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White matter measures are near normal in controlled HIV infection except in those with cognitive impairment and longer HIV duration

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Abstract The objective of the current study was to quantify the degree of white matter (WM) abnormalities in chronic and virally suppressed HIV-infected (HIV+) persons while carefully taking into account demographic and disease factors. Diffusion tensor imaging (DTI) was conducted in 40 HIV− and 82 HIV+ men with comparable demographics and life style factors. The HIV+ sample was clinically stable with successful viral control. Diffusion was measured across 32 non-colinear directions with a *b*-value of 1000 s/mm^2; fractional anisotropy (FA) and mean diffusivity (MD) maps were quantified with Itrack IDL. Using the ENIGMA DTI protocol, FA and MD values were extracted for each participant and in 11 skeleton regions of interest (SROI) from standard labels in the JHU ICBM-81 atlas covering major striato-frontal and parietal tracks. We found no major differences in FA and MD values across the 11 SROI between study groups. Within the HIV+ sample, we found that a higher CNS penetrating antiretroviral treatment, higher current CD4+ T cell count, and immune recovery from the nadir CD4+ T cell count were associated with increased FA and decreased MD ($p < 0.05$–0.006), while HIV duration, symptomatic, and asymptomatic cognitive impairment were associated with decreased FA and increased MD ($p < 0.01$–0.004). Stability of HIV treatment and antiretroviral CNS penetration efficiency in addition to current and historical immune recovery were related to higher FA and lower MD ($p = 0.04$–$p < 0.01$). In conclusion, WM DTI measures are near normal except for patients with neurocognitive impairment and longer HIV disease duration.

Keywords HIV/AIDS · HIV-associated neurocognitive disorder · Diffusion tensor imaging · Antiretroviral treatment · Immune functions

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Introduction

HIV-related white matter (WM) neuropathological changes are common. Yet, at the macroscopic level when using MRI techniques such as diffusion tensor imaging (DTI), the location and magnitude of WM damage measured [using common parameters such as by fractional anisotropy (FA) and mean diffusivity (MD)] are far from being consistent across neuro-HIV studies (Masters and Ances 2014).

Variations in DTI acquisition parameters, each of which affects the rendering WM fiber-crossing (Mukherjee et al. 2008a, b), and the sensitivity to indicate pathological changes, in addition to heterogeneity in analytic approaches have potentially

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resulted in inconsistent results. Moreover, variations in the studied HIV+ participants in relation to their combination antiretroviral (cART) status, level of viral suppression, the nature of HIV disease (Cysique and Brew 2014), and their demographics (mainly age) (Towgood et al. 2012; Chang et al. 2008; Gongvatana et al. 2011; Seider et al. 2015) have probably contributed to a lack of consistency. Altogether, while DTI may be useful at characterizing the extent of WM injury in cases with moderate to severe HIV-associated neurocognitive disorder (HAND) (Chang et al. 2008; Chen et al. 2009; Gongvatana et al. 2011) or advanced untreated HIV infection (Hoare et al. 2011; Leite et al. 2013; Pfefferbaum et al. 2009) (when HIV replication is the cause of the WM damage), its use at elucidating what may be the HIV-related neuropathology substrate in virally suppressed HIV infection is less clear.

We sought to address these issues by comparing the WM DTI measures of FA and MD in fronto-striatal and fronto-parietal WM connections using 32-direction DTI in virally suppressed HIV+ adults relative to age-comparable HIV− controls. We hypothesized that the magnitude of abnormal WM DTI measures in the HIV+ group would correspond to a maximum of a medium effect size (d = 0.50-0.60) compared to the HIV− control group, reflecting the high degree of viral suppression and the potential lesser degree of HIV-related brain injury. Further, we determined which demographic, clinical factors, and treatment moderated WM DTI measures in the HIV+ group. We hypothesized that the main drivers of abnormal WM DTI measures would be HAND severity and an aging and HIV interaction.

Methods

Participants

The current investigation is based on the Australian HIV and Brain Aging research, a prospective study investigating the effects of HIV infection on the brain in HIV+ and HIV− adults aged 45+ (>85% of men who have sex with men (MSM) and >95% HIV RNA undetectable, which is representative of Australian HIV demographic and clinical characteristics) (The Kirby Institute 2015). Study details and enrolment procedures have already been reported (Cysique et al. 2013, 2014). The current study sample included 40 HIV−, and 82 HIV+ men who completed a MRI scan in addition to a standard neuropsychological assessment (see Tables 1 and 2).

Diffusion-weighed imaging acquisition

Diffusion-weighed imaging (DWI) was acquired on a 3-T Achieva TX MRI system (Philips Medical Systems, Best, the Netherlands) with an eight-channel SENSE head coil. Images were acquired in the axial plane using a single-shot EPI sequence optimized for use on this scanner (TR/TE short/68 ms; b = 1000 s/mm^2; 2.5-mm isotropic resolution; flip angle 90°; FOV 240 × 240; 55 slices) in 32 diffusion gradient directions, plus a B_0 image averaged from two acquisitions for diffusion tensor fitting.

Table 1 Demographic characteristics in the study groups

	HIV−	HIV+	p
N	40	82	–
Age (years)	54.5 (6.5) (45–67)	55.1 (6.7) (45–69)	0.65
Male (%)	100%	100%	–
Education level (years)	15.1 (2.7)	14.0 (2.9)	0.05
Urban dwelling MSM	90%	90%	–

MSM men who have sex with men, mean (SD) (range)

DTI processing

MD and FA maps were calculated after eddy current and head motion correction. DTI data were corrected by applying an affine transformation of each image to the B_0 image using the Oxford FSL toolkit (http://www.fmrib.ox.ac.uk/fsl/fdt/index.html). For each voxel, tensor eigenvectors and corresponding eigenvalues as well as FA and MD values were computed in Itrack IDL (Bucci et al. 2013; Chung et al. 2006). FA and MD maps were then fed into standard

Table 2 HIV disease and HIV-associated neurocognitive disorder characteristics in the HIV+ group

	HIV+ group
Median nadir CD4 cells/mL	180
Median current CD4 cells/mL	528
Median HIV duration (years)	20
Historical AIDS (%)	71%
Plasma HIV RNA undetectable (%)	99%
CSF HIV RNA undetectable (%) (N = 36)	97%
Median current CART duration (months)	29
CPE 2010 rank score	8.2 (2.2)
Asymptomatic neurocognitive impairment (ANI) (%)	38%
Mild neurocognitive disorder (MND) (%)	11%
HIV-associated dementia (HAD) (%)	7%

Ninety-nine percent were successfully treated with evidence of viral control; the single detectable case was a viral blip as he was undetectable prior to the study as well as after. The neuropsychological assessment consisted in a standard neuropsychological test battery covering seven ability domains (Lane et al. 2012). To classify overall neurocognitive impairment and determine the presence of HAND, we used a standard definition of impairment based upon a battery-wide summary score, the Global Deficit Score (GDS). Impairment classification (GDS ≥ 0.5) was based on the HIV− standard z-scores (Cysique et al. 2014)

CSF cerebrospinal fluid, *CART* combined antiretroviral therapy, *CPE* CNS Penetration Efficiency rank score 2010 version (Letendre et al. 2010)

tract-based spatial statistic (TBSS) skeletonization using the Oxford FSL toolkit (Smith et al. 2004). Next, each subject's FA and MD data was projected onto ENIGMA standard space (DTI) (ENIGMA DTI protocol, 2015). Each subject's FA and maps were inspected for quality of this registration, and all passed the ENIGMA protocol QC threshold (DTI) (ENIGMA DTI protocol, 2015). Then, TBSS skeletons were segmented according to the Johns Hopkins ICBM-DTI-81 atlas; average FA and MD values were computed in 11 selected skeleton regions of interest (SROI) (see Fig. S1 for the illustration of the skeletonization and ENIGMA protocol results in one HIV+ case). The rationale for the SROI selection was that HIV-related brain injury predominantly impacts striato-frontal and striato-parietal circuits (Ellis et al. 2007). Normal aging is also associated with lower FA and higher MD primarily in the same regions (Cysique and Brew 2014). Thus, we selected SROI that included major projection fibers with a mostly retrolandic distribution, anterior and superior corona radiata (ACR, SCR), anterior limb of internal capsule (ALIC), and posterior limb of internal capsule (PLIC); major association fibers that have a substantial striato-frontal-parietal distribution, superior longitudinal fasciculus (SLF), superior fronto-occipital fasciculus (SFO), external capsule (EC), cingulum (CG), and fornix (FX); and the main anterior commissural fibers, corpus callosum (genu body and body of corpus callosum [GCC, BCC]). Note that right and left SROI FA and MD values were averaged, as there were no within-subject differences.

Statistical analyses

Averaged FA and MD values in the 11 SROI were compared between HIV− and HIV+ samples using a series of ANOVAs. False discovery rate (FDR) p values were computed using the Benjamini-Hochberg technique (default in JMP JMP® 12.0.1) to control the FDR for multiple tests. With a sample size of 82 HIV+ and 40 HIV−, we had 82–93% power to detect a d = 0.50-0.60 difference (one-tailed) between the study groups. WM damage of a medium-size maximum (as opposed to large $d \geq 0.80$) was hypothesized due to ongoing viral suppression in the HIV+ sample.

Effects of age and education on DTI parameters were tested using a linear regression with an interaction term.

Neurocognitive functioning relation to DTI parameters was evaluated using a clinically relevant classification, neurocognitively normal (NR normal; N = 26) versus ANI (N = 31), versus mild neurocognitive disorder (MND) + HIV-associated dementia (HAD) (N = 15), and compared with ANOVA and Dunnett's controls with the NR normal as the reference. Alpha was set at $p < 0.05$ for these analyses.

Effects of HIV disease biomarkers (nadir CD4-T cell count, current CD4-T cell count, difference between current and nadir CD4-T cell count, CD4-T cell count recovery, HIV duration, AIDS status) and HIV treatment (cART current duration, CNS Penetration Effectiveness (CPE version 2010; Letendre et al. 2010) rank score) on DTI parameters were tested using univariate and multivariate linear regression. Alpha was set at $p < 0.05$ for these analyses and effect sizes are provided.

Statistical analyses were conducted in JMP® 12.0.1 (SAS Inc.)

Ethics

All individuals signed an informed consent before study participation, and the St. Vincent's Hospital and The University of New South Wales Human Research Ethics Committees approved our protocol.

Results

Comparisons between HIV− and HIV+ groups (Fig. 1)

We detected only trends for lower FA values in HIV+ group relative to the HIV− group in the FX (p = 0.062) and the SFO (p = 0.056). There also was a trend for higher FA values in the HIV+ group relative to the HIV− group in the PLIC (p = 0.057). We also only detected trends for higher MD values in the HIV+ relative to the HIV− in the FX (p = 0.109) and the BCC (p = 0.11). All effect sizes were below the hypothesized clinically relevant medium magnitude.

Age and education effects

Older age was associated with lower FA values in the ACR ($p < 0.009$), EC ($p < 0.02$), and FX ($p < 0.001$). There was only a trend for an age × HIV status interaction on the FA values in the CG (p = 0.07, standard beta = 0.18), where HIV+ cases had lower FA as a function of older age compared to the HIV− group. Older age was associated with higher MD values in the ACR ($p < 0.04$), BCC ($p < 0.002$), GCC ($p < 0.04$), FX (p = 0.0006), SRC ($p < 0.002$), and SLF ($p < 0.004$). There were no interaction effects. There was no overall significant education effect. However, there were interactions of HIV status × education in the SCR and SLF ($p < 0.03$, standard beta = −0.22), showing that in these regions, FA was higher as function of education in HIV+ group compared to the HIV− group.

Neurocognitive impairment effect in the HIV+ group only

Individuals with MND + HAD had lower FA values in the CG (p = 0.04) and FX (p = 0.007) compared to both NR normal and ANI cases. Individuals with MND + HAD and ANI had higher MD values in the ALIC (p = 0.03) compared to the NR normal group. Individuals with MND + HAD has higher MD

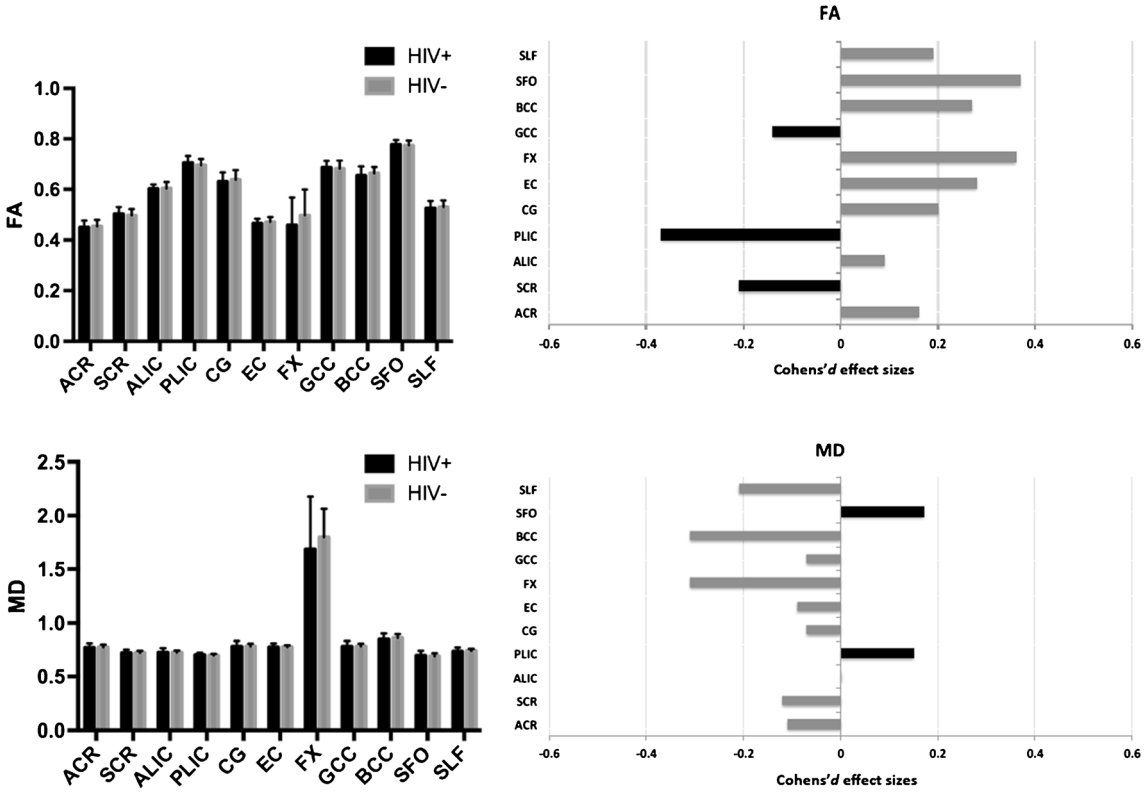


Fig. 1 FA and MD mean and standard errors in the HIV+ and HIV− study groups. *ACR* anterior corona radiata, *SCR* superior corona radiata, *ALIC* anterior limb of internal capsule, *PLIC* posterior limb of internal capsule, *SLF* superior longitudinal fasciculus, *SFO* superior fronto-occipital fasciculus, EC external capsule, *CG* cingulum, *FX* fornix, *GCC* genu corpus callosum, *BCC* body of corpus callosum. FDR-based values were $p \leq 0.056$ for FA and $p \leq 0.109$ for MD. Cohen's *d* effect sizes are provided for both FA and MD measures. For FA, a positive effect size means lower FA in the HIV+ group compared to the HIV− group (*light gray*); reserve is colored with *black bars*. For MD, a negative effect means higher MD in the HIV+ group compared to the HIV− group (*light gray*); reserve is colored with *black bars*

values in the FX ($p = 0.04$) and EC ($p < 0.05$) compared to both the ANI and NR normal groups.

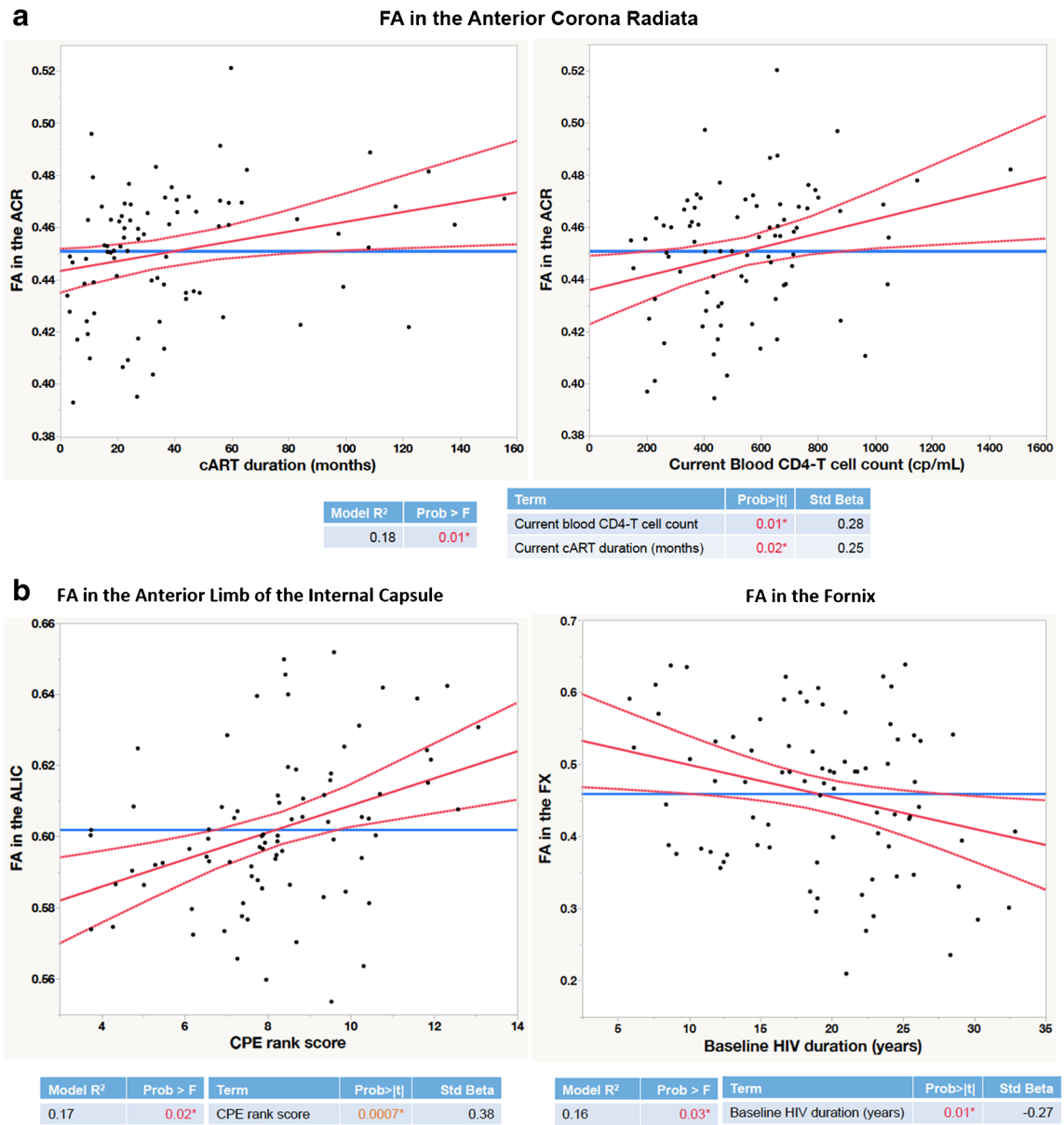
HIV biomarkers and HIV treatment effects in the HIV+ group only

Univariate analyses showed that higher CD4-T cell count was associated with higher FA in the ACR ($r = 0.30$, $p = 0.0006$), FX ($r = 0.25$, $p = 0.03$), and SLF ($r = 0.25$, $p = 0.03$). A CD4-T cell count recovery was associated with higher FA in the ACR ($r = -0.25$, $p < 0.03$). HIV duration was associated with lower FA in the FX ($r = -0.31$, $p = 0.004$) and higher FA in the SCR ($r = 0.25$, $p = 0.03$). cART duration ($r = 0.27$, $p = 0.01$) was associated with higher FA in the ACR ($r = 0.27$, $p = 0.01$). CPE rank score was associated with higher FA in the ALIC ($r = 0.38$, $p = 0.0004$) and PLIC ($r = 0.23$, $p = 0.04$) and GCC ($r = 0.23$, $p = 0.04$). A multivariate model showed that each of the tested biomarkers (note that CD4-T cell count recovery was not included to avoid collinearity with the current CD4-T cell count) and treatment effects remained independently associated with FA values in specific SROI (detailed results are presented in Fig. 2 with illustrations for whole models that reached significance at $p < 0.05$).

Similar analyses with the MD values showed that a higher CD4-T cell count was associated with lower MD in the EC ($r = -0.23$, $p = 0.03$) and in the FX (-0.30, $p = 0.006$). A higher CD4-T cell count recovery was associated with lower MD in the FX ($r = -0.25$, $p < 0.03$). A longer HIV duration was associated with higher MD in the FX ($r = 0.28$, $p = 0.01$). Lastly, a higher CPE was associated with a lower MD in the ALIC ($r = -0.22$, $p < 0.05$), the PLIC ($r = -0.25$, $p < 0.03$), and the SFO (-0.30, $p = 0.006$). A multivariate model showed that each of the tested biomarkers (again, CD4-T cell count recovery was not included to avoid collinearity) and treatment effect remained independently associated with MD values in specific SROI (detailed results are presented in Fig. 3 with illustrations for whole model that reached significance at $p < 0.05$).

Discussion

The current study sought to determine the level of WM abnormalities in HIV+ men who were clinically stable and virally suppressed relative to age-comparable men. The main study finding is that there were no differences of clinically relevant level (hypothesized as at least medium effect size WM



Model R^2	Prob > F
0.18	0.01*

Term	Prob>|t|	Std Beta
Current blood CD4-T cell count	0.01*	0.28
Current cART duration (months)	0.02*	0.25

Model R^2	Prob > F	Term	Prob>|t|	Std Beta
0.17	0.02*	CPE rank score	0.0007*	0.38

Model R^2	Prob > F	Term	Prob>|t|	Std Beta
0.16	0.03*	Baseline HIV duration (years)	0.01*	-0.27

Fig. 2 Multivariate regression models for FA and the effects of HIV biomarkers and treatment leverage plots are presented. Models included in order nadir CD4 T-cell count, current CD4-T cell count, HIV duration, current cART duration, and CPE rank score. Models with a significant R^2 and at least one significant predictor at $p < 0.05$ are illustrated. **a** Results in the ARC. **b** Results in the IC and FX. There were also significant predictors that were significant at $p < 0.05$, but when the model R^2 was not significant, model $R^2 = 0.08$, $p = 0.13$. Significant predictor CPE rank score (standard beta = 0.23, $p < 0.04$) on FA in the GCC. Model $R^2 = 0.06$, $p = 0.29$. Significant predictor CPE rank score (standard beta = 0.23, $p < 0.05$) on FA in the PLIC. Model $R^2 = 0.09$, $p = 0.09$. Significant predictor CD4-T cell count (standard beta = 0.23, $p < 0.04$) on FA in the SLF

damage in HIV+ group) in both FA and MD values between the HIV+ and HIV− groups. In the HIV context and based on DTI acquisition parameters, we interpret FA as relating to the degree of axonal structural integrity primarily reflecting membrane and cytoskeleton integrity (Winston 2012) and possibly astrocytic structural integrity (Lentz et al. 2014). MD reflects aspects of WM microstructure density (Winston 2012; Zhu et al. 2013), such as degree of extracellular space between WM tracts (Lentz et al. 2014) that may be affected by neuroinflammation including glial involvement and myelin loss to a lesser extent. In this case, myelin contribution to MD changes is debatable (Lentz et al. 2014) as massive demyelination is not a common feature of HIV-related brain injury even in those who had severe HIV encephalopathy (Pelle et al. 2008).

The finding of no significant difference between the study groups in both FA and MD reflects near-normal WM in the HIV+ sample at the group level. While DTI could not be performed in this sample at the time they were severely

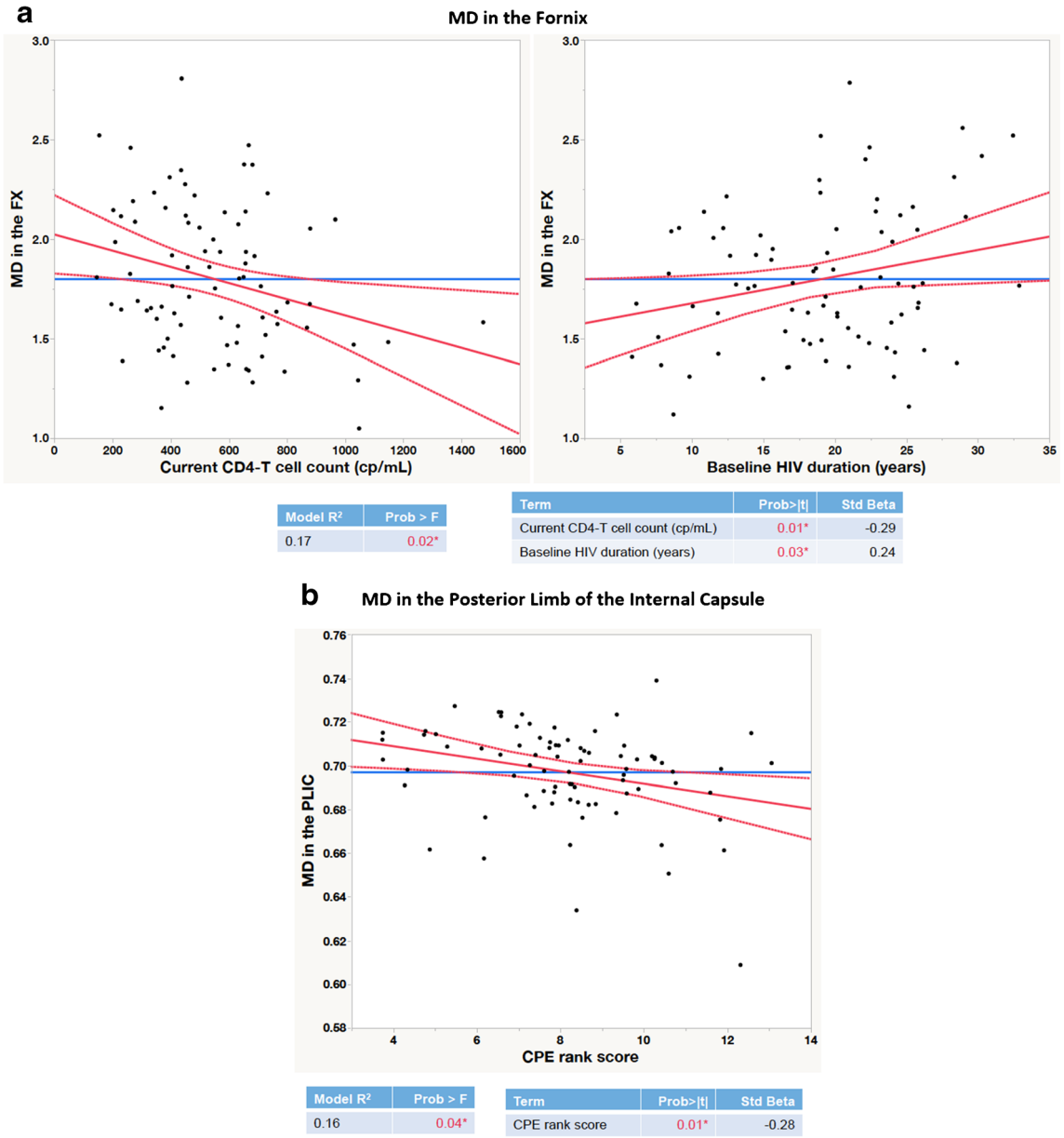


Fig. 3 Multivariate regression models for MD and the effects of HIV biomarkers and treatment leverage plots are presented. Models included in order nadir CD4 T-cell count, current CD4-T cell count, HIV duration, current cART duration, and CPE rank score. Models with a significant R^2 and at least one significant predictor at $p < 0.05$ are illustrated. **a** Results in the FX. **b** Results in the IC. There were also significant predictors that were significant at $p < 0.05$, but when the model R^2 was not significant, model $R^2 = 0.08$, $p < 0.16$. Significant predictor CPE rank score (standard beta = −0.25, $p < 0.03$) on the MD in the ALIC. Model $R^2 = 0.11$, $p = 0.06$. Significant predictor CD4-T cell count (standard beta = −0.22, $p < 0.05$) on the MD in the EC. Model $R^2 = 0.09$, $p = 0.10$. Significant parameter CPE rank score (standard beta = −0.30, $p = 0.007$) on the MD in the SFO

immuno-compromised (20 years with median nadir CD4 T cells = 180), it is highly likely that most individuals experienced some level of WM injury, since WM injury in immune-compromised HIV+ individuals represents one of the hallmarks of HIV-related brain injury (Jernigan et al. 1993). Yet without individual rating of the level of WM injury at that time, we can only infer that near-normal DTI measures in the current sample reflect the long-term benefit of immune recovery and controlled viral replication leading to potential WM repair. Another explanation could be that the current sample was initially particularly resistant to HIV-related CNS involvement and because of this, survived the pre-cART era without any major brain insult. We believe that this is an improbable explanation, as 71% had historical AIDS.

In multivariate models, we found robust independent treatment and HIV disease markers' effects on in the direction of normal WM DTI measures. These treatment and HIV disease markers have been robustly associated with significant

improvement in brain functions in naive HIV+ patients initiating treatment and in patients with HIV-associated dementia after cART initiation (Langford et al. 2003). Typically, viral suppression and immune recovery afforded by cART were significantly associated with reduction of WM inflammation (Sailasuta et al. 2012; Young et al. 2014) and diffuse WM matter alterations (Langford et al. 2003). In the current study, we showed that the longer the current cART stability is, the greater the axonal structural integrity is. In addition, greater immune recovery and current immune status were associated both with improved measures of axonal structural integrity and density. Those three findings support the interpretation that improvement in systemic immune functions afforded by cART improve HIV-related brain injury in the WM. Evidence for such a level of benefit in DTI studies has, however, never been as strongly documented in a stable and virally suppressed cohort (Correa et al. 2016; Su et al. 2016). We note that relative lack of differences between the HIV− and HIV+ samples is unlikely to be explained by an abnormal amount of WM damage in the HIV− sample as those were screened for any history of neurological, psychiatric, alcohol, and substance misuse as well as acute cardio-vascular diseases (Cysique et al. 2013), while their neurocognitive functions are within the expected normal range (Cysique et al. 2014). Moreover, another study also detected no differences in MD and FA between their HIV− and HIV+ sample (Towgood et al. 2012). Because their main study aim was to assess the effects of HIV and aging on brain functions, they did not explore further reasons for this lack of difference. Interestingly, however, all their HIV+ participants were virally suppressed and their HIV− sample had been screened for medical, psychiatric, alcohol, and drug misuse, identically to our cohort. Finally, the HIV+ sample obtained higher FA value in the PLIC, and this was of a small to medium effect size compared to the HIV− sample ($d = -0.37$, $p = 0.057$). But, this was driven by four HIV+ cases which all had FA between.076 and 0.79. Potential explanation for such cases, although speculative, is that WM repair afforded by viral suppression and cART could lead to greater degree of axonal structural integrity that is above normal effects of aging. This may be particularly possible in the internal capsule as it is a region that is sensitive to WM damage in those with cognitive impairment and AIDS (Gongvatana et al. 2009). These possibilities will need to be explored with more advanced diffusion imaging including tracktography.

We found that the cART CPE rank score was also strongly and independently associated with better axonal structural integrity and density, suggesting that on the top of the systemic-related brain repair, a unique effect of cART CPE is present within the WM. The strongest of effect of the CPE was found in the internal capsule, a major WM nexus. The independent and strong effect of the CPE suggests that DTI parameters are potentially more specific indicators of the CPE effect than neurocognitive testing, where inconsistent findings have been found (Cysique et al. 2011). Long-term repair in chronic HIV+ persons is suspected but difficult to demonstrate. It is possible that we were able to detect near-normal WM DTI measures more so than in other DTI studies (Correa et al. 2016; Su et al. 2016) of HIV+ cohorts because the current study HIV+ participants were selected to be clinically stable and successfully treated with long-term HIV infection from 45 years of age onwards. With a median HIV duration of 20 years, it is undeniable that many in the current study are survivors from the pre-cART era. They therefore represent a group of persons that may be particularly resilient as well as somewhat resistant to the effect of HIV. Yet, the results of the current study, in addition to the findings from our neuropsychological study (Cysique et al. 2014) and ^{1}H-MRS study (Cysique et al. 2013) in the same cohort, show that while brain repair processes are ongoing, there is also ongoing HIV-related brain injury.

The current study also investigated which demographic and clinical factors may moderate WM DTI measures in HIV+ persons with viral suppression. We detected no age and HIV status interaction on the DTI parameters. This is similar to the Dutch-British study (Su et al. 2016) but different from a recent American study (Seider et al. 2015). The American study included a much wider range of ages (23–79), but only 68% were undetectable; while both our study and the European study focused on undetectable middle-aged HIV+ persons (mean age 55 and 53, respectively). Studies with virally undetectable HIV+ individuals may include cases who are particularly resistant to the effect of HIV × aging (Correa et al. 2016). Indirectly supporting this interpretation is that in our sample, while age × HIV status interaction was not a factor on the level of axonal structural integrity, education was. This effect may be associated with long-term brain resilience due to greater cognitive reserve in some HIV+ persons. Better cognitive health in HIV+ persons as a function of education is not a new finding (Cysique and Brew 2011), but better WM DTI measures as a function of education in adults is, to the best of our knowledge. A study in adolescents has also demonstrated such a positive effect of education on FA (Noble et al. 2013).

Results of a cross-sectional study (Su et al. 2016) in 100 virally suppressed HIV+ persons differ quite substantially from ours as they found significant and diffuse FA reduction and MD increase across the brain WM relative to 70 age-comparable HIV− men. Several methodological differences may explain the discrepant findings. First, it is possible that because the HIV duration was 10 years longer in our study (median or 20 versus 13 years), the brain repair reached in a higher level in our HIV+ participants. Second, we carefully excluded participants with current substance use disorders, while this Dutch-British study did not. Third, our analytical approach was different. The Dutch-British study used the

entire TBSS FSL toolkit, which represents a voxel-wise analysis of WM DTI parameter (Smith et al. 2004), which has however several well-recognized limitations (Bach et al. 2014). The main concern is that TBSS as a whole brain voxel-based analysis has potentially limited anatomical specificity and consequently may over-estimate DTI parameter abnormalities especially in clinical populations (Bach et al. 2014). One way to overcome this issue, and as applied in the current study, is to determine *individual* skeleton regions of interest for FA and MD values and retain those as the primary outcomes as opposed to the merged FA and MD values within the HIV− and HIV+ samples. As such, the individual anatomy is not truncated in the process of merging FA or MD maps across different subjects. Our method is therefore more conservative, and merged brain illustrations cannot be presented. On the flip side, the European study based their DTI parameters on 50–64 directions, while ours was based on 32. It remains possible that subtle WM damage was missed in our sample because the 32-direction protocol has inherently less resolution to detect crossing fibers. A longitudinal study (Correa et al. 2016) in a small sample ($N = 21$) of virally suppressed HIV+ participants (no controls), who were tested at baseline and 26.6 months, on a 30 non-colinear directions DTI (b-value = 900 s/mm^2; 1.5-T scanner) and TBSS skeletonization showed no change in FA and MD across this time period and interpreted their results as potential stabilizing/beneficial effect of cART (from baseline where some patients had WM abnormalities detected; Correa et al. 2015). Although cART effect was not directly tested and the sample was small, their results are indirectly supportive of the beneficial effect of cART as demonstrated in our study.

While at the group level, there was no significant difference between the HIV− and HIV+ groups, we still detected abnormal WM DTI measures as a function of HAND severity, demonstrating the clinical relevance of our 32-direction DTI protocol and associated analytical approach. The majority of our HIV+ cases with cognitive impairment had ANI. The level of WM damage was fairly restricted in the ANI cases to one projection bundle, the ALIC, and only for MD, suggesting mild WM atrophy. This finding may reflect that ANI indeed represents a mild level of HIV brain involvement. It could also reveal that DTI FA and MD measures may not be the most sensitive to ongoing brain injury that have been shown using CSF analyses (Jessen Krut et al. 2014) and ^{1}H-MRS (Cysique et al. 2013; Winston et al. 2015) in virally suppressed and ANI patients. The WM alterations were more widespread in the MND + HAD consistent with the greater severity of the underlying HIV brain involvement (major association fibers, EC, FX, and CG, for both MD and FA suggesting both atrophy and axonal injury).

The current main study limitation is that this is a cross-sectional investigation only in men. Our findings' generalizability is not fully certain, especially in the more international context of the HIV epidemics (more diverse sample in terms of gender, ethnicity, and different HIV risk factors). HIV and aging effects on the brain in aging women using DTI remain to be explored and could be influenced by hormonal changes in mid-life. While we detected evidence from immune recovery and treatment duration supporting mechanisms of brain repair, further longitudinal study would more fully establish the extent to which the CPE and HIV disease biomarker changes directly relate to WM repair.

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Author contributions (1) Conception and design of the study: LAC, BJB, CR, RGH.

(2) Acquisition and analysis of data: JS, JG, MS, KM.

(3) Drafting a significant portion of the manuscript or figures: LAC, BJB, CR, JS, MG, RGH.

Compliance with ethical standards

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