Title: No evidence for accelerated ageing-related brain pathology in treated HIV: longitudinal neuroimaging results from the Comorbidity in Relation to AIDS (COBRA) project.

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Summary

Longitudinal neuroimaging and neuropsychological assessments were used to study ageing-related brain changes in people living with HIV (PLWH) on successful antiretroviral therapy. No differences in brain structure or cognitive function were seen over two years, compared to closely-matched HIV-negative controls.

Short title: Neuroimaging of ageing in treated HIV

Abstract

Background

Despite successful antiretroviral therapy people living with HIV (PLWH) experience higher rates of age-related morbidity, including abnormal brain structure, brain function and cognitive impairment. This has raised concerns that PLWH may experience accelerated ageing-related brain pathology.

Methods

We performed a multi-centre longitudinal study of 134 virologically-suppressed PLWH (median age = 56.0 years) and 79 demographically-similar HIV-negative controls (median age = 57.2 years). To measure cognitive performance and brain pathology, we conducted detailed neuropsychological assessments and multi-modality neuroimaging (T1-weighted, T2-weighted, diffusion-MRI, resting-state functional-MRI, spectroscopy, arterial spin labelling) at baseline and after two-year follow-up. Group differences in rates of change were assessed using linear mixed effects models.

Results

123 PLWH and 78 HIV-negative controls completed longitudinal assessments (median interval = 1.97 years). There were no differences between PLWH and HIV-negative controls in age, sex, years of education, smoking, alcohol use, recreational drug use, blood pressure, body-mass index or cholesterol levels.

At baseline, PLWH had poorer global cognitive performance ($P<0.01$), lower grey matter volume ($P=0.04$), higher white matter hyperintensity load ($P=0.02$), abnormal white-matter microstructure ($P<0.005$) and greater 'brain-predicted age difference' ($P=0.01$). Longitudinally, there were no significant differences in rates of change in any neuroimaging measure between PLWH and HIV-negative controls ($P>0.1$). Cognitive performance was stable across the study period in both groups.

Conclusions

Our finding indicate that when receiving successful treatment, middle-aged PLWH are not at increased risk of accelerated ageing-related brain changes or cognitive decline over two years, when compared to closely-matched HIV-negative controls.

Keywords: HIV; ageing; neuroimaging; brain structure; cognitive function

Introduction

Combination anti-retroviral therapy (cART) has drastically reduced AIDS-associated morbidity and mortality [1], dramatically improving life-expectancy for people living with HIV (PLWH) [2]. Nevertheless, higher comorbidity rates have been observed in PLWH, including neurological, cardiovascular, renal, hepatic and pulmonary disease, cancer, osteoporosis and physical frailty [3-6]. Given that higher rates of such conditions are typically seen with increasing age, this has raised the concern that PLWH may have an accelerated risk of age-related deterioration in health, despite effective cART.

Higher rates of cognitive impairment are reported in PLWH compared to HIV-negative individuals [7, 8]. These are associated with neuroimaging abnormalities including: lower brain volume, increased white matter hyperintensities (WMHs), abnormal white-matter microstructure, lower cerebral perfusion, changes to neural metabolite concentrations and altered functional connectivity [9-19]. Potentially, this pathology interacts with normal age-related changes to the brain, leading to an apparent 'acceleration' of brain ageing [20]. Accelerated brain changes and cognitive decline may significantly increase the risk of neurodegenerative diseases, substantially impacting public health, given the approximately 35 million PLWH worldwide [21]. However, abnormal brain structure and function in PLWH does not necessarily indicate an accelerated pathological process; brain injury may be either static or progressive, something that cannot be disentangled using cross-sectional studies.

During the cART-era only a small number of longitudinal neuroimaging investigations of PLWH have been conducted [16, 22-29]. Some, but not all, have provided evidence for accelerated brain pathology [23-26, 28, 29]. However, these studies have key limitations. Firstly, some did not include longitudinal assessment of controls. When controls were included, they often differed from PLWH on important factors such as rates of smoking and alcohol use. Hence, the influence of ageing and lifestyle factors associated with 'accelerated' ageing were not controlled for. As these factors influence brain structure and function independently from HIV [30-32], apparent brain abnormalities can be difficult to interpret. Secondly, studies have often included a high proportion of PLWH with unsuppressed plasma HIV RNA. These studies may not be representative of PLWH in resource-rich settings, of whom the vast majority receive suppressive cART. Hence, previous longitudinal studies have not clarified whether brain pathology and cognitive impairment seen in PLWH on suppressive cART are static or accelerating.

Here, we sought to definitively address whether PLWH on cART show accelerated changes in age-related neuroimaging measures. Detailed multi-modal measures of brain structure and function were derived on two occasions in a large cohort assessed longitudinally with a two-year interval. Our hypotheses were: (1) magnetic resonance imaging (MRI) measures of brain structure and function are abnormal at baseline in PLWH; (2) greater rates of change over two years in MRI metrics will be present in PLWH compared to HIV-negative participants, indicating the presence of accelerated brain pathology; and (3) progressive change in MRI metrics will be associated with a decline in cognitive function, despite successful cART.

Methods

Participants

This longitudinal, observational study included 134 PLWH and 79 HIV-negative controls with similar demographic and lifestyle characteristics (Table 1). Recruitment of HIV-negative individuals was via social networks of PLWH participants and through sexual health clinics, to reduce recruitment biases that may cause important demographic or lifestyle differences. Participants were recruited from London and Amsterdam as part of the COmorBidity in Relation to AIDS (COBRA) collaboration [14, 20, 33]. Assessments were conducted at baseline and after two years. Exclusion criteria were: age <45 years, current depressive symptoms (Patient Health Questionnaire-9 score ≥15), neurological or psychiatric diagnosis, previous cerebral infections (including AIDS-defining central nervous system [CNS] diseases), self-reported intravenous drug use within the past six months, daily recreational drugs use (except cannabis), excess alcohol intake (>48 units/week) and MRI contraindications. All PLWH were required to be on cART, with undetectable plasma HIV RNA (<50 copies/mL) for ≥12 months prior to enrolment. The study was approved by the Academic Medical Center, Amsterdam (#NL 30802.018.09) institutional review board and a UK Research Ethics Committee (#13/LO/0584 Stanmore, London). All participants provided written informed consent.

Neuropsychological assessment

Participants completed a comprehensive neuropsychological assessment, testing six cognitive domains: attention, executive function, information processing speed, language fluency, memory and motor function (Supplementary material Table S1). Raw scores were standardised as T-scores, adjusted for age, sex and education level, with higher T-scores representing better cognitive function. T-scores were averaged within domains to calculate domain specific T-scores and across domains to calculate a global T-score. Three PLWH participants were missing data for one or more domains. Follow-up data were missing for one HIV-negative participant.

Neuroimaging acquisition

Multiple modalities of MRI data were acquired at both sites. These were T1-weighted MRI, T2-FLAIR, diffusion-MRI, resting-state fMRI, arterial spin labelling (ASL) and magnetic resonance spectroscopy (MRS), using standard sequences (see Supplementary material for acquisition details). The scanner in London was a Siemens 3T Verio. In Amsterdam a Philips 3T Intera was used initially, then upgraded to a Philips 3T Ingenia during the baseline assessment period.

Neuroimaging processing

Six different MRI modalities were processed to generate measures of brain structure and function. These included both voxelwise measures and regional or global summary metrics (full details in Supplementary material). The measures used were: brain volume, cortical thickness, brain-predicted age difference (brain-PAD) [20], WMH load, white-matter microstructure (i.e., fractional anisotropy, mean diffusivity), resting state functional connectivity, cerebral perfusion and concentrations of neural metabolites (choline [Cho], creatine [Cr], glutamate/glutamine [Glx], myo-inositol [MI], N-acetyl-aspartate [NAA]). Longitudinal neuroimaging data were unavailable for three PLWH and four controls. Further exclusions were made after quality control, per modality (Supplementary material).

Statistical analysis

Univariate analyses were conducted on metrics derived from the neuroimaging processing, resulting in 23 variables. Data normality was determined and non-normal variables were log transformed. Cross-sectional baseline analysis used linear regressions with each neuroimaging measure as the outcome variable and HIV status, age, scanner and intracranial volume (ICV) as predictors. For longitudinal analysis, linear mixed effects models were used with each neuroimaging metric as the outcome variable, fixed effects for visit (i.e., baseline and follow-up), HIV status, age, scanner and ICV and a random effect identifying each subject, which modelled repeated measures. Differences in rates of change were evaluated by assessing the significance of group-by-visit interactions. Least-squares mean estimates were derived from linear mixed models to provide group means adjusted for covariates. The same approach was used to assess neuropsychological data, using HIV status, age and study site as predictors. These analyses were conducted using R v3.2 [34].

Voxelwise analyses were conducted for longitudinal assessment of brain volume, white-matter microstructure and cerebral perfusion, using spatially normalised data. For brain volume, the Jacobian determinants representing volumetric expansions and contractions between baseline and follow-up were analysed. For white-matter microstructure and perfusion, baseline DTI or blood-flow values were subtracted from follow-up values, giving a 'difference' map. General linear models to compare groups used FSL 'Randomise' [35], covarying for age, scanner and ICV. Multiple comparison correction involved 10,000 permutations and threshold-free cluster enhancement.

Results

Characteristics of PLWH and HIV-negative controls

The PLWH and HIV-negative controls were similar in terms of age and sex (table 1), though there was a higher proportion of people of Black-African ethnicity in the PLWH group (P = 0.02). The groups were had similar years of education, smoking and alcohol intake, use of recreational drugs, blood pressure, cholesterol levels and body-mass index. All PLWH were receiving cART and had plasma HIV RNA levels of below 50 copies/ml. The median CD4+ T lymphocyte count was 618 cells/μL. The median time since HIV diagnosis with 15 (inter-quartile range=9-20) years and the median duration of cART was 13 (7-17) years.

Longitudinal participation

Of the 213 participants who took part in the baseline assessment, N=12 did not complete the follow-up (PLWH N=11, HIV-negative N=1), due to death (N=3), time commitments (N=2) or health complications (N=7). This left a total of 201 participants with longitudinal data, a retention rate of 94.3%.

Baseline cognitive function

At baseline, PLWH showed poorer global cognitive performance in neuropsychological tests, relative to HIV-negative controls (P<0.001, Table 2), as previously reported in this cohort [14, 20, 33]. Differences were observed in the domains of attention (P<0.001), information processing speed (P=0.001), executive function (P=0.02) and motor function (P<0.001). Language (P=0.15) and memory performance (P=0.15) did not differ significantly between the groups.

Abnormal brain structure is seen in PLWH

Baseline analysis indicated that PLWH had significantly lower grey matter volume (*P*=0.04) and greater WMH load (*P*=0.02), compared to HIV-negative controls (Table 2, Fig 1). PLWH had a significantly greater brain-PAD (*P*=0.01). Measures of white-matter microstructure were also significantly different between the groups, with PLWH showing lower fractional anisotropy (*P*=0.01) and higher mean diffusivity (*P*=0.01) in the whole-brain white-matter 'skeleton'. There were no group differences in cortical thickness, white-matter volume, cerebrospinal fluid volume, cerebral perfusion, neural metabolite concentrations or functional network connectivity (Table 2).

Changes in cognitive function

Generally, both PLWH and HIV-negative controls showed similar changes in neuropsychological tests between baseline and follow-up (Table 3, Fig 2), except for attention and memory T-scores. For memory, significant improvements were observed across both groups (*P*<0.001). For attention, there was a significant interaction between group and visit (*P*=0.02), which was driven by a reduction in performance in the HIV-negative group.

Rates of change in brain measures are similar in PLWH and controls

Both PLWH and HIV-negative participants showed longitudinal reductions in brain volumes, altered white matter structure, increased WHM load and reduced cerebral perfusion using univariate neuroimaging measures of brain structure (Table 3). However, group-by-visit interactions were not significant for any of the neuroimaging modalities and brain-PAD (all *P*>0.1), indicating that the rates of change did not differ between groups (Fig 3). Within PLWH there were no significant relationships between longitudinal neuroimaging changes and current CD4 count, nadir CD4 count, time since HIV diagnosis, cART duration or past AIDS diagnosis (all *P*>0.1). Voxelwise analysis of changes in brain volumes, white-matter microstructure and cerebral perfusion showed no significant longitudinal differences between PLWH and HIV-negative controls.

Discussion

In PLWH with suppressed viraemia on cART, we found no evidence for accelerated changes in brain structure, function or brain-predicted age and no evidence for cognitive decline over two years. Cross-sectional abnormalities in brain structure, brain-predicted age, and cognitive function were apparent in our group of PLWH compared to controls. Importantly, these differences between the two groups remained stable over time. Overall, we observed longitudinal changes in neuroimaging measures, likely related to ageing. Importantly, studies of other neurological or neurodegenerative conditions (e.g., Alzheimer's disease, traumatic brain injury) commonly report changes above and beyond expected ageing-related declines in shorter timescales [36, 37]. Hence, our results indicate that in PLWH on suppressive cART, any progressive changes to the brain that occur are no greater than those seen in appropriately-matched HIV-negative controls.

Our comprehensive multi-modality neuroimaging study clarifies previous reports that suggested progressive brain injury was occurring in PLWH, perhaps due to key methodological differences. For example, Gongvatana and colleagues reported two-year progressive changes in neural metabolites in PLWH, but this MRS study did not include an HIV-negative control group [25]. We also observed reductions in NAA over time, but these were similar in PLWH and HIV-negative controls and so are

likely a consequence of ageing or secondary factors other than HIV-infection. Pfefferbaum and colleagues [26] reported greater reductions in regional brain volumes in PLWH. Their control group had more years of education, fewer depressive symptoms, higher socioeconomic status, higher IQ and lower rates of smoking. These factors may have introduced bias as they are known to interact with age-related volume loss [30, 32]. Most recently, Clifford and colleagues reported more rapid rates of brain volume loss in PLWH aged ≥60 years compared to healthy controls. The controls here had lower rates of current and past smoking, and showed very little change in brain volumes over time (e.g., 0.16% annualised grey matter volume reduction, compared to 0.65% in PLWH). Studies in the general population report around 0.4%/year [38]. By contrast, the HIV-negative controls in the current study lost a mean 0.89% of grey matter volume per year. Potentially, Clifford and colleagues' controls had particularly good brain health for their age, which may not be attributable solely to the absence of HIV. This highlights the importance of using appropriate controls, not only for HIV research, but for any studies considering brain health during ageing. Limiting the impact of lifestyle and demographic factors is essential to disentangle the effects of ageing, disease and common comorbidities, particularly for such longitudinal studies.

The composition of the HIV-positive group can also strongly influence neuroimaging results. We restricted analysis to PLWH on cART with suppressed viraemia and no history of CNS infections. This is broadly representative of PLWH in Europe and North America and allows us to address the natural history of chronic HIV infection when HIV viraemia is adequately suppressed. This has not been possible in all previous longitudinal studies. For example, whilst Cardenas and colleagues [24] reported results in comparison with a control group, only 53% of PLWH were virally-suppressed at both time points and people with a history of CNS opportunistic infections were included. Untreated HIV or a history of CNS infections likely leads to progressive neural deterioration. In our study of virologically-suppressed PLWH this progression was not observed.

We observed baseline differences in brain structure between PLWH and controls in several neuroimaging modalities. These included evidence of lower grey matter volume, higher WHM load and extensive areas of white matter abnormalities, in line with previous reports [9, 13, 14]. Cross-sectional differences were not observed in measures of resting-state functional connectivity (fMRI), neural metabolite concentrations (MRS) or cerebral perfusion (ASL), contrary to previous reports during the cART era [11, 12, 15]. The discrepancy between our findings and these studies could be explained by differing samples or scanner-related and MRI-protocol differences. Additionally, these modalities may measure more dynamic aspects of HIV-associated brain injury that may improve with successful treatment compared with volumetric measures (e.g., grey-matter volume loss), which may be irreversible. An important contributing factor could be the noise levels in fMRI, MRS and ASL, relative to diffusion-MRI, T2-FLAIR and particularly T1-weighted MRI. As our study pooled data from three scanners, scanner-related variability is also an issue. That structural MRI measures were sensitive to group differences in this multi-centre study is important when considering future study design, particularly regarding longitudinal assessment and clinical trials.

PLWH had poorer cognitive performance at baseline, as previously observed [14, 20]. Longitudinally, performance was generally stable, though improvements were seen in memory, potentially reflecting practice effects. Interestingly, performance in measures of attention decreased in HIV-negative controls, but not in PLWH. The noticeably high baseline attention scores of HIV-negative

participants suggests that this reduction could be a regression towards the 'true' group mean. Overall, the cognitive performance of both groups was high, compared to the normative average T-score of 50. This may reflect a 'research participant' effect; that nearly half had tertiary-level education illustrates the difficulties faced in recruiting truly representative study samples. To better understand trajectories of cognitive ageing in PLWH, future studies would benefit from larger numbers, more frequent assessments and run-in periods to mitigate practice effects.

Differences in longitudinal rates of changes in brain structure or brain function between the two groups were absent. This suggests that the cross-sectional neuroimaging and cognitive deficits observed are the result of historical pathological processes, such as the direct pathogenic effect of HIV on the CNS or the initial immune response to infection, rather than an ongoing pathological process. Further work to clarify the pathogenic processes leading to these abnormalities is warranted. Nevertheless, the presence of the cross-sectional differences in PLWH is important, as it could mean that PLWH reach a symptomatic threshold for age-related cognitive decline earlier that HIV-negative people, despite appearing to be on the same trajectory.

Some strengths and weakness of our study should be noted. Strengths include the large sample size for a longitudinal neuroimaging study, which gave sufficient statistical power to detect even small changes in brain structure over two years. The two-year follow-up period limits extrapolation to longer-term changes, however the consistently parallel nature of the group 'slopes' of change suggests that brain changes in PLWH with suppressed viraemia are not diverging from those seen in HIV-negative people. Our control group was similar to the PLWH group, ethnicity notwithstanding, potentially reducing confounding factors that may independently affect brain ageing. Another strength is the use of six neuroimaging modalities, sensitive to many different neurobiological phenomena, allowing a comprehensive assessment of the brain. Weaknesses include the potential for recruitment bias whereby PLWH may be higher functioning that the majority of the HIV population, reflected by a willingness to enter a longitudinal study. Also, we specifically recruited non-depressed PLWH to be able to interpret the cognitive findings. However, depression is commonly reported in PLWH [39] and brain ageing may differ in subjects with depressive illnesses. The use of three different scanners, including a system upgrade during the baseline phase, may have influenced some neuroimaging measures. However, we explicitly controlled for this in our statistical analysis, and crucially, proportions of PLWH and HIV-negative controls scanned at each site were similar. The proportion of PLWH and HIV-negative controls affected by the upgrade (i.e., different scanners at baseline and follow-up) was similar to the overall group proportions in the study, thus are representative and did not introduce bias. To investigate scanner effects further, we analysed data from London only (Supplementary Table S2). There were only minor differences in cross-sectional results and the longitudinal results were the same as the overall results, providing further reassurance that the combination of scanners did not influence our findings. Another potential limitation is survivor bias, whereby only unrepresentatively healthy individuals completed longitudinal assessment. However, we found no systematic differences between participants who dropped out and participants who completed both assessments (Supplementary Table S3) and given the high retention rate (94.4%), any residual survivor bias is likely to be minimal.

In conclusion, our study finds no evidence of accelerated ageing-related changes in brain structure, function, and brain-predicted age in well-treated PLWH. This provides reassurance that, with

virological suppression, PLWH in middle-age are not at increased risk of progressive cognitive decline and abnormal deterioration to brain health over two years.

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Potential conflicts of interest:

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Tables

Table 1. Characteristics of PLWH and HIV-negative participants

	PLWH	HIV-negative	*p* value
N	134	79	
Age at baseline - years	56 (51-62)	57 (52-65)	0.22
Sex			0.79
Female	9 (7%)	6 (8%)	
Male	125 (93%)	73 (92%)	
Ethnicity			0.02
White	118 (88%)	77 (97%)	
Black-African	16 (12%)	2 (3%)	
Study site			0.31
Amsterdam	75 (63%)	50 (56%)	
London	59 (37%)	29 (44%)	
Scanner system at baseline*			0.04
Siemens Verio 3T	59 (44%)	29 (37%)	
Philips Intera	46 (34%)	20 (25%)	
Philips Ingenia	29(22%)	30 (38%)	
Completed follow-up	123 (92%)	78 (99%)	0.83
Assessment interval - years	1.97 (1.77-2.12)	1.91 (1.76-2.07)	0.20
Years of education	14 (13-16)	16 (14-16)	0.21
Smoking status			0.26
Current smoker	40 (30%)	20 (25%)	
Ex-smoker	58 (43%)	29 (37%)	
Never smoked	36 (27%)	30 (38%)	
Alcohol consumption - units per week	5.5 (1.5-15.0)	7.5 (1.5-17.5)	0.37
Use of recreational drugs in past 6 months	44 (33%)	18 (23%)	0.16
Systolic blood pressure - mmHg	131 (124–140)	130 (123–142)	0.63
Diastolic blood pressure - mmHg	85 (78–93)	84 (77–91)	0.55
Total cholesterol/HDL cholesterol ratio	4.1 (3.4–5.1)	4.0 (3.5–4.8)	0.63
Body Mass Index - kg/m^2	24.6 (22.6–27.4)	24.6 (23.2–28.4)	0.29
Time since HIV diagnosis - years	15 (9-20)	-	
Duration of antiretroviral therapy - years	13 (7-17)	-	
Plasma HIV-RNA <50 copies/mL	134 (100%)	-	
History of clinical AIDS	42 (31%)	-	
Nadir CD4+ count - cells/µL	180 (90-250)	-	
CD4+ count - cells/µL	618 (472-806)	-	
CD4+:CD8+ cell count ratio	0.84 (0.60-1.12)	-	
Anti-retroviral regimens			
Currently on NRTIs, n (%)	124 (92.5%)	-	
On 1 NRTI, n (%)	11 (8.9%)	-	
On 2 NRTIs, n (%)	109 (87.9%)	-	
On 3 NRTIs, n (%)	2 (3.2%)	-	
Currently on PIs, n (%)	68 (50.7%)	-	
Currently on NNRTIs, n (%)	74 (55.2%)	-	
Currently on other drugs, n (%)	21 (15.7%)	-	

Data are median (inter-quartile range) or N (%). P values refer to group comparison Wilcoxon rank-sum or Fisher exact tests where appropriate. NRTIs = Nucleoside reverse-transcriptase inhibitors, PIs = Protease inhibitors, NNRTIs = Non-nucleoside reverse-transcriptase inhibitors. *All participants were scanned on the same scanner system at baseline and follow-up, except for those scanned on the Philips Intera at baseline, who were scanned on the Philips Ingenia at follow-up.

Table 2. Baseline cross-sectional analysis of neuropsychological and neuroimaging measures

Measure	*Baseline*			
	Group means [95% CI]		**Parameter estimate [95% CI]**	***P* value**
	PLWH (N)	**HIV-negative (N)**		
Neuropsychology	**131**	**79**		
Attention	49.68 [47.86, 51.50]	56.04 [53.64, 58.43]	-0.13 [-0.20, -0.06]	<0.001
Executive function	48.58 [47.14, 50.02]	51.25 [49.36, 53.14]	-0.06 [-0.11, -0.01]	0.02
Language fluency	51.81 [50.17, 55.45]	53.58 [51.44, 55.73]	-0.04 [-0.09, 0.01]	0.15
Memory	55.49 [54.10, 56.88]	57.38 [55.55, 59.21]	-0.03 [-0.07, 0.01]	0.16
Motor function	46.80 [45.34, 48.27]	50.56 [48.64, 52.48]	-0.10 [-0.15, -0.04]	<0.001
Processing speed	50.50 [49.15, 51.85]	54.18 [52.41, 55.96]	-0.08 [-0.12, -0.03]	0.001
Global cognitive performance	50.46 [49.38, 51.54]	53.81 [52.39, 55.23]	-0.07 [-0.11, -0.03]	<0.001
Neuroimaging				
T1-MRI	**134**	**78**		
Grey matter volume (L)	0.66 [0.65, 0.67]	0.68 [0.67, 0.69]	-0.01 [-0.03, 0.00]	0.035
White matter volume (L)	0.48 [0.47, 0.49]	0.48 [0.47, 0.50]	0.00 [-0.01, 0.01]	0.46
Cerebrospinal fluid volume (L)	0.36 [0.34, 0.37]	0.36 [0.34, 0.38]	0.01 [-0.01, 0.03]	0.26
Cortical thickness (mm)	2.36 [2.35, 2.38]	2.38 [2.36, 2.40]	-0.02 [-0.05, 0.01]	0.15
Brain-PAD (years)	1.60 [0.19, 3.01]	-0.88 [-2.61, 0.85]	3.04 [0.75, 5.32]	0.01
Diffusion-MRI	**119**	**73**		
Whole brain fractional anisotropy	0.55 [0.54, 0.55]	0.55 [0.55, 0.56]	-0.01 [-0.01, 0.00]	0.005
Whole brain mean diffusivity†	0.74 [0.74, 0.75]	0.73 [0.72, 0.74]	0.02 [0.00, 0.03]	0.012
T2-FLAIR	**132**	**71**		
WMH load - mm^3 †	1126.20 [932.6, 1360.1]	823.6 [653.3, 1038.4]	0.38 [0.08, 0.69]	0.015
Resting-state fMRI	**103**	**61**		
Default mode network	4.66 [4.35, 4.97]	5.09 [4.71, 5.48]	-0.39 [-0.88, 0.10]	0.12
Executive control network	7.46 [7.04, 7.88]	7.56 [7.03, 8.09]	-0.14 [-0.79, 0.52]	0.68
Fronto-parietal network left†	4.99 [4.70, 5.28]	4.81 [4.44, 5.17]	0.04 [-0.05, 0.12]	0.39
Fronto-parietal network right	6.10 [5.78, 6.43]	6.11 [5.70, 6.53]	-0.07 [-0.61, 0.47]	0.80
Sensorimotor	9.32 [8.72, 9.91]	8.67 [7.93, 9.41]	0.62 [-0.25, 1.50]	0.16
Auditory†	7.87 [7.33, 8.41]	7.61 [6.94, 8.23]	0.01 [-0.08, 0.10]	0.76
Visual – medial	12.23 [11.72, 12.85]	12.05 [11.35, 12.75]	0.31 [-0.59, 1.21]	0.50
Visual – lateral	6.36 [60.4, 6.69]	6.28 [5.87, 6.68]	0.06 [-0.46, 0.58]	0.81
Visual – occipital	6.92 [6.57, 7.28]	6.56 [6.12, 7.00]	0.33 [-0.23, 0.90]	0.25
Cerebellar	7.99 [7.40, 8.58]	8.33 [7.60, 9.07]	-0.38 [-1.27, 0.50]	0.39
MRS – Frontal white matter	**111**	**58**		
N-Acetyl Aspartate	1.39 [1.35, 1.44]	1.43 [1.37, 1.49]	-0.03 [-0.11, 0.04]	0.37
Myo-inositol	0.64 [0.6, 0.69]	0.67 [0.61, 0.73]	-0.03 [-0.09, 0.03]	0.26
Choline	0.36 [0.35, 0.37]	0.38 [0.36, 0.39]	-0.02 [-0.04, 0.00]	0.09
Glutamate/Glutamine†	1.02 [0.92, 1.12]	1.07 [0.94, 1.20]	-0.03 [-0.20, 0.14]	0.72
ASL	**128**	**71**		
Grey matter perfusion (mL/100g/min)	62.41 [60.00, 64.83]	62.39 [59.38, 65.40]	0.49 [-3.44, 4.42]	0.81

Group means values are least square means (i.e., adjusted for covariates) in the appropriate units. CI = confidence intervals. P values reported are derived from analyses of group factors on the outcome variables, using linear regression. Mean diffusivity values are reported in units of $x10^{-3}mm^2/s^{-1}$. Resting-state fMRI values represents relative measures of within-network connectivity. MRS values are reported as a ratio of creatine. Neuropsychological tests are reported at T-scores, based on normative data adjusted for age, sex and education. †Indicates that the variable was log transformed due to having a non-normal distribution.

Table 3. Longitudinal analysis of neuropsychological and neuroimaging measures

Measure	*Rates of longitudinal change*			
	Group means [95% CI]		**Parameter estimate [95% CI]**	***P* value**
	PLWH (N)	**HIV-negative (N)**		
Neuropsychology	**123**	**77**		
Attention	0.71 [-2.65, 4.07]	-2.19 [-6.48, 2.09]	2.69 [0.52, 4.87]	0.02
Executive function	0.04 [-2.64, 2.72]	0.94 [-2.48, 4.37]	-0.85 [-2.53, 0.83]	0.33
Language fluency	0.25 [-2.78, 3.29]	0.29 [-3.57, 4.16]	-0.18 [-1.89, 1.53]	0.84
Memory	2.19 [-0.41, 4.80]	3.45 [0.13, 6.78]	-1.19 [-2.69, 0.31]	0.12
Motor function	0.43 [-2.37, 3.24]	0.39 [-3.19, 3.98]	0.04 [-1.69, 1.77]	0.97
Processing speed	0.84 [-1.66, 3.33]	0.42 [-2.77, 3.60]	0.45 [-0.78, 1.68]	0.48
Global cognitive performance	0.75 [-1.23, 2.72]	0.55 [-1.98, 3.08]	0.18 [-0.59, 0.96]	0.64
Neuroimaging				
T1-MRI	**120**	**74**		
Grey matter volume (L)	-0.01 [-0.03, 0.01]	-0.01 [-0.04, 0.01]	-0.001 [-0.10, 0.01]	0.70
White matter volume (L)	-0.01 [-0.02, 0.01]	0.00 [-0.03, 0.02]	0.00 [-0.05, 0.03]	0.78
Cerebrospinal fluid volume (L)	0.01 [-0.01, 0.04]	0.01 [-0.02, 0.05]	-0.001 [-0.01, 0.10]	0.85
Cortical thickness (mm)	-0.03 [-0.06, 0.01]	-0.02 [-0.06, 0.02]	-0.005 [-0.07, 0.06]	0.55
Brain-PAD (years)	-0.17 [-2.70, 2.36]	-0.47 [-3.69, 2.75]	0.30 [-1.86, 8.79]	0.70
Diffusion-MRI	**119**	**71**		
Whole brain fractional anisotropy	-0.01 [-0.02, 0.00]	-0.01 [-0.02, 0.00]	-0.001 [-0.01, 0.02]	0.64
Whole brain mean diffusivity†	0.02 [0.00, 0.03]	0.01 [-0.01, 0.03]	0.007 [-0.04, 0.02]	0.15
T2-FLAIR	**119**	**74**		
WMH load (mm^3)†	749.3 [-506.4, 2004.9]	442.3 [-1184.7, 2069.4]	0.10 [0.04, 1.52]	0.12
Resting-state fMRI	**104**	**57**		
Default mode network	0.37 [-0.18, 0.92]	0.46 [-0.26, 1.19]	-0.01 [-1.14, 0.76]	0.75
Executive control network	0.04 [-0.77, 0.85]	0.44 [-0.63, 1.50]	-0.29 [-1.58, 0.98]	0.49
Fronto-parietal network left†	0.18 [-0.34, 0.69]	0.52 [-0.16, 1.20]	0.31 [-1.52, 0.37]	0.24
Fronto-parietal network right	0.09 [-0.50, 0.68]	0.35 [-0.42, 1.13]	-0.29 [-1.80, 0.29]	0.34
Sensorimotor	0.66 [-0.41, 1.73]	1.66 [0.25, 3.07]	-0.91 [-2.89, 0.70]	0.13
Auditory†	0.56 [-0.55, 1.67]	1.74 [0.28, 3.20]	-0.93 [-3.14, 0.01]	0.22
Visual – medial	-0.02 [-1.07, 1.02]	0.32 [-1.06, 1.69]	-0.35 [-2.46, 1.33]	0.45
Visual – lateral	0.12 [-0.49, 0.73]	0.41 [-0.40, 1.21]	-0.23 [-1.93, 0.14]	0.43
Visual – occipital	0.14 [-0.54, 0.82]	0.55 [-0.35, 1.45]	-0.32 [-2.08, 0.19]	0.32
Cerebellar	0.11 [-0.99, 1.20]	0.85 [-0.60, 2.29]	-0.70 [-2.05, 1.66]	0.21
MRS – Frontal white matter	**115**	**71**		
N-Acetyl Aspartate	-0.03 [-0.13, 0.06]	-0.08 [-0.20, 0.04]	0.04 [-0.16, 0.11]	0.44
Myo-inositol	-0.06 [-0.14, 0.01]	-0.03 [-0.13, 0.07]	-0.03 [-0.16, 0.08]	0.54
Choline	0.00 [-0.03, 0.02]	-0.02 [-0.05, 0.01]	0.02 [-0.04, 0.04]	0.16
Glutamate/Glutamine†	-0.01 [-0.21, 0.18]	-0.04 [-0.29, 0.22]	0.02 [-0.24, 0.34]	0.89
ASL	**92**	**60**		
Grey matter perfusion (mL/100g/min)	-5.45 [-10.05, -0.86]	-7.58 [-13.39, -1.76]	1.32 [-6.49, 10.51]	0.55

Group means values are least square means (i.e., adjusted for covariates) in the appropriate units. Longitudinal group difference parameter estimates are from linear mixed effects models of group-by-visit interactions. P values reported are derived from analyses of group factors on the outcome variables, using linear mixed effects models (interaction group*time). N are the number of participants included after quality control, for each modality. CI = confidence intervals. Mean diffusivity values are reported in units of $x10^{-3}mm^2/^{s-1}$. Resting-state fMRI values represents relative measures of within-network connectivity. MRS values are reported as a ratio of creatine. Neuropsychological tests are reported at T-scores, based on normative data adjusted for age, sex and education. †Indicates that the variable was log transformed due to having a non-normal distribution.

Figure Legends

Fig 1. Summary of voxel-wise and vertex-wise neuroimaging results

A) Significant baseline differences between PLWH and HIV-negative controls were seen in four neuroimaging modalities (displayed voxelwise or on the cortical surface). Highlighted regions show where PLWH showed lower brain volume (red-yellow areas), lower fractional anisotropy (red), higher white matter hyperintensities and lower cortical thickness (red-yellow areas, left hemisphere displayed). B) Longitudinal data for these four neuroimaging modalities are displayed to illustrate significant changes between baseline and follow-up within each group. Longitudinal changes are seen for T1-MRI with increased lateral ventricles volume (red areas) and reduced grey matter volume (blue areas). For diffusion-MRI reductions in fractional anisotropy (red-yellow) were observed, overlaid on the mean white matter 'skeleton' (green) and an average fractional anisotropy image. For assessment of white-matter hyperintensities, a moderate increase in average distribution was seen in both groups. For cortical thickness, changes are plotted on an inflated cortical surface (left hemisphere) for both groups.

Fig 2. Longitudinal changes in neuropsychological tests

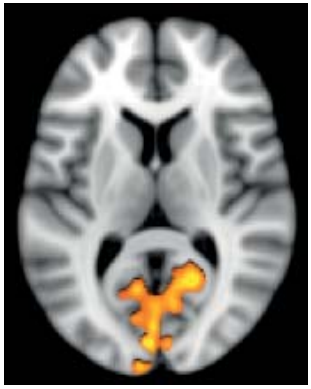
Within-group change between baseline and follow-up in cognitive domain summary T-scores for PLWH and HIV-negative controls. Values plotted are the least-square means and associated 95% confidence intervals, adjusted for covariates. A) Attention, B) Executive function, C) Language fluency, D) Memory, E) Motor function, F) Processing speed, G) Global cognitive performance (average across the six domains).

Fig 3. Longitudinal changes in neuroimaging measures

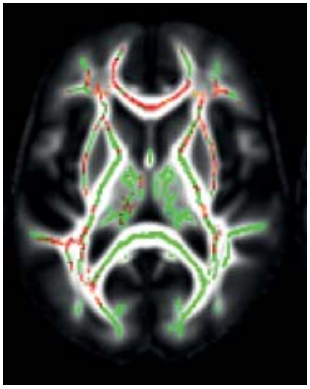
Within-group change between baseline and follow-up in neuroimaging measures for PLWH and HIV-negative controls. Values plotted are the least-square means and associated 95% confidence intervals, adjusted for covariates. A) Grey matter volume, B) White matter volume, C) Brain-predicted age difference (Brain-PAD), D) cortical thickness averaged across both hemispheres, E) White matter hyperintensity load, F) Cerebral blood flow averaged across cortical grey matter, G) Fractional anisotropy averaged across the whole brain white matter skeleton, H) Mean diffusivity averaged across the whole brain white matter skeleton, I) N-acetyl aspartate concentration (as a ratio of creatine) in frontal white matter, J) Default mode within-network connectivity, K) Sensorimotor within-network connectivity, L) Choline concentration (as a ratio of creatine) in frontal white matter.

A) Baseline group differences between PLWH and HIV-negative controls

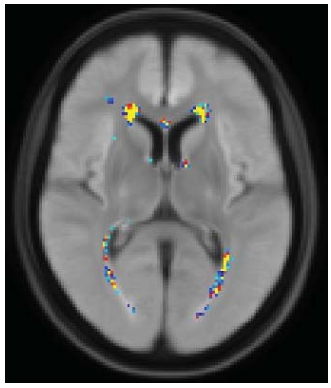
T1-MRI
Brain volume



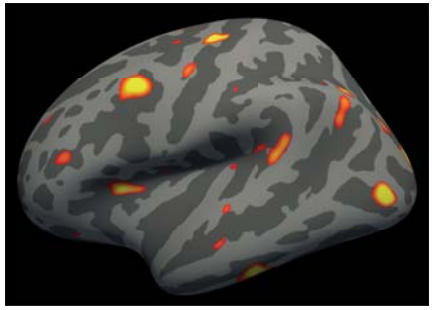
Diffusion-MRI
White matter structure



T2 FLAIR
White matter hyperintensities

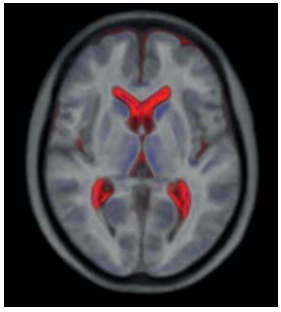


T1-MRI
Cortical thickness

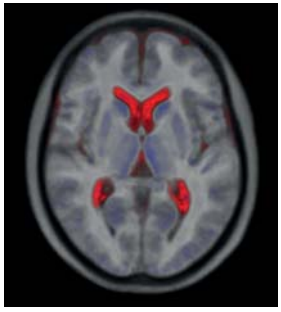


B) Longitudinal changes by group

T1-MRI
Brain volume

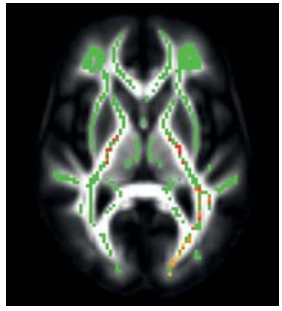


PLWV

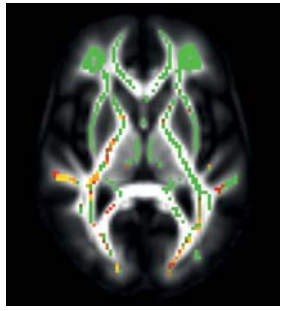


HIV- controls

Diffusion-MRI
White matter structure

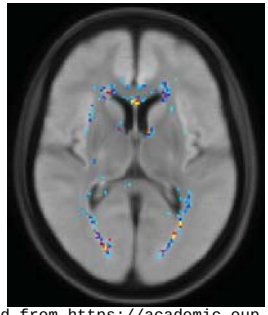


PLWH

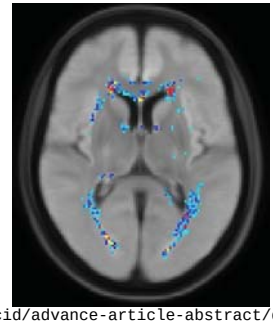


HIV- controls

T2 FLAIR
White matter hyperintensities



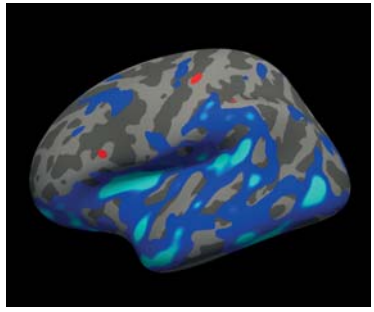
PLWH



HIV- controls

T1-MRI
Cortical thickness

PLWH



HIV- controls