

Determinants of reduced cognitive performance in HIV-1-infected middle-aged men on combination antiretroviral therapy

**Short title:** Determinants of cognitive dysfunction

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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/ART-specific factors, but also other comorbid conditions. The purpose of this current study was to explore possible determinants for decreased cognitive performance.

### **Design and methods:**

Neuropsychological assessment was performed on 103 HIV-1-infected men with suppressed viraemia on cART for  $\geq 12$  months and 74 HIV-uninfected highly similar male controls, all aged  $\geq 45$  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-1-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 CD4-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.

**Key words:** HIV infection, cognitive impairment, HAND, risk factors, determinants.

Number of Tables: 3

Appendix with study group members of the AGE<sub>h</sub>IV Study Group

## **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.[1–3] 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.[4–9]

To classify this broad clinical spectrum of HIV-associated neurocognitive disorders (HAND), a set of diagnostic criteria, commonly referred to as Frascati criteria, was developed.[10] These criteria however appear oversensitive, not only resulting in 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.[11]

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>h</sub>IV 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. [11]

In the pre-cART era HIV-specific factors such as HIV viral load and CD4-count were most strongly associated with CI.[12] In cART-treated (and aging) 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.[13] 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 abovementioned AGE<sub>h</sub>IV Cohort Study population. Within this study, which investigates age-associated comorbidity among middle-aged individuals with and without HIV-1-infection, a nested substudy was established focussing 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 aging-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 been previously described.[14] 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.[11]

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-1-infected group, sustained suppression of HIV-1 viraemia on antiretroviral treatment (plasma HIV-1 RNA <40 copies/mL) for at least 12 months; the presence of so-called viral ‘blips’ (transient low-level viraemia between 40-200 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 (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.

Individuals with a previous or current diagnosis of HAD were excluded from participation as they most likely already underwent interventions (adaptation of their antiretroviral treatment for example), 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 nine-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 participation in the substudy.[15]

The in/exclusion criteria with regards to illicit drug use (allowing weekly to monthly use of cocaine or ecstasy, as well as daily cannabis use) were implemented in order to minimize selection bias and were based on illicit drug use prevalence data obtained previously 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-1-infected participants attending the HIV outpatient department and HIV-uninfected controls.[14]

### **Standard Protocol Approvals, Registrations, and Patient Consents**

The protocol of the AGE<sub>h</sub>IV Cohort Study, including the abovementioned 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 (NPA)**

As part of the substudy, NPA 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).[11] Depressive symptoms were assessed using the Beck Depression Inventory (BDI)[16], and subjective cognitive complaints with the Cognitive Failures Questionnaire (CFQ)[17]. Everyday functioning was assessed using the Instrumental Activities of Daily Living (IADL)[18] questionnaire and pre-morbid intelligence was estimated by the Dutch Adult Reading Test (DART)[19]. Use of psychotropic medication was assessed and included antidepressants, benzodiazepines, and methylphenidate.

### **Definitions**

All definitions of investigated variables are provided as footnotes in Tables 1 and 2.

## **CI diagnosis by MNC**

MNC is a statistical method that may be seen as a multivariate version of Student's t-test for one sample.[11,20] 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-1-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.[11] 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.

## **MNC: cognitive impairment as a dichotomous measure and cognitive performance as a continuous measure**

Applying MNC as described above provides a dichotomous result (CI versus no CI). The number of cognitively impaired participants in our cohort, as diagnosed by MNC, being 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-1-infected participant compared to the HIV-uninfected control group as a whole.

The direction of the deviation (better or worse cognitive performance compared to 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 non-parametric test for trend, chi-square, Fisher's exact, or Wilcoxon rank-sum test as appropriate.

Determinants for decreased cognitive performance were analyzed 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 analyzed by logistic regression. All regression analyses were restricted to the HIV-1-infected study group.

Plausible determinants of cognitive performance were analyzed using a forward stepwise model selection with  $p < 0.05$  as entry and  $p > 0.1$  as exit criterion, exploring the following categories of variables:

- demographic factors (age, premorbid IQ, educational level, Dutch as native language)
- co-infections (chronic hepatitis B/C virus co-infections)
- factors related to psychiatric comorbidity (depressive symptoms, psychotropic medication use)
- use of illicit drugs (cannabis/cocaine/ecstasy) and/or alcohol

- cardiovascular and metabolic factors (hypertension, smoking, diabetes mellitus type 2, body mass index (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)
- markers of inflammation, monocyte activation, and coagulation (high-sensitivity C-reactive protein (hsCRP), soluble CD14 (sCD14), soluble CD163 (sCD163), D-dimer)
- HIV/ART-related factors (time since HIV-1 diagnosis, HIV-1 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 (CPE) 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, Texas, USA) was used.

## **Results**

### **Participants' characteristics**

103 HIV-1-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 1. Both groups were highly comparable, with a median age of 54 in both groups, the majority of whom were men who have sex with men (MSM). HIV-1-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-count of 170 cells/mm<sup>3</sup>, current median CD4-count of 625 cells/mm<sup>3</sup>, and undetectable plasma viral load for a median 8 years.

Factors related to cognition, behaviour, comorbidity, and inflammation are presented in Table 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 (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 lipid-lowering 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 >10 mg/L were also significantly more prevalent among HIV-positives (10% vs. 0%, p=0.005). D-dimer and sCD163 levels were comparable between the two study groups.

### **CI as diagnosed by MNC**

As reported previously, using MNC, CI was detected in 17 (17%) HIV-1-infected men. Transformed Hotelling's  $T^2$  statistics of the HIV-1-infected men ranged between -2.39 and 1.90, with a median of -0.15 (IQR -0.87-+0.48).

### **Determinants of decreased cognitive performance by MNC in HIV-1-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-count to be independently associated with poorer cognitive performance (Table 3, Model 1).

### **Determinants of CI as dichotomized by MNC 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 3, Model 2).

## **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-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>h</sub>IV Cohort Study and its nested substudy are the large similarity between the HIV-1-infected and the HIV-uninfected study group, 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-1-infected and HIV-uninfected study groups 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 Caucasian 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 analyzing 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.[21,22] 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).[23,24] Besides direct effects of cannabis on cognition, the observed association could also be partly explained by some of the abovementioned 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 cognitive impairment 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.[25–35]

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.[28,31,36–38]



Additionally, 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.[38–40]

Interpreting these results, cardiovascular/metabolic factors may contribute substantially 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.[41–43]

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.[44] Among HIV-infected individuals, depressive symptoms have also been associated with decreased cognitive function[45], although one study did not report an association between cognitive function and depressive symptoms.[46]

We also found severity of prior immune deficiency, as reflected in a lower nadir CD4-count, to be associated with decreased cognitive performance, which is also consistent with earlier findings.[12,47–49]

Although HIV-infection is known to cause immune deficiency by depleting CD4-cells, it is also associated with activation of the immune system and inflammation. This is partly driven by depletion of CD4-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 which persists, albeit at a reduced level, among cART-treated HIV-infected patients with suppressed viraemia.[50,51]

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).[52] 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-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 performance in HIV-1-infected men with sustained suppressed viraemia 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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#### **Contributions made by each of the authors:**

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.

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 manuscript.

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

MC contributed to 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.

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

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

PP contributed to the study design, 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.

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ACCEPTED

## **Appendix:**

### **AGE<sub>h</sub>IV Cohort Study Group members**

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**Table 1: Baseline demographic and HIV-1-related characteristics.**

	<b>HIV- uninfected (n=74)</b>	<b>HIV-1-infected (n=103)</b>	<b>p- value</b>
Age (years)	54 (49-61)	54 (49-62)	0.94 <sup>a</sup>
MSM (%) <sup>1</sup>	90%	93%	0.48 <sup>b</sup>
Dutch origin (%)	89%	86%	0.63 <sup>b</sup>
Hepatitis C virus RNA positive (%)	0%	1%	1.00 <sup>c</sup>
Hepatitis B virus antigen and/or hepatitis B virus DNA positive (%)	0%	2%	0.51 <sup>c</sup>
Time since HIV-1 diagnosis (years)	-	13.5 (7.4-17.1)	-
Diagnosed with HIV-1 prior to 1996 (%)	-	35%	-
CD4 count at enrolment (cells/mm <sup>3</sup> )	-	625 (475-800)	-
Nadir CD4 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>2</sup>	-	8.3 (3.5-11.2)	-
Time since ART was first initiated (years)	-	11.6 (4.9-14.9)	-
Naïve at start of cART (%) <sup>3</sup>	-	80%	-
Prior clinical AIDS (%) <sup>4</sup>	-	35%	-
Use of efavirenz			
Prior use	-	47%	-
Current use	-	21%	-
Central nervous system penetration effectiveness score of current cART regimen <sup>5</sup>	-	7 (7-8)	-

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

Test used: <sup>a</sup> Wilcoxon rank-sum test, <sup>b</sup> Chi-square test, <sup>c</sup> Fisher's exact test

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

<sup>2</sup> Duration of undetectable plasma viral load was defined as: number of years since last plasma viral load >200 copies/mL.

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

<sup>4</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>5</sup> Central nervous system penetration effectiveness (CPE) score of the cART regimen of each HIV-1-infected participant was calculated using the algorithm as proposed by Letendre et al. in 2010.[53]



**Table 2: Baseline characteristics related to cognition, behaviour, comorbidity, and inflammation**

	<b>HIV-uninfected (n=74)</b>	<b>HIV-1-infected (n=103)</b>	<b>p-value</b>
Dutch as native language (%)	95%	91%	0.56 <sup>a</sup>
Education (ISCED level) <sup>1</sup>	6 (5-6)	6 (5-6)	0.50 <sup>b</sup>
Premorbid intelligence (IQ) <sup>2</sup>	103 (96-112)	101 (95-111)	0.48 <sup>c</sup>
Subjective cognitive complaints (%) <sup>3</sup>	5%	13%	0.13 <sup>a</sup>
Mild to moderate depressive symptoms (%) <sup>4</sup>	4%	6%	0.74 <sup>a</sup>
Severe depressive symptoms (%) <sup>5</sup>	0	0	-
Use of psychotropic medication (%) <sup>6</sup>	14%	16%	0.71 <sup>d</sup>
Level of daily functioning (IADL score) <sup>7</sup>	0	0	-
Weekly to monthly use of ecstasy (%) <sup>8</sup>	13%	2%	0.008 <sup>a</sup>
Weekly to monthly use of cocaine (%) <sup>8</sup>	4%	4%	1.00 <sup>a</sup>
Daily to monthly use of cannabis (%) <sup>8</sup>	15%	16%	0.96 <sup>d</sup>
Past intravenous drug use (%) <sup>8</sup>	0	0	-
Use of methamphetamine (%) <sup>9</sup>	1%	0	1.00 <sup>a</sup>
Alcohol intake (units per week)	5 (3-12)	6 (2-14)	0.89 <sup>c</sup>
Smoking status			0.048 <sup>b</sup>
Never smoked	36%	24%	
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>c</sup>
Body mass index (kg/m <sup>2</sup> )	25.4 (23.7-27.5)	24.1 (22.2-26.0)	0.003 <sup>c</sup>

Body mass index categories			0.002 <sup>b</sup>
<20 kg/m <sup>2</sup>	1.4%	9.7%	
20-<25 kg/m <sup>2</sup>	44.4%	55.3%	
25-<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>c</sup>
Waist-to-hip ratio higher than normal (%) <sup>10</sup>	70%	85%	0.02 <sup>d</sup>
HDL cholesterol (mmol/L)	1.32 (1.01-1.58)	1.27 (1.02-1.52)	0.60 <sup>c</sup>
LDL cholesterol (mmol/L)	3.35 (2.80-3.84)	3.25 (2.39-3.79)	0.26 <sup>c</sup>
Total cholesterol (mmol/L)	5.39 (5.07-6.15)	5.40 (4.57-6.22)	0.37 <sup>c</sup>
Lipoprotein(a) (mg/L)	86 (46-205)	87 (43-324)	0.57 <sup>c</sup>
Triglycerides (mmol/L)	1.61 (1.09-2.43)	1.85 (1.20-2.81)	0.29 <sup>c</sup>
Use of lipid-lowering medication (%)	11%	12%	0.91 <sup>d</sup>
Statins (%)	11%	11%	0.90 <sup>d</sup>
Fibrates (%)	0%	2%	0.51 <sup>a</sup>
Physical activity (%) <sup>11</sup>	46%	47%	0.87 <sup>d</sup>
Positive family history for myocardial infarction, hypertension, diabetes mellitus type 2, or hypercholesterolemia (%) <sup>12</sup>	66%	64%	0.74 <sup>d</sup>
Diabetes mellitus type 2 (%) <sup>13</sup>	4%	6%	0.74 <sup>a</sup>
Hypertension (%) <sup>14</sup>	38%	39%	0.86 <sup>d</sup>
Renal function class, by albumin-to-creatinine ratio (ACR)			0.01 <sup>b</sup>
Normal (<3 mg/mmol)	94.2%	80.9%	
Moderately impaired (3-30 mg/mmol)	5.8%	19.2%	

Severely impaired (>30mg/mmol)	0%	0%	
Renal function class, by estimated glomerular filtration rate (eGFR)			0.76 <sup>b</sup>
Normal (≥90 ml/min)	54.9%	50.5%	
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%	
Cardiovascular disease (%) <sup>15</sup>	6%	8%	0.76 <sup>a</sup>
Angina pectoris (%)	1%	5%	0.40 <sup>a</sup>
Myocardial infarction (%)	3%	2%	1.00 <sup>a</sup>
Peripheral arterial disease (%)	1%	3%	0.65 <sup>a</sup>
hsCRP (mg/L)	1.1 (0.6-2.1)	1.5 (0.7-3.3)	0.02 <sup>c</sup>
hsCRP >10 mg/L (%)	0%	10%	0.005 <sup>a</sup>
D-dimer (mg/L)	0.27 (0.20-0.40)	0.21 (0.20-0.33)	0.06 <sup>c</sup>
D-dimer >0.5 mg/L (%)	15%	10%	0.27 <sup>d</sup>
sCD14 (ng/mL)	1207 (995-1558)	1548 (1318-2025)	<0.001 <sup>c</sup>
sCD163 (ng/mL)	242 (186-343)	273 (205-417)	0.12 <sup>c</sup>

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

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

Chi-square test

<sup>1</sup> Educational level was defined using the International Standard Classification of Education (ISCED) 2011.

<sup>2</sup> Premorbid intelligence quotient (IQ) was estimated using the Dutch Adult Reading Test (DART). One of in total 74 HIV-uninfected controls and 6 of in total 103 HIV-1-infected individuals were unable to complete this test due to dyslexia.

<sup>3</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. Among HIV-1-infected participants with cognitive impairment as diagnosed by MNC (n=17, 17%), 4 participants (24%) fulfilled the criteria for experiencing subjective cognitive complaints and 13 participants did not (76%).

<sup>4</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-1-infected individuals did not complete this test.

<sup>5</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-1-infected individuals did not complete this test.

<sup>6</sup> Psychotropic medication included: antidepressants, benzodiazepines, methylphenidate.

<sup>7</sup> Level of day-to-day functioning was defined using the Independent Activities of Daily Living (IADL) questionnaire.

<sup>8</sup> 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).

<sup>9</sup> Participants were asked by questionnaire about any illicit drug use (type and frequency) during the 6 months prior to completing the questionnaire. Use of methamphetamine was reported by only a single HIV-1-infected participant. This participant reported to have used methamphetamine sporadically (less than once a month) during the 6 months prior to

completing the questionnaire. This participant was not diagnosed with cognitive impairment by MNC.

<sup>10</sup> The waist-to-hip ratio was considered higher than normal if it was  $\geq 0.9$ .<sup>[54]</sup>

<sup>11</sup> Physical activity was defined following the Dutch guidelines for healthy physical activity ('Combinorm'): at least 5 days per week at least 30 minutes of moderate physical activity or at least twice per week at least 20 minutes of heavy physical activity.<sup>[55]</sup>

<sup>12</sup> Participants were considered to have a positive family history for myocardial infarction/hypertension/diabetes mellitus type 2/hypercholesterolemia when they stated in the questionnaire to have a first degree family member that experienced a myocardial infarction before the age of 60, or to have a first degree family member suffering from hypertension, diabetes mellitus type 2, or hypercholesterolemia.

<sup>13</sup> Diabetes mellitus type 2 was considered present if HbA1c (IFCC)  $\geq 48$  mmol/mol and/or elevated blood glucose (non-fasting  $\geq 11.1$  mmol/L or fasting  $\geq 7.0$  mmol/L), or if on antidiabetic medication.<sup>[56]</sup>

<sup>14</sup> Hypertension was considered present if diastolic blood pressure  $\geq 90$  mmHg and/or systolic blood pressure  $\geq 140$  mmHg in all three measurements (Omron 705IT) with a one-minute interval, or if on antihypertensive medication.<sup>[57]</sup>

<sup>15</sup> 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>

**Table 3: Determinants for cognitive performance/impairment as determined by MNC**

	<b>Model 1</b>		<b>Model 2</b>	
	<b>Continuous outcome measure:</b> <b>Decreased cognitive performance</b> <b>as determined by MNC</b>		<b>Dichotomous outcome measure:</b> <b>Cognitive impairment</b> <b>as determined by MNC</b>	
	<b>Beta coefficient</b> <b>95% CI</b>	<b>P-value</b>	<b>Odds ratio</b> <b>95% CI</b>	<b>p-value</b>
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>1</sup>	-0.64 -1.32, 0.04	0.066	17.71 1.81, 173.42	0.014
Impaired renal function (y/n) <sup>2</sup>	-0.36 -0.79, 0.07	0.096	8.76 1.47, 52.36	0.017
Diabetes mellitus type 2 (y/n) <sup>3</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>4</sup>	-0.46 -0.93, 0.01	0.055	-	-
Presence of depressive symptoms (y/n) <sup>5</sup>	-0.69 -1.42, 0.03	0.061	-	-
Nadir CD4-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-1-infected study group.

Abbreviations: 95% CI=95% confidence interval, MNC=multivariable normative comparison.

<sup>1</sup> The variable past cardiovascular disease included angina pectoris, myocardial infarction, and peripheral arterial disease.

<sup>2</sup> Impaired renal function as determined by albumin-to-creatinine ratio in urine of  $\geq 3$  mg/mmol.

<sup>3</sup> Diabetes mellitus type 2 was considered present if HbA1c (IFCC)  $\geq 48$  mmol/mol and/or elevated blood glucose (non-fasting  $\geq 11.1$  mmol/L or fasting  $\geq 7.0$  mmol/L), or if on antidiabetic medication.

<sup>4</sup> The waist-to-hip ratio was considered higher than normal if it was  $\geq 0.9$ .

<sup>5</sup> Depressive symptoms were considered present with a BDI-score  $> 13$ .