

Cerebral blood flow and cognitive function in HIV-infected men with sustained suppressed viremia on combination antiretroviral therapy

Tanja Su^a, Henri J.M.M. Mutsaerts^a, Matthan W.A. Caan^a, Ferdinand W.N.M. Wit^{b,c,j}, Judith Schouten^{b,d}, Gert J. Geurtsen^e, David J. Sharp^f, Maria Prins^g, Edo Richard^{d,h}, Peter Portegies^{d,i}, Peter Reiss^{b,c,j}, Charles B. Majoie^a, on behalf of the AGE_hIV Cohort Study

Objective: To assess if HIV-infected patients on long-term successful combination antiretroviral therapy show cerebral blood flow (CBF) alterations in comparison with HIV-uninfected, otherwise similar controls. To explore whether such alterations are associated with HIV-associated cognitive impairment and to explore potential determinants of CBF alterations in HIV.

Design: Cross-sectional comparison of CBF in an observational cohort study.

Methods: Clinical, cognitive and MRI data of 100 middle-aged aviremic HIV-infected men on combination antiretroviral therapy and 69 HIV-uninfected controls were collected and compared. From pseudocontinuous arterial spin labeling MRI data, CBF-maps were calculated. The associations of mean gray matter CBF with clinical and cognitive parameters were explored in regression models, followed by a spatial delineation in a voxel-based analysis.

Results: CBF was decreased in HIV-infected patients compared with HIV-uninfected controls ($P=0.02$), adjusted for age, ecstasy use and waist circumference. Spatially distinct and independent effects of total gray matter volume and HIV-serostatus on CBF were found. Within the HIV-infected group, decreased CBF was associated with increased triglyceride levels ($P=0.005$) and prior clinical AIDS ($P=0.03$). No association between CBF and cognitive impairment was found.

Conclusion: Decreased CBF was observed among HIV-infected patients, which was associated with both vascular risk factors as well as with measures of past immune deficiency. These results provide support for increased vascular disease in HIV-infected patients as represented by hemodynamic alteration, but without overt cognitive consequences within the current cohort of patients on long-term successful treatment.

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^aDepartment of Radiology, ^bDepartment of Global Health, Academic Medical Center, Amsterdam Institute for Global Health and Development (AIGHD), ^cCenter for Infection and Immunity Amsterdam (CINIMA), Division of Infectious Diseases, Department of Internal Medicine, ^dDepartment of Neurology, ^eDepartment of Medical Psychology, Academic Medical Center, Amsterdam, The Netherlands, ^fThe Computational, Cognitive, and Clinical Neuroimaging Laboratory, Department of Medicine, Imperial College London, London, UK, ^gPublic Health Service Amsterdam, Infectious Diseases Research, Amsterdam, ^hDepartment of Neurology, Radboud University Medical Centre, Nijmegen, ⁱDepartment of Neurology, Onze Lieve Vrouwe Gasthuis (OLVG Hospital), and ^jHIV Monitoring Foundation, Amsterdam, The Netherlands.

Correspondence to Prof Dr Charles B. Majoie, MD, PhD, Department of Radiology, Academic Medical Center, Location C1-426, PO Box 22660, 1100 DD Amsterdam, The Netherlands.

Tel: +0031 205669111; fax: +0031 205669119; e-mail: c.b.majoie@amc.uva.nl

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Introduction

With the introduction of combination antiretroviral therapy (cART), the incidence of severe HIV-related complications has decreased substantially and life expectancy has been prolonged [1]. Despite these advances, HIV-associated cognitive impairment is commonly reported, even among patients with a systemically well controlled HIV infection by cART [2–4]. The pathogenesis of such HIV-associated cognitive impairment is poorly understood, but the increased cardiovascular risk factors among long-term HIV-infected and treated patients may play a role.

Several factors may contribute to vascular disease in HIV-infected patients, either due to the virus itself or to cART therapy. The HIV-infection may directly affect blood vessels by the release of toxic viral proteins and induce host proinflammatory responses [5]. Although such deleterious effects of HIV can be attenuated by successful suppression of viral replication by cART, immune activation and inflammation may persist [6,7]. In addition, HIV infection may indirectly cause vessel wall damage, due to the use of particular cART regimens [8] and associated metabolic complications including dyslipidemia, insulin resistance and hypertension [9–11]. Furthermore, certain lifestyle risk factors (e.g. smoking, alcohol and recreational drug use) are more prevalent among HIV-infected patients and may further contribute to vascular disease, and as the HIV-infected population ages, vascular disease may further accumulate by age-related risk factors [12].

In HIV-uninfected individuals, white matter hyperintensities (WMH), neuroimaging correlates of cerebrovascular disease, have been associated with cognitive decline, particularly among individuals at increased risk for cardiovascular disease (CVD) [13]. Such WMH have also been reported to be more extensive in HIV and associated with cognitive deficits [14–18]. Cerebrovascular disease and cerebral blood flow (CBF) are assumed to be interrelated, and the extent of WMH burden is associated with CBF decline over time [19].

To date, a few studies have examined CBF in HIV-infected patients and their results vary widely [20–23]. Reports on associations between CBF alterations and cognitive impairment are contradictory as well [21,24]. It thus remains unclear how CBF is affected in HIV-infected patients with suppressed viremia on cART, and if an association with cognitive impairment exists. Therefore, we have compared a group of middle-aged HIV-infected men with long-term and successful cART with HIV-uninfected controls with similar sociodemographic backgrounds and lifestyles to investigate potential associations between CBF and HIV status and to assess whether CBF is associated with HIV-associated cognitive impairment. Furthermore, potential determinants of CBF alterations in HIV were explored.

Methods

Study population

The current study is nested within the AGE_hIV cohort study. This is an ongoing study on the prevalence, incidence and risk factors of age-associated comorbidities in HIV-infected patients and comparable HIV-uninfected controls (e.g. same sociodemographic background and lifestyle), aged 45 years or older [25]. Eligible male participants from this cohort were approached to participate in a nested neuroimaging substudy. For the HIV-infected patients, sustained suppressed viremia on cART (plasma HIV-RNA < 40 copies/ml) for at least 12 months was required (transient low-level viremia, i.e. 40–200 copies/ml was allowed). Exclusion criteria were current or past neurological disorders including stroke, seizure disorder, multiple sclerosis, (HIV-associated) dementia and traumatic brain injury (defined as loss of consciousness >30 min), (HIV-associated) central nervous system infection or tumor, current severe psychiatric disorders (e.g. psychosis and major depression), injecting drug use, daily use of noninjecting illicit drugs (daily cannabis use was permissible), excessive alcohol consumption (>48 units of alcohol/week), insufficient command of the Dutch language, mental retardation and MRI contraindications.

Standard protocol approval, registrations and patient consents

The AGE_hIV cohort study and the nested neuroimaging substudy were both approved by the institutional review board of the Academic Medical Center and are registered at www.clinicaltrials.gov (identifier: NCT01466582). From all participants, written informed consent was obtained separately for the cohort study and the nested neuroimaging study.

Clinical parameters

Participants completed standardized questionnaires on demographics, medical characteristics and lifestyle factors. Standardized screenings for age-associated comorbidity and organ dysfunction were performed, and blood and urine samples were collected for laboratory testing. Information on HIV and cART history was obtained. Detailed description of these procedures was published previously [25].

Cognitive parameters

A full neuropsychological assessment was performed, covering six cognitive domains (fluency, attention, processing speed, memory, executive function and motor function). Test scores were adjusted for age and education effects using normative standards. Multivariate normative comparison (MNC) was performed to identify cognitive impairment, as this method has been shown to be a more reliable method for detecting cognitive impairment than the Frascati criteria [26]. The MNC method is designed to control the false positive rate while retaining sensitivity

[27]. It identifies HIV-infected patients as cognitively impaired if their cognitive profile deviates significantly from those of the HIV-uninfected control group, based on the Hotelling's T^2 statistic. This statistic was used to create a continuous measure to reflect cognitive function. In addition, the Global Deficit Score (GDS) [28] was computed. Details on the test battery and MNC method were published previously [26,29].

MRI protocol

MRI was performed on two Philips 3T scanners (Intera and Ingenia, Philips Healthcare; Best, The Netherlands) due to a scanner replacement midway through the study. The number of patients and controls scanned on the two systems was comparable (Table 1). All patients were requested to abstain from nicotine (≥ 2 h), caffeine-containing beverages (≥ 5 h) and noninjecting illicit drugs (≥ 14 days) prior to the MRI examination to minimize physiological CBF fluctuations.

The scanning protocol consisted of pseudocontinuous arterial spin labeling (ASL) for measuring CBF [echo time (TE)/repetition time (TR) = 14/4000 ms, $240 \times 240 \text{ mm}^2$ field of view (FOV), 17 slices, $3 \times 3 \times 7 \text{ mm}^3$ resolution labeling duration = 1650 ms, initial postlabeling delay = 1525 ms, 30 control and label pairs] and a T_1 -weighted magnetization prepared rapid gradient echo (3D-magnetization prepared rapid gradient echo) for anatomical reference (TE/TR = 3.1/6.8 ms, $256 \times 256 \times 204 \text{ mm}^3$ FOV, $1.1 \times 1.1 \times 1.2 \text{ mm}^3$ in-plane resolution).

Image processing

After acquisition, ASL postprocessing was performed to obtain quantitative CBF maps using the in-house developed 'ExploreASL' toolbox based on SPM12 (Statistical Parametric Mapping, Wellcome Trust Centre for Neuroimaging; London, UK). All control-label pairs were corrected for head motion, whereby pairs with severe head motion were excluded. The perfusion-weighted map was obtained by averaging the control minus label pairs, and converted to CBF values using the single compartment quantification model that has been previously described in detail [30]. A single M0 value was used for all participants, obtained in a previous study of healthy volunteers [31]. Considering the possible effect of HIV on hematocrit, and the influence that hematocrit has on the T_1 relaxation rate of blood, CBF was quantified with patient-specific blood T_1 values derived from the individual hematocrit measurements, instead of a commonly used reference value [32].

Gray matter probability maps were obtained from the anatomical reference scans by SPM12. In addition, the CBF map was registered to the gray matter probability map by rigid body registration (Fig. 1). Because of the relatively low signal of ASL in the white matter, white matter CBF was not analyzed. Gray matter volume was

computed relative to the total intracranial volume to assess possible partial volume effects on CBF [33].

Gray matter probability maps were then used to create a population-based template in common space using diffeomorphic anatomical registration analysis using exponentiated Lie algebra, to which CBF maps were transformed using the same transformation parameters [34]. Based on this population-based gray matter probability template, a common gray matter mask was created (thresholded on 0.4).

To correct for CBF quantification differences between scanners (e.g. M0), mean gray matter CBF values were computed and used to linearly scale CBF maps per scanner, such that the mean gray matter CBF of the Ingenia patients was the same as the mean gray matter CBF of the Intera patients (after validating that both scanners had a comparable number of cases and controls). For voxel-based analysis (VBA), CBF maps were smoothed with a 7 mm full width at half maximum Gaussian kernel.

Statistical analysis

Characteristics of HIV-infected participants and uninfected controls were compared using a chi-square test or Fisher's exact test (in cases in which $n < 5$ for at least one group) for categorical variables. Normally distributed continuous variables were compared using a Student's t test, and nonnormally distributed continuous variables were compared using a Mann-Whitney U test.

To reveal potential associations between CBF and HIV, the data were analyzed in two ways. First, using the mean gray matter CBF value as a measure for global brain perfusion and second using smoothed CBF-maps in a VBA to spatially delineate effects within the brain's gray matter. In addition, the association between mean gray matter CBF and cognitive function was assessed within the HIV-infected patient group.

The mean gray matter CBF value (hereafter referred to as CBF) was used as dependent variable in a regression model, with HIV-serostatus, age and scanner system as independent variables. In addition, identified determinants and confounders were incorporated into our statistical models as independent variables to adjust for their effects.

Two models were derived, with model 1 including both HIV-infected patients and HIV-uninfected controls and model 2 including HIV-infected patients only.

Biologically plausible determinants and confounders of CBF were selected from the cohort database, and their relations with CBF were probed by a stepwise regression model selection approach, with P less than 0.05 to enter and P more than 0.1 to remove.

Table 1. Demographic and clinical characteristics.

	HIV-uninfected controls (n = 69)	HIV-infected patients (n = 100)	P
Demographics			
Age (years)	53 (49–60)	54 (48–61)	0.85 ^a
MSM ^b	60/67 (90%)	94/100 (94%)	0.29 ^c
Intoxicants			
Cannabis use ^d	10/67 (15%)	16/100 (16%)	0.85 ^c
Ecstasy use ^d	8/67 (12%)	2/100 (2%)	0.02^e
Alcohol intake (unit/week)	5 (3–13)	6 (2–14)	0.72 ^a
Smoking tobacco (pack-years)	8.0 (2.4–19.5)	19.8 (7.7–34.5)	0.01^a
Comorbid conditions and medication use			
Diabetes mellitus type 2 ^f	3/69 (4%)	6/100 (5%)	0.74 ^e
Hypertension ^g	24/69 (35%)	39/100 (39%)	0.58 ^c
Past cardiovascular disease ^h	4/66 (6%)	8/98 (8%)	0.76 ^e
Lipid-lowering medication ⁱ	7/68 (10%)	12/100 (12%)	0.71 ^c
Psychotropic medication ^j	10/69 (14%)	15/100 (15%)	0.93 ^c
Vascular disease risk factors			
BMI	25 (24–28)	24 (22–26)	0.001^a
Waist-to-hip-ratio (>0.9)	46/66 (70%)	85/100 (85%)	0.02^e
Waist circumference (cm)	92 (87–103)	92 (88–98)	0.29 ^a
SBP (mmHg)	134 (128–146)	136 (128–147)	0.40 ^a
DBP (mmHg)	81 (74–86)	82 (77–88)	0.31 ^a
Vascular stiffness (m/s) ^k	7.7 (7.2–8.8)	7.9 (7.1–8.7)	0.69 ^a
Hematocrit (l/l)	0.46 (0.44–0.50)	0.43 (0.41–0.44)	<0.001^a
Total HDL cholesterol (mmol/l)	1.3 (1.0–1.6)	1.3 (1.0–1.5)	0.80 ⁱ
Total LDL cholesterol (mmol/l)	3.4 (2.9–4.0)	3.2 (2.3–3.8)	0.12 ^j
Triglycerides (mmol/l)	1.6 (1.1–2.4)	1.8 (1.2–2.7)	0.32 ^a
Lipoprotein(a) (mmol/l)	0.7 (0.6–0.8)	0.8 (0.6–0.9)	0.41 ^j
Hemoglobin A1c (IFCC – mmol/mol)	37 (35–41)	35 (32–39)	0.007^a
SCORE-low ^m	1.8 (1.0–3.4)	2.0 (1.2–4.3)	0.39 ^j
Inflammation, immune activation and coagulation			
High-sensitivity C-reactive protein (mg/l)	1.1 (0.6–2.2)	1.4 (0.7–3.3)	0.04^a
Soluble CD14 (ng/ml)	1193 (990–1534)	1542 (1313–2021)	<0.001^a
Soluble CD163 (ng/ml)	245 (184–359)	275 (205–418)	0.16 ^a
CD4 ⁺ /CD8 ⁺ ratio	1.7 (1.3–2.3)	0.7 (0.5–1.0)	<0.001^a
D-dimer (mg/l)	0.26 (0.20–0.40)	0.21 (0.20–0.33)	0.11 ^a
Neuroimaging factors			
Scanner ⁿ			0.32 ^c
Philips Intera	44/69 (64%)	71/100 (71%)	
Philips Ingenia	25/69 (36%)	29/100 (29%)	
Gray matter CBF ^o	46.4 (41.4–55.5)	44.0 (38.7–52.4)	0.06 ^a
Gray matter volume ^p (ml)	693 (648–731)	661 (621–710)	0.03^a
HIV disease and treatment factors			
Known duration of HIV-1-infection (years)	–	13.4 (7.3–17.1)	–
Duration since start of first ART (years)	–	11.4 (4.9–14.9)	–
Naïve at start of cART ^q	–	80/100 (80%)	–
Duration of undetectable plasma viral load ^r (years)	–	10.5 (4.4–13.4)	–
Known duration of CD4 ⁺ cell count < 500 cells/μl (years)	–	3.8 (1.6–6.9)	–
Current CD4 ⁺ cell count (cells/μl)	–	628 (476–797)	–
Nadir CD4 ⁺ cell count (cells/μl)	–	170 (60–248)	–
Prior clinical AIDS ^s	–	38/100 (38%)	–
Protease inhibitors	–	47/100 (47%)	–
Cognitively impaired ^t	–	17/100 (17%)	–

Data presented median and interquartile ranges or *n* of total *n* and percentage. Bold indicates statistical significance. ART, antiretroviral therapy; cART, combination antiretroviral therapy; CBF, cerebral blood flow.

^aMann–Whitney *U* test.

^bThe term MSM applied to male patients who stated in the questionnaire that they felt mostly or exclusively sexually attracted to men.

^cChi-square test.

^dCannabis and ecstasy use were assessed by a questionnaire and assigned to either absent (i.e. 'none') or present (i.e. 'daily', 'weekly' or 'monthly' basis).

^eFisher's exact test.

^fDiabetes mellitus type 2 was considered present if HbA1c (IFCC) ≥48 mmol/mol and/or elevated blood glucose (nonfasting ≥11.1 mmol/l or fasting ≥7.0 mmol/l), or if on antidiabetic medication.

^gHypertension was considered present if DBP ≥90 mmHg and/or SBP ≥140 mmHg in all three measurements (Omron 7051T; Hoofddorp, The Netherlands) with a 1-min interval, or if on antihypertensive medication.

^hThe variable past cardiovascular disease included angina pectoris, myocardial infarction and/or peripheral arterial disease.

ⁱPsychotropic medication included antidepressants, benzodiazepines and methylphenidate.

^jLipid-lowering medication consisted mainly of the use of statins that is atorvastatin, fluvastatin, pravastatin, rosuvastatin and simvastatin, except for one HIV-infected patient who used a combination of rosuvastatin and ciprofibrate and another HIV-infected patient who solely used ciprofibrate.

^kVascular stiffness: Pulse wave velocity as measured by Arteriograph.

In model 1, the following candidate variables were selected and examined:

- (1) Intoxicants: reported cannabis use on daily to monthly basis (transformed to binary variable of absent or present), reported cocaine or ecstasy use on weekly to monthly basis (transformed to binary variables of absent or present), average units of alcohol consumed per week and tobacco consumption in pack-years;
- (2) Comorbid conditions: history of CVD; including angina pectoris, myocardial infarction, or peripheral arterial disease (absent or present); hypertension (absent or present); diabetes mellitus type 2 (absent or present) and use of psychotropic medication (absent or present);
- (3) Vascular disease risk factors: SBP and DBP, arterial stiffness based on pulse wave velocity [35,36], hemoglobin A1c, waist circumference, hip circumference, BMI, waist-to-hip ratio, HDL cholesterol and LDL cholesterol, triglycerides and lipoprotein(a); SCORE-low: a CVD score for low-risk regions in Europe [37], log-transformed to obtain a normal distribution for statistical analysis.
- (4) Inflammation, immune activation and coagulation markers: high-sensitivity C-reactive protein, soluble CD14 and CD16 or D-dimer.

To identify additional HIV disease and antiretroviral therapy (ART)-related determinants of CBF, the following variables were additionally examined in model 2, that is within the HIV-infected population: known duration of HIV infection, being treatment-naïve when starting cART (absent or present), duration of ART use, current and nadir CD4⁺ cell counts, the time spent with a CD4⁺ cell count below 500 cells/ μ l (lower boundary of normal range of CD4⁺ cell count), duration of undetectable plasma viral load, prior AIDS according to the Centers for Disease Control and Prevention classification (absent or present) and current/prior/duration of/use of individual antiretroviral agents.

To assess the association between CBF and cognitive function, regression analyses were performed within the HIV-infected group, with CBF as independent variable (adjusted for determinants and confounders as identified

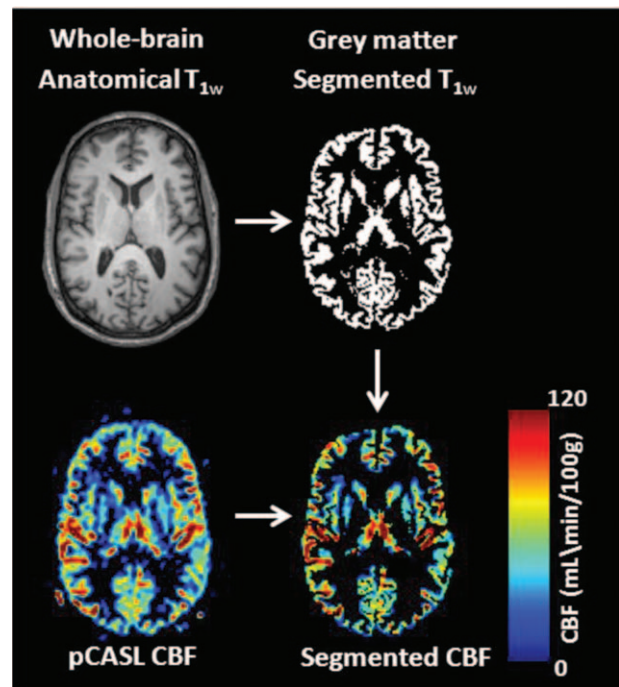


Fig. 1. Workflow gray matter cerebral blood flow quantification. From anatomical T_1 -weighted images, the gray matter was segmented and registered to the calculated pseudocontinuous arterial spin labeling cerebral blood flow maps to create segmented gray matter cerebral blood flow maps.

in the previous analyses) and cognitive function as dependent variable.

The VBA included variables that were found to have a significant effect on CBF in the preceding stepwise linear regression analysis. Gray matter volume was added as a nuisance variable to adjust for partial volume effects. Voxels were considered significant at family-wise error corrected P less than 0.05 using a P less than 0.001 (primary) cluster-forming threshold [38].

MNC was performed using R statistical software (R Developmental Core Team; Vienna, Austria), VBA in SPM12 and the remaining analyses were performed with SPSS (IBM SPSS Statistics for Windows, Version 20.0.; IBM Corp., Armonk, New York, USA).

¹Student's t test.

^mSCORE-low: a cardiovascular disease (CVD)-score for low-risk regions in Europe (4).

ⁿScans were performed on either a Philips 3T Intera scanner or 3T Philips Ingenia system (Philips Healthcare; Best, The Netherlands), using the exact same ASL protocol.

^oFor each individual patient, cerebral blood flow (CBF) was calculated while accounting for hematocrit levels.

^pAbsolute volumes. For statistical analyses, the ratio of total gray matter volume over intracranial volume was taken.

^qThe term 'cART' was used for a combination of ≥ 3 antiretroviral drugs, other than ritonavir used as a pharmacologic booster.

^rDuration of undetectable plasma viral load was defined as: number of years since last plasma viral load > 200 copies/ml.

^sThe term 'prior clinical AIDS' was used in case of a previous AIDS-defining condition according to the United States Centers for Disease Control and Prevention (CDC) classification.

^tCognitive impairment was identified by multivariate normative comparison method, a statistical method designed to control the false positive rate while retaining sensitivity. HIV-infected patients were identified as cognitively impaired if their cognitive profile significantly deviates from those of the HIV-uninfected control group.

Results

Patient characteristics

A total of, 103 HIV-infected patients and 74 HIV-uninfected controls were enrolled between December 2011 and August 2013. Neuroimaging data were incomplete for three HIV-infected patients and five controls, such that data from 100 HIV-infected patients and 69 uninfected controls were used for analysis.

Table 1 provides an overview of the patient characteristics, including HIV disease and treatment factors. Although some differences were detected, HIV-infected patients and uninfected controls were generally comparable in terms of demographics, clinical characteristics and lifestyle-related factors (Table 1).

The HIV-infected patients were known to have been infected for a long period (median duration 13.4 years) and to have been treated with ART for most of this time (median duration 11.4 years). They had achieved substantial immune recovery on treatment, from a median nadir CD4⁺ cell count of 170 cells/ μ l to a current median CD4⁺ cell count of 628 cells/ μ l.

Mean gray matter cerebral blood flow per group and its confounders and determinants

The median and interquartile ranges for CBF were 46.4 ml/100 g min (41.4–55.5) in HIV uninfected controls and 44.0 ml/100 g min (38.7–52.4) in HIV patients ($P=0.06$; Table 1). When adjusting for potential confounders in model 1 (Table 2), HIV-seropositive

status was significantly associated with lower CBF ($P=0.02$). Ecstasy use and greater waist circumference were both associated with lower CBF ($P=0.04$, $P=0.02$, respectively). SCORE-low was significantly associated with CBF ($P=0.05$), but was no longer an independent risk factor after adding waist circumference to the model ($P=0.17$). A trend was found for higher age and lower CBF ($P=0.07$). Normalizing measurements on both scanners for mean population gray matter CBF removed the effect of scanner system on CBF (model 1, Table 2).

When restricting the analysis to the HIV-infected patient group in model 2 (Table 2), greater waist circumference remained associated with lower CBF ($P=0.04$). In addition, prior AIDS and higher triglyceride levels were associated with lower CBF ($P=0.03$, $P=0.005$, respectively). Higher D-dimer levels and longer time spent with a CD4⁺ cell count below 500 cells/ μ l were not significantly associated with lower CBF, although a trend was found for both associations. Increased age was not related to CBF, nor was the effect of scanner system.

No association was found between CBF and current or prior use of particular antiretroviral drugs. No associations were found between CBF and markers of innate immune activation. No other HIV-related or ART-related determinants of CBF were identified.

Replacing the variable waist circumference by the variable BMI resulted in comparable effects on CBF

Table 2. Determinants and confounders of gray matter cerebral blood flow as identified by multiple regression models.

	Outcome measure GM CBF ^a					
	Model 1 ^b			Model 2 ^c		
	β (95% CI)	<i>P</i>	η^2	β (95% CI)	<i>P</i>	η^2
Age (years)	-0.183 (-0.383, 0.018)	0.07	0.02	-0.179 (-0.419, 0.060)	0.14	0.02
Scanner system (1/2) ^d	2.346 (-0.870, 5.561)	0.15	0.01	2.546 (-1.522, 6.615)	0.22	0.02
HIV serostatus (0/1) ^e	-3.873 (-7.027, -0.719)	0.02	0.04	-	-	-
Ecstasy use (0/1) ^f	-6.791 (-13.228, -0.353)	0.04	0.03	-	-	-
Waist circumference (cm)	-0.168 (-0.310, -0.026)	0.02	0.03	-0.190 (-0.376, -0.005)	0.04	0.04
Prior clinical AIDS (0/1) ^g	-	-	-	-4.346 (-8.142, -0.550)	0.03	0.05
Triglycerides (mmol/l)	-	-	-	-1.881 (-3.185, -0.577)	0.005	0.08
D-dimer (mg/l)	-	-	-	-7.139 (-15.216, 0.938)	0.08	0.03
CD4 ⁺ cell count < 500 cells/ μ l (years)	-	-	-	-0.328 (-0.707, 0.051)	0.09	0.03

η^2 , partial eta squared; CI, confidence interval; GM CBF, gray matter cerebral blood flow; β , beta coefficients. Bold indicates statistical significance.

^aFor each individual patient, GM CBF was calculated while accounting for hematocrit levels.

^bModel 1 included data for 66 HIV-infected patients and 100 HIV-uninfected controls. Variables from the categories of intoxicants, comorbid conditions, vascular disease risk factors, inflammation, immune activation and coagulation were selected and examined by a stepwise regression model approach.

^cModel 2 was restricted to 100 HIV-infected patients. HIV disease and treatment factors were selected in addition to the parameters of model 1 and examined by a stepwise regression model approach.

^dScans were performed on either a 3T Intera (1) or 3T Ingenia (2) systems (Philips Healthcare; Best, The Netherlands), using the exact same neuroimaging protocol.

^eParticipants of this study were either HIV-uninfected (0) or HIV-infected (1).

^fEcstasy use was assessed by a questionnaire and assigned to either absent (i.e. 'none') or present (i.e. 'daily', 'weekly' or 'monthly' basis).

^gThe term 'prior clinical AIDS' was used in case of a previous AIDS-defining condition according to the United States Centers for Disease Control and Prevention (CDC) classification.

(effect of BMI on CBF in model 1 $P=0.02$; model 2 $P=0.04$).

HIV, cerebral blood flow and cognition

Only 17% of HIV-infected patients were considered cognitively impaired, and none of them had HIV-associated dementia. HIV-seropositive status was significantly associated with poorer cognitive function ($P=0.04$). No association was found between CBF (adjusted for age, scanner, ecstasy use and waist circumference) and cognitive function within the HIV-infected patient group ($P=0.64$). Similarly, no association with GDS was found ($P=0.94$).

Voxel-based analysis

HIV-infected patients had lower gray matter volumes compared with the HIV-uninfected controls ($P=0.03$, Table 1). The relation between lower gray matter volumes and CBF was addressed in the VBA. HIV-seropositive status remained associated with lower CBF after adjustment for age, ecstasy use, waist circumference and gray matter volume. The effect of HIV serostatus on CBF was found in different regions than the effects of gray matter-volume and age on CBF (Fig. 2).

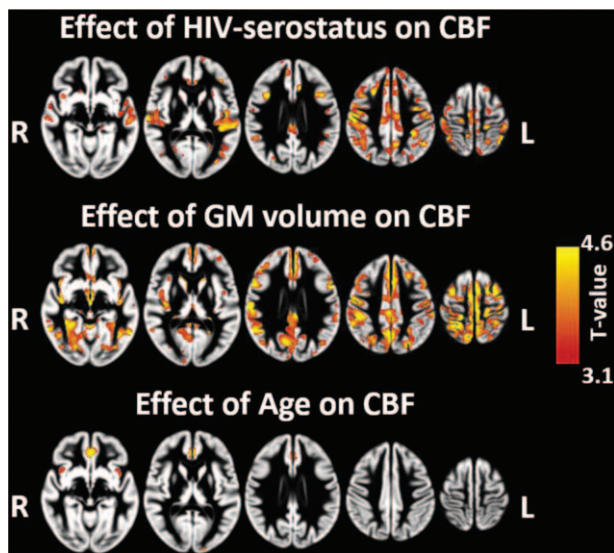


Fig. 2. Voxel-based analysis showing spatial effects of model parameters on cerebral blood flow. Voxel-based analysis showed that HIV-seropositive status remained independently associated with widespread pattern of reduced gray matter cerebral blood flow, after adjustment of age, scanner system, ecstasy use, waist circumference and gray matter volume. Significant independent effects of HIV serostatus, gray matter volume and age on reduced cerebral blood flow are presented. T -statistics are color-coded and shown in voxels in which $P < 0.05$ (family-wise error rate).

Discussion

Main results and general interpretation

We found that HIV-seropositive status was associated with decreased CBF, after adjusting for age, ecstasy use and waist circumference. The spatial analysis showed that the pattern of decreased CBF was widespread throughout the brain's gray matter. Within the HIV-infected group, decreased CBF was significantly associated with higher triglyceride levels and prior clinical AIDS. Although HIV-seropositive status was associated with both poorer cognitive function and decreased CBF, no association between CBF and cognition was found within the current cohort of HIV-infected patients on long-term successful treatment.

So far, few studies have examined CBF in HIV-infected patients within the cART era. One study reported increased CBF among treatment-naïve HIV-infected patients, which suggested increased metabolic activity due to HIV-associated inflammation [23]. Conversely, two studies on HIV-infected patients, of whom the majority were treated by cART, reported decreased CBF [20,24]. However, another study of HIV-infected patients on successful treatment reported a general association between increased age and decreased CBF within the frontal lobes of the brain but no group differences [22]. The latter may be explained by their smaller sample size; however, the general age effect that they observed was replicated in the current study.

Determinants of cerebral blood flow

From the HIV-related parameters, we found that having experienced prior clinical AIDS was associated with decreased CBF. Lower CD4⁺ cell counts and higher plasma viral load have been associated with decreased CBF in earlier studies. This has been suggested to reflect injury associated with immune status or disease severity [39]. Within the current study, a trend was found between the time spent with reduced CD4⁺ cell counts and decreased CBF. The underlying pathological mechanism of such associations between measures of past immune deficiency and decreased CBF remains poorly understood, but may reflect vascular insult from direct toxic effects of HIV to the vessel walls and indirect proinflammatory responses of the vessel wall, reinforcing atherosclerosis [5]. Raised concentrations of endothelial activation and inflammation markers in HIV-infected patients provide support for vessel wall inflammation in HIV [40]. Such vascular insult may have developed particularly in the period between HIV infection and initiation of effective ART, when viral toxicity and immune activation were at their peak (i.e. legacy effect).

Among HIV-infected patients, we found that higher triglyceride levels were associated with decreased CBF. This has previously been reported in studies concerning patients with metabolic syndrome and diabetes mellitus

[41,42]. Elevated triglyceride levels are frequent in the context of HIV because of the increasingly recognized risk of metabolic complications in HIV, such as dyslipidemia, insulin resistance and hypertension [5,25,43]. This may suggest that lipid changes, including those that may be seen in conjunction with the use of certain cART regimens, may affect cerebral arteries and microcirculation and lead to hemodynamic changes [11,44].

We observed a significant association between a larger waist circumference and lower CBF, whereas there was no significant association with hip circumference ($P=0.19$) and a borderline association with waist-to-hip ratio ($P=0.07$). Visceral adiposity as a cardiovascular risk factor generally is known to be related to lower CBF [41]. These findings may suggest that, with respect to prior exposure to older antiretrovirals, exposure to some of the older HIV protease inhibitors known to be associated with lipohypertrophy, in this context may be more relevant than exposure to older thymidine analogue reverse transcriptase inhibitors that were associated with peripheral lipoatrophy. Both lipohypertrophy and lipoatrophy as part of the lipodystrophy syndrome have been associated with increased cardiovascular and metabolic risk. [45] Although lipoatrophy has become rare, weight gain and abdominal obesity continues to be frequently observed in patients first initiating treatment with contemporary ART regimens [46,47].

Ecstasy use was more prevalent among the controls than among the HIV-infected patients, although the occurrence was low in both groups (12 vs. 2%). The confounding result of ecstasy use that we have found on decreased CBF may result from ecstasy-induced subacute and prolonged vasoconstriction [48,49].

Cerebral blood flow and HIV-associated cognitive impairment

The associations of CBF and cognitive impairment in HIV that have been reported thus far varied widely. One study examined patients with different degrees of HIV-related minor motor deficits and reported increased CBF among patients with early psychomotor slowing compared with patients with normal motor function or sustained pathological psychomotor slowing. This finding was suggested to reflect increased metabolic demand to compensate for cognitive decline [21]. In contrast, another study reported decreased CBF in patients with early stages of HIV-cognitive motor complex in comparison with controls [39]. Finally, one study specifically compared cognitively impaired with unimpaired HIV-infected patients and reported no differences in CBF [24]. Likewise, we were also unable to detect an association between CBF and cognitive function amongst our HIV-infected cohort. This lack of association could possibly be explained by the fact that only 17% of patients

had cognitive deficits in this well defined cohort of aviremic HIV-infected patients on long-term cART. Also, given that the differences in CBF values were subtle, a possible relationship between CBF and cognitive impairment would be difficult to detect. Follow-up of our cohort of HIV-infected patients could provide more insight in the time course of hemodynamic alterations in relation to cognitive deterioration in the context of the aging HIV-infected population on long-term successful treatment.

Strengths and limitations

Strengths of the current study include the comparison of our cohort of HIV-infected patients with a (demographic and lifestyle factor) comparable HIV-uninfected control group and the various assessments performed to characterize the participants. This allowed us to study the relationships of a broad range of clinical factors with CBF and to assess the effects of HIV more thoroughly. In addition, HIV-infected patients had significantly lower hematocrit values compared with HIV-uninfected controls. As the ASL signal is hematocrit-dependent, we have used the patient-specific hematocrit values in the CBF quantification rather than a commonly used reference value. Our study was limited by a scanner upgrade midway through the study, which was adjusted for methodologically by scaling CBF maps and statistically by incorporating it as a covariate in the analyses. Furthermore, the HIV-infected population examined within the current study consisted of exclusively male participants who were HIV-infected for a long period of time with sustained suppressed viremia on cART. Therefore, we are unable to make generalizations to other HIV-infected populations.

Conclusion

When adjusting for age, ecstasy use and waist circumference, we observed decreased CBF in middle-aged HIV-infected men with sustained suppressed viremia on cART in comparison with HIV-uninfected, otherwise similar controls. Decreased CBF was associated with both vascular risk factors as well as with measures of past immune deficiency, but not with cognitive impairment. The results from the current study are suggestive for increased vascular disease in HIV, which may affect hemodynamic changes, but without overt cognitive consequences within the current cohort of patients on long-term successful treatment. Longitudinal follow-up studies are needed to provide further insight in the progression of such hemodynamic changes in relation to HIV-associated cognitive impairment.

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Author contributions: T.S. contributed to the study design, was responsible for the data collection, data analysis and interpretation and conception of the manuscript. H.J.M.M.M. processed the MRI data, contributed to the interpretation of the data and reviewed and approved the final manuscript. M.W.A.C. 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. F.W.N.M.W. contributed to the study design, supervised data analysis, reviewed and approved the final manuscript. J.S. and M.P. contributed to the study design, the data collection, data interpretation and reviewed and approved the final manuscript. G.J.G. contributed to the study design, supervised neuropsychological data acquisition, contributed to data interpretation and reviewed and approved the final manuscript. D.J.S. contributed to the study design, data interpretation and reviewed and approved the final manuscript. E.R. contributed to the interpretation of the data, critically revised the manuscript and approved the final manuscript. P.P. contributed to the study design, data interpretation and reviewed and approved the final manuscript. P.R. conceived the main cohort study and the substudy, contributed to both study designs, to data interpretation and reviewed and approved the final manuscript. C.B.M. conceived the substudy, acquired funding for the substudy, contributed to its design, contributed to data interpretation and reviewed and approved the final manuscript.

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Conflicts of interest

F.W.N.M.W. has received travel grants from Gilead Sciences, ViiV Healthcare, Boehringer Ingelheim, Abbvie and Bristol-Myers Squibb. J.S. has received travel grants from Gilead Sciences, ViiV Healthcare and Boehringer Ingelheim. D.J.S. is funded by a National Institute of Health Research Professorship (NIHR-RP-011-048) and has received an investigator-led grant from Pfizer, unrelated to the current work. P.P. has been an ad-hoc advisor to or speaking at various events sponsored by ViiV Healthcare, Gilead Sciences, Abbvie and Bristol-Myers Squibb. P.R. through his institution has received independent scientific grant support from Gilead Sciences, Janssen Pharmaceuticals Inc., Merck&Co, Bristol-Myers Squibb and ViiV Healthcare. He has served on scientific advisory board for Gilead Sciences; he has served on a data safety monitoring committee for Janssen Pharmaceuticals Inc and has chaired a scientific symposium organized by ViiV Healthcare, for which his institution has received remuneration.

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