

## Magnetic resonance imaging of cerebral small vessel disease in men living with HIV and HIV negative men aged 50 and above

**Running title:** Cerebral small vessel disease in HIV

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## Abstract

We assessed whether HIV status was associated with white matter hyperintensities (WMH), a neuroimaging correlate of cerebral small vessel disease, in men aged  $\geq 50$  years. A cross-sectional substudy was nested within a larger cohort study. Virologically suppressed men living with HIV (MLWH) and demographically matched HIV negative men aged  $\geq 50$  underwent magnetic resonance imaging (MRI) at 3 Tesla. Sequences included volumetric 3D T1-weighted, FLAIR and pseudocontinuous arterial spin labelling. Regional segmentation by automated image processing algorithms was used to extract WMH volume (WMHV) and resting cerebral blood flow (CBF). The association between HIV status and WMHV as a proportion of intracranial volume (ICV; log-transformed) was estimated using a multivariable linear regression model. Thirty-eight MLWH (median age 59 years [interquartile range, IQR 55, 64]) and 37 HIV negative (median 58 years [54, 63]) men were analyzed. MLWH had median CD4+ count 570 (470, 700) cells/microliter and a median time since diagnosis of 20 (14, 24) years. Framingham 10-year risk of cardiovascular disease was 6.5% in MLWH and 7.4% in controls. Two (5%) MLWH reported a history of stroke or transient ischaemic attack (TIA) and five (13%) reported coronary heart disease (CHD) compared to none of the controls. The total WMHV in MLWH was 1696 microliters (IQR 1229, 3268 microliters) or 0.10% of ICV compared to 1627 microliters (IQR 1032, 3077 microliters), also 0.10% of ICV, in the HIV negative group ( $p=0.43$ ). In the multivariable model, WMHV/ICV was not associated with HIV status ( $p=0.86$ ). There was an age-dependent decline in cortical CBF ( $-3.9$  ml/100ml/min per decade of life [95% CI 1.1 to 6.7 ml]) but no association between CBF and HIV status ( $p>0.2$  in all brain regions analyzed). In conclusion, we found no quantitative MRI evidence of an increased burden of cerebral small vessel disease in MLWH aged 50 years and over.

## Introduction

An increasing proportion of people living with HIV are over the age of 50, largely due to increased survival,<sup>1, 2</sup> and they are therefore at greater risk of comorbidities of older age such as cardiovascular disease, dementia, hypertension and type 2 diabetes. It is possible that HIV infection in middle and older age is associated with an increased risk of cerebral small vessel disease (CSVD) in addition to that seen in the general population. CSVD is associated with circulating inflammatory mediators,<sup>3, 4</sup> and chronic immune activation and corresponding elevations of systemic inflammatory markers are seen in HIV even after virological suppression has been achieved with antiretroviral therapy (ART).<sup>5</sup> An association between HIV infection and CSVD would be of major importance to those living with HIV because of the increased risk of stroke, dementia, and death in individuals with white matter hyperintensities (WMH) of presumed vascular origin,<sup>6</sup> one of the key quantifiable neuroimaging features of CSVD.<sup>7</sup> It is recognized that vascular pathology makes a substantial contribution to cognitive impairment and dementia in the general population.<sup>8</sup> Cognitive impairment is thought to be highly prevalent in people living with HIV,<sup>9-11</sup> and CSVD is one plausible mechanism for this.

While epidemiological evidence suggests higher rates of large vessel ischemic stroke in HIV infection,<sup>12-17</sup> it cannot be assumed that a similar association exists for CSVD as it is likely to be a different disease process. Early studies of mainly untreated people living with HIV using older neuroimaging techniques found little evidence of an association between HIV and WMH.<sup>18, 19</sup> Furthermore, an autopsy study found no association between CSVD and HIV status.<sup>20</sup> More recently, two other studies measuring the total volume of WMH found no overall difference between people living with HIV and HIV negative participants.<sup>21, 22</sup> Other authors have suggested that there is an association between HIV and CSVD but did not compare neuroimaging and neuropathological measures to HIV negative controls.<sup>23, 24</sup> However, a recent neuroimaging study of Dutch men living with HIV (MLWH) aged over 45 and HIV negative controls reported a greater burden of WMH of presumed vascular origin on brain magnetic resonance imaging (MRI).<sup>25</sup> There is also some evidence to support an association between HIV infection and lower cerebral blood flow (CBF),<sup>26, 27</sup> although this is not consistent across all studies.<sup>28, 29</sup> A recent meta-analysis of studies of HIV negative

individuals showed that lower CBF is associated with higher numbers of WMH, at least in cross-sectional studies.<sup>30</sup>

In the current study, we aimed to assess whether HIV status was associated with an increased volume of WMH in men aged  $\geq 50$  years at two clinics in London, UK. We also sought to compare CBF between MLWH and HIV negative men, and estimate the association between CBF and WMH burden.

## Materials and Methods

### *Participant selection*

We conducted a cross-sectional neuroimaging sub-study within the Pharmacokinetic and Clinical Observations in People over fifty (POPPY) study (ClinicalTrials.gov Identifier NCT01737047).<sup>31</sup> Male participants enrolled in POPPY aged 50 or over at two study sites in London, United Kingdom, were recruited between October 2013 and September 2015. Only those self-identifying with a white ethnic group were recruited. Participants gave written, informed consent and the study was approved by the National Research Ethics Service Committee London – Central, reference 13/LO/1088. Our target sample size was 40 in each group. We excluded participants with current active neurological, psychiatric or substance use disorders.

### *Data collection*

The POPPY cohort database was used to obtain clinical information relating to previous CVD, cardiovascular risk factors, current and previous ART, lymphocyte subset counts, anthropometric data, serum lipids, syphilis and hepatitis C (HCV) serology, and recreational drug use. Framingham 10-year cardiovascular disease risk was calculated using the *framingham* STATA module.<sup>32</sup> Clinical assessments included frailty measurements, namely timed 5 meter walk and grip strength.

### *Magnetic resonance image processing*

Participants underwent a 1-hour imaging protocol including volumetric T1- and T2-weighted sequences, 3D fluid-attenuated inversion recovery (FLAIR), pseudocontinuous arterial spin labelling (pCASL), and a 3D multiple echo recombined gradient echo (MERGE) sequence of the carotid arteries. Images were obtained on the same Phillips 3 Tesla system

for all participants. Individuals with metallic implants such as coronary stents were only imaged if the safety of undergoing MRI could be confirmed.

WMH volume (WMHV) was extracted from volumetric 3D T1 and FLAIR images using automated image processing algorithms.<sup>33</sup>

CBF was calculated from pCASL sequence data after partial volume correction and adjustment for hematocrit.<sup>34</sup> CBF maps were co-registered to structural sequences and CBF was measured in the following regions using automated segmentation algorithms: supratentorial grey matter, deep grey matter (basal ganglia and thalamus), frontal cortex, occipital cortex, parietal cortex, cingulate cortex, and total white matter.

#### *Statistical analysis*

MLWH who did not have a suppressed HIV-1 viral load (<50 copies/mL) on antiretroviral therapy were excluded from the analysis. The main outcome variable of WMHV was expressed as a proportion of intracranial volume [ICV] and log-transformed. This was compared between the MLWH and HIV negative groups using the Mann-Whitney test. Other variables were compared between groups using the Mann-Whitney test and analyzed for their association with WMHV using linear regression; these included linear continuous variables (body mass index, waist circumference, diastolic and systolic blood pressure, and concentrations of total, high density lipoprotein and low density lipoprotein cholesterol) and binary variables (smoking status, self-reported history of ischemic heart disease, stroke or transient ischemic attack [TIA], injection drug use, psychoactive recreational drug use in the past 6 months, hepatitis C antibody status, and previous syphilis). HIV status, age and any other factors found to have at least a modest association ( $p < 0.2$ ) with WMHV in bivariate models were analyzed in multivariable linear regression models.

Additional linear regression analyses including the subgroup of MLWH only explored associations between WMHV and HIV-related factors (linear continuous variables of CD4+ and CD8+ lymphocyte counts and CD4:CD8 ratio, time since HIV diagnosis, time between diagnosis and first initiation of antiretroviral therapy, and total duration of antiretroviral therapy, and binary categorization of current use of all antiretroviral drug classes as well as abacavir and tenofovir), adjusted for age. Finally, linear regression was used to assess the

relationship between CBF in each region of interest and HIV status, and between CBF in each region of interest and WMHV.

## Results

### *Participant characteristics*

There were 38 MLWH and 37 HIV negative controls in the final analysis (Fig. 1); their characteristics are shown in Table 1. The two groups were similar in cardiovascular risk factors, although HIV negative participants had higher low density lipoprotein concentration. MLWH had more previous syphilis (47% vs. 22%;  $p=0.02$ ), but the prevalence of hepatitis C was low in both groups (5% vs. 0%;  $p=0.49$ ). Two (5%) MLWH reported a history of stroke or transient ischemic attack (TIA) and five (13%) reported coronary heart disease (CHD) compared to none of the controls ( $p=0.49$  for stroke/TIA;  $p=0.05$  for CHD). MLWH had a markedly slower 5 meter walk than controls (median 6.3 s [interquartile range (IQR) 5.7, 8.3] vs. 4.5 s [IQR 3.0, 5.6],  $p<0.001$ ) although there was no difference in grip strength ( $p=0.61$ ). The most frequently reported recreational drugs were ketamine (16%), methamphetamine (“crystal meth”, 15%), amphetamine (15%) and cannabis (13%), with no differences between groups. MLWH had a median duration of infection of 20 years (IQR 14, 24 years), a median duration of treatment of 15 years (IQR 12, 20 years), and 17 (45%) had a history of AIDS. Antiretroviral therapy regimens included nucleoside reverse transcriptase inhibitor(s) in 34 (89%), non-nucleoside reverse transcriptase inhibitors in 19 (50%), protease inhibitors in 19 (50%) and an integrase inhibitor in one man (3%).

### *Factors associated with volume of white matter hyperintensities*

There were no differences in WMHV between MLWH and HIV negative participants, whether analyzed by absolute volume or the proportion of total ICV (Table 2). Age was associated with WMHV (unadjusted;  $p=0.02$ ). Ten-year Framingham cardiovascular risk was also associated ( $p=0.049$ ), most likely due to the contribution of age to the risk calculation. Weaker associations ( $0.2 > p \geq 0.05$ ) were present for body mass index (BMI) ( $p=0.13$ ), waist circumference ( $p=0.09$ ) and low density lipoprotein cholesterol concentration (LDL) ( $p=0.10$ ). Adjusting for age, BMI and LDL (Fig. 2), WMHV was not associated with HIV status ( $p=0.86$ ), BMI ( $p=0.20$ ) or LDL ( $p=0.16$ ) but there was an

association with increasing age (1.4-fold increase in WMHV/ICV per decade, 95% confidence interval [CI] 1.0-2.0,  $p=0.038$ ).

Among the subgroup of MLWH, there was no association between WMHV and any of the exposure variables analyzed (absolute and percentage CD4 count, absolute CD8 count, CD4/CD8 ratio, time since HIV diagnosis, time since starting ART, history of AIDS, HCV status, and ART drug class).

#### *Regional distribution of white matter hyperintensities*

The anatomical distribution of WMH was compared between MLWH and controls. To do this, white matter and subcortical structures were subdivided by region (left and right frontal, parietal, occipital and temporal lobes, and a combined basal ganglia / inferotemporal region) and by distance from the ventricular surface (in four concentric layers, numbered from 1 at the periventricular surface to 4 at the juxtacortical margin). The rationale and procedures for this subdivision method have been recently developed and the results can be displayed graphically as “bullseye” plots<sup>35</sup>. Comparison of the median WMHV as a proportion of segmental brain volume between groups was done independently for each of the 36 subregions, using the Mann-Whitney test. After applying Bonferroni’s correction for multiple comparisons, one subregion reached statistical significance, namely layer 4 of the combined basal ganglia / inferotemporal region (WMHV median 0.07% in MLWH [IQR 0.02%, 0.10%] compared to median 0.009% in controls [IQR 0%, 0.04%],  $p=0.0009$ , critical  $p$ -value by Bonferroni’s correction 0.0010). Three other subregions reached conventional statistical significance ( $p<0.05$ ) but were deemed not statistically significant after Bonferroni’s correction. These were layer 4 of the left occipital white matter ( $p=0.02$ ), layer 3 of the basal ganglia / inferotemporal region ( $p=0.04$ ) and layer 3 of the left occipital white matter ( $p=0.04$ ), and in all three subregions there was a greater proportion of WMH in brain tissue in MLWH than in controls.

#### *Cerebral blood flow in HIV positive and HIV negative participants*

Mean resting CBF, measured using pCASL, was compared between MLWH and HIV negative men, in the grey matter of the frontal, occipital, parietal and cingulate cortices, in subcortical grey matter, and in white matter. There were 34 participants in each group with pCASL images of sufficient quality. There was no difference between groups in any of these regions (Table 3). The same null findings were seen after

adjustment for age. Age itself was the only significant factor associated with CBF, with a 3.9 ml/100ml/min decrease in cortical CBF per decade of life (95% CI 1.1 to 6.7 ml) and a 2.8 ml/100ml/min decrease in subcortical CBF per decade of life (95% CI 0.1 to 5.5 ml). Similar associations were seen between age and CBF in all cortical regions. There was no association between CBF and the total volume of white matter hyperintensities in cortical grey matter ( $p=0.52$ ), subcortical grey matter ( $p=0.37$ ) or white matter ( $p=0.48$ ).

### Discussion

We found no difference in the burden of cerebral small vessel disease, estimated by the volume of WMH, between MLWH aged 50 and above and demographically matched HIV negative controls. Previous evidence in the literature offers a mixture of results. In the Netherlands, Su et al used similar techniques to ours to investigate 103 MLWH and 70 HIV negative men aged 45 and above, and found a higher volume of WMH in MLWH (1.0 milliliters of white matter hyperintensity compared to 0.7 milliliters in controls,  $p=0.008$ ).<sup>25</sup> Their HIV positive participants had more tobacco consumption (expressed as pack-years) and a higher waist-hip ratio than controls. Cole and colleagues also found a higher burden of WMH in PLWH than in controls (1126 microliters compared to 824 microliters,  $p=0.015$ ).<sup>29</sup> Their cohort, based in the United Kingdom and the Netherlands, was more than 90% male and there may have been some overlap in the participant pool with the preceding Dutch study. However, they observed that there was no difference between groups in the rate of change over a two-year period. In France, a study using the Fazekas and Schmidt radiological rating scale reported a prevalence of cerebral SVD of 52% in 456 people living with HIV (PLWH) compared to 36% in 154 HIV negative controls (odds ratio 2.3, 95% CI 1.5 to 3.6).<sup>36</sup> The PLWH in their sample were 85% male compared to 77% in the control group ( $p=0.03$ ) and the positive group had statistically significantly higher rates of hypertension, hyperlipidemia, and previous CVD. Null findings come from two other MRI studies, both conducted in the United States and again using measures of WMHV similar to ours. In one there was no difference between the PLWH ( $n=88$ ) and the control group ( $n=49$ ) ( $p=0.49$ ) but neither the average WMHV, the gender, nor the cardiovascular risk profile of the participants were reported.<sup>21</sup> Watson et al also found no difference in WMHV between 65 PLWH and 29 HIV negative controls (2.78 milliliters of WMH compared

to 3.18 milliliters,  $p=0.69$ ).<sup>22</sup> All participants in the latter study were aged over 60, with the control group being on average 2 years older, and there were higher rates of smoking and hypercholesterolemia in the PLWH, but no difference in the prevalence of hypertension. Some studies measuring other biomarkers of CSVD such as retinal vessel calibers<sup>37, 38</sup> and cerebrovascular histology<sup>20</sup> have similarly found no association with HIV.

We also found no evidence of an association between HIV status and resting CBF, supporting the findings of several other groups as well as our own unpublished arterial spin labelling data.<sup>28, 29, 39</sup> However in a study of cerebral vasoreactivity, a dynamic measure of cerebrovascular endothelial function, an attenuated response to hypercapnia was found in HIV-positive participants.<sup>40</sup> We observed decreasing grey matter perfusion with age, which is expected in the normal ageing process.<sup>41</sup>

Our study focused on a specific demographic group and recruited from two locations in the same city, which may limit the generalizability of our findings to other settings. We chose to restrict our sample by ethnic group and gender to eliminate the well-described and powerful confounding effects of these variables on vascular disease markers; the study would likely have been insufficiently powered to perform gender and ethnicity subgroup analyses. We chose an older male cohort because such individuals are at greater risk of vascular disease, and we targeted the predominant demographic and transmission risk group (white men who have sex with men) attending our local clinics and living with HIV in the UK and many high-income settings. It is a strength of our study that the HIV negative controls had similar measured characteristics to the MLWH, and their attendance for sexual health and HIV testing at the same centers as the MLWH should have reduced the effect of unmeasured confounders. There was an incidental finding of a slower timed walk in the HIV positive group, possibly indicating occult brain insult from another etiology unrelated to the primary outcome measure.

The study's power to detect associations between HIV and cerebral SVD was limited by its small sample size. Age was the only variable found to have a strong association with the outcome measures. Most participants were in middle age and therefore at lower risk of cerebrovascular disease than an older-aged group. However the difference observed between the two groups was very small compared to the overall distribution of values, and

despite the small sample the evidence for there being no true difference in WMH volume on the basis of HIV status is strong.

The results of this study contribute to the accumulated evidence so far in this field. In summary, this evidence does not consistently find HIV to be a risk factor for cerebral SVD. The inconsistencies between published studies may in part be due to differences in the method for measuring WMH and the lack of a gold standard.<sup>7</sup> But our findings do not diminish the need for assessment and optimization of individual patients' cardiovascular risks. Of the MLWH, 24% had a 10-year risk of cardiovascular disease (Framingham model) of above 10% (the threshold above which the UK National Institute for Health and Care Excellence recommends statin therapy for primary prevention<sup>42</sup>). Signs, symptoms and risk factors associated with CSVD should be addressed in people living with HIV in the same manner as in HIV negative individuals.

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**Authorship:** LJH conceived and led the study. CS, MS, SO and MJC extracted and processed the magnetic resonance imaging data. All authors contributed to the analysis and interpretation of data. LJH conducted the main analyses and wrote the manuscript. All authors read and approved the final version.

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**Table 1:** Characteristics of enrolled participants and comparison between the two groups

Variable	MLWH (n=38)	HIV negative (n=37)	P value for group-wise comparison *
Age, median years (IQR)	59 (55, 64)	58 (54, 63)	0.61
Blood pressure, median mm Hg (IQR)			
Systolic	125 (117, 131)	126 (116, 138)	0.45
Diastolic	79 (73, 84)	76 (72, 82)	0.37
Smoking, n (%)			
Current	9 (24)	7 (19)	0.82
Previous	15 (39)	14 (38)	
Alcohol/week, median units (IQR)	6 (2, 15)	14 (4, 21)	0.12
Psychoactive drug use in past 6 months, n (%)	11 (29)	9 (24)	0.65
Medical history, n (%)			
Coronary heart disease	5 (13.2)	0	0.05
Stroke or TIA	2 (5.3)	0	0.49
Syphilis	18 (47.4)	8 (21.6)	0.02
Hepatitis C	2 (5.3)	0	0.49
Framingham 10-year CVD risk, median % (IQR) **	6.5 (5.0, 9.4)	7.4 (6.0, 10.4)	0.41
Framingham 10-year CVD risk above 10%,	9/37 (24.3)	8/29 (27.6)	0.78

n/N (%) **			
Body mass index, median kg/m <sup>2</sup> (IQR)	25.4 (23.5, 28.2)	25.0 (23.3, 28.0)	0.66
Waist circumference, median cm (IQR)	93 (86, 100)	90 (86, 100)	0.56
Lipids, median mmol/L (IQR) ***			
Total cholesterol	4.8 (3.9, 5.4)	5.1 (4.2, 5.8)	0.14
HDL	1.3 (1.0, 1.5)	1.3 (1.1, 1.6)	0.55
LDL	2.4 (1.8, 3.3)	3.1 (2.6, 3.5)	0.06
Cholesterol:HDL ratio	3.4 (2.8, 5.0)	4.0 (3.2, 4.6)	0.74
Years since tested HIV positive, median (IQR)	20.4 (14.2, 24.4)	n/a	-
Years since first started ART, median (IQR)	15.1 (11.7, 20.2)	n/a	-
Lymphocytes, median cells/mm <sup>3</sup> (IQR)			
Absolute CD4+	570 (470, 700)	n/a	
Percentage CD4+	34 (23, 39)		
CD8+	1010 (690, 1320)		
CD4+/CD8+ ratio	0.61 (0.39, 0.89)		

\* Groups were compared using the Mann-Whitney test for continuous variables and the Chi-squared test for categorical variables.

\*\* Framingham risk could not be calculated in 9 participants, due to missing lipid results.

\*\*\* Lipid concentrations are presented in SI units (mmol/L). Values can be converted to mg/dL by multiplying by 38.67. A total cholesterol concentration of 4.8 mmol/L equates to around 186 mg/dL.

Abbreviations: ART, antiretroviral therapy; CVD, cardiovascular disease; HDL, high density lipoprotein; IQR, inter-quartile range; LDL, low density lipoprotein; TIA, transient ischemic attack.

**Table 2:** White matter hyperintensity volume in study participants, expressed as median (IQR)

	<b>MLWH (n=38)</b>	<b>HIV negative (n=37)</b>	<b>p *</b>
WMHV, microliters	1696 (1229, 3268)	1627 (1032, 3077)	0.43
Total intracranial volume (ICV), mL	1639 (1577, 1711)	1682 (1574, 1764)	0.33
WMHV, % of ICV	0.10 (0.08, 0.20)	0.10 (0.06, 0.19)	0.36

\* Groups were compared by Mann-Whitney's test. When compared using Student's t-test, p was equal to 0.69, 0.22 and 0.64, respectively.

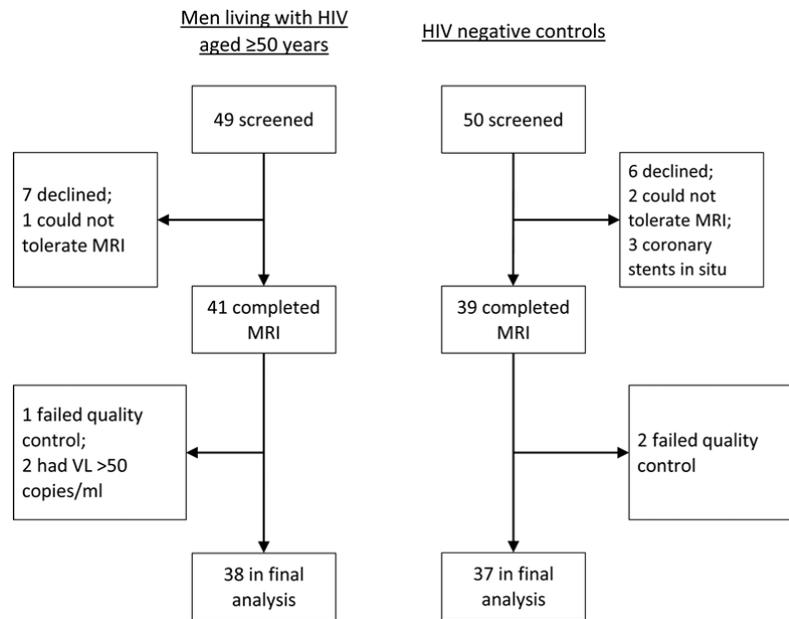
Abbreviations: ICV, intracranial volume; IQR, interquartile range; WMHV, white matter hyperintensity volume.

**Table 3:** Resting cerebral blood flow in MLWH and HIV negative men aged 50 and above

<b>Brain region</b>	<b>MLWH (n=34) *</b>	<b>HIV negative (n=34) *</b>	<b>p **</b>
Frontal cortex	50.1 (43.5, 56.4)	48.6 (44.4, 55.5)	0.90
Occipital cortex	47.4 (40.2, 51.4)	45.0 (39.8, 52.9)	0.69
Parietal cortex	47.9 (40.4, 51.9)	46.6 (41.2, 52.5)	0.92
Cingulate cortex	49.3 (45.4, 56.0)	49.7 (45.5, 53.9)	0.84
Subcortical grey matter	41.7 (37.4, 46.8)	40.4 (37.9, 44.8)	0.49
White matter	26.6 (25.0, 29.2)	26.6 (23.2, 29.0)	0.59

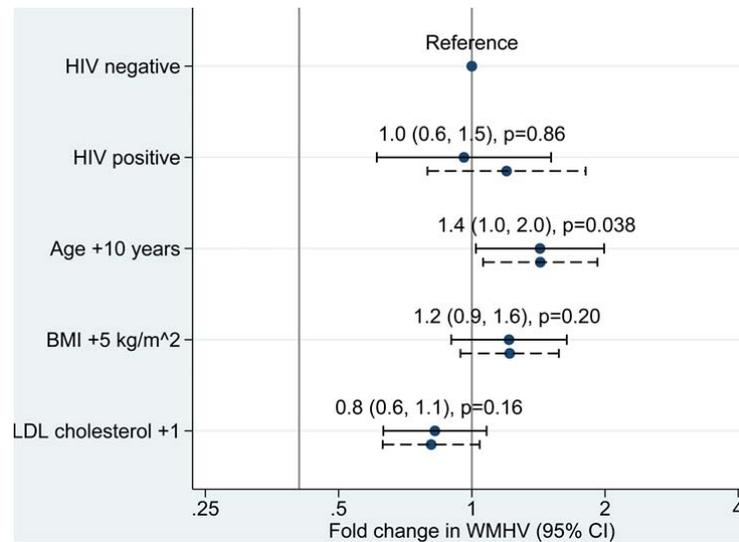
\* Cerebral blood flow is expressed in units of ml of blood per 100 ml of brain tissue per minute, and displayed as median (interquartile range).

\*\* Groups were compared by Mann-Whitney's test.



**Figure 1:** Participant flowchart

Abbreviations: MRI, magnetic resonance imaging; VL, viral load.



**Figure 2:** Association between white matter hyperintensity volume, HIV status and other factors

Footnote: Broken lines denote unadjusted (bivariate) analyses. Solid lines denote adjusted (multivariable) analyses. Factors were included in the multivariable model if they showed  $p < 0.2$  in bivariate models. HIV status was also included. Deflections to the right denote a positive association (higher value of exposure variable correlated with larger volume of white matter hyperintensities). Lipid concentrations were analyzed in SI units (mmol/L). One mmol/L of LDL is equal to 38.67 mg/dL.

Abbreviations: BMI, body mass index; CI, confidence interval; LDL, high density lipoprotein; WMHV, white matter hyperintensity volume (as a proportion of total intracranial volume).