Title: White Matter Hyperintensities in Relation to Cognition in HIV-infected Men with Sustained Suppressed Viral Load on cART (max. 120 characters; now 119)

Running head: White Matter Hyperintensities in HIV (max. 40 characters; now 37)

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Abstract:

Objectives: The objective of this study was to assess whether HIV-infected patients on long-term successful combination antiretroviral therapy (cART) have more extensive white matter hyperintensities (WMH) of presumed vascular origin compared with uninfected controls and whether these intensities are associated with cognitive impairment. Furthermore, we explored potential determinants of increased WMH load in long-term suppressed HIV infection.

Design: A cross-sectional comparison of WMH in an observational cohort.

Methods: Clinical, cognitive and magnetic resonance imaging data were collected from 103 middle-aged, aviremic HIV-infected men on cART and 70 HIV-uninfected, otherwise similar controls. In the MRI data, WMH load was quantified by automated approaches and qualitatively reviewed by an experienced neuroradiologist using the Fazekas scale.

Results: HIV-infected men had an increased WMH load. Among HIV-infected patients, increased WMH load was independently associated with older age, higher diastolic blood pressure and D-dimer levels, and longer time spent with a CD4 count below 500 cells/mm³. HIV-associated cognitive deficits were associated with increased WMH load.

Conclusions: WMH are more extensive and associated with cognitive deficits in middle-aged, aviremic cART-treated HIV-infected men. The extent of WMH load was associated with both cardiovascular risk factors and past immune deficiency. Since cognitive impairment in these same patients is also associated with these risk factors, this may suggest that in the setting of HIV, WMH and cognitive deficits share a common etiology. This supports the importance of optimizing cardiovascular risk management, and early, effective treatment of HIV infection.

Key words: HIV-1-infection; antiretroviral therapy; aging; HIV-associated cognitive impairment; white matter hyperintensities; cerebral small vessel disease

Introduction

The incidence of HIV-associated dementia and other severe HIV-related central nervous system (CNS) complications has decreased dramatically with the introduction of combination antiretroviral therapy (cART).[1–4] However, cognitive impairment remains common, even among HIV-infected patients with systemically well-suppressed HIV replication on cART.[5–8] Such HIV-associated cognitive impairment is characterized by a wide range of deficits

regarding attention, memory, information processing and executive function,[9] impacting quality of life.[10] The pathogenesis of this cognitive impairment is poorly understood, and the deficits may affect medication adherence, jeopardizing long-term successful suppression of HIV-infection and immune restoration by cART.[11] Furthermore, as the HIV-infected population ages and the proportion of older HIV-infected patients continues to increase, cognitive impairment may be further accentuated by age-related cognitive decline.[7]

HIV-infected patients on long-term cART are at increased risk of cardiovascular disease (CVD), particularly those infected and treated for a long period.[12–14] Exposure to some, but not all, cART regimens has been associated with an increased incidence of clinical CVD events as well as increases in CVD risk factors such as hypertension, dyslipidemia and diabetes.[15–18] CVD risk may be further increased by certain lifestyle factors (e.g., smoking) and aging of the HIV-infected population.[19] Furthermore, the risk of cerebral small vessel disease (cSVD) may also be increased in HIV.[15,20] In HIV-uninfected elderly individuals, white matter hyperintensities (WMH), detected using MRI, are thought to reflect ischemic consequences of hypoperfusion [21] or consequences of leakage of fluid into the brain parenchyma as a result of blood-brain-barrier (BBB) dysfunction [22], and have been associated with cognitive impairment.[23] So far, only two studies have specifically studied WMH of presumed vascular origin in HIV-infected patients during the cART era, hereafter referred to simply as WMH.[24,25] These studies reported that WMH were commonly observed in HIV-infected patients. However, as these studies did not include HIV-uninfected controls it is unclear whether their findings were a specific effect of HIV or merely related to aging.

The purpose of the current study was to examine the prevalence and sequelae of WMH in HIV. Using a well-defined cohort of middle-aged HIV-infected patients with durable virological suppression on cART and HIV-uninfected controls with a similar geographic and sociodemographic background and lifestyle, we investigated how WMH relates to HIV-associated cognitive impairment and other potential biological or clinical determinants of cSVD. We hypothesized that WMH may be more extensive in HIV-infected patients compared to controls and that the extent of WMHs would be related to cognitive performance in middle-aged HIV-infected men.

Material and methods

Study Population

HIV-infected patients and HIV-uninfected but otherwise similar controls aged 45 years or older with comparable sociodemographics and behavioral risk factors from the AGE_hIV cohort study were consecutively invited to participate in a nested neuroimaging sub-study[14]. Specific inclusion criteria for the sub-study were: male gender and, for the HIV-infected patients, sustained suppression of HIV viremia on cART (plasma HIV RNA <40 copies/mL) for ≥ 12 months, though transient low-level viremia (40-200 copies/mL) was permissible. Specific exclusion criteria were current or past severe neurological disorders including traumatic brain injury (i.e., with loss of consciousness >30 minutes), diagnosis of (HIV-associated) dementia and (HIV-associated) CNS infections or tumors. Other reasons for exclusion were current injecting drug use, daily use of non-injecting illicit drugs with the exception of daily cannabis use, excessive alcohol consumption (i.e., >48 units of alcohol/week) and significant psychiatric disorders. Finally, subjects with insufficient command of the Dutch language to undergo a neuropsychological assessment, intellectual disability and MRI contraindications were also excluded.

Standard Protocol Approvals, Registrations, and Patient Consent

The parent AGE_hIV cohort study and the sub-study were both approved by the institutional review board of the Academic Medical Center and have been registered at www.clinicaltrials.gov (identifier: NCT01466582). Written informed consent was obtained separately from all participants for the parent study and sub-study.

Clinical Parameters

Participants completed a standardized questionnaire on demographics, lifestyle factors and medical characteristics. Standardized screening for aging-associated comorbidity and organ dysfunction was performed and blood and urine samples were collected for laboratory testing. Detailed information concerning HIV and cART history was obtained. The details can be found in a previous publication.[14] To identify determinants of WMH, candidate variables (listed in the statistical analyses section) were selected from the parent study and their effects on WMH burden were examined.

Neuropsychological Assessment

All subjects underwent comprehensive neuropsychological assessment, covering six domains (see Supplementary Table 2). A detailed overview of the test battery and normative standards used can be found in a previous publication.[8] Cognitive impairment was identified by multivariate normative comparison (MNC), a statistical method designed to control the false-positive rate, while retaining sensitivity.[26] MNC identifies HIV-infected patients as cognitively impaired if their cognitive profile significantly deviates from those of the HIV-uninfected control group.

Imaging Protocol

MRI was performed using two Philips 3T scanners (Intera and Ingenia systems, Philips Healthcare, Best, the Netherlands) due to a scanner upgrade midway through the study. The numbers of patients and controls scanned on the two systems were similar (Table 2). The scanning protocol included a three-dimensional T₁-weighted MPRAGE sequence, 1.1x1.1x1.2mm² resolution, a three-dimensional fluid-attenuated inversion recovery (3D-FLAIR) sequence, 1x1x1.12mm² resolution, and T₂-weighted fast spin-echo (FSE) and fast low angle shot (FLASH) sequences.

Automated Image Processing

The workflow of WMH segmentation is illustrated in Figure 1A. Some examples of WMH segmentation are shown in Figure 1B. WMH segmentation was based on relative 3D-FLAIR intensity, an anatomical prior, and white matter probability. To train a classifier, an independent manual annotation was made on a set of 20 hypertensive individuals between 72 and 80 years old with varying lesion load. An anatomical prior was included using the normalized spatial coordinates of the annotated maps.[27] A random forest classifier was then trained,[28] consisting of an ensemble of 200 decision trees. This classifier outputs a probabilistic WMH map. A probability threshold value of 90% was chosen, which maximized the average dice coefficient of the computed and manually annotated WMH maps. The trained classifier was then applied to all subjects' data to obtain WMH maps. Periventricular- and deep WMH maps were then generated.[29] WMH loads relative to the intracranial volumes were computed and log transformed to obtain a normal distribution.

Expert Visual Image Assessment

A neuro-radiologist (CBM: 15 years of experience), blinded to all clinical parameters, visually inspected all MRI examinations. WMH, lacunes and microbleeds were assessed according to the standards for reporting vascular changes on neuroimaging (STRIVE) recommendations.[30] WMH was rated qualitatively on axial FLAIR images using the 4-point (0-3) Fazekas rating scale,[31] with the burden of periventricular- and deep WMH assessed separately. Lacunes and microbleeds were recorded as either present or absent. Twenty-three images were randomly selected and rated twice, by the same neuro-radiologist (CBM), to determine the intra-observer reliability.

Statistical Analyses

Statistical analyses were performed in SPSS (IBM SPSS Statistics for Windows, Version 20.0., IBM Corp., Armonk, NY, USA). Intra-observer reliability was assessed by Cohen's weighted kappa coefficients.

Group comparison of clinical characteristics and neuroimaging findings by visual rating between HIV-infected patients and HIV-uninfected controls were performed using Chi-square or Fisher's exact tests for dichotomous and categorical variables and Student's T and Mann-Whitney U tests for continuous variables.

Two-way ANOVAs were performed to examine effects of HIV serostatus, age and scanner system on WMH load. Interaction effects of HIV serostatus and age on WMH load were evaluated in separate two-way ANOVAs, in which the variables of HIV serostatus, age and scanner system were also included.

Possible confounders of WMH load were identified by a stepwise regression model selection approach with $p < 0.05$ probability to enter and $p > 0.1$ probability to remove, in which the variables of HIV serostatus, age and scanner system were constantly included in the model. Variables from the following categories were selected and examined.

- Intoxicants: reported cannabis use on daily to monthly basis, reported cocaine or ecstasy use on weekly to monthly basis and the average units of alcohol consumed per week.
- Vascular, metabolic and other comorbidities and its risk factors: past cardiovascular disease (including angina pectoris, myocardial infarction and peripheral arterial disease), hypertension, use of anti-hypertensives, systolic and diastolic blood pressure,

vascular stiffness based on pulse wave velocity, smoking (pack-years), diabetes mellitus type 2, hemoglobin A1c, body mass index (BMI), waist-to-hip ratio, total, high, and low density lipoprotein cholesterol, triglycerides, lipoprotein a, use of lipid lowering medication, renal disease, use of psychotropic medication and hepatitis B/C virus coinfections.

- Biomarkers of inflammation (high-sensitivity C-reactive protein (hs-CRP)), macrophage / monocyte activation and microbial translocation (soluble CD14 and CD163), coagulation activation (D-dimer), and the time spent with a CD4 count below 500. Until recently, this value was used as the threshold value below which there is an indication to start cART, and is the lower boundary of the normal range of CD4 count.

To identify additional HIV-related and treatment-related determinants of WMH load, the following variables were additionally examined in the HIV-infected patients: known duration of HIV-infection, being treatment-naïve at the start of cART, duration of ART use, duration/degree of immune deficiency, duration of having detectable or undetectable plasma viral load, prior AIDS diagnosis and current/prior/duration of/use of individual antiretroviral agents.

Cognitive impairment was identified by MNC.[26] The test statistic of the MNC method is the Hotelling's T^2 statistic, which was transformed to create a continuous measure of cognitive function.[32] Regression analyses were performed to assess effects of HIV serostatus, WMH load and age on cognitive function. Previously identified determinants of cognitive function were included, i.e. reported cannabis use on daily to monthly basis, prior diagnosis of cardiovascular disease (including angina pectoris, myocardial infarction and peripheral arterial disease), diabetes mellitus type 2, having an abnormal waist-to-hip ratio (≥ 0.9), and presence of depressive symptoms (Beck Depression Inventory score between 13 and 29 reflecting mild-to-moderate depressive symptoms).[32,33] An interaction effect of HIV serostatus and adjusted WMH load on cognitive function was evaluated in separate two-way ANOVA. Explorative MANOVAs were performed to determine relationships between domain-specific aspects of cognitive performance and WMH load within the HIV-infected population.

Results

Clinical Characteristics

103 HIV-infected patients and 74 HIV-uninfected controls were enrolled between December 2011 and August 2013. Neuroimaging data were not available for four controls. An overview of the clinical characteristics is provided in Table 1. The group of patients (median age: 54 (IQR 49-61) years) was highly comparable to the control group. However, ecstasy use (on a weekly to monthly basis) was more common in controls, while greater lifetime tobacco exposure was found in patients. Patients had higher levels of hemoglobin A1c, hs-CRP and soluble CD14, and lower CD4 over CD8 ratios compared to controls. HIV-infected patients also had a lower BMI, although they were more likely to have a high waist-to-hip ratio (>0.9). HIV disease and treatment characteristics are listed in Table 1. Cognitive impairment was identified by MNC in 17% of the HIV-infected patients, none of whom had HIV-associated dementia.

Prevalence Of WMH

WMH load was 1.0ml in HIV-infected patients and 0.7ml in healthy controls. HIV serostatus and age were found to have significant main effects on total WMH load ($p=0.008, \eta^2=0.04$; $p<0.001, \eta^2=0.29$, respectively), with HIV infection and older age being associated with increased WHM. Full details of neuroimaging measures are presented in Table 2. Effect of scanner type on total WMH load was non-significant ($p>0.1$). There was no evidence for statistical interaction effects of age and HIV serostatus on total WMH load ($p=0.42$). A significantly higher proportion of HIV-infected patients had more visible periventricular WMH, but not deep WMH, as assessed by the Fazekas score compared to controls ($p=0.045$, Cramers $V=0.22$) (see also Table 2). In both groups, the Fazekas scores increased with older age (see Supplementary Figure 1). However, group differences in Fazekas score within each age category were all non-significant (results not shown). The number of lacunes and microbleeds did not differ significantly between HIV-infected patients and controls (Table 2). For the Fazekas scale, intra-observer agreement was 83% and 91%, with a Cohen's weighted kappa of 0.76 and 0.92 for rating periventricular and deep WMH, respectively.

Determinants of WMH

Using regression analysis, higher diastolic blood pressure and D-dimer levels were significantly associated with increased total WMH load ($p=0.008, \eta^2=0.04$; $p=0.004, \eta^2=0.05$, respectively), while HIV-seropositive status and age also remained significantly associated (see Table 3, model 1). No other confounders or determinants of total WMH load were identified.

In the HIV-infected patients only, longer time spent with a CD4 count below 500 cells/mm³ was found to be significantly associated with increased total WMH load ($p=0.009, \eta^2=0.07$), independent of age, diastolic blood pressure and D-dimer levels ($p<0.001, \eta^2=0.26$; $p=0.02, \eta^2=0.06$; $p=0.02, \eta^2=0.05$, respectively), see Table 3, model 2.

We did not find any association between total WMH load and either current or prior (duration of) use of particular (classes of) antiretroviral drugs (results not shown). No associations were observed between total WMH load and markers of innate immune activation (e.g., sCD163, sCD14). No other HIV-related determinants of total WMH load were identified.

HIV, WMH and Cognition

HIV seropositive status was significantly associated with poorer cognitive function ($p=0.03, \eta^2=0.03$), also after adjusting for other possibly impacting factors ($p=0.03, \eta^2=0.03$). When adding WMH load to the model, the effect of HIV serostatus was attenuated ($p=0.07, \eta^2=0.02$). Moreover, higher total WMH load was significantly associated with poorer global cognitive function. After adjusting for age, diabetes, diastolic blood pressure and D-dimer levels, no interaction effect of HIV serostatus with total WMH load on cognitive function was found ($p>0.1$).

Poorer performances on the fluency domain were significantly associated with increased total WMH load in HIV-infected patients ($p=0.03$). Of the remaining cognitive domains assessed, no associations with total WMH load were found, see Supplementary Table 2.

Discussion

Main Findings

Both quantitative and qualitative assessments of white matter hyperintensities (WMH) showed that WMH were more prevalent in middle-aged HIV-infected patients with durable viral suppression on cART than in HIV-uninfected, otherwise similar, controls. In addition to HIV-seropositive status, older age, higher diastolic blood pressure and D-dimer levels were associated with increased WMH burden. Furthermore, among HIV-infected patients, longer time spent with reduced CD4 count was also associated with increased WMH burden. Finally, more extensive WMH was also associated with HIV-associated cognitive deficits.

Interpretation

Although the present finding of increased WMH burden in HIV-infected patients is in agreement with findings from earlier studies, the patient populations in these earlier studies were different.[24,25] In particular, earlier studies included severely immunocompromised patients with detectable HIV viral loads, some of whom had neurological signs and symptoms. Therefore, these earlier findings could not be generalized to the HIV-infected patients included in our study, who have sustained suppressed viral load and immune recovery on cART. Moreover, these previous studies lacked appropriate control groups and whether HIV-seropositive status was independently associated with the extensive WMH burden observed remained unknown. By including a comparison with HIV-uninfected, but otherwise similar, controls, we were able to demonstrate an independent effect of HIV-seropositive status on increased WMH burden among aviremic HIV-infected patients on cART.

The radiological appearance, location and pattern of WMH in HIV-infected patients was similar to that seen in the controls and also corresponds closely to WMH seen in HIV-infected patients with vascular cognitive impairment.[23] This suggests that the WMH observed in HIV-infected patients may reflect the ischemic consequences of cSVD, which is thought to be among the main causes of vascular cognitive impairment. In contrast, HIV encephalopathy has a different radiological appearance and is not expected to occur in the HIV-infected patients in our study who were relatively immunocompetent, had long-term suppressed viral load on cART and had no acute or sub-acute neurological signs and symptoms.[34]

Previous studies support the concept that the increased WMH burden found in HIV-infected patients has a vascular origin and suggest an increased risk of cSVD in HIV. For example, research in immunocompromised and less effectively-treated HIV-infected patients found WMH to be associated with older age, systolic blood pressure, hypertension, diabetes and past cardiovascular events.[24,25] Moreover, older age, hypertension and dyslipidemia, low CD4 count and detectable HIV viral load have also been associated with a moderately increased risk of stroke.[35] Furthermore, in HIV-uninfected individuals, higher levels of the coagulation marker D-dimer have been associated with cSVD.[36] This argument is further supported by the report of small vessel pathology that closely resembles arteriolosclerosis of cSVD in HIV-infected patients on protease inhibitor-based cART regimens.[20,23]

The mechanisms underlying the increased risk of cardiovascular disease and cSVD in HIV remain unclear, but may originate from HIV-associated vasculopathy.[37] This refers to (peri)vascular changes mediated by direct infection of the vessel walls by HIV,[38] and indirect HIV-mediated pro-inflammatory responses of the vessel walls.[39] Such vessel wall inflammation may be further exacerbated by direct toxicity of cART or indirectly by cART-related cardiovascular complications.[40] On the other hand, It is possible that most of the vasculopathy may also occur have developed during the period between identification of HIV infection and initiation of effective antiretroviral therapy (i.e., a ‘legacy effect’). This hypothesis appears to be supported by our observation that the extent of WMH was associated with older age and with the time spent with reduced CD4 counts.

Despite the numerous arguments to support the vascular origin of the WMH, we cannot rule out that damage to the brain parenchyma was caused by factors associated with the direct effects of HIV or by immune responses to the virus. For example, the association between increased WMH burden and time spent with reduced CD4 observed in our study may reflect irreversible white matter injury. Appearing as WMH, this injury would have occurred during periods of immune deficiency when viral toxicity and host-inflammatory responses were at their peak. In addition, low-level viral replication and persistent inflammation within the brain of HIV-infected patients cannot be ruled out either, but is unlikely given that the CSF viral load is generally <40 copies/mL on modern cART regimens and decreases in parallel with the decline of plasma viral load. Moreover, we did not find any association of WMH burden with soluble serum markers of innate immune activation, which has been suggested to be related to CNS markers of immune activation.[41]

Thus far, reports of a relationship between WMH and HIV-associated cognitive impairment have been inconsistent.[42,43] Our study helps clarify this relationship by showing that the significant effect of HIV serostatus on cognitive function was attenuated when taking WMH load into account suggesting WMH may mediate the association between HIV seropositivity and poorer cognitive function, and that WMH load may serve as a biomarker for cognitive impairment in HIV-infected patients with systemically well-controlled HIV infection on cART.

Implications

We found an association between the time spent with reduced CD4 count and increased WMH burden in aviremic HIV-infected patients on cART. This underscores the importance of early detection of HIV infection and initiation of cART in all HIV-infected patients irrespective of CD4 count to prevent immune deficiency, in line with the recommendations of current HIV treatment guidelines.[44] This should improve the general clinical outcome of HIV-infected patients, reduce HIV transmission risk and potentially also reduce the development of WMH and cognitive impairment. Our results also suggest that clinicians should approach HIV-infected patients similarly to HIV-uninfected individuals at increased risk for vascular disease. This is based on the assumption that WMH represent ischemic consequences of cSVD, and on the association of WMH with increased risk of stroke, dementia and death.[45] Such an approach should include prompt and detailed screening and treatment of vascular risk factors as well as promoting a healthy lifestyle.[46]

Strengths/Limitations

The strength of the current study is the inclusion of HIV-uninfected, otherwise similar controls. We also had access to a broad range of clinical factors, which allowed us to probe their relationships with WMH, as well as to examine the independent relationship between WMH and HIV. Our high-resolution MRI protocol and automated WMH segmentation method enabled us to identify an association with HIV-seropositive status and older age, and a relation with subtle cognitive deficits. The burden of WMH found in HIV-infected patients, albeit higher than in the controls, was mild. This is a clinically important positive message, but restricted us from making strong implications. As the aviremic HIV-infected population on cART is aging, the subject of cSVD could become a more important point of research and

clinical care in the context of HIV. Although the HIV-infected patients and HIV-uninfected controls studied were highly comparable, certain vascular risk factors were more prevalent within the HIV-infected patient group, albeit not significantly. The same is true for the prevalence of lacunes, which was more than twice as high in the HIV-infected group than in the control group, but not statistically significantly different, possibly due to our limited sample size. Furthermore, this study was limited by the inclusion of only male participants, most of whom were Caucasians, reflecting the majority of our cohort.

Conclusion

MRI findings of WMH of presumed vascular origin are more extensive and associated with cognitive impairment in middle-aged HIV-infected patients with durable viral suppression on cART compared to healthy controls. The burden of WMH was associated with both cardiovascular risk factors and past immune deficiency. These findings suggest the relevance of WMH MRI findings in HIV, which could reflect an increased risk of cSVD, and the importance of optimizing cardiovascular risk management and of early, effective treatment of HIV infection. Longitudinal studies are warranted to monitor progression of WMH and its relationship with cognitive impairment, to reveal its overt clinical significance and further implications.

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Author Contributions:

Ms. Su: contributed to the study design, was responsible for the data collection, data analysis and interpretation and conception of the manuscript. Dr. Wit: contributed to the study design, supervised data analysis, reviewed and approved the final manuscript. Dr. Caan: contributed to the study design, supervised MRI data acquisition, processed the MRI data, contributed to data analysis and interpretation, and reviewed and approved the final manuscript. Ms. Schouten and Dr. Stolte contributed to the study design, the data collection, data interpretation, and reviewed and approved the final manuscript. Dr. Geurtsen, contributed to the study design, supervised neuropsychological data acquisition, contributed to data interpretation, and reviewed and approved the final manuscript. Dr. Cole and Prof. Sharp contributed to the study design, data interpretation, and reviewed and approved the final manuscript. Dr. Richard contributed to the study design, the interpretation of the data, and reviewed and approved the final manuscript. Dr. Reneman, contributed to the study design, data interpretation, and reviewed and approved the final manuscript. Prof. Portegies contributed to the study design, data interpretation, and reviewed and approved the final manuscript. Prof. Reiss conceived the main cohort study and the sub-study, contributed to both study designs, to data interpretation, and reviewed and approved the final manuscript. Prof. Majoie conceived the sub-study, contributed to its design, assessed all MRI scans by

visual rating, contributed to data interpretation and revised all drafts, and reviewed and approved the final manuscript.

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Table 1: Clinical characteristics

	HIV-uninfected controls (n=70)	HIV-infected patients (n=103)	p
<i>Demographics</i>			
Age (years)	53 (49-59)	54 (49-61)	0.81 ^d
“Men having sex with men” ¹	90	93	0.41 ^b
Premorbid intelligence ² (intelligence quotient)	103 (96-112)	101 (95-112)	0.68 ^c
<i>Intoxicants</i>			
Cannabis use ³	15	16	1.00 ^b
Cocaine use ³	4	4	0.86 ^a
Ecstasy use ³	12	2	0.02^b
Alcohol intake (units per week)	5 (3-12)	6 (2-14)	0.75 ^d
<i>Vascular risk factors</i>			
Smoking tobacco (pack-years)	2.3 (0.0-11.8)	9.0 (0.0-31.6)	0.005^d
Vascular stiffness ⁴ (m/s)	7.8 (7.2-8.9)	7.9 (7.2-8.8)	0.41 ^d
Systolic blood pressure (mmHg)	134 (128-145)	136 (128-147)	0.38 ^d
Diastolic blood pressure (mmHg)	81 (75-86)	82 (77-88)	0.35 ^d
Hemoglobin A1c (mmol/L)	37 (35-41)	35 (32-39)	0.01^d
Body Mass Index (score)	26 (24-28)	24 (22-26)	0.001^d
Waist-to-hip ratio >0.9	72	85	0.03^a
Cholesterol (mmol/L)	5.4 (5.1-6.2)	5.5 (4.6-6.2)	0.41 ^d
High density lipoprotein cholesterol (mmol/L)	1.3 (1.0-1.6)	1.3 (1.0-1.5)	0.91 ^c
Low density lipoprotein cholesterol (mmol/L)	3.4 (2.9-3.9)	3.3 (2.4-3.8)	0.20 ^c
Triglycerides (mmol/L)	1.6 (1.1-2.4)	1.9 (1.2-2.8)	0.23 ^d
Lipoprotein(a) (mmol/L)	0.7 (0.6-0.9)	0.8 (0.6-0.9)	0.11 ^d
<i>Comorbidities</i>			
Diabetes mellitus type 2 ⁵	4	6	0.65 ^a
Hypertension ⁶	36	40	0.59 ^a
Past cardiovascular disease ⁷	6	8	0.76 ^b
Renal disease ⁸	4	0	0.06 ^b
Past non-AIDS cancer	7	7	0.89 ^a
Current chronic Hepatitis B virus infection	0	2	0.20 ^a

Current chronic/active hepatitis C virus infection	0	1	1.00 ^b
<i>Medication use</i>			
Antihypertensive medication	15	17	0.63 ^a
Lipid-lowering medication ⁹	11	11	1.00 ^b
Psychotropic medication ¹⁰	11	15	0.82 ^a
<i>Biomarkers</i>			
High sensitivity C-reactive protein (mg/L)	1.1 (0.6-2.2)	1.5 (0.7-3.4)	0.02^d
CD4 ⁺ /CD8 ⁺ T cell count ratios	1.7 (1.3-2.3)	0.7 (0.5-1.0)	<0.001^d
Soluble CD14 (ng/mL)	1196 (992-1509)	1548 (1318-2025)	<0.001^d
Soluble CD163 (ng/mL)	242 (182-356)	273 (205-417)	0.15 ^d
D-dimer (mg/L)	0.26 (0.20-0.40)	0.21 (0.20-0.33)	0.13 ^d
<i>HIV and treatment related factors</i>			
Duration known HIV infection (years)	-	13.5 (7.4-17.1)	-
Duration since start of first ART (years)	-	11.6 (4.9-14.9)	-
Naive at start of cART ¹¹	-	80	-
Protease inhibitors	-	47	-
Duration of undetectable plasma viral load ¹² (years)	-	10.4 (4.2-13.5)	-
Duration of detectable plasma viral load ¹³ (years)	-	2.3 (0.9-5.2)	-
Current CD4 ⁺ T-cell count (cells/mm ³)	-	625 (475-800)	-
Nadir CD4 ⁺ T-cell count (cells/mm ³)	-	170 (60-250)	-
Duration CD4 ⁺ T-cell counts < 500 cells/mm ³ (years)	-	3.9 (1.7-6.9)	-
Prior AIDS ¹⁴	-	35	-
Cognitive impairment	-	17	-

Data presented as percentages or as median and IQR

^aChi-square test, ^bFisher's exact test, ^cStudents T-test, ^dMann-Whitney U test. ¹Premorbid intelligence quotient was estimated using the Dutch adult reading test.

²The term "Men having Sex with Men" applied to male participants who stated in the questionnaire that they felt mostly or exclusively sexually attracted to men.

³Cannabis, cocaine and ecstasy use were assessed by a questionnaire as either absent (i.e. "none") or present ("i.e. "daily", "weekly" or "monthly" basis).

⁴Vascular stiffness: Pulse wave velocity as measured by Arteriograph®

⁵Diabetes mellitus type 2 was considered present if hemoglobin A1c (IFCC) ≥ 48 mmol, or elevated blood glucose (non-fasting ≥ 11.1 mmol/L or fasting) ≥ 7.0 mmol/L), or if on antidiabetic medication.

⁶Hypertension was considered if diastolic blood pressure ≥ 90 mmHg or systolic blood pressure ≥ 140 mmHg, or if on antihypertensive medication.

⁷The variable past cardiovascular disease included angina pectoris, myocardial infarction and peripheral arterial disease.

⁸Renal disease was defined if estimated glomerular filtration rate < 60 mL/min

⁹Lipid-lowering medication included: statins and fibrates

¹⁰Psychotropic medication included: antidepressants, benzodiazepines and methylphenidate.

¹¹The term cART was used for a combination of ≥ 3 antiretroviral drugs from at least two classes, other than ritonavir used as a pharmacologic booster.

¹²Duration of undetectable plasma viral load was defined as: number of years since last plasma viral load < 200 c/mL.

¹³Duration of detectable plasma viral load was defined as: number of years with plasma HIV viral load > 200 c/mL.

¹⁴The term “prior AIDS” was used in case of a previous AIDS-defining condition according to the United States Centers for Disease Control and Prevention classification.

Table 2: Neuroimaging factors and measures

	HIV-uninfected controls (n=70)	HIV-infected patients (n=103)	p
<i>Scanner system</i>			
Philips Intera	66	72	0.41 ^a
Philips Ingenia	34	28	
<i>Quantitative assessment</i>			
Total WMH load (ml)*	0.7 (0.3-1.5)	1.0 (0.5-2.2)	0.008^c
Periventricular WMH load (ml)*	0.4 (0.2-1.2)	0.8 (0.4-1.9)	0.002^c
Deep WMH load (ml)*	0.2 (0.1-0.3)	0.2 (0.1-0.4)	0.55 ^c
<i>Qualitative assessment</i>			
Fazekas scale, periventricular WMH			0.045^a
(1) Absent	30	21	
(2) Caps and bands	60	54	
(3) Smooth halo	3	16	
(4) Extending into deep white matter	7	9	
Fazekas scale, deep WMH			0.93 ^b
(1) Absent	42	40	
(2) Punctate lesions	47	48	
(3) Beginning confluent	7	9	
(4) Large confluent areas	4	3	
Other			
Lacunes	3	7	0.31 ^b
Cerebral microbleeds	4	6	0.74 ^b

Data presented as percentages or as median and IQR

^aChi-square test, ^bFisher's exact test, ^cTwo-way ANOVA

*Absolute volumes. For statistical analyses, the ratio of WMH volumes over intracranial volumes was taken and log transformed and defined as dependent variable within two-way ANOVA, including HIV serostatus, age and scanner system as independent variables. P-value provided for HIV serostatus.

Table 3: Determinants of white matter hyperintensity load as identified by multiple regression models

	Outcome measure: Total WMH load ^a					
	Model 1 (HIV+ and HIV-)*			Model 2 (HIV+ only) [#]		
	β (95% CI)	p	η^2	β (95% CI)	p	η^2
Age (years)	0.034 (0.026-0.042)	<0.001	0.29	0.031 (0.21-0.43)	<0.001	0.26
Scanner system ^b (1/2)	0.075 (-0.059-0.209)	0.27	0.01	0.072 (-0.116-0.260)	0.45	0.01
HIV serostatus ^c (0/1)	0.18 (0.05-0.30)	0.006	0.04	-	-	-
D-dimer (mg/L)	0.44 (0.14-0.74)	0.004	0.05	0.44 (0.068-0.817)	0.02	0.05
Diastolic blood pressure (mmHg)	0.009 (0.002-0.015)	0.008	0.04	0.011 (0.002-0.019)	0.02	0.06
CD4 < 500 cells/ mm ³ (years)	-	-		0.023 (0.006-0.040)	0.009	0.07

β = beta coefficient; CI = confidence interval; η^2 = partial eta squared; WMH: white matter hyperintensity

*Model 1 included both HIV-infected patients and HIV-uninfected controls. Variables from the categories of intoxicants, vascular, metabolic and other comorbidities and its risk factors and biomarkers of inflammation, were selected and examined by a stepwise regression model approach.

[#]Model 2 was restricted to HIV-infected patient group. Variables from the categories of intoxicants, vascular, metabolic and other comorbidities and its risk factors, biomarkers of inflammation and HIV/ART-related factors were selected and examined by a stepwise regression model approach.

^aThe ratio of WMH volumes over intracranial volumes was taken and log transformed.

^bScans were performed on either a 3T Intera (1) or 3T Ingenia (2) system (Philips Healthcare, Best, The Netherlands), using the exact same MRI protocol.

^cParticipants of this study were either HIV seronegative (0) or HIV seropositive (1).

Table 4: White matter hyperintensity load in relation to global cognitive function as determined by multiple regression models.

	Outcome measure: Cognitive function ^a								
	Model 1			Model 2 [*]			Model 3 [#]		
	β (95% CI)	<i>p</i>	η^2	β (95% CI)	<i>p</i>	η^2	β (95% CI)	<i>p</i>	η^2
HIV serostatus (0/1) ^b	-0.29 (-0.55 - -0.03)	0.03	0.03	-0.29 (-0.55 - -0.04)	0.03	0.03	-0.23 (-0.49 - 0.02)	0.07	0.02
Diabetes mellitus (0/1) ^c	-	-	-	-0.60 (-1.17 - -0.03)	0.04	0.02	-0.56 (-1.13 - 0.01)	0.05	0.02
Age (years)	-	-	-	-0.011 (-0.028 - 0.006)	0.20	0.01	0.00 (-0.020 - 0.020)	0.99	0.0001
D-dimer (mg/L)	-	-	-	-0.43 (-1.04 - 0.19)	0.17	0.01	-0.28 (-0.90 - 0.34)	0.37	0.005
Diastolic blood pressure (mmHg)	-	-	-	0.004 (-0.010 - 0.017)	0.60	0.00 2	0.006 (-0.007 - 0.020)	0.35	0.005
Total WMH load ^d	-	-	-	-	-	-	-0.33 (-0.64 - -0.02)	0.04	0.03

β = beta coefficient; CI = confidence interval; η^2 = partial eta squared

In all models, both HIV-infected patients and HIV-uninfected controls were included.

^aRegression analyses were performed to assess effects of HIV serostatus and age on cognitive function, including previously identified determinants of cognitive function (i.e., reported cannabis use, past cardiovascular disease, diabetes mellitus type 2, having an abnormal waist-to-hip ratio, presence of depressive symptoms) [32].

[#]Model 2 with total WMH load added.

^aBased on transformed Hotelling's T^2 statistic

^bParticipants of this study were either HIV seronegative (0) or HIV seropositive (1).

^cDiabetes mellitus type 2 was considered not present (0) or present (1) if haemoglobin A1c (IFCC) ≥ 48 mmol, or elevated blood glucose (non-fasting ≥ 11.1 mmol/L or fasting) ≥ 7.0 mmol/L), or if on antidiabetic medication.

^dThe ratio of WMH volumes over intracranial volumes was taken and log transformed.

Figure legends

Figure 1

Figure Title: Workflow of automated segmentation of white matter hyperintensities and segmentation examples

Figure Legend: **A.** White matter hyperintensity (WMH) segmentation was based on normalized 3D-FLAIR intensity, an anatomical prior (based on the normalized spatial coordinates of the annotated maps) and white matter probability map (obtained by segmenting the T₁-weighted scan). The T₁-weighted scan was used to segment the ventricles, which was subsequently dilated by 10 mm. This ventricle distance map was intersected with the WMH map to generate the periventricular and deep WMH maps (i.e., WMH located within a distance of 10 mm or outside the range of 10 mm from the ventricles, respectively).

B. Visual rating was performed by assessing transversal slices of 3D-FLAIR scans. This image shows an example of an HIV-infected patient with a visible lacune; periventricular and deep WMH is shown. Automated segmentation of the WMH of this example resulted in a high total WMH load of 24.8 ml. Next, median WMH loads of HIV-infected patient group and HIV-uninfected controls group is illustrated (1.1 ml and 0.8 ml, respectively).

Supplementary Figure 1

Figure Title: Quantitative and qualitative assessment of white matter hyperintensity per age category

Figure Legend: Quantitative and qualitative assessment of periventricular and deep white matter hyperintensity (WMH). Results displayed by HIV serostatus in relation to age. **A.** Quantitative segmentation of periventricular WMH load. **B.** Quantitative segmentation of deep WMH load. **C.** Qualitative assessment of periventricular WMH by Fazekas scale. **D.** Qualitative assessment of deep WMH by Fazekas scale.

Supplementary Table 1: White matter hyperintensity load in relation to individual cognitive domains in HIV-infected patients

	Total WMH load		
Cognitive domain	F	p	η^2
Fluency Category fluency – Animals Category fluency - Occupations Letter fluency (D-A-T)	3.003	0.03	0.083
Executive Function Trial Making Test, part B Wisconsin Card Sorting Test Stroop Colour-Word test	0.771	0.47	0.025
Processing Speed Trail Making Test, part A Digit symbol (WAIS-III) Symbol search (WAIS-III)	0.841	0.51	0.023
Attention Paced Auditory Serial Addition Task 3.2 Paced Auditory Serial Addition Task 2.8 Letter-number sequencing (WAIS-III)	1.458	0.23	0.042
Memory Rey's Verbal Learning Test learning Rey's Verbal Learning Test recall Visual Reproduction learning (WMS-IV) Visual Reproduction recall (WMS-IV)	1.648	0.17	0.063
Fine Motor Function Grooved Pegboard dominant hand Grooved Pegboard non-dominant hand Finger Tapping dominant hand Finger Tapping non-dominant hand	0.656	0.62	0.026

*Multivariate analysis of variance was performed for each individual cognitive domain in relation to WMH load among HIV-infected patients. These analyses are uncorrected for multiple comparisons.

Figure 1

A

B

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Supplemental Data File (.doc, .tif, pdf, etc.)

Figure2_descriptive age effects by visual rating and
quantification.pdf

Supplementary material: AGE_nIV Cohort Study Group

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