White matter hyperintensities in relation to cognition in HIV-infected men with sustained suppressed viral load on combination antiretroviral therapy

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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 long-term suppressed HIV infection.

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

Methods: Clinical, cognitive, and MRI 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 DBP, higher D-dimer levels, and longer time spent with a $CD4^+$ cell count below 500 cells/μl. 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. As 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 cause. This supports the importance of optimizing cardiovascular risk management, and early, effective treatment of HIV infection.

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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 continue 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 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 WHM 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 with 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 substudy [14]. Specific inclusion criteria for the substudy were: male gender and, for the HIV-infected patients, sustained suppression of HIV viremia on cART (plasma HIV RNA < 40 copies/ml) for less than 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 min), diagnosis of (HIV-associated) dementia, and (HIV-associated) CNS infections or tumors. Other reasons for exclusion were current injecting drug use, daily use of noninjecting illicit drugs with the exception of daily cannabis use, excessive alcohol consumption (i.e. >48 units of alcohol/week) and significant psychiatric disorders. Finally, study participants 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 substudy 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 substudy.

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 study participants underwent comprehensive neuropsychological assessment, covering six domains (see Supplementary Tables 1 and 2, http://links.lww.com/

QAD/A925). 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) because of a scanner upgrade midway through the study. The numbers of patients and controls scanned on the two systems were similar (Table 1). The scanning protocol included a three-dimensional T_1-weighted magnetization prepared gradient echo sequence, $1.1 \times 1.1 \times 1.2\,mm^2$ resolution, a three-dimensional fluid-attenuated inversion recovery (3D-FLAIR) sequence, $1 \times 1 \times 1.12\,mm^2$ resolution, and T_2-weighted fast spin-echo and fast low-angle shot sequences.

Automated image processing

The workflow of WMH segmentation is illustrated in Fig. 1a. 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).

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.

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).

Some examples of WMH segmentation are shown in Fig. 1b. Visual rating was performed by assessing transversal slices of 3D-FLAIR scans. Figure 1b shows

Table 1. Neuroimaging factors and measures.

	HIV-uninfected controls ($n = 70$)	HIV-infected patients ($n = 103$)	P
Scanner system			
Philips intera	66	72	0.41[a]
Philips ingenia	34	28	
Quantitative assessment			
Total WMH load (ml)[d]	0.7 (0.3–1.5)	1.0 (0.5–2.2)	**0.008**[c]
Periventricular WMH load (ml)[d]	0.4 (0.2–1.2)	0.8 (0.4–1.9)	**0.002**[c]
Deep WMH load (ml)[d]	0.2 (0.1–0.3)	0.2 (0.1–0.4)	0.55[c]
Qualitative assessment			
Fazekas scale, periventricular WMH			**0.045**[a]
Absent	30	21	
Caps and bands	60	54	
Smooth halo	3	16	
Extending into deep white matter	7	9	
Fazekas scale, deep WMH			0.93[b]
Absent	42	40	
Punctate lesions	47	48	
Beginning confluent	7	9	
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. Bold indicates statistical significance. WMH, white matter hyperintensity.
[a]χ^2 test.
[b]Fisher's exact test.
[c]Two-way ANOVA.
[d]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.

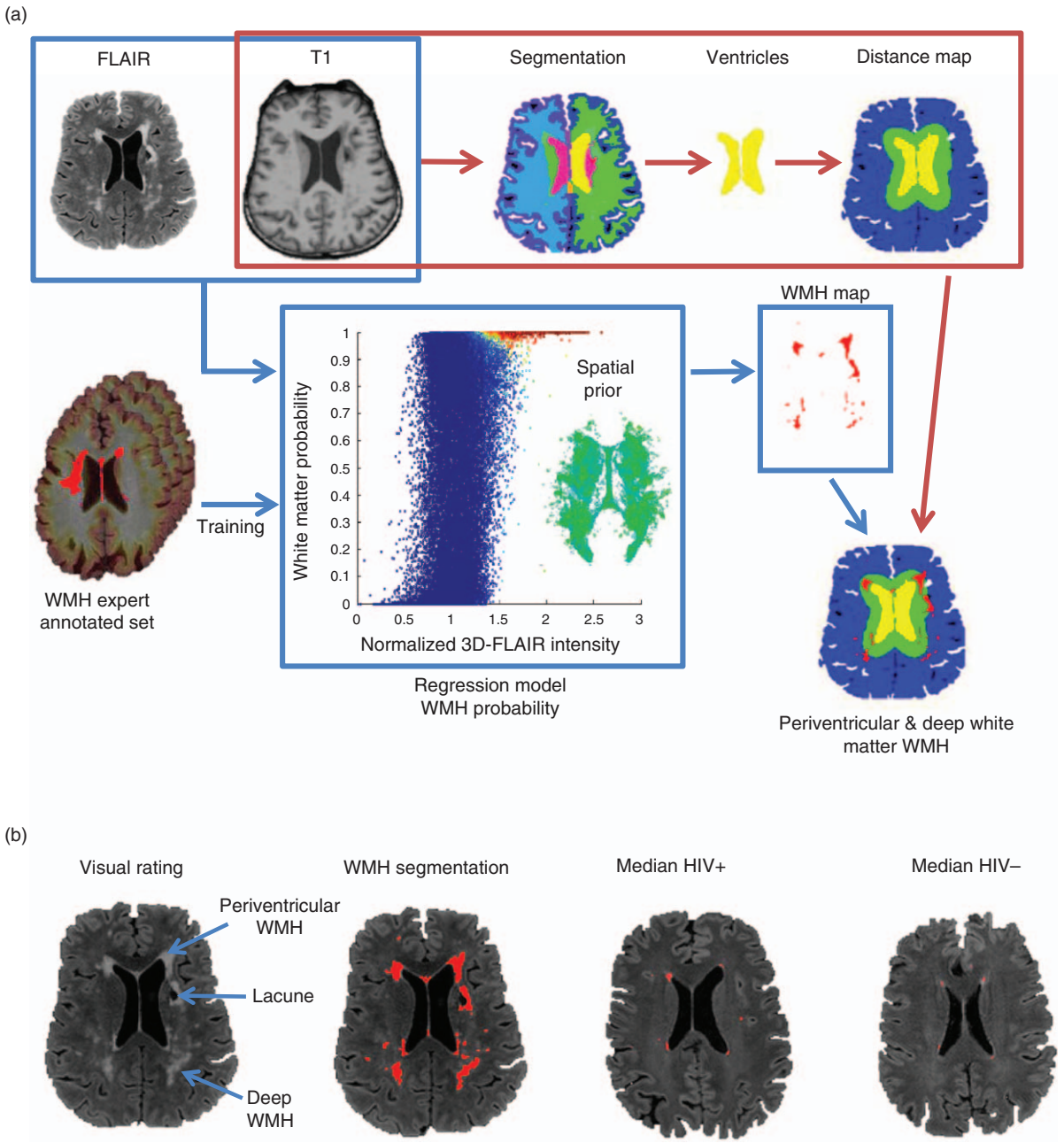

Fig. 1. Workflow of automated segmentation of white matter hyperintensities and segmentation examples. See the text for details.

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. Furthermore, Fig. 1b displays median WMH loads of the HIV-infected patient group and HIV-uninfected controls group (1.1 and 0.8 ml, respectively).

Expert visual image assessment

A neuro-radiologist (C.B.M.: 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 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 (C.B.M.), to determine the intraobserver reliability.

Statistical analyses

Statistical analyses were performed in SPSS (IBM SPSS Statistics for Windows, Version 20.0.; IBM Corp., Armonk, New York, USA). Intraobserver reliability was assessed by Cohen's-weighted κ coefficients.

Group comparison of clinical characteristics and neuroimaging findings by visual rating between HIV-infected patients and HIV-uninfected controls were performed using χ^2 or Fisher's exact tests for dichotomous and categorical variables and Student's *t* and Mann–Whitney U tests for continuous variables.

Two-way analyses of variance (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* less than 0.05 probability to enter and *P* more than 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 CVD (including angina pectoris, myocardial infarction, and peripheral arterial disease), hypertension, use of antihypertensives, SBP, and DBP, vascular stiffness based on pulse wave velocity, smoking (pack-years), diabetes mellitus type 2, hemoglobin A1c, BMI, waist-to-hip ratio, total, high, and low-density lipoprotein cholesterol, triglycerides, lipoproteina, 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), macrophage/monocyte activation, and microbial translocation (soluble CD14 and CD163), and coagulation activation (D-dimer).

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 naive 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, and the time spent with a $CD4^+$ cell count below 500 cells/μl. 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 $CD4^+$ cell count.

Cognitive impairment was identified by MNC [26]. The test statistic of the MNC method is the Hotelling's T^2statistic, 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, that is, reported cannabis use on daily to monthly basis, prior diagnosis of CVD (including angina pectoris, myocardial infarction, and peripheral arterial disease), diabetes mellitus type 2, having an abnormal waist-to-hip ratio ($\geq$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

A total of 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 2. The group of patients [median age: 54 (inter-quartile range 49–61) years] was highly comparable with the control group. However, ecstasy use (on a weekly to monthly basis) was more common in controls, whereas greater lifetime tobacco exposure was found in patients. Patients had higher levels of hemoglobin A1c, high-sensitivity C-reactive protein and soluble CD14, and lower $CD4^+$ over $CD8^+$ ratios compared with 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 2. Cognitive impairment was identified by MNC in 17% of the HIV-infected patients, none of whom had HIV-associated dementia.

Prevalence of white matter hyperintensities

WMH load was 1.0 ml in HIV-infected patients and 0.7 ml 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

Table 2. Clinical characteristics.

	HIV-uninfected controls ($n = 70$)	HIV-infected patients ($n = 103$)	P
Demographics			
Age (years)	53 (49–59)	54 (49–61)	0.81[d]
'MSM'[1]	90	93	0.41[b]
Premorbid intelligence[2] (intelligence quotient)	103 (96–112)	101 (95–112)	0.68[c]
Intoxicants			
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]
Vascular risk factors			
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]
SBP (mmHg)	134 (128–145)	136 (128–147)	0.38[d]
DBP (mmHg)	81 (75–86)	82 (77–88)	0.35[d]
Hemoglobin A1c (mmol/l)	37 (35–41)	35 (32–39)	**0.01**[d]
BMI (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]
Comorbidities			
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]
Medication use			
Antihypertensive medication	15	17	0.63[a]
Lipid-lowering medication[9]	11	11	1.00[b]
Psychotropic medication[10]	11	15	0.82[a]
Biomarkers			
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]
HIV and treatment-related factors			
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/μl)	–	625 (475–800)	–
Nadir $CD4^+$ T-cell count (cells/μl)	–	170 (60–250)	–
Duration $CD4^+$ T-cell counts <500 cells/μl (years)	–	3.9 (1.7–6.9)	–
Prior AIDS[14]	–	35	–
Cognitive impairment	–	17	–

Data presented as percentages or as median and IQR. Bold indicates statistical significance.
[a]χ^2 test.
[b]Fisher's exact test.
[c]Students t test.
[d]Mann–Whitney U test.
[1]The term 'MSM' applied to men participants who stated in the questionnaire that they felt mostly or exclusively sexually attracted to men.
[2]Premorbid intelligence quotient was estimated using the Dutch adult reading test.
[3]Cannabis, cocaine, and ecstasy use were assessed by a questionnaire as either absent (i.e., 'none') or present '(i.e., 'daily', 'weekly', or 'monthly' basis).
[4]Vascular stiffness: pulse wave velocity as measured by arteriograph.
[5]Diabetes mellitus type 2 was considered present if hemoglobin A1c (IFCC) ≥48 mmol, or elevated blood glucose (nonfasting ≥11.1 mmol/l or fasting) ≥7.0 mmol/l), or if on antidiabetic medication.
[6]Hypertension was considered if DBP ≥90 mmHg or SBP ≥140 mmHg, or if on antihypertensive medication.
[7]The variable past cardiovascular disease included angina pectoris, myocardial infarction, and peripheral arterial disease.
[8]Renal disease was defined if estimated glomerular filtration rate< 60 ml/min.
[9]Lipid-lowering medication included: statins and fibrates.
[10]Psychotropic medication included: antidepressants, benzodiazepines, and methylphenidate.
[11]The term cART was used for a combination of at least three antiretroviral drugs from at least two classes, other than ritonavir used as a pharmacologic booster.
[12]Duration of undetectable plasma viral load was defined as: number of years since last plasma viral load <200 copies/ml.
[13]Duration of detectable plasma viral load was defined as: number of years with plasma HIV viral load >200 copies/ml.
[14]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 classification.

with increased WHM. Full details of neuroimaging measures are presented in Table 1. Effect of scanner type on total WMH load was nonsignificant ($P > 0.1$). 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 with controls ($P = 0.045$, Cramers $V = 0.22$) (Table 1). In both groups, the Fazekas scores increased with older age (see Supplementary Figure 1, http://links.lww.com/QAD/A925). However, group differences in Fazekas score within each age category were all nonsignificant (results not shown). The number of lacunes and microbleeds did not differ significantly between HIV-infected patients and controls (Table 1). For the Fazekas scale, intraobserver agreement was 83 and 91%, with a Cohen's-weighted κ of 0.76 and 0.92 for rating periventricular and deep WMH, respectively.

Determinants of white matter hyperintensities

Using regression analysis, higher DBP 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), HIV-seropositive status and age also remained significantly associated (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 $CD4^+$ cell count below 500 cells/μl was found to be significantly associated with increased total WMH load ($P = 0.009$, $\eta^2 = 0.07$), independent of age, DBP, 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) (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 and sCD14). No other HIV-related determinants of total WMH load were identified.

HIV, white matter hyperintensities, 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 ($P = 0.03$, $\eta^2 = 0.03$) (Table 4, model 1 and 2). When adding WMH load to the model, the effect of HIV serostatus was attenuated ($P = 0.07$, $\eta^2 = 0.02$) (Table 4, model 3). Moreover, higher total WMH load was significantly associated with poorer global cognitive function, after adjusting for age, diabetes, DBP, and D-dimer levels ($P = 0.04$) (Table 4, model 3). 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, http://links.lww.com/QAD/A925.

Discussion

Main findings

Both quantitative and qualitative assessments of WMHs showed that WMH were more prevalent in middle-aged

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)#		
	ß (95% CI)	*P*	η^2	ß (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 to 0.209)	0.27	0.01	0.072 (−0.116 to 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
DBP (mmHg)	0.009 (0.002–0.015)	**0.008**	0.04	0.011 (0.002–0.019)	**0.02**	0.06
$CD4^+$ <500 cells/μl (years)	–	–		0.023 (0.006–0.040)	**0.009**	0.07

η^2, partial eta squared; CI, confidence interval; ß, beta coefficient; WMH, white matter hyperintensity. Bold indicates statistical significance.
[a]The ratio of WMH volumes over intracranial volumes was taken and log transformed.
[b]Scans were performed on either a 3T Intera (1) or 3T Ingenia (2) system (Philips Healthcare, Best, The Netherlands), using the exact same MRI protocol.
[c]Participants of this study were either HIV seronegative (0) or HIV seropositive (1).
*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 the 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.

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#		
	ß (95% CI)	*P*	η^2	ß (95% CI)	*P*	η^2	ß (95% CI)	*P*	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
DBP (mmHg)	–	–	–	0.004 (−0.010–0.017)	0.60	0.002	0.006 (−0.007–0.020)	0.35	0.005
Total WMH load[d]	–	–	–	–	–	–	−0.33 (−0.64−−0.02)	**0.04**	0.03

η^2, partial eta squared; CI, confidence interval; ß, beta coefficient. Bold indicates statistical significance. In all models, both HIV-infected patients and HIV-uninfected controls were included.
*Regression 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.
[a]Based on transformed Hotelling's T^2 statistic.
[b]Participants of this study were either HIV seronegative (0) or HIV seropositive (1).
[c]Diabetes mellitus type 2 was considered not present (0) or present (1) if hemoglobin A1c (IFCC) ≥48 mmol, or elevated blood glucose (nonfasting ≥11.1 mmol/l or fasting) ≥7.0 mmol/l), or if on antidiabetic medication.
[d]The ratio of WMH volumes over intracranial volumes was taken and log transformed.

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 DBP, and higher D-dimer levels were associated with increased WMH burden. Furthermore, among HIV-infected patients, longer time spent with reduced $CD4^+$ cell 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 subacute 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, SBP, hypertension, diabetes, and past cardiovascular events [24,25]. Moreover, older age, hypertension and dyslipidemia, low $CD4^+$ cell 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 disease 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 CVD 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 proinflammatory 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]. It is possible that most of the vasculopathy may also 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 $CD4^+$ cell 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 more than 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, studies 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 $CD4^+$ cell 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 $CD4^+$ cell 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 study participant 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 because of 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.

In 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 with 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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Conflicts of interest

F.W. has received travel grants from Gilead Sciences, ViiV Healthcare, Boehringer Ingelheim, Abbvie, and Bristol-Myers Squibb.

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