbiditeiten, vasculaire ziekte en cognitieve stoornissen, valt op dat zij vele gedeelde risicofactoren hebben en onderling ook verband houden met elkaar.

Twee categorieën risicofactoren komen herhaaldelijk en consequent aan de oppervlakte, zowel binnen de AGE<sub>h</sub>IV Cohort Studie als binnen de gepubliceerde wetenschappelijke literatuur: immuundeficiëntie en metabole/vasculaire risicofactoren. Vermijden, optimaliseren, of behandelen van deze factoren is dan ook van zeer groot belang om de kans op comorbiditeit te verminderen.

## The bigger picture: veerkrachtig oud worden met HIV

Het feit dat mensen met HIV ouder kunnen worden, kan worden beschouwd als één van de grootste verworvenheden in de geschiedenis van de geneeskunde. De uitdagingen van vandaag de dag zijn om het verschil in levensverwachting tussen mensen met en zonder HIV te doen verdwijnen, en om ervoor te zorgen dat mensen met HIV veerkrachtig oud kunnen worden.

Een essentiële stap om deze doelen te bereiken, zoals in de paragraaf hiervoor benoemd, is om twee belangrijke risicofactoren voor comorbiditeit, immuundeficiëntie en metabole/vasculaire risicofactoren, te behandelen, te optimaliseren, of te vermijden.

Om immuundeficiëntie te vermijden is zo vroeg mogelijk testen, diagnosticeren, en behandelen van de HIV infectie van groot belang. Dit is niet alleen belangrijk om het risico op AIDS-gerelateerde com-

plicaties te verkleinen, maar ook om de kans op lange-termijn niet-AIDS/verouderings-gerelateerde comorbiditeiten te verkleinen. Behalve dit individuele voordeel is er ook een belangrijk positief gevolg vanuit volksgezondheids-perspectief, omdat vroege adequate behandeling de kans op overdracht van de infectie vermindert, hetgeen bijdraagt aan het beteugelen van de HIV epidemie.

Cardiovasculaire ziekte komt veelvuldig voor onder HIV-geïnfecteerden, maar ook in de algemene bevolking, aangezien vasculaire ziekte de nummer één doodsoorzaak is wereldwijd. Optimale primaire en secundaire preventie van vasculaire ziekte is dan ook essentieel. Ondanks de vele inspanningen en verbeteringen die zijn bewerkstelligd de afgelopen decades, is er nog een lange weg te gaan en zijn er nog vele hordes te nemen.

Optimalisering van het vasculaire risicoprofiel is gefundeerd op twee pijlers: medische behandeling van afwijkingen die gerelateerd zijn aan een verhoogde kans op vasculaire ziekte (hypertensie, dyslipidemie, nierfunctiestoornissen, en onder HIV-geïnfecteerden: HIV replicatie en immuundeficiëntie), en optimaliseren van leefstijl-gerelateerde risicofactoren (roken, dieet, lichaamsbeweging).

Wat betreft de eerste pijler is frequente monitoring en strikte behandeling van vasculaire risicofactoren zeer belangrijk. Ondanks dat een nadelig risicoprofiel voor cardiovasculaire ziekte vaak door een dokter wordt vastgesteld, vindt het optimaliseren van het cardiovasculaire risicoprofiel grotendeels thuis plaats (therapietrouw wat betreft de medicatie, gezonde leefstijl). Aangezien vasculaire ziekte en de bijbehorende risicofactoren nog steeds zeer veel voorkomend zijn, lijkt het huidige, vooral ziekenhuis-gebaseerde systeem, te falen. Home-based of zelfs community-based benaderingen zouden beter kunnen werken, met gebruikmaking van kennis en expertise vanuit andere gebieden waaronder bijvoorbeeld de gedragspsychologie.

Wereldwijd zullen cardiovasculaire ziekte en HIV in toenemende mate twee overlappende en verstrengelde epidemieën zijn. Beide vereisen langdurige monitoring en behandeling, zowel op individueel als op maatschappelijk niveau. Daarbij dient optimaal gebruik gemaakt te worden van vooruitgang die geboekt is binnen elk van beide epidemieën, niet alleen op medisch en wetenschappelijk gebied, maar ook wat betreft

voortschrijdend inzicht ten aanzien van het optimaal aanbieden van langdurige preventie en zorg.

In minder welvarende delen van de wereld hebben de laatste tientallen jaren vele inspanningen plaatsgevonden om een infrastructuur te creëren voor HIV zorg. Verdere verbetering van de volksgezondheid zou daar kunnen worden bewerkstelligd wanneer cardiovasculaire en basisgezondheidszorg geïntegreerd kunnen worden in deze bestaande infrastructuur.

Cognitieve stoornissen en vasculaire ziekten delen vele risicofactoren, waarbij immunodeficiëntie en metabole/vasculaire risicofactoren met beide comorbiditeiten geassocieerd zijn. Dientengevolge gelden dezelfde aanbevelingen als boven beschreven wat betreft risicoreductie voor cognitieve stoornissen in de context van chronische HIV infectie.

In het geval van cognitieve klachten moeten patiënten worden verwezen naar een neuroloog, en bij voorkeur een met kennis en ervaring op het gebied van HIV. De differentiaaldiagnose van cognitieve stoornissen omvat niet alleen de boven beschreven HIV-gerelateerde cognitieve stoornissen maar ook cerebrale schade door zogenaamde 'viral escape' in het centrale zenuwstelsel. Dit is een situatie waarbij er een discordantie bestaat tussen de virale replicatie in het hersenvocht en het bloed, en de virale lading in het hersenvocht disproportioneel hoger is dan in het bloed.

Verder dienen in de verouderende HIV-geïnfecteerde populatie ook de meer algemene oorzaken van cognitieve achteruitgang in toenemende mate te worden overwogen.

Aangezien co- en multimorbiditeit reeds zo veel voorkomend zijn onder HIV-geïnfecteerde mensen van middelbare leeftijd, valt te voorzien dat complexe multimorbiditeit en polyfarmacie in oudere HIV-patiënten eerder de norm dan de uitzondering zullen zijn. Het valt ernstig te betwijfelen of de (Nederlandse) gezondheidszorg in haar huidige vorm op deze situatie is voorbereid.

Behalve de eerdergenoemde strikte monitoring en behandeling van vasculaire risicofactoren, dient er ook actief te worden gescreend op andere comorbiditeiten, en indien nodig, adequaat te worden behandeld. Zorg voor mensen met HIV moet multidisciplinair zijn, met integratie van kennis en ervaring vanuit andere specialisaties, waaronder de huis-

artsgeneeskunde. Het is dan ook essentieel dat HIV behandelcentra beschikking hebben over een variëteit aan medisch specialisten (zoals een neuroloog, vasculair geneeskundig specialist, cardioloog, psychiater) die affiniteit hebben met HIV infectie.

Polyfarmacie moet actief worden gemanaged teneinde 'medicatievermoeidheid', ernstige interacties, ziekenhuisopnames, en sterfte te voorkómen. Zorg- en verpleeginstellingen zullen voorbereid moeten worden en werknemers beschikbaar moeten hebben met kennis van zaken over HIV, alsook over geschikte middelen moeten kunnen beschikken om optimale zorg te kunnen leveren.

Op weg naar deze doelen zullen inzichten verkregen door onderzoek gericht op veroudering en comorbiditeit binnen de context van HIV infectie hopelijk ook aanvullende kennis en voordelen bieden voor de algemeen ouder wordende bevolking, en vice versa.

## The biggest picture: een wereld zonder HIV/AIDS

Veerkrachtig oud kunnen worden met HIV is een geweldige prestatie, maar veerkrachtig oud kunnen worden zonder HIV zou nog veel beter zijn. Naar schatting zijn er 36 miljoen mensen wereldwijd geïnfecteerd met HIV, waarvan meer dan de helft op dit moment nog steeds geen toegang heeft tot adequate behandeling. Het doen van onderzoek zowel naar een genezing van HIV als naar een preventief vaccin blijft dan ook van het grootste belang. Alleen dan zal er sprake kunnen zijn van een toekomstige generatie zonder AIDS, en uiteindelijk een wereld zonder HIV.



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*AMC, Department of Gastroenterology*

E. Dekker

*AMC, Division of Geriatric Medicine*

N. van der Velde

*AMC, Division of Nephrology*

L. Vogt, J.M.R. Willemsen

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P. Portegies, J. Schouten

*AMC, Department of Medical Psychology*

B.A. Schmand, G.J. Geurtsen, P.T. Nieuwkerk, N. Langebeek

*AMC, Department of Ophthalmology*

F.D. Verbraak, N. Demirkaya

*AMC, Department of Psychiatry*

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*Free University Medical Center Amsterdam, Department of Psychiatry*

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H.W. van Lunsen, M.A.F. Nievaard

*AMC, Division of Vascular Medicine*  
B.J.H. van den Born, E.S.G. Stroes

*AMC, Department of Nuclear Medicine*  
H.J. Verberne, M. de Jong

*HIV Vereniging Nederland*  
W.M.C. Mulder

### Past members

I. Stolte, M. Martens, S. Moll, L. Möller, C. Welling, E. Hoornenborg, S. Mooij (*Public Health Service Amsterdam, Infectious Diseases Research Cluster*)

T. Woudstra, T. Rutkens (*HIV Monitoring Foundation*)

M. Heidenrijk, J.H.N. Schrijver, R. Meester, L. Boumans, S. van den Bersselaar, E. Dekker (*AIGHD*)

M. Joerink †, A.B. van 't Wout, H. Schuitemaker, R. Lutter, J. Hamann, V. Cobos Jiménez, E.M. van Leeuwen (*AMC, Laboratory for Viral Immune Pathogenesis and Department of Experimental Immunology*)

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S.E.J.A. de Rooij (*AMC, Division of Geriatric Medicine*)

R. Krediet (*AMC, Division of Nephrology*)

J.A. ter Stege, M. Klein Twennaar (*AMC, Department of Medical Psychology*)

H.G. Ruhé (*AMC, Department of Psychiatry*)

B. van den Bogaard, E. de Groot (*AMC, Division of Vascular Medicine*)

B.L.F. van Eck-Smit (*AMC, Department of Nuclear Medicine*)

D. Richel (*AMC, Division of Oncology*)



# List of publications

- **J. Schouten**, P. Cinque, M. Gisslen, P. Reiss, and P. Portegies, "HIV-1 infection and cognitive impairment in the cART era: a review," *AIDS*, vol. 25, no. 5, pp. 561–575, Mar. 2011.
- **J. Schouten**, C. L. Gorter de Vries, B. Jaeger, P. Reiss, and P. Portegies, "HIV-1 infectie en cognitieve stoornissen," *Tijdschrift voor Neurologie en Neurochirurgie*, vol. 113, no. 4, pp. 168–177, Aug. 2012.
- I. G. Stolte, J. Kuiper, **J. Schouten**, F. W. Wit, P. Reiss, J. Sluiter, and M. Prins, "Prevalence and determinants of unemployment among ageing HIV-1-infected and HIV-uninfected individuals," *Journal of the International AIDS Society*, vol. 15, no. 6(Suppl 4), Nov. 2012.
- M. V. Zanni, **J. Schouten**, S. K. Grinspoon, and P. Reiss, "Risk of coronary heart disease in patients with HIV infection," *Nature Reviews Cardiology*, vol. 11, no. 12, pp. 728–741, Oct. 2014.
- **J. Schouten**, F. W. Wit, I. G. Stolte, N. Kootstra, M. van der Valk, S. G. Geerlings, M. Prins, P. Reiss, on behalf of the AGE<sub>h</sub>IV Cohort Study Group, "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," *Clinical Infectious Diseases*, Sep. 2014.
- K. W. Kooij, F. W. Wit, P. H. Bisschop, **J. Schouten**, I. G. Stolte, M. Prins, M. van der Valk, J. M. Prins, B. L. F. van Eck-Smit, P. Lips, P. Reiss, on behalf of the AGE<sub>h</sub>IV Cohort Study Group, "Low bone mineral density in patients with well-suppressed HIV infection: association with body weight, smoking, and prior advanced HIV disease," *Journal of Infectious Diseases*, vol. 211, no. 4, pp. 539–548, Feb. 2015.
- T. Su, **J. Schouten**, G. J. Geurtsen, F. W. Wit, I. G. Stolte, M. Prins, P. Portegies, M. W. A. Caan, P. Reiss, C. B. Majoie, B. A. Schmand, on behalf of the AGE<sub>h</sub>IV Cohort Study Group, "Multivariate normative comparison, a novel method for more reliably detecting cognitive impairment in HIV infection," *AIDS*, vol. 29, no. 5, pp. 547–557, Mar. 2015.
- T. Su, **J. Schouten**, G. J. Geurtsen, F. W. Wit, I. G. Stolte, M. Prins, P. Portegies, M. W. A. Caan, P. Reiss, C. B. Majoie, and B. A. Schmand, "Novel method for more reliably estimating the burden of cognitive impairment in HIV," *Experimental Gerontology*, vol. 68, p. 103, Aug. 2015.
- T. Su, M. W. A. Caan, F. N. Wit, **J. Schouten**, G. J. Geurtsen, B. Schmand, F. Vos, P. Portegies, P. Reiss, and C. B. Majoie, "White matter abnormalities

- in males with suppressed HIV-infection on cart compared to representative controls," *Experimental Gerontology*, vol. 68, p. 97, Aug. 2015.
- K. W. Kooij, F. W. Wit, **J. Schouten**, M. van der Valk, I. Stolte, and P. Reiss, "HIV infection is independently associated with frailty in middle-aged HIV-infected individuals compared to uninfected controls," *Experimental Gerontology*, vol. 68, pp. 95–96, Aug. 2015.
  - K. W. Kooij, F. W. Wit, **J. Schouten**, M. van der Valk, M. H. Godfried, I. G. Stolte, M. Prins, J. Falutz, P. Reiss, on behalf of the AGE<sub>HIV</sub> Cohort Study Group, "HIV infection is independently associated with frailty in middle-aged HIV type 1-infected individuals compared with similar but uninfected controls," *AIDS*, vol. 30, no. 2, pp. 241–250, Jan. 2016.
  - T. Su, M. W. A. Caan, F. W. Wit, **J. Schouten**, G. J. Geurtsen, J. H. Cole, D. J. Sharp, F. M. Vos, M. Prins, P. Portegies, P. Reiss, C. B. Majoie, on behalf of the AGE<sub>HIV</sub> Cohort Study, "White matter structure alterations in HIV-1-infected men with sustained suppression of viraemia on treatment," *AIDS*, vol. 30, no. 2, pp. 311–322, Jan. 2016.
  - **J. Schouten**, T. Su, F. W. Wit, N. A. Kootstra, M. W. A. Caan, G. J. Geurtsen, B. A. Schmand, I. G. Stolte, M. Prins, C. B. Majoie, P. Portegies, P. Reiss, on behalf of the AGE<sub>HIV</sub> Study Group, "Determinants of reduced cognitive performance in HIV-1-infected middle-aged men on combination antiretroviral therapy," *AIDS*, vol. 30, no. 7, pp. 1027–1038, Apr. 2016.
  - L. M. Möller, R. Brands, J. K. Sluiter, **J. Schouten**, F. W. Wit, P. Reiss, M. Prins, and I. G. Stolte, "Prevalence and determinants of insufficient work ability in older HIV-positive and HIV-negative workers," *International Archives of Occupational and Environmental Health*, vol. 89, no. 4, pp. 699–709, May 2016.
  - V. Cobos Jiménez, F. W. Wit, M. Joerink, I. Maurer, A. M. Harskamp, **J. Schouten**, M. Prins, E. M. M. van Leeuwen, T. Booiman, S. G. Deeks, P. Reiss, N. A. Kootstra, on behalf of the AGE<sub>HIV</sub> Study Group, "T-Cell Activation Independently Associates With Immune Senescence in HIV-Infected Recipients of Long-term Antiretroviral Treatment," *Journal of Infectious Diseases*, vol. 214, no. 2, pp. 216–225, Jul. 2016.
  - R. A. van Zoest, F. W. Wit, K. W. Kooij, M. van der Valk, **J. Schouten**, N. A. Kootstra, W. J. Wiersinga, M. Prins, B.-J. H. van den Born, and P. Reiss, "Higher Prevalence of Hypertension in HIV-1-Infected Patients on Combination Antiretroviral Therapy Is Associated With Changes in Body Composition and Prior Stavudine Exposure," *Clinical Infectious Diseases*, vol. 63, no. 2, pp. 205–213, Jul. 2016.
  - K. W. Kooij, F. W. Wit, R. A. van Zoest, **J. Schouten**, N. A. Kootstra, M. van Vugt, M. Prins, P. Reiss, M. van der Valk, on behalf of the AGE<sub>HIV</sub> Cohort Study Group, "Liver fibrosis in HIV-infected individuals on long-term antiretroviral therapy: associated with immune activation, immuno-



deficiency and prior use of didanosine," *AIDS*, vol. 30, no. 11, pp. 1771–1780, Jul. 2016.

- K. W. Kooij, **J. Schouten**, F. W. Wit, M. van der Valk, N. A. Kootstra, I. G. Stolte, J. T. M. van der Meer, M. Prins, D. E. Grobbee, B.-J. H. van den Born, and P. Reiss, "Difference in Aortic Stiffness Between Treated Middle-Aged HIV Type 1-Infected and Uninfected Individuals Largely Explained by Traditional Cardiovascular Risk Factors, With an Additional Contribution of Prior Advanced Immunodeficiency," *Journal of Acquired Immune Deficiency Syndromes*, vol. 73, no. 1, pp. 55–62, Sep. 2016.
- T. Su, F. W. Wit, M. W. A. Caan, **J. Schouten**, M. Prins, G. J. Geurtsen, J. H. Cole, D. J. Sharp, E. Richard, L. Reneman, P. Portegies, P. Reiss, C. B. Majoie, and AGE<sub>HIV</sub> Cohort Study, "White matter hyperintensities in relation to cognition in HIV-infected men with sustained suppressed viral load on combination antiretroviral therapy," *AIDS*, vol. 30, no. 15, pp. 2329–2339, Sep. 2016.



# PhD portfolio

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Name PhD student: Judith Schouten

PhD period: 2010-2017

Name PhD supervisors: Peter Reiss, Peter Portegies, Ferdinand Wit, Marc van der Valk

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	Year	Workload (hours/ECTS)
<b>Courses</b>		
- BROK	2010/2015	1.0
- Clinical data management	2010	0.3
- Practical biostatistics	2010	1.1
- Clinical Epidemiology 1-4	2011	2.8
- Advanced immunology	2011	2.9
Total		8.1
<b>Seminars, workshops and master classes</b>		
- Masterclass Linda Partridge	2011	0.2
- Masterclass Richard Koup	2012	0.2
- Ruysch Lectures	2010	1
- HIV Masterclass (one year course)	2013	4
- VasCog Workshop	2016	0.2
Total		5.6
<b>Presentations</b>		
- Comorbidity and aging with HIV (oral): NCHIV 2011	2011	0.5
- Comorbidity and aging with HIV(oral): IWHOD 2012	2012	0.5
- Comorbidity and aging with HIV (oral): IWADR 2012	2012	0.5
- Comorbidity and aging with HIV (oral): IAS 2012	2012	0.5
- Novel method for more reliably estimating the burden of cognitive impairment in HIV (poster): CROI 2014	2014	0.5
- Determinants of cognitive impairment in HIV-positive men on cART and uninfected controls (poster): CROI 2014	2014	0.5
- Novel method for more reliably estimating the burden of cognitive impairment in HIV (poster): NNA 2014	2014	0.5
- Determinants of cognitive impairment in HIV-positive men on cART and uninfected controls (poster): NNA 2014	2014	0.5
- Cognitive impairment in The Netherlands (oral): ARROW 2015	2015	0.5
Total		4.5

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	Year	Workload (hours/ECTS)
<b>(Inter)national conferences</b>		
- EACS (11-14 Nov 2009, Cologne, Germany)	2009	1
- CROI (25-29 Feb 2010, San Francisco, USA)	2010	1.25
- NCHIV (23 Nov 2010, Amsterdam, The Netherlands)	2010	0.25
- HANSA (26-28 May 2011, Gothenburg, Sweden)	2011	0.75
- IWADR (15-16 Jul 2011, Rome, Italy)	2011	0.50
- IAS (17-20 Jul 2011, Rome, Italy)	2011	1
- NCHIV (29 Nov 2011, Amsterdam, The Netherlands)	2011	0.25
- IWHOD (29-31 Mar 2012, Athens, Greece)	2012	01.75
- CROI (5-8 Mar 2012, Seattle, USA)	2012	0.75
- HANSA (3-5 May 2012, Hamburg, Germany)	2012	0.75
- IWADR (19-21 Jul 2012, Washington DC, USA)	2012	1.5
- IAS (22-27 Jul 2012, Washington DC, USA)	2013	1
- WHCAE (14-17 Oct 2013, Brussels, Belgium)	2013	1.25
- EACS (15-19 Oct 2013, Brussels, Belgium)	2013	0.25
- NCHIV (19 Nov 2013, Amsterdam, The Netherlands)	2013	1
- CROI (3-6 Mar 2014, Boston, USA)	2014	0.75
- HANSA (8-10 May 2014, Berlin, Germany)	2014	1.5
- NNA (27 Jul-1 Aug 2014, Bregenz, Austria)	2014	0.50
- ARROW (5-6 Oct 2015, Bucharest, Romania)	2015	16
Total		
<b>Lecturing</b>		
- Neurological Complications of HIV infection (Hogeschool van Amsterdam)	2010	1
- Neurological Complications of HIV infection (Hogeschool van Amsterdam)	2011	1
- Neurological Complications of HIV infection (HIV Consulenten Nederland Onderwijs)	2011	1
- Neurological Infectious Diseases (Amstel Academy)	2014	1
- Neurological Infectious Diseases (Amstel Academy)	2016	1
Total (max 4)		4

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gedegen wetenschappelijke en klinische vorming en onvoorwaardelijke  
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*Ferdinand Wit, Marc van der Valk*

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Amsterdamse authenticiteit en teamspirit

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Afdeling Experimentele Immunologie/Laboratorium voor Virale  
Immuun Pathogenese AMC – voor geweldige samenwerking en  
onmisbare wetenschappelijke en logistieke bijdragen

*Neeltje Kootstra, Agnes Harskamp-Holwerda, Irma Maurer, Margareta  
Mangas Ruiz, Arginell Girigorie, Brigitte Boeser-Nunnink*

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*Ben Schmand, Gert Geurtsen*

Afdeling Radiologie AMC – voor deskundigheid, enthousiasme en fantastische scans  
*Charles Majoie, Matthan Caan*

Afdeling Oogheelkunde AMC – voor een verruimende blik, prachtige beeldvorming en een altijd goed humeur  
*Frank Verbraak, Nazli Demirkaya*

Onderafdeling Vasculaire Geneeskunde AMC – voor het gulle delen van grote vasculaire en methodologische vakkennis  
*Bert-Jan van den Born*

Gehele AGE<sub>h</sub>IV studie groep – voor de vrijgeveige, constructieve en inspirerende samenwerking

Studiedeelnemers – voor bereidheid tot deelname, pionierschap en indrukwekkende levensverhalen

Co-auteurs – voor zinvolle, kritische en verdiepende inbreng

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overige neurologen, collega arts-assistenten*

Paranimfen – voor vriendschap 'hors catégorie', immer beschikbare  
steun en adviezen en al dan niet politiek incorrecte geestigheid  
*Marleen Hendriks, Marie-Hélène Hylkema-Berghuijs*

Vrienden en familie – voor grote en bijzondere harten en hoofden,  
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*Ruud en Marja Schouten, Ria en Fré Huizinga  
Martijn Schouten, Annebeth Flinterman, Dries, Guusje*

Wouter, Isolde en Nynke – de zonnen en manen aan mijn hemel,  
mijn alles





## About the author

Judith Schouten was born on February 12<sup>th</sup> 1980 in Dordrecht. In 1998 she graduated from secondary school at the Johan de Witt Gymnasium in Dordrecht. Between 1998 and 2000 she studied violin at the Conservatory of Amsterdam.

In 2000 she started her medical training at the Vrije Universiteit (VU) in Amsterdam. In 2004 she completed a scientific internship in endocrinology at the Joslin Diabetes Center, affiliated with Harvard Medical School, in Boston, USA (supervisors Prof. Horton and Prof. Heine). In 2006 she completed an elective surgical internship at the Queen Elizabeth Hospital in Blantyre, Malawi (supervisor Dr. Jiskoot). She also completed additional electives in radiology (Onze Lieve Vrouwe Gasthuis (OLVG) in Amsterdam) and neurology (Spaarne Ziekenhuis in Hoofddorp).

In 2007 she obtained her medical degree (cum laude) and started working as a resident not in training at the neurology department of the OLVG in Amsterdam (supervisor Prof. Portegies).

In 2008 she started her residency in neurology at the Academic Medical Center (AMC) in Amsterdam (supervisors Prof. Stam/Prof. Roos, Prof. Vermeulen/Prof. van Schaik, and Dr. Koelman) and the OLVG (supervisor Dr. Kwa).

In 2010 she interrupted her residency temporarily to start a PhD on the subject of treated HIV infection and ageing-associated comorbidities, at the AMC and the Amsterdam Institute for Global Health and Development (AIGHD), which led to the current thesis. The research was conducted between 2010 and 2014 within the framework of the AGE<sub>H</sub>IV Cohort Study which she assisted in designing and initiating (supervisors Prof. Reiss, Prof. Portegies, Dr. Wit, and Dr. van der Valk). In 2014 she resumed her residency in neurology, while continuing to work on the AGE<sub>H</sub>IV Cohort Study. She plans to complete her residency in 2018.

Judith lives in Nederhorst den Berg, together with Wouter Huizinga and their two daughters Isolde and Nynke.

