Title: Longitudinal Trajectories of Brain Volume and Cortical Thickness in Treated and Untreated Primary HIV Infection

Running title: Longitudinal brain changes in early HIV

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Summary: We examined longitudinal brain changes in treated and untreated primary HIV infection participants. Before treatment, we observed significant brain volume loss and cortical thinning. After treatment brain atrophy was no longer observed. This highlights the importance of early treatment.
Abstract

**Background:** HIV penetrates the brain in early infection. We used neuroimaging to longitudinally examine the impact the virus and combination antiretroviral therapy (cART) has on brain structure in treated and untreated participants starting in primary HIV infection (PHI).

**Methods:** Sixty-five participants enrolled during PHI and underwent longitudinal MRI, 30 of whom commenced cART during follow-up. Cross-sectional MRI was acquired from 16 chronic HIV infection (CHI) and 19 HIV-negative participants. Brain volume and cortical thickness were estimated using tensor-based morphometry (TBM) and cortical modeling, respectively. Mixed-effects models mapped brain morphometric changes before and after cART initiation. The relationships between brain morphometry estimates and blood and CSF biomarkers were also tested. Region-of-interest analyses were performed to compare brain volume and cortical thickness between the groups cross-sectionally.

**Results:** Prior to cART initiation, longer duration of untreated infection correlated with volume loss in the thalamus, caudate, and cerebellum, and with cortical thinning in the frontal and temporal lobes, and cingulate cortex. After cART, no further volume loss was found by TBM. However, small increases of cortical thickness in the right frontal and temporal lobe correlated with longer cART duration. No correlations were observed with blood or CSF measures. The PHI group had cortical thickness reductions only in the right frontal lobe compared with the HIV-negative group, but had larger volumes in the thalamus, caudate, putamen, brainstem, and cortical gray matter compared with CHI group.

**Conclusion:** Subcortical atrophy and cortical thinning occur during untreated infection, but may be arrested by cART. These findings emphasize the importance of early cART.
Introduction

HIV penetrates the central nervous system (CNS) soon after seroconversion. Despite the use of combination antiretroviral therapy (cART), people living with HIV (PLWH) continue to experience neurological impairment[1-3]. The etiology of this mild, but quality-of-life limiting brain dysfunction in most of these individuals is unclear.

Recently, a longitudinal study demonstrated that well-treated, aviremic chronic HIV infection (CHI) participants had significant brain volume reductions compared to HIV-negative controls at all visits, but changes in brain volume over time were similar in both groups[3]. Although this result argues against an active, destructive process to be the cause of the brain alterations, the timing and cause of these changes remain uncertain.

It has been hypothesized that structural brain alterations may occur in primary HIV infection (PHI; defined as <1 year after exposure), possibly before cART initiation. Several studies have demonstrated that prominent inflammation[4-6], immune activation[7-9] and blood-brain barrier (BBB) disruption[10] were evident during the first year of infection, and progressively worsened in the absence of cART[4,8,10]. All of these factors have been linked to neuronal injury during this period[11], and could have contributed, in part, to brain volume reductions previously reported in PHI individuals[12-14]. However, the natural course of structural brain changes that occur in early infection, and the impact cART has on these changes have not been well-characterized[12-15].

In this longitudinal study, we: sought to map the trajectory of structural brain changes in early infection; assessed whether cART stabilizes or reverses structural alterations; and explored the relationship between brain morphometric measures and blood and cerebrospinal fluid (CSF) biomarkers. A large sample of treated and untreated PHI participants, and smaller samples of CHI participants and HIV-negative controls, underwent structural MRI. Regional brain volume and cortical thickness was characterized with advanced neuroimaging processing tools tensor-based morphometry (TBM) and cortical modeling, respectively. These approaches are useful for examining regional brain morphometry because they do not require a priori anatomical hypotheses, and they have previously been shown to be effective in detecting brain changes in PLWH[2,3,16,17]. It is advantageous to use these methods in tandem because they provide complimentary information; while TBM is best suited to detect spatially localized volume changes in subcortical regions[17], cortical modeling is well-suited extracting cortical morphometric measures (i.e. thickness)[2].

Methods

Standard protocol approvals, registrations, and patient consents

The Institutional Review Board at University of California San Francisco (UCSF) approved the study. Written informed consent was obtained from all participants.

Participants

Sixty-five PHI, 19 HIV-negative, and 16 CHI participants were studied at UCSF from December 14th, 2005 to December 22nd, 2011. PHI was defined as infection within 12 months prior to enrolment, confirmed by the Serological Testing Algorithm for Recent HIV Seroconversion[18]. HIV transmission date was estimated as 14 days before onset of
Seroconversion symptoms, or as the date halfway between the last negative and first HIV positive test[11]. Sixty-one PHI participants (94%) were cART-naïve at enrolment. CHI participants had a history of HIV diagnosis for at least 3 years, and were either cART-naïve (n=9) or had elected to interrupt therapy for at least 3 months before entering the study (n=7; mean time off therapy: 11.7 months). HIV-negative controls were recruited from the San Francisco community, and matched to PHI participants for age, sex and education. Exclusion criteria included confounding active neurologic illness, active substance use (except tobacco, marijuana and alcohol), and hepatitis B or C co-infection.

**Specimen Sampling, Processing, and Laboratory Analysis**

Participants underwent detailed medical and neurological examinations, and collection of blood and CSF specimens at each visit. Details of the laboratory analysis have been described previously[19]. In brief, blood samples were analyzed for CD4+ and CD8+ T-lymphocyte counts using flow cytometry. CSF samples were analyzed for neurofilament light chain (NFL), at enrolment in PHI only, with the NF-light enzyme-linked immunosorbent assay kit (UmanDiagnostics, Umeå, Sweden)[11]. Paired blood and CSF samples were analyzed for white blood cell (WBC) count and albumin, while paired blood and cell-free CSF samples were analyzed for HIV RNA and neopterin concentrations[19]. These measures are considered biomarkers of viral burden (blood and CSF HIV RNA), immune status (CD4+ and CD8+ T-lymphocyte counts), inflammation (blood and CSF neopterin, and WBC counts), BBB permeability (CSF:blood albumin ratio), and neuronal injury (CSF NFL).

**MRI acquisition**

All participants underwent MRI using the same Bruker (Billerica, MA) MedSpec 4T with Siemens (Munich, Germany) TRIO console in San Francisco. The scanning protocol included a T1-weighted three-dimensional magnetization-prepared rapid acquisition gradient echo sequence [repetition time (TR)/echo time (TE)/inversion time (TI)=2300/3.0/950ms; voxel=1.0mm³]. PHI participants completed longitudinal MRI scans, whereas HIV-negative and CHI participants only completed a baseline scan. MRI and laboratory data were acquired at enrolment, 6 weeks, and every 6 months thereafter. While some MRI and laboratory data were not acquired on the same date, they were associated with the same study interval. MRIs were obtained a median of 12 days (IQR: 6, 23.5) from each associated laboratory visit.

**MRI processing**

All PHI T1-weighted data was processed using a longitudinal processing pipeline, as previously described[3,20]. Pre-processing included denoising[21], intensity inhomogeneity removal[22] and brain masking[23]. Images were linearly registered to the Montreal Neurological Institute ICBM152 template using a nine-parameter affine transform[24]. To ensure the registrations to the ICBM152 space were consistent across all time points, a subject-specific template was created using an unbiased template creation approach[25]. This process yielded nonlinear transformations that mapped each visit to the ICBM152 space in a consistent manner reducing the intra-subject variability in brain volume measures across visits, increasing the statistical power to detect within-subject changes[20,26]. Cross-sectional T1-weighted data for HIV-negative and CHI participants followed similar processing procedures, except the scans were
nonlinearly registered directly to the ICBM152 template[26]. All data were carefully inspected for unacceptable processing outcomes. All data passed visual quality control and were available for TBM and cortical modeling.

**Tensor-based morphometry**

TBM provides a voxel-wise estimate of brain structure volume relative to the ICBM152 template, corrected for overall brain size. Structural volumes were calculated by taking the Jacobian determinant of the deformation field from the nonlinear transform[27].

**Cortical modeling**

Cortical modeling provides a quantitative measure of cortical thickness. Cortical thickness estimates were extracted with Fast Accurate Cortical Extraction by deforming polygonal meshes to fit the gray-white matter and pial surface boundaries[28]. Thickness estimates were mapped to the ICBM152 average cortical template using an iterative feature-based registration algorithm[29], and blurred with a 20-mm surface-based kernel.

**Statistical analysis**

To assess the longitudinal trajectory of brain volume and cortical thickness before and after cART initiation in PHI, a piecewise mixed-effects model was applied to the whole brain. This involved fitting a linear model to the visits before cART initiation, and a different linear model to the visits after cART initiation. Both models were constrained to meet at cART initiation[4]. Age was included as a fixed-effect, as well as a participant-specific random intercept.

At the visits before cART initiation in PHI, a multivariable mixed-effects model assessed the relationship between the brain morphometric measures and markers of inflammation, immune status, viral burden and BBB integrity. This model included age, CD4+ and CD8+ T-lymphocyte counts, blood and CSF HIV RNA and neopterin, CSF WBC, and albumin ratio as fixed effects, and a participant-specific random intercept. Given that CSF NFL was acquired at enrolment, a fixed-effects model was constructed to assess the correlation of the HIV-related factors at baseline, including CSF NFL, with baseline brain morphometric measures. All whole brain statistical maps were corrected for multiple comparisons using the standard false discovery rate (FDR) with a false-positive rate of 5%[30].

The small HIV-negative and CHI groups limited the ability to perform meaningful whole brain comparisons. Instead, we performed region-of-interest analyses based on prior hypotheses to compare brain volumes and cortical thickness between the groups. Volumes from the thalamus and caudate, and cortical thickness from the right frontal and temporal lobes were extracted from the baseline scan in untreated PHI, HIV-negative and CHI participants. These regions were chosen because we found that they were significantly correlated with the duration of untreated infection in the piecewise mixed-effects model in the PHI group (see Results). Volumes were also extracted from the putamen, 3rd ventricle, brainstem and cortical gray matter because these regions were shown in previous work to be affected in PHI[12-14]. General linear models were used to cross-sectionally compare these brain volumes between the groups at baseline, while controlling for age.
Results

Participants

Table 1 summarizes baseline demographic and clinical characteristics of the participants. The PHI group completed a total of 184 MRI scans over 6 years. Treatment was commenced in 30 PHI participants during follow-up, independent of the study, at a median of 10.9 months (IQR: 6.2, 22.0) after HIV transmission. Those that started treatment were significantly older ($p=0.04$) and more educated ($p<0.001$) at the initial visit compared to those that deferred treatment. At enrolment, the median duration of infection after HIV transmission was 3.7 months in the PHI group, while the CHI participants had a median duration of 90 months since HIV diagnosis, though initial infection date was unknown. The HIV-negative participants were comparable to the PHI participants with respect to age, sex and education, while CHI participants were only comparable to the PHI participants with respect to sex and education.

By the study conclusion, the PHI participants who initiated cART had been receiving treatment for a median of 7.9 months (IQR: 3.7, 29.9). Significant improvements in almost all blood and CSF biomarkers, except albumin ratio, were observed when comparing the first and last MRI visit (Table 2).

Table 1: Baseline demographic and clinical characteristics of study participants.

<table>
<thead>
<tr>
<th></th>
<th>HIV-negative (n=19)</th>
<th>Primary HIV infection (n=65)</th>
<th>Chronic HIV infection (n=16)</th>
<th>PHI vs HIV-</th>
<th>PHI vs CHI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [years, mean (SD)]</td>
<td>34.5 (9.9)</td>
<td>36.9 (9.0)</td>
<td>45.1 (10.3)</td>
<td>0.36</td>
<td>0.01</td>
</tr>
<tr>
<td>Sex [n, (% male)]</td>
<td>19 (100)</td>
<td>65 (100)</td>
<td>15 (94)</td>
<td>1.00</td>
<td>0.17</td>
</tr>
<tr>
<td>Education [years, mean (SD)]</td>
<td>16.1 (2.6)</td>
<td>15.4 (2.3)</td>
<td>14.3 (2.3)</td>
<td>0.34</td>
<td>0.09</td>
</tr>
<tr>
<td>Duration of HIV infection [months, median (IQR)]</td>
<td>NA</td>
<td>3.7 (2.0, 5.2)</td>
<td>90 (48, 220)</td>
<td>NA</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CD4+ T-lymphocyte count [cells/µl, median (IQR)]</td>
<td>790 (745, 1003)</td>
<td>579 (412, 748)</td>
<td>223 (145, 310)</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CD8+ T-lymphocyte count [cells/µl, median (IQR)]</td>
<td>476 (335, 732)</td>
<td>901 (640.5, 1151)</td>
<td>1029 (695, 1366)</td>
<td>&lt;0.01</td>
<td>0.50</td>
</tr>
<tr>
<td>CD4/CD8 ratio [median (IQR)]</td>
<td>1.91 (1.25, 2.42)</td>
<td>0.61 (0.41, 0.92)</td>
<td>0.23 (0.15, 0.29)</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Blood HIV RNA [log_{10}, mean (SD)]</td>
<td>NA</td>
<td>4.3 (0.9)</td>
<td>4.6 (0.8)</td>
<td>NA</td>
<td>0.32</td>
</tr>
<tr>
<td>CSF HIV RNA [log_{10}, mean (SD)]</td>
<td>NA</td>
<td>2.5 (0.8)</td>
<td>3.9 (1.1)</td>
<td>NA</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Blood neopterin [nmol/L, median (IQR)]</td>
<td>NC</td>
<td>13.0 (7.8, 20.4)</td>
<td>18.4 (11.1, 22.3)</td>
<td>NA</td>
<td>0.13</td>
</tr>
<tr>
<td>CSF neopterin [nmol/L, median (IQR)]</td>
<td>NC</td>
<td>9.0 (6.6, 12.8)</td>
<td>23.9 (19.4, 43.6)</td>
<td>NA</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CSF white blood cell count [cells/µl, mean (SD)]</td>
<td>1.8 (1.5)</td>
<td>7.7 (8.3)</td>
<td>8.3 (6.8)</td>
<td>&lt;0.01</td>
<td>0.80</td>
</tr>
<tr>
<td>CSF blood albumin ratio [mean (SD)]</td>
<td>5.3 (1.9)</td>
<td>5.6 (2.3)</td>
<td>7.6 (3.5)</td>
<td>0.60</td>
<td>0.05</td>
</tr>
<tr>
<td>CSF neurofilament light chain [pg/mL, median (IQR)]</td>
<td>NC</td>
<td>519 (408, 793)</td>
<td>NC</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Table 2: Comparison of first and last MRI visit in the PHI participants who initiated cART.

| CD4+ T-lymphocyte count [cells/µl, median (IQR)] | 441.0 (371.5, 628.3) | 531.5 (469.8, 758.0) | 89.5 (26.0, 176.0) | 0.03 |
| CD8+ T-lymphocyte count [cells/µl, median (IQR)] | 881.0 (618.8, 1142.3) | 693.0 (574.0, 913.5) | -225.2 (-3.79, -60.0) | 0.004 |
| CD4/CD8 [median (IQR)] | 0.46 (0.35, 0.91) | 0.89 (0.58, 1.14) | 0.31 (0.18, 0.44) | <0.001 |
| Blood HIV RNA [log₁₀, mean (SD)] | 4.40 (0.97) | 1.82 (0.62) | -2.59 (-3.05, -2.13) | <0.001 |
| CSF HIV RNA [log₁₀, mean (SD)] | 2.65 (0.68) | 1.72 (0.15) | -0.98 (-1.34, -0.62) | <0.001 |
| Blood neopterin [nmol/L, median (IQR)] | 11.5 (8.0, 21.0) | 6.2 (4.6, 9.0) | -7.51 (-11.2, -3.5) | <0.001 |
| CSF neopterin [nmol/L, median (IQR)] | 9.0 (6.8, 11.6) | 7.1 (5.2, 9.5) | -1.96 (-7.5, 0.3) | 0.07 |
| CSF white blood cell count [cells/µl, mean (SD)] | 7.68 (6.35) | 3.33 (4.41) | -3.78 (-7.76, -0.11) | 0.03 |
| CSF blood albumin ratio [mean (SD)] | 5.78 (1.85) | 5.50 (1.91) | -0.24 (-1.07, 0.58) | 0.54 |

Abbreviations: SD = standard deviation; IQR = Interquartile range

Table 2: Comparison of first and last MRI visit in the PHI participants who initiated cART.

| Abbreviations: NA = not applicable; SD = standard deviation; IQR = Interquartile range
| a| Comparisons were made using Wilcoxon signed-rank test for continuous variables and chi-square test for categorical variables.
| b| Duration of HIV infection for PHI was estimated by date of recent seroconversion as confirmed by laboratory measures. CHI duration of infection reflects the period since known HIV diagnosis.
| c| CSF NFL only measured in PHI participants at baseline.

**Cross-sectional comparison of brain volumes and cortical thickness**

Volumes in the thalamus, caudate, putamen, 3rd ventricle, cortical gray matter and brainstem, and cortical thickness in the right temporal and frontal lobes were extracted from the baseline MRI scan in untreated PHI, HIV-negative and CHI participants (Table 3). The cortical thickness in the right frontal lobe was significantly reduced in the PHI compared to HIV-negative controls, whereas the other regions did not significantly differ. Significant differences in most
Brain regions were observed when PHI and CHI groups were compared, where CHI participants had smaller brain volumes, thinner cortices, and enlarged 3rd ventricle.

Table 3: Cross-sectional comparison of brain volumes at baseline.

<table>
<thead>
<tr>
<th></th>
<th>HIV- [mean (SD)]</th>
<th>PHI [mean (SD)]</th>
<th>CHI [mean (SD)]</th>
<th>PHI vs HIV-</th>
<th>PHI vs CHI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thalamus (mm³)</td>
<td>7663.0 (877.2)</td>
<td>7816.0 (661.0)</td>
<td>7108.3 (954.2)</td>
<td>0.25</td>
<td>0.02</td>
</tr>
<tr>
<td>Caudate (mm³)</td>
<td>5871.8 (666.9)</td>
<td>5744.7 (598.6)</td>
<td>5215.0 (435.0)</td>
<td>0.61</td>
<td>0.03</td>
</tr>
<tr>
<td>Putamen (mm³)</td>
<td>5395.9 (531.7)</td>
<td>5188.4 (494.0)</td>
<td>4661.2 (688.1)</td>
<td>0.22</td>
<td>0.07</td>
</tr>
<tr>
<td>3rd Ventricle (mm³)</td>
<td>1513.5 (491.2)</td>
<td>1691.5 (521.9)</td>
<td>2310.1 (1054.8)</td>
<td>0.28</td>
<td>0.03</td>
</tr>
<tr>
<td>Brainstem (mm³)</td>
<td>3389.9 (3792.3)</td>
<td>3489.5 (3408.7)</td>
<td>3275.5 (3335.7)</td>
<td>0.31</td>
<td>0.06</td>
</tr>
<tr>
<td>Cortical gray matter (mm³)</td>
<td>609.920 (7399.6)</td>
<td>616.862 (60774.6)</td>
<td>558.856 (71997.4)</td>
<td>0.71</td>
<td>0.008</td>
</tr>
<tr>
<td>Right superior temporal lobe</td>
<td>3.10 (0.55)</td>
<td>3.12 (0.57)</td>
<td>2.76 (0.53)</td>
<td>0.78</td>
<td>0.13</td>
</tr>
<tr>
<td>Right frontal lobe (mm)</td>
<td>3.23 (0.50)</td>
<td>2.92 (0.49)</td>
<td>2.47 (0.52) (d)</td>
<td>0.03</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Abbreviations: SD = standard deviation

\(a\) p values calculated from a general linear model while controlling for age.

\(b\) Volumes from the left and right hemisphere were averaged.

\(c\) Average cortical thickness within anatomical brain region.

\(d\) One CHI participant was considered an outlier and removed because cortical thickness was \(>5.9\sigma\).

**Brain volume and cortical thickness changes prior to cART initiation in PHI**

When brain volume and cortical thickness estimates were evaluated by TBM and cortical modeling, respectively, we observed that before cART initiation longer duration of untreated infection was significantly correlated with volume loss in the right cerebellum, bilateral thalami, left caudate and left temporal lobe (Figure 1A), and with cortical thinning in bilateral frontal and temporal lobes, and the cingulate cortex (Figure 1B). Within these regions, atrophy rates ranged from 0.27% to 1.22%/year (median: 0.63%/year [IQR: 0.55-0.73%/year]) (see Supplementary Figure 1) when volumes were estimated by TBM, and cortical thinning rates ranged from 0.02mm to 0.48mm/year (median: 0.25 mm/year [IQR: 0.22-0.30mm/year]) (see Supplementary Figure 2).

**Brain volume and cortical thickness changes after cART initiation in PHI**

After cART, no further significant brain volume loss was found by TBM (Figure 1A). However, small but significant increases of cortical thickness in the right frontal and temporal lobes were correlated with longer cART duration (Figure 1B).

**Brain volume and cortical thickness correlations with blood and CSF measures in untreated PHI**

At the visits before cART in PHI, the relationship between brain volumes and blood and CSF measures were tested. Increasing CSF HIV RNA tended to be correlated with volume loss in
the thalamus, while CD4+ cell count was positively correlated with cortical thickness in small areas of the left frontal lobe. However, these correlations did not reach statistical significance after multiple comparisons correction. The remaining blood and CSF measures, including CSF NFL, were not significantly associated with any brain morphometric measures.

Figure 1: A) Upper row: Brain volume loss associated with longer duration of untreated infection as revealed with TBM. Lower row: Volume of the right cerebellum (left plot) and bilateral thalami (right plot) in relation with the duration of infection. B) Upper row: Cortical thinning associated with longer duration of untreated infection (left) and longer cART duration (right). Lower row:
Cortical thickness of the right superior temporal gyrus (left) and right frontal lobe (right) in relation to the duration of infection for all PHI participants.

Discussion

While several studies have investigated the effects of HIV on the brain in early infection, the natural course of structural changes that occur during this period, and the impact cART has on these changes are not completely characterized. We observed that before cART initiation, PHI participants experienced progressive subcortical atrophy and cortical thinning that worsened in the absence of treatment. However, after cART was commenced structural deterioration was no longer observed.

We demonstrated that during untreated infection, PHI participants had a median atrophy and thinning rate of 0.63%/year and 0.25mm/year, respectively. These rates were of greater magnitude than that of normal ageing with previous studies reporting atrophy rates of 0.2%/year[31] and thinning rates of 0.01mm/year[32] in similarly aged healthy individuals, suggesting that untreated PHI individuals may experience greater-than-age related brain changes. However, given that longitudinal control data was not acquired, we cannot definitively conclude that the observed atrophy is a result of the infection. Future studies with longitudinal data on treated and untreated PHI participants and matched HIV-negative controls are warranted to clarify whether structural atrophy is caused by the virus or confounded by general processes such as ageing.

These findings, along with reports from previous studies[4,6-8,10,11,15], support the presumed initiation of HIV neuropathogenesis in early infection. The virus penetrates the CNS after initial systemic viral infection, infecting and activating local immune cells within the CNS compartment. These cells begin to release viral proteins and produce inflammatory factors resulting in prominent inflammation[4-6], immune activation and suppression[7-9], and BBB disruption[10], all of which facilitate neuronal injury and brain volume loss[1,11]. If the infection remains untreated, we demonstrate here that brain atrophy and cortical thinning continues, while others report that markers of immune status, inflammation and BBB permeability progressively worsen[4,8,10]. Although the observed atrophy was not associated with any blood or CSF biomarkers, this could be an indication that a single biomarker is insufficient to infer underlying structural brain alterations; instead, a combination of these biomarkers may be more suitable for neurological prognosis[33].

The brain regions affected during untreated infection correspond with previous studies that examined PLWH. These studies reported volume reductions throughout the subcortical regions[2,3,34-37] and cerebellum[38,39], and cortical thickness reductions in the frontal and temporal lobes, and cingulate cortex[2,3,16,40]. The data in aggregate add to the growing body of evidence that demonstrate the brain is not spared in early infection. This suggests that the brain volume reductions reported in PLWH may reflect changes that occurred, in part, during the period of untreated infection.

In the subset of PHI participants that commenced cART, we observed improvements in markers of inflammation, immune status and viral burden, which demonstrates the effectiveness of cART. While this is not surprising, given that cART has been successful in treating systemic HIV infection, the impact it has on the brain is less known. Here, we did not find brain atrophy nor cortical thinning after cART initiation. Notably, we did not observe further subcortical atrophy
with TBM. This is worth noting because while this suggests that treatment prevents additional subcortical atrophy, it points to possible permanent and irreversible changes. Interestingly, small increases in cortical thickness were found in the right frontal and temporal lobes suggesting that cortical volume partially recovers with treatment. This finding is consistent with a previous magnetic resonance spectroscopy study performed in acute HIV, where increased N-acetylaspartate (NAA) in the frontal gray and white matter was associated with cART[6]. However, extra caution must be exercised when interpreting these results, considering that 30 PHI participants started cART during follow-up, yielding only 61 post-cART scans, the likelihood of false-positive errors increases. Nevertheless, our findings suggest that cART can arrest structural atrophy, emphasizing the importance of early treatment. It also lends further support to the idea that events prior to cART may be responsible, in part, for the structural differences found in CHI[3].

To further assess the existence and extent of brain changes in PHI, we extracted volumes from the thalamus, caudate, putamen, 3rd ventricle, cortical gray matter and brainstem, and cortical thickness from the right temporal and frontal lobes in the PHI, HIV-negative and CHI groups. In contrast to previous PHI work[12-14], only the cortical thickness in the right frontal lobe was significantly reduced in the PHI group compared to HIV-negative controls. Discrepancies with prior work most likely reflect differences in sample size[13,14], duration of untreated infection[12,13], number of participants receiving cART[12,13], and the brain regions investigated[12-14]. As expected, the untreated CHI group had smaller brain volumes, thinner cortices and enlarged 3rd ventricle compared to PHI participants. This result is not surprising given that the CHI group had been infected for a median of 90 months and most participants were cART-naive. This is in agreement with a large body of cross-sectional work that reported smaller subcortical volumes[2,3,34-36] and thinner cortices[2,3,16,40] in CHI individuals. Taken together, these findings provide further insight into the natural course of brain volume changes in untreated PHI, where it appears that the right frontal lobe may be preferentially susceptible to the virus, whereas macroscopic changes as seen on MRI in the remaining brain regions may not be detectable after a median time of 3.7 months of infection. Meanwhile, the CHI group consistently had worse brain volume and cortical thickness measures. Presumably, brain volumes in the PHI group would approach the volumes observed in the CHI group in the continued absence of treatment. Although causal inferences cannot be made with cross-sectional data, these conclusions are supported by our longitudinal data; altogether, the data provides additional suggestive evidence for brain atrophy progressing in the absence of treatment, arguing for early treatment.

This study has limitations. First, the HIV-negative and CHI groups were small, limiting the ability to perform exploratory whole brain statistics, reducing the generalizability, and exposing the results to false-positive errors. Second, since longitudinal control data was not acquired, we cannot determine when the structural brain changes begin to occur or estimate any component related to ageing. Third, given that data on lifestyle was not acquired, we cannot definitively exclude that lifestyle modifications after cART initiation contributes to the post-cART results. Finally, the study participants were young adult men with a history of drug use, limiting the generalizability of the results to individuals with similar characteristics.

In conclusion, our findings provide a unique narrative regarding the natural course of brain volume changes in early HIV infection. We reported that subcortical atrophy and cortical thinning
begins early in HIV infection, principally during untreated infection, and worsens in the continued absence of cART. However, initiating cART may halt further structural deterioration. These results highlight the importance of early cART, and demonstrate that effective cART in PHI may be instrumental in preserving long-term brain health.

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**Conflicts of Interest**
All authors report no disclosures or potential conflicts of interests.
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