Structural brain abnormalities in successfully treated HIV infection: associations with disease and cerebrospinal fluid biomarkers

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Summary

Lower grey matter volume and white matter microstructural abnormalities in persons with HIV on suppressive cART likely reflect historical injury that occurred during untreated infection, as well as more general influence of systemic factors such as hypertension and ongoing neuroinflammation.

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Abstract

Background

Brain structural abnormalities have been reported in persons with HIV (PWH) on suppressive combination antiretroviral therapy (cART), but their pathophysiology remains unclear.

Methods

We investigated factors associated with brain tissue volumes and white matter microstructure (fractional anisotropy) in 134 PWH on suppressive cART and 79 comparable HIV-negative controls, aged ≥45 years from the Co-morBidity in Relation to AIDS (COBRA) cohort, using multimodal neuroimaging and cerebrospinal fluid (CSF) biomarkers.

Results

Compared to controls, PWH had lower grey matter volumes (-13.7 mL [95%-confidence interval -25.1, -2.2 mL]) and fractional anisotropy (-0.0073 [-0.012, -0.0024]), with the largest differences observed in those with prior clinical AIDS. Hypertension and CSF soluble CD14 concentration were associated with lower fractional anisotropy. These associations were independent of HIV serostatus ($P_{interaction}$=0.32 and $P_{interaction}$=0.59, respectively) and did not explain the greater abnormalities in brain structure in relation to HIV.

Conclusions

The presence of lower grey matter volumes and more white matter microstructural abnormalities in well-treated PWH partly reflect a combination of historical effects of AIDS, as well as the more general influence of systemic factors such as hypertension and ongoing neuroinflammation. Additional mechanisms explaining the accentuation of brain structure abnormalities in treated HIV infection remain to be identified.

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Key words: HIV; neuroimaging; neurofilament light chain; cerebrospinal fluid; biomarkers

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Introduction

Despite the use of combination antiretroviral therapy (cART), widespread brain grey and white matter abnormalities have been reported in persons with HIV (PWH) [1-3], including those with viral suppression [4,5]. For example, we have recently shown that PWH on suppressive cART who participated in the CO-morBidity in Relation to AIDS (COBRA) study demonstrated lower grey matter volumes and more white matter microstructural abnormalities than HIV-negative controls [4]. The pathophysiology of these abnormalities among well-treated PWH remains to be fully elucidated, and likely reflects a range of factors. Untreated HIV, severe immunodeficiency, ill health, e.g. manifested by prior AIDS, as well as ongoing central nervous system (CNS) HIV replication, immune activation, and inflammation despite cART may all contribute [5-8]. Cardiovascular risk factors (e.g. hypertension) and lifestyle factors (e.g. alcohol or recreational drug use) may also play a role [9-12]. Elucidating which of these factors contribute to grey and white matter abnormalities is important as this may provide future therapeutic targets. Conceptually, brain injury could be ongoing (i.e. active) and/or historical (i.e. static). Distinguishing between these possibilities is important as they have different management strategies and prognostic implications.

Cerebrospinal fluid (CSF) biomarkers provide additional temporal information about neuroinflammation and neuronal damage and so can inform whether brain injury visible on neuroimaging is active [13,14]. Various types of CSF biomarkers exist: protein biomarkers commonly identified in neurodegenerative diseases (herein referred to as 'neuronal damage biomarkers') and neuroinflammatory biomarkers. Elevated CSF neurofilament light chain concentrations are linked to active neuronal damage, and are a highly sensitive biomarker of HIV-associated neuronal damage [13,15-17], with highest concentrations found in those with HIV-associated dementia and lowest in those on suppressive cART [16-18]. Associations with other neuronal damage biomarkers, such as total tau (t-tau), are less consistent, but

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some studies found increased concentrations, especially in PWH with HIV-associated dementia [18,19]. CSF phosphorylated-tau (p-tau) and amyloid beta 1-42 fragment (Aβ1-42) reflect Alzheimer-type neurofibrillary tangle pathology and senile plaque pathology respectively. Most studies suggest that these biomarkers are not associated with HIV-associated CNS disease [18,19]. Neuroinflammatory biomarkers have also shown inconsistent results and their relationship to brain injury in well-treated PWH is unclear. Soluble (s)CD14, sCD163, neopterin, and kynurenine:tryptophan (K:T) ratio are all monocyte activation markers. Elevated concentrations of these biomarkers have been observed in PWH on cART [8,20-22], but results are inconsistent [20,23].

The current study aimed to investigate the pathophysiology of the lower grey matter volume and white matter microstructural abnormalities in well-treated PWH compared to appropriately matched HIV-negative controls participating in the COBRA study [4]. We investigated a wide range of factors, including demographics, alcohol and recreational drug use, cardiovascular disease risk factors, plasma/CSF monocyte activation biomarkers, CSF neuronal damage biomarkers, and HIV-related factors.

Methods

Study participants

PWH (n=134) and HIV-negative controls (n=79) were recruited at HIV outpatient clinics, sexual health clinics and from targeted community groups in Amsterdam (n=125) and London (n=88). Inclusion criteria were age ≥45 years (London: ≥50 years), laboratory-confirmed presence or absence of HIV-1 infection and PWH were required to have plasma HIV-RNA<50 copies/mL for ≥12 months on cART. Exclusion criteria were: (history of) confounding neurological diseases, severe head injury (loss of consciousness for ≥30 minutes), infections or tumors involving the CNS (including AIDS-defining illnesses), current major depression (PHQ-9 questionnaire score ≥15), self-reported intravenous drug use in the past six

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months, daily recreational drug (except for cannabis), excess alcohol consumption (>48 units/week), severe psychiatric disorders, insufficient command of the Dutch/English language, or contraindication to magnetic resonance imaging (MRI) or lumbar puncture [4,24]. The primary data for participants included in the current study have been published previously [4].

The study was approved by the local ethics review board of the Academic Medical Center (reference number NL 30802.018.09) and a UK Research Ethics Committee (reference number 13/LO/0584 London – Stanmore). All participants provided written informed consent.

Study visits

Baseline COBRA study visits took place between December 2011 and December 2014, and included neuropsychological assessment, cerebral MRI, and lumbar puncture. CSF data from two participants were incomplete (due to contraindications to lumbar puncture identified after study inclusion). Useable T1 MRI data and complete MRI diffusion data lacked for one and four participants respectively, due to incomplete acquisition (n=1) or excessive movement (n=3).

Blood samples and data regarding age-associated comorbidities, organ dysfunction, and risk factors were collected as described previously [25,26]. In addition, participants were asked to complete a standardized questionnaire from which we obtained information regarding demographic characteristics, medication use, medical history, smoking status, and alcohol/recreational drug use. (Historical) information regarding HIV infection and antiretroviral therapy was obtained from existing databases [27,28].

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Neuroimaging data acquisition and imaging processing

High resolution MRI T1-weighted and diffusion-weighted images were acquired at 3T at both sites along 64 non-collinear directions. In London, images were acquired using a Siemens Verio scanner (Siemens AG, Erlangen, Germany), and in Amsterdam initially with a Philips Intera and later using a Philips Ingenia scanner (both Philips Healthcare, Best, The Netherlands) due to scanner replacement. At both sites, imaging was acquired with comparable acquisition parameters [4,24] (see supplementary methods).

Image processing has been described in detail previously [4,24]. Briefly, 3D T1-weighted images were bias corrected and segmented using SPM12 (University College London, London, UK), and total grey matter volume, white matter volume, and intracranial volume were calculated. Diffusion-weighted data were pre-processed and registered to a custom template and standard space, using Diffusion Tensor Imaging Toolkit v2.3.1 [29,30] to estimate the amount of water diffusion in multiple directions. Fractional anisotropy maps for each participant were then 'skeletonised' using FMRIB Software Library v5.0.6 (FSL, FMRIB, University of Oxford) and thresholded (≥0.2) to exclude areas with considerable inter-individual variability prior to performing tract based spatial statistics [31]. Mean fractional anisotropy values over the skeleton were calculated for each participant. Fractional anisotropy describes the degree of directionality of diffusion by water molecules, and is expressed as a scalar value between zero (representing an isotropic medium with diffusion occurring equally in all directions) and one (representing maximum anisotropy). Hence, a higher fractional anisotropy value represents a more coherent white matter structure [32].

Laboratory assessments

CSF and serum albumin, serum lipids, glycated haemoglobin (HbA1c), glucose, CD4+ and CD8+ T lymphocyte counts, plasma HIV-1 RNA concentrations, and hepatitis B and C virus (HBV/HCV) status

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were measured in fresh samples by local clinical laboratories using routine methods. Other laboratory measurements were performed centrally on cell-free CSF/plasma samples (stored at -80^{o}C).

The CSF to serum albumin ratio (albumin ratio) was calculated from paired samples as an indicator of blood-brain barrier integrity [33]. sCD14 and sCD163 concentrations in plasma and CSF were determined by enzyme-linked immunosorbent assay (ELISA) (CD14/CD163 DuoSet ELISAs, R&D Systems, Minneapolis, Minnesota). CSF neopterin was quantified using ELISA (BRAHMS Diagnostics, Berlin, Germany) [34]. CSF kynurenine and tryptophan were determined by high-performance liquid chromatography [35]. Kynurenine concentrations below the detection limit of 0.1 µmol/L (n=89) were set to half of the detection limit (0.05 µmol/L) to calculate CSF K:T ratio.

CSF neurofilament light chain concentrations were measured by sandwich ELISA (neurofilament light chain ELISA kit; UmanDiagnostics AB, Umeå, Sweden) and upper age-related reference values were calculated [36]. Aβ1-42, p-tau, and t-tau were measured using INNOTEST ELISAs (Fujirebio, Ghent, Belgium) [37–39].

CSF HIV-1 RNA concentration was measured by Abbott RealTime M2000 assay (Abbot, Chicago, USA) with a lower limit of detection of 40 copies/mL.

Statistical analysis

Stata software (version 12.1; StataCorp, USA) was used for all statistical analyses except voxelwise analysis (FSL v5.0.6). Group comparisons were performed using Fisher's exact, or Wilcoxon rank sum tests, as appropriate. Since neuronal damage biomarkers increase with age, multiple linear regression models adjusted for age were used to assess differences between PWH and HIV-negative participants.

Multiple linear regression models were used to identify factors associated with whole brain grey matter volume and white matter skeleton fractional anisotropy. All models were adjusted for a priori defined

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confounders: age, scanner type, and intracranial volume. Scanner type was entered into the model as a three level factor to remove variance associated with potential scanner differences [1,40]. All models were adjusted for intracranial volume as is recommended in volumetric and diffusion tensor imaging analyses [41,42]. Other factors potentially associated with grey matter volume and fractional anisotropy were analyzed using a stepwise model selection approach. Variables were entered into the model when statistically significant ($P<0.05$) in the initial model adjusted for age, scanner type, and intracranial volume. A variable was considered a confounder or mediator if its addition to the model resulted in a change in the coefficient of HIV serostatus of >10%. Continuous variables were $\log_{10}$-transformed to improve normality and/or linearity between independent and dependent variables if necessary. In addition, clinically plausible interactions between associated factors and HIV serostatus were explored.

The following factors were investigated in all individuals:

(1) Demographic factors

(2) Use of recreational drugs and/or alcohol

(3) Cardiovascular disease risk factors

(4) Biomarkers of monocyte activation in plasma and CSF

(5) Albumin ratio

(6) Chronic HBV (defined as detectable hepatitis B surface antigen) or HCV infection (defined as detectable HCV RNA)

(7) Neuronal damage biomarkers

In addition, we explored associations with HIV-specific factors among PWH only:

(8) Prior immunodeficiency (nadir CD4+ T lymphocyte count)

(9) Current CD4+ T lymphocyte count, CD4:CD8 ratio

(10) CSF HIV-RNA

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Lastly, we classified participants into three groups based on their HIV serostatus and prior diagnosis of AIDS-defining illness: HIV-negative, PWH without prior AIDS, and PWH with prior AIDS, since trends towards lower grey matter volume and greater white matter microstructural abnormalities in those with prior AIDS were found in previous work [4]. PWH were classified as having experienced prior AIDS when a category C event as per the Centers for Disease Control and Prevention's classification system for HIV infection was reported, regardless of their CD4+ T lymphocyte count.

Factors identified by linear regression analysis as independently associated with whole brain structural imaging measures were carried forward to perform a voxelwise regression in order to obtain additional spatial information (using tract based spatial statistics for fractional anisotropy and voxel-based morphometry for grey matter volume). These localized associations were calculated using non-parametric permutation testing with 10,000 replications [43], adjusting for age, intracranial volume, and scanner type. Threshold-free cluster enhancement was used to account for spatial dependency of the tests and only corrected p-values <0.05 were considered statistically significant [44].

Results

Cohort characteristics (Table 1)

PWH and HIV-negative participants were of comparable age (median 57 years, interquartile range 51-63 years), gender (93% male), and showed similar cardiovascular disease risk factors, and recreational drug use. More PWH were of African descent. All PWH had plasma HIV RNA <50 copies/mL on cART, median CD4+ T lymphocyte count was 618 cells/µL, and 31% had a prior clinical AIDS diagnosis. CSF HIV RNA was <50 copies/mL in all apart from two participants (59 and 1,043 copies/mL).

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Plasma and CSF biomarkers (Figures 1-3, Supplementary figure)

PWH had higher concentrations of plasma sCD14, plasma sCD163, CSF neopterin, and CSF K:T ratio compared to controls (Figure 1A-B, Figure 2C-D). No group differences were observed for albumin ratio, CSF sCD14, and CSF sCD163 (Figure 1C, Figure 2A-B).

CSF neurofilament light chain concentrations were comparable in the HIV-positive and HIV-negative groups (Figure 3A), but there was a trend towards slightly higher CSF neurofilament light chain concentrations among PWH after adjustment for age (Supplementary figure; +10% higher CSF neurofilament light chain concentrations among PWH; 95% confidence interval (95%-CI) -1%, +23%; P=0.07). No group differences were found in the prevalence of CSF neurofilament light chain concentrations above age-related reference values (PWH: 3%; HIV-negative participants: 4%), nor for Aβ1-42 (Figure 3B). T-tau and p-tau CSF concentrations were lower among PWH (Figure 3C-D), even after adjustment for age.

Factors associated with grey matter volume (Table 2, Figure 4A)

As previously described [4], PWH had lower total grey matter volume than HIV-negative controls (Table 2, Model 1). Classification by HIV serostatus and prior AIDS demonstrated that grey matter volume was only significantly lower in PWH with prior AIDS (Table 2, Model 2) compared to controls.

Across the entire study population, an independent association was found between total grey matter volume and t-tau (+1.4 mL per 10% increase in t-tau; 95%-CI: +0.01, +2.8 mL; P=0.05). There were no associations observed between grey matter volume and other factors (all P>0.1), and no factors significantly mediated the association between HIV and grey matter volume. In linear regression analysis among PWH only, nadir CD4+ T lymphocyte count, current CD4+ T lymphocyte count, CD4:CD8 ratio, and CSF HIV-RNA were not associated with grey matter volume (all P>0.1).

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PWH with prior AIDS had significantly lower grey matter volume than HIV-negative individuals in various locations, including –but not limited to– the postcentral gyrus, paracingulate gyrus, and Heschl's gyrus (Figure 4A).

Factors associated with microstructural white matter abnormalities (Table 2, Figure 4B-5)

PWH also had lower fractional anisotropy than HIV-negative controls (Table 2, Model 1), as previously described [4]. Classification by HIV serostatus and prior AIDS demonstrated that both subgroups of PWH (those with and without prior AIDS) had white matter microstructural abnormalities, which were greatest amongst those with prior AIDS (Table 2, Model 2).

Across the entire study sample, fractional anisotropy was independently and negatively associated with the presence of hypertension and higher CSF sCD14 concentrations, although these factors did not influence the strength of the association between HIV serostatus and fractional anisotropy (Table 2, Model 3). There were no interactions between HIV serostatus and either hypertension (P=0.32) or CSF sCD14 (P=0.59). No association was found between fractional anisotropy and CSF neurofilament light chain or other biomarkers (all P>0.1), and the biomarkers did not mediate HIV-related differences in fractional anisotropy. In linear regression analysis among PWH only, nadir CD4+ T lymphocyte count, current CD4+ T lymphocyte count, CD4:CD8 ratio, and CSF HIV-RNA were not associated with fractional anisotropy (all P>0.1).

Regardless of the presence or absence of a prior AIDS diagnosis, PWH had lower fractional anisotropy than HIV-negative individuals in many white matter tracts, including –but not limited to– the corpus callosum, and corona radiata. Differences were more pronounced for the prior AIDS group (Figure 4B). Both hypertension and sCD14 were negatively associated with fractional anisotropy in various locations

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(Figure 5). For CSF sCD14 these included the corpus callosum, superior fronto-occipital fasciculi, and corona radiata; for hypertension the anterior corona radiata and external capsule.

Discussion

Despite effective cART, PWH had lower grey matter volume, widespread white matter microstructural abnormalities, and persistent systemic immune activation. Greatest abnormalities were found in subjects with prior clinical AIDS. In addition, white matter microstructural abnormalities were associated with hypertension and higher concentrations of CSF monocyte activation biomarkers. Our findings suggest that abnormalities in brain structure in virally suppressed PWH are likely to reflect historical effects of prolonged untreated infection rather than ongoing injury, combined with the influence of systemic factors such as hypertension and ongoing neuroinflammation independent of HIV.

Several findings in our study suggest a "legacy effect" of prolonged untreated HIV infection, with the risk of further HIV-associated brain injury likely being mitigated by effective cART. Firstly, PWH with prior AIDS had the most severe structural abnormalities. Grey matter volume was reduced in PWH, but only significantly so among those with prior AIDS, suggesting that grey matter loss may be predominantly associated with prolonged untreated infection. This grey matter volume loss for PWH in general was more modest than previous studies [1] perhaps because all PWH in this study had suppressed plasma HIV replication. However, these grey matter changes, which were most pronounced in PWH with prior AIDS, may have cognitive sequelae similar to other neurodegenerative diseases if sufficient in magnitude. White matter microstructural abnormalities were present in all PWH, but were most pronounced in those with prior AIDS, suggesting that white matter microstructural abnormalities occur earlier in the course of infection than grey matter volume loss. Secondly, CSF neurofilament light chain was not significantly elevated among PWH, with virtually all measurements below upper age-related

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reference values. Since CSF neurofilament light chain provides information regarding the presence of active brain injury this suggests a lack of substantial ongoing HIV-associated neuronal damage. Thirdly, CSF neuronal damage biomarkers were not associated with imaging measures of brain structure, nor were they mediators of the observed HIV-related differences in brain structure, which again suggests static rather than active brain injury. This conclusion is supported by previous work reporting greater structural abnormalities in PWH with longer known duration of HIV infection (and hence probably longer duration of untreated HIV infection) [7,45,46] and immunodeficiency [5], and by studies in neuroasymptomatic untreated PWH reporting substantially higher CSF neurofilament light chain concentrations mainly among those with low CD4+ T lymphocyte counts [16-18]. Our results reinforce current recommendations of early cART initiation, as this is likely to limit structural brain damage from progressing.

Our results suggest that systemic factors such as hypertension also contribute to observed white matter microstructural abnormalities. Across all our study participants, hypertension was independently associated with fractional anisotropy. This is unsurprising, as hypertension is a well-known cause of white matter abnormalities [47]. For example, patients with uncontrolled hypertension show more white matter microstructural abnormalities than those with controlled hypertension [9]. This relationship emphasizes the importance of treating hypertension, especially in PWH, and future studies should address the effect of antihypertensive treatment on neuroimaging abnormalities in PWH.

CSF sCD14 concentrations were negatively associated with fractional anisotropy. This relationship was independent of HIV serostatus and did not explain HIV-related reductions in white matter integrity. Across the whole group this relationship was seen across large parts of the white matter, suggesting a potentially important link between levels of monocyte activation and white matter integrity. Underlying mechanisms of this correlation are unclear, but could reflect either neurotoxic or neurotrophic effects of microglial or macrophage activation within the brain [48]. Spill-over into the CSF of plasma monocyte

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activation biomarkers is less likely, given the fact that CSF sCD14 concentrations were not significantly correlated with albumin ratio or plasma sCD14 (data not shown). In the future, more specific biomarkers might be able to further elucidate the mechanisms through which immune activation within the CNS affects white matter structure and its implications for preserving brain health.

We found evidence for persistent systemic immune activation in PWH (i.e. higher plasma sCD14 and sCD163 concentrations). These specific monocyte activation markers were not elevated in the CSF. Since other CSF monocyte activation biomarkers were elevated amongst PWH (neopterin concentration and K:T ratio) but were not associated with neuroimaging abnormalities, the precise nature of the intrathecal immune activation remains unclear. This might indicate activation of a specific interferon-gamma-induced pathway in PWH, which induces neopterin and indolamine-2,3-dioxygenase expression (which in turn results in elevated K:T ratios) but does not per se result in increased shedding of CSF sCD14 and sCD163 [49,50].

Our study is representative of most PWH in developed healthcare systems. We only recruited PWH on suppressive cART for ≥12 months, removing potentially confounding effects of ongoing untreated HIV infection. Another major strength was our recruitment of well-matched HIV-negative controls from sexual health clinics and targeted community groups. The control group is critical to the interpretation of our results, because HIV-specific effects can only be identified by comparison of PWH with controls with similar lifestyles and demographic characteristics. Our control group did not reflect the general population, but was instead highly comparable to the HIV-positive group regarding demographic characteristics, (sexual) risk behavior, and cardiovascular disease risk factors. Lastly, the robust statistical analyses are a strength of the study. Through linear regression analyses in all PWH and HIV-negative participants jointly we were able to investigate associations between structural imaging measures and a wide range of factors, study interactions with HIV serostatus, and identify potential

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confounders or mediators. In addition, voxelwise analyses allowed us to investigate focal associations with brain structure.

Despite its strengths, this study has several limitations. First, as a cross-sectional analysis this work can merely report associations. Longitudinal data are needed to elucidate whether the observed structural abnormalities are progressive. Second, CSF albumin concentrations were not measured among HIV-negative participants recruited in London, resulting in 14% of the cohort missing albumin ratios. Multiple imputation did not significantly change the associations between albumin ratio and imaging measures (not reported). Third, the current work was exploratory and multiple statistical tests were performed, which might have resulted in type I errors. Fourth, cognitive function was not included in the current analysis. However, previously we found that white matter microstructural injury in affected tracts was associated was poorer cognitive function [4]. Lastly, due to the large proportion of Caucasian men who have sex with men in this study, it is unclear whether our results are generalizable to other populations with greater proportions of non-Caucasian and/or female PWH with incomplete cART use.

In conclusion, the presence of lower grey matter volume and widespread white matter microstructural abnormalities in PWH on suppressive cART partly reflect a combination of historical injury that occurred during untreated HIV infection, as well as the more general influence of systemic factors such as hypertension and ongoing neuroinflammation. Appropriate blood pressure management and early cART initiation may therefore both contribute to safeguarding brain health and cognitive function in PWH.

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Footnotes

Conflicts of interest

RZ has received travel grants from Gilead Sciences, and was a speaker at an event sponsored by Gilead Sciences for which her institution received remuneration. CS has received funding from Gilead Sciences, ViiV Healthcare and Janssen-Cilag for membership of Data Safety and Monitoring Boards, Advisory Boards, Speaker Panels and for the development of educational materials. FW has received travel grants from Gilead Sciences, ViiV Healthcare, Boehringer Ingelheim, Abbvie, and Bristol-Myers Squibb. CM's institution (AMC) received funds from Stryker for consultations by CM. HZ is co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg. AW has received honoraria or research grants from or been a consultant or investigator in clinical trials sponsored by Abbott, Boehringer Ingelheim, Bristol-Myers Squibb, Gilead Sciences, GlaxoSmithKline, Janssen-Cilag, Roche, Pfizer and ViiV Healthcare. MG reports grants from Gilead Sciences, and has received honoraria for advisory boards or lectures from Gilead Sciences, Janssen Pharmaceutica, Bristol Myers Squibb, Merck & Co, and ViiV Healthcare. PR reports grants from Gilead Sciences, grants from ViiV Healthcare, grants from Janssen Pharmaceutica, grants from Bristol Myers Squibb, grants from Merck & Co, during the conduct of the study; other from Gilead Sciences, other from Janssen Pharmaceutica, other from ViiV Healthcare, outside the submitted work. For the other authors none were declared.

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Table 1. Baseline characteristics of PWH and HIV-negative individuals participating in COBRA

{Insert Table 1}

Data are presented as median (interquartile range) or number (%) as appropriate.

Type test used: [a] Wilcoxon rank sum test, [b] Fisher's exact test.

Abbreviations: BMI, body mass index; HBV, hepatitis B virus; HCV, hepatitis C virus; MSM, men who have sex with men.

* One unit of alcohol equals one glass of beer (200-250 mL), a small glass of wine (100-125 mL), or a small glass of spirit (25 mL).

† Hypertension was defined as use of antihypertensive drugs, all available systolic blood pressure measurements ≥140 mmHg, and/or all available diastolic blood pressure measurements ≥90 mmHg.

‡ Blood pressure was measured three times; the reported systolic and diastolic blood pressure measurements represent the calculated mean of available measurements.

¥ Chronic HBV infection was defined as detectable hepatitis B surface antigen (HBsAg).

§ Chronic HCV infection was defined as detectable HCV RNA.

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Table 2. Associations of HIV serostatus and diagnosis of prior clinical AIDS, with i) grey matter volume and ii) fractional anisotropy from linear regression models[1]

{Insert Table 2}

Abbreviations: 95%-CI, 95% confidence interval; CSF, cerebrospinal fluid; sCD14, soluble CD14.

[1] Multiple linear regression models were constructed to identify factors associated with whole brain grey matter volume and white matter skeleton mean fractional anisotropy. All models were adjusted for a priori defined confounders: age, scanner type, and intracranial volume. Other factors potentially associated with grey matter volume and fractional anisotropy were analyzed using a stepwise model selection approach. Associations with HIV-specific factors were explored among PWH only.

[2] All models were adjusted for age, scanner type and intracranial volume.

[3] Reference group consists of HIV-negative controls.

[4] Model 3 included 205 individuals due to missing CSF sCD14 data. Log_{10}-transformed soluble CD163 CSF was also negatively associated with fractional anisotropy, but not included into the model because of collinearity.

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Figure 1. Jitterplots of plasma monocyte activation biomarkers (A-B), and albumin ratios† (C) in PWH and HIV-negative participants. The black lines denote medians with the red circles representing HIV-negative participants and the green triangles PWH. A color version of this figure is available online.

{Insert Figure 1}

Abbreviations: Albumin ratio, CSF:plasma albumin ratio; p, p-value; sCD14, soluble CD14; sCD163, soluble CD163. P-values were calculated using Wilcoxon rank sum test.

† Albumin ratios were missing among 30 HIV-negative controls and 5 PWH. CSF albumin concentrations were not measured in HIV-negative individuals at the study sites in London.

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Figure 2. Jitterplots of CSF monocyte activation biomarkers in PWH and HIV-negative participants. The black lines denote medians with the red circles representing HIV-negative participants and the green triangles PWH. A color version of this figure is available online.

{Insert Figure 2}

Abbreviations: CSF, cerebrospinal fluid; K:T ratio, kynurenine : tryptophan ratio; p, p-value; sCD14, soluble CD14; sCD163, soluble CD163.

P-values were calculated using Wilcoxon rank sum test.

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Figure 3. Jitterplots of CSF neuronal damage biomarkers in PWH and HIV-negative participants. The black lines denote medians with the red circles representing HIV-negative participants and the green triangles PWH. A color version of this figure is available online.

{Insert Figure 3}

Abbreviations: Aβ1-42, Amyloid-beta 1 fragment 42; CSF, cerebrospinal fluid; p, p-value; p-tau, phosphorylated tau; t-tau, total tau.

P-values were calculated using Wilcoxon rank sum test.

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Figure 4. Grey matter voxel-based morphometry and white matter tract-based spatial statistics of PWH with and without prior AIDS compared to HIV-negative individuals. Figure 5A illustrates the regions where grey matter volume was significantly lower among PWH with prior clinical AIDS (reference group: HIV-negative individuals). Figure 5B shows the areas where fractional anisotropy was significantly lower among PWH without prior clinical AIDS (red) and with prior clinical AIDS (blue) compared to HIV-negative individuals. Areas are colored by t-statistic, corrected for multiple comparisons and adjusted for age, scanner type, and intracranial volume. Significant differences ($P<0.05$) overlaid on the grey matter or the mean fractional anisotropy image (grey scale), and white matter skeleton (green).

{Insert Figure 4}

Abbreviations: CSF, cerebrospinal fluid; sCD14, soluble CD14.

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Figure 5. White matter tract-based spatial statistics of hypertension and CSF sCD14 in all participants. Figure illustrates the regions where fractional anisotropy negatively correlates with hypertension (upper panel), and CSF sCD14 (lower panel) for all participants ($P<0.05$). Areas are colored red (hypertension) or blue (CSF sCD14) by t-statistic, corrected for multiple comparisons and adjusted for age, scanner type, intracranial volume, and HIV serostatus. Significant differences overlaid on the white matter skeleton (green) and the mean fractional anisotropy image (grey scale).

{Insert Figure 5}

Abbreviations: CSF, cerebrospinal fluid; sCD14, soluble CD14.

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Table 1.

	PWH (n=134)	HIV-negative (n=79)	P-value
Demographic characteristics			
Age (years)	55 (51–62)	57 (52–64)	0.24[a]
Male gender	125 (93%)	73 (92%)	0.79[b]
African descent	16 (12%)	2 (3%)	0.02[b]
MSM	114 (85%)	62 (78%)	0.26[b]
Substance use			
Current alcohol consumption (units/week)*	2 (0–8)	6 (2–15)	0.02[a]
Use of recreational drugs in past 6 months	44 (33%)	18 (23%)	0.16[b]
Current smoking status			0.26[b]
Never smoked	36 (27%)	30 (38%)	
Ex-smoker	58 (43%)	29 (37%)	
Current smoker	40 (30%)	20 (25%)	
Cardiovascular disease risk factors			

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Hypertension †	56 (42%)	30 (38%)	0.67[b]
Systolic blood pressure (mmHg) ‡	131 (124–140)	130 (123–142)	0.63[a]
Diastolic blood pressure (mmHg) ‡	85 (78–93)	84 (77–91)	0.55[a]
Total cholesterol/HDL cholesterol ratio	4.1 (3.4–5.1)	4.0 (3.5–4.8)	0.63[a]
BMI (kg/m^2)	24.6 (22.6–27.4)	24.6 (23.2–28.4)	0.29[a]
Co-infections			
HBV, chronic infection ¥	7 (6%)	0	0.05[b]
HCV, chronic infection §	5 (4%)	0	0.16[b]
HIV specific characteristics			
Years since HIV diagnosis	15 (9–20)		
Duration of antiretroviral therapy (years)	13 (7–17)		
Plasma HIV-RNA <200 copies/mL	134 (100%)		
History of clinical AIDS	42 (31%)		
Nadir CD4 cell count (cells/µL)	180 (90–250)		
Current CD4 cell count (cells/µL)	618 (472–806)		
Current CD4:CD8 ratio	0.84 (0.60-1.12)		

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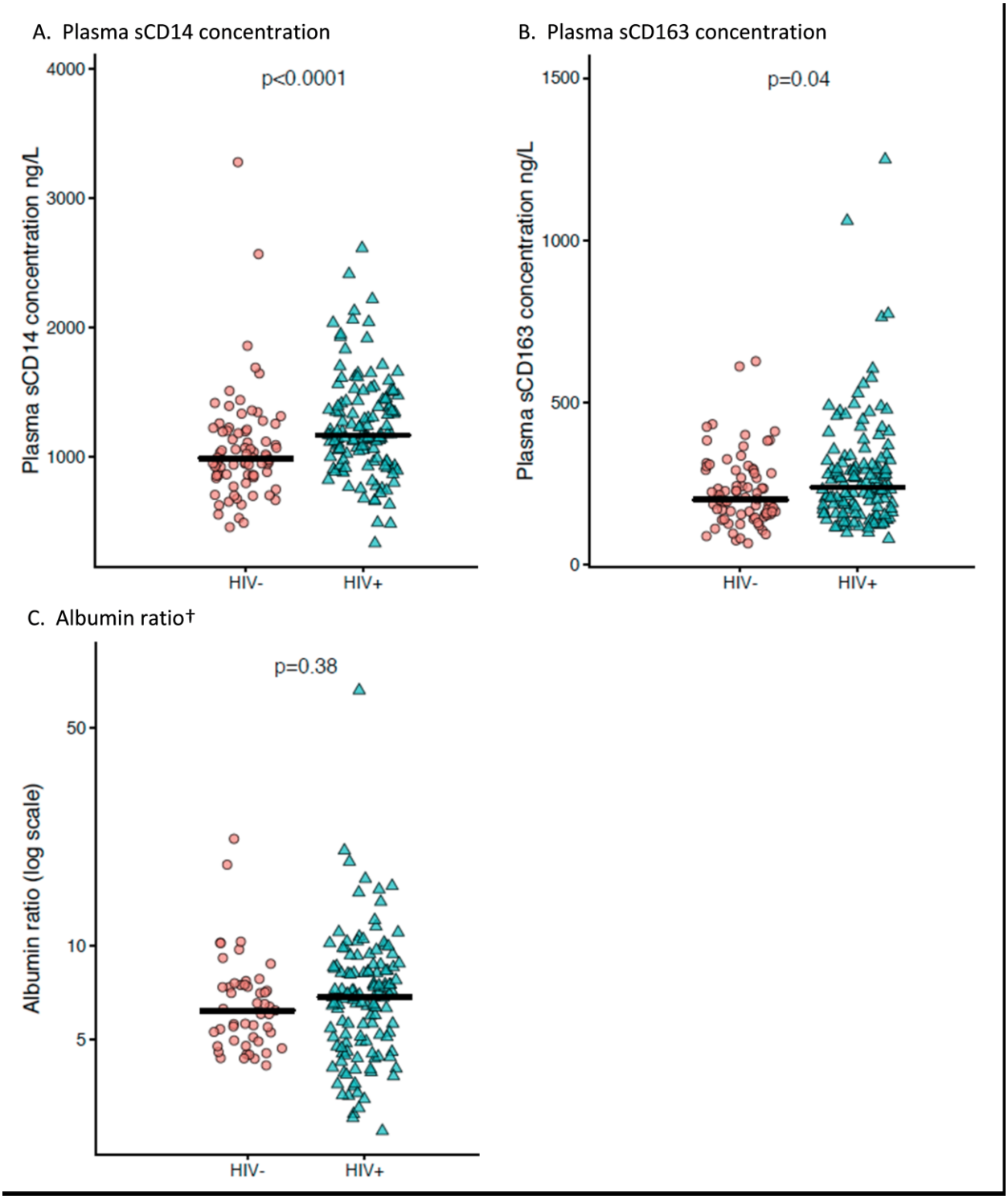
Table 2.

		Grey matter volume in mL[2] (n=212)		Fractional anisotropy[2] (n=208)	
		Coefficient (95%-CI)	P	Coefficient (95%-CI)	P
Model 1	HIV-positive serostatus[3]	-13.7 (-25.1, -2.2)	0.02	-0.0073 (-0.012, -0.0024)	0.004
Model 2	HIV-positive, no prior clinical AIDS[3]	-9.6 (-21.7, 2.5)	0.12	-0.0056 (-0.011, -0.0004)	0.04
	HIV-positive, prior clinical AIDS[3]	-23.4 (-38.7, -8.1)	0.003	-0.011 (-0.018, -0.0047)	0.001
Model 3	HIV-positive, no prior AIDS[3]	-	-	-0.0058 (-0.011, -0.0006)	0.03
	HIV-positive, prior AIDS[3]	-	-	-0.011 (-0.017, -0.0041)	0.002
	Hypertension	-	-	-0.0054 (-0.010, -0.0005)	0.03
	sCD14 CSF (per 10% increase)[4]	-	-	-0.0002 (-0.0004, -0.00004)	0.01

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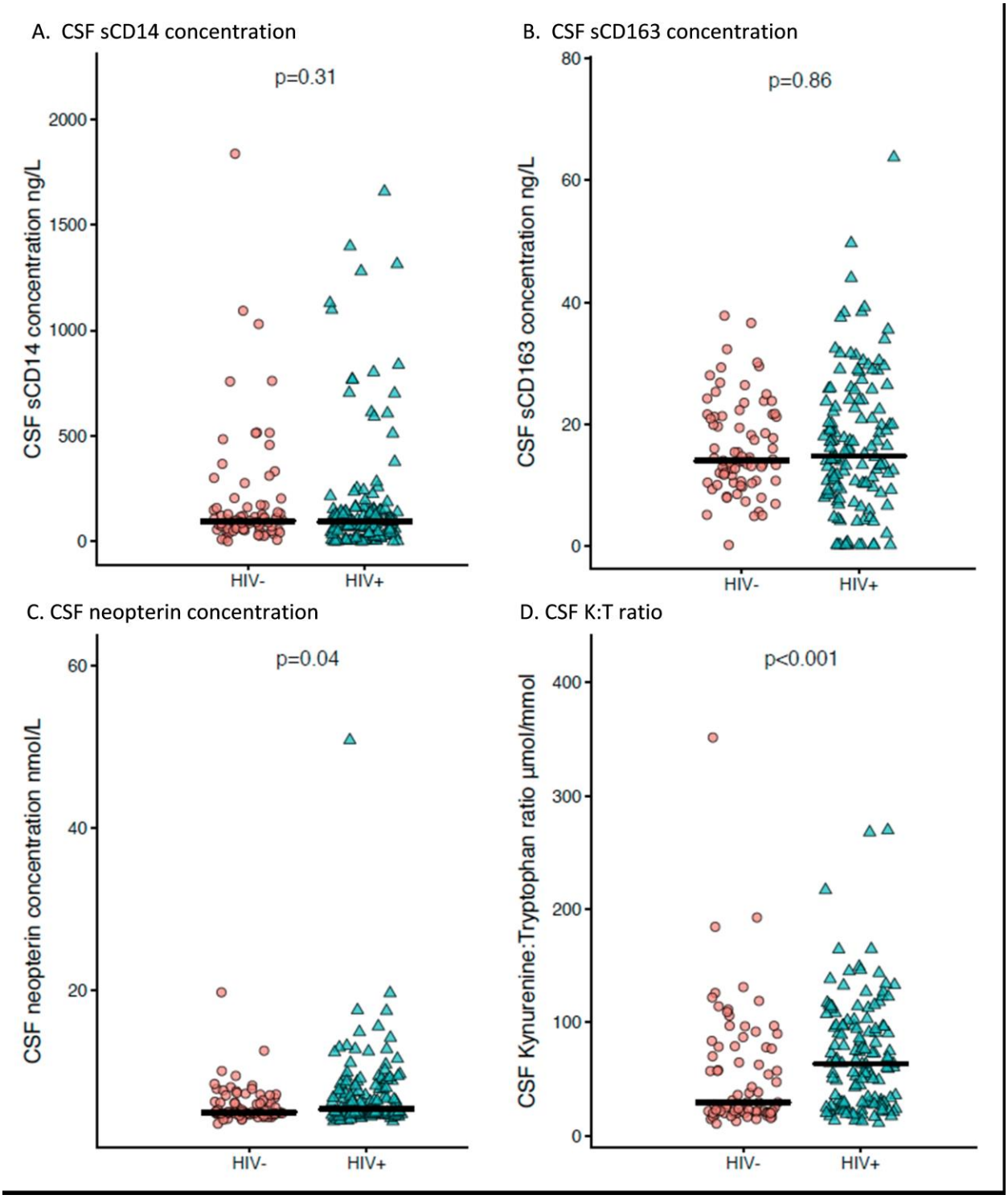
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Figure 1.



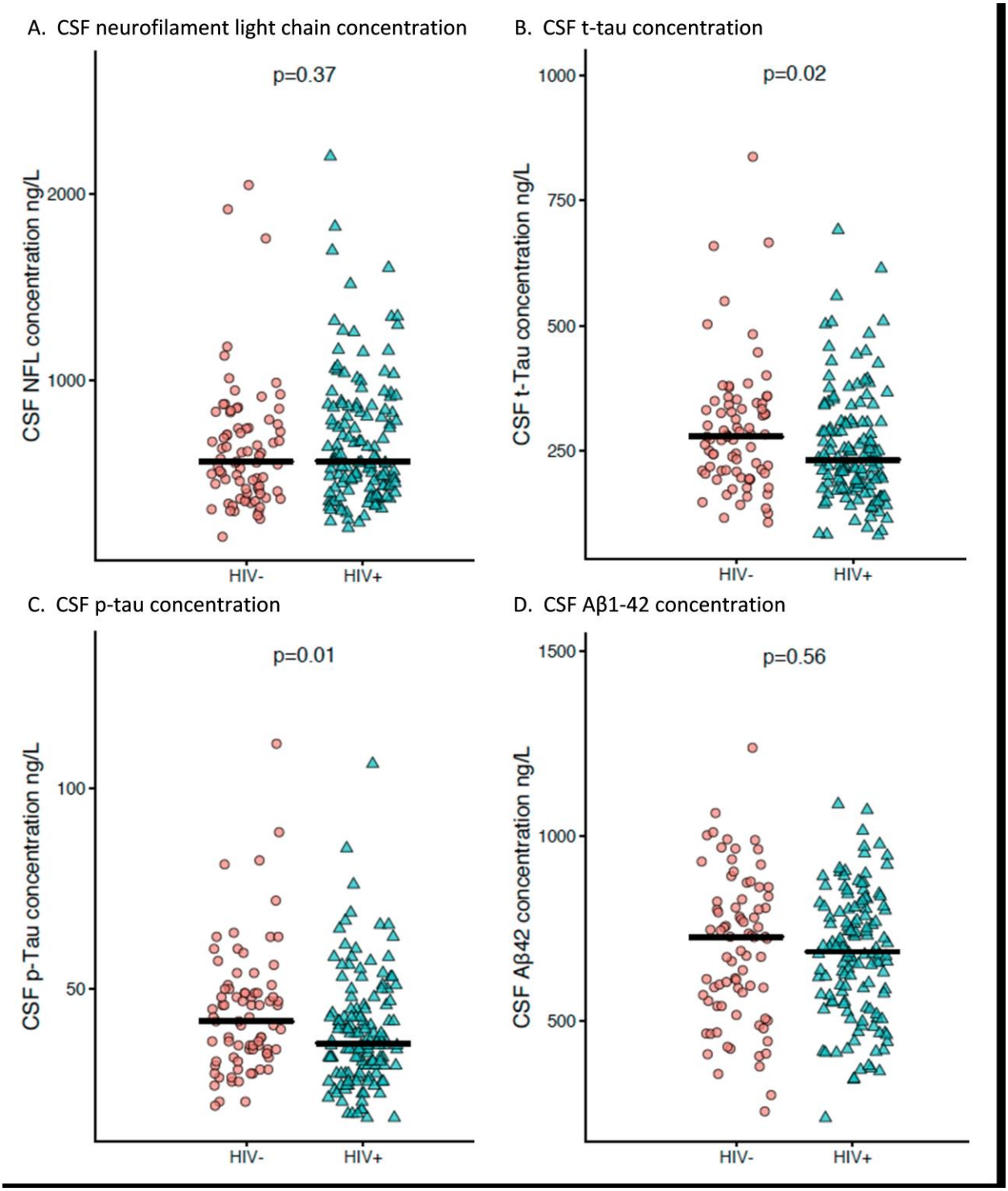
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Figure 2.



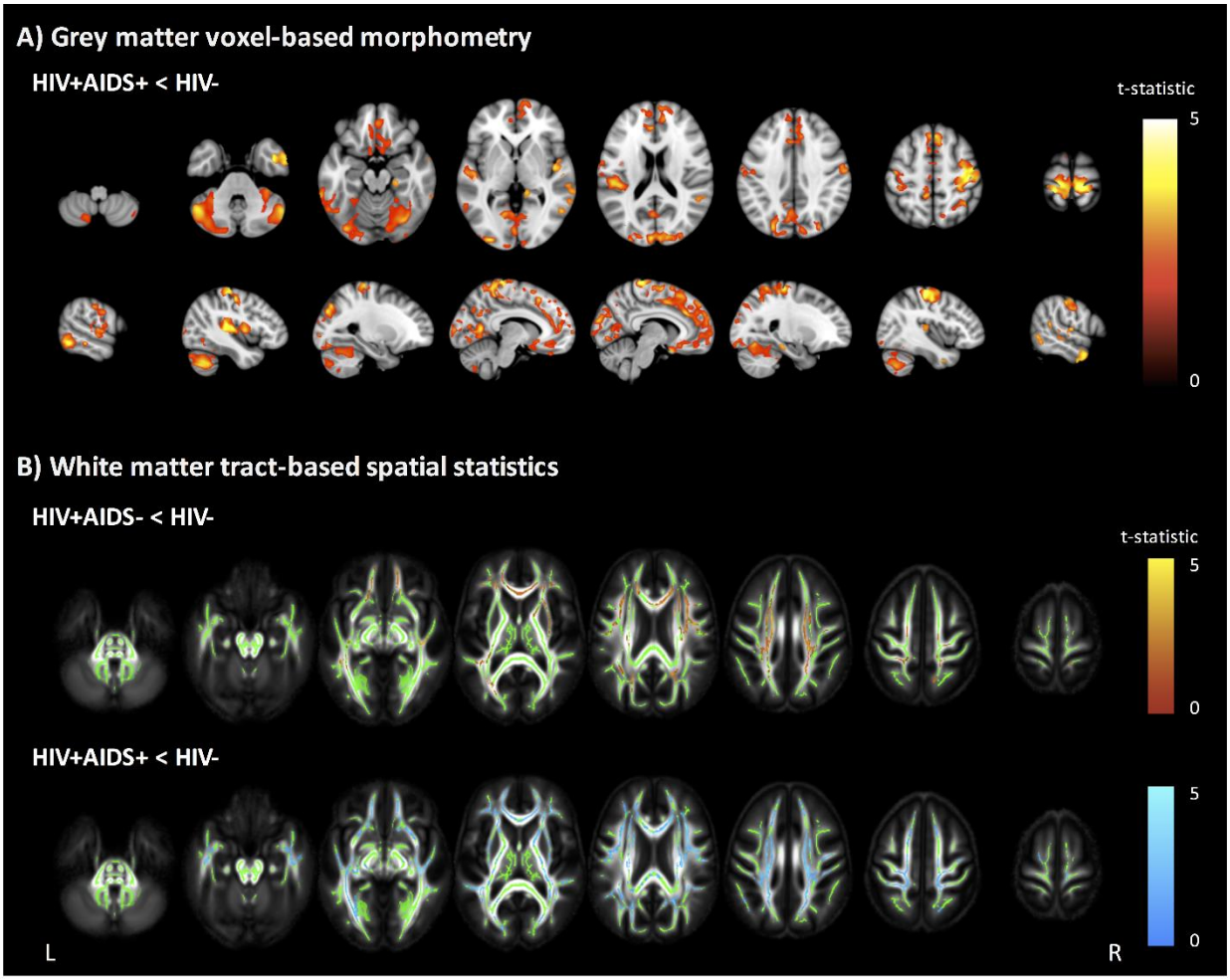
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Figure 3.



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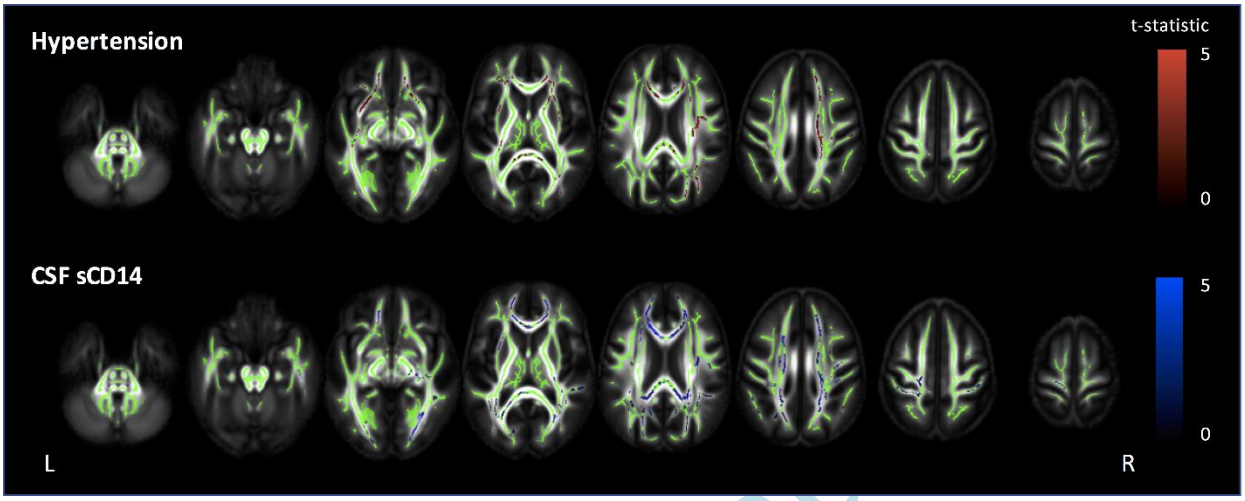
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Figure 5.



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