Gray and White Matter Alterations in Early HIV-Infected Patients: Combined Voxel-Based Morphometry and Tract-Based Spatial Statistics

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Purpose: To investigate both the gray matter (GM) and whiter matter (WM) alterations in a homogeneous cohort of early HIV-infected patients by combining voxel-based morphometry (VBM) and tract-based spatial statistics (TBSS).

Materials and Methods: Twenty-six HIV and 26 control subjects enrolled in this study with 3D T1 and diffusion-tensor imaging acquired on a 3.0T Siemens scanner. Group differences in regional GM were assessed using VBM analysis, while differences in fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and relative anisotropy (RD) of WM were evaluated using TBSS analysis. After that, interactions between GM changes and white matter alterations were investigated by using a correlation analysis.

Results: The HIV-infected patients displayed decreased GM volume, mainly located in the bilateral frontal cortices, bilateral anterior cingulate cortex, and left supplementary motor area (P < 0.05, false discovery rate-corrected). Meanwhile, the patient group showed decreased FA in the genu of capsule callosum, body of capsule callosum, and bilateral anterior corona radiate (P < 0.05, family wise error [FEW]-corrected). Areas of increased MD, RD, and AD in HIV patients were more extensive and observed in most skeleton locations (P < 0.05, FEW-corrected). The interaction analysis in the patient group revealed that there were no significant correlations between GM changes and WM alterations (P > 0.05).

Conclusion: Our results indicate that structural brain alterations occurred early in HIV-infected patients. The current study may shed further light on the potential brain effects of HIV.

Magnetic resonance imaging (MRI) provides a noninvasive examination of brain structure changes in HIV infection.8 Diffusion tensor imaging (DTI) is one of the most rapidly evolving MRI techniques, which is more sensitive in detecting subtle white matter (WM) changes.9 Derived from DTI, changes in four parameters, including fractional

Aquired immune deficiency syndrome (AIDS) is a disease spectrum of the human immune system caused by infection with human immunodeficiency virus (HIV).1 Although it has been established that HIV invasion of the brain occurs soon after initial infection, possible brain changes during this period are not well characterized.2 The most common histopathological findings in autopsy studies of HIV-infected patients show that brain atrophy, white matter alterations, demyelination, and injury to subcortical regions, occur in the later stages of infection.3–5 HIV-associated neurocognitive disorders (HAND) and HIV-associated dementia (HAD) have been strongly associated with an increased risk of death.6 However, the onset of a neurologic disorder still has not been determined.7 Therefore, a clearer understanding of brain injury with HIV infection, particularly in the early stages, is critical.

Magnetic resonance imaging (MRI) provides a noninvasive examination of brain structure changes in HIV infection.8 Diffusion tensor imaging (DTI) is one of the most rapidly evolving MRI techniques, which is more sensitive in detecting subtle white matter (WM) changes.9 Derived from DTI, changes in four parameters, including fractional
anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD), may reflect microstructural abnormality of WM.9 Using a region of interest (ROI)-based method, brain abnormalities have been reported in multiple regions in HIV-infected patients, including the genu of corpus callosum,10–12 frontal WM,13–15 hippocampi,10 lentiform nucleus,11 temporal WM,14 internal capsule,13 basal ganglia,15 parietal and periventricular WM.11 Besides ROI-based analysis, there were also many studies of WM abnormality using whole-brain-based DTI analysis.16–19

In addition, another MRI analysis method called voxel-based morphometry (VBM) has revealed widespread gray matter (GM) and WM changes in HIV patients.20 A recent VBM study by Küper et al showed that HIV leads to regional atrophy of cerebral GM and WM structures, which particularly affected nigrostriatal and frontostriatal circuits when comparing HIV-positive individuals with concomitant cognitive decline with controls.21 More recently, Sarma et al investigated the regional brain GM and WM changes in perinatally HIV-infected adolescents receiving antiretroviral therapy (ART) with VBM analyses.22 They showed that both decreased WM and increased GM volume appeared in perinatally HIV-infected youths for several brain regions when compared to controls.

Although many previous studies have reported brain alterations in patients with HIV, few studies focused on the early stages of HIV infection. In this study we sought to characterize possible brain changes during the early period of HIV-infection by combining VBM and tract-based spatial statistics (TBSS).23 In taking this approach, our study had two primary goals. First, we tried to detect GM and WM alterations in the early HIV-infected patients, where mean length of infection was estimated as less than 1 year based on assay results. Second, we sought to investigate the interactions between GM changes and WM alterations through a correlation analysis.

Materials and Methods
This study was approved by the Beijing You’an Hospital of Capital Medical University. All participants provided written informed consent.

Subjects
Twenty-six HIV-infected patients (23 males, 3 females; mean age: 38.0 ± 11.3 years) and 26 healthy controls (19 males, 7 females; mean age: 34.0 ± 9.4 years) were enrolled in our study. The inclusion criteria for patients were: all of their mean length of infection was estimated as less than 1 year based on assay results, and HAD stage was 0 or 0.5. Exclusion criteria for all the subjects included any drug abuse history and any obvious brain lesions, such as stroke or tumors assessed on the basis of medical history and conventional MRI. All healthy controls were enrolled from the same urban areas as the infected subjects. Demographic and clinical information of HIV-infected and control subjects are presented in Tables 1 and 2.

Data Acquisition
All MRIs were acquired on a 3.0T Siemens scanner (Allegra, Siemens Medical System, Erlangen, Germany) at the Beijing You’an Hospital of Capital Medical University. A standard birdcage head coil was used, along with restraining foam pads to minimize head motion and to diminish scanner noise. First, high-resolution 3D T₁-weighted images (T₁/WI) were obtained with a spoiled gradient recall sequence and the following parameters: repetition time (TR) = 1900 msec; echo time (TE) = 2.52 msec; field of view (FOV) = 250 × 250 mm; in-plane matrix = 256 × 256; slice thickness = 1 mm; number of slices = 176; acquisition time (TA) = 4.18 minutes. Second, DTIs were obtained along 20 non-collinear directions with b = 1000 s/mm² and one b = 0 s/mm². The parameters were: TR/TE = 3300/90 msec; slice thickness = 4 mm; number of slice = 63; FOV = 230 × 230 mm; acquisition matrix = 128 × 128; number of excitation (NEX) = 3; TA = 3.39 minutes.

Data Processing
For T₁-weighted images, VBM processing was performed with the VBM8 toolbox (http://dlimb.cenPts.jena.de/vbm8/) which runs within the statistical parametric mapping package (SPM8, http://www.fil.ion.ucl.ac.uk/spm/). VBM involves a voxel-wise comparison of the local concentration of GM between two groups of subjects. Our processing included three steps. First, T₁-weighted images were corrected for any bias, and partitioned into GM, WM, and cerebrospinal fluid (CSF) tissue types using a unified segmentation approach.24 GM and WM were normalized to the standard Montreal Neurological Institute (MINI) space with linear (12-parameter affine) and nonlinear transformation (warping). Second, analysis was performed on GM tissue separately, which was multiplied by the nonlinear components derived from the normalization matrix in order to preserve actual GM value locally (modulated GM volume). Finally, the modulated volumes were smoothed using an isotropic Gaussian kernel of 6 mm full-width at half maximum (FWHM).25

For DTI images, TBSS processing was performed with FSL5.0 (FMRIB Image Analysis Group, Oxford, UK, http://www.fmrib.ox.ac.uk/fsl). Our analysis involved two stages. The first stage was to create FA data from DTI data. First, the DTI was corrected for the effects of eddy currents and head movement using eddy current correction within FDT (FMRIB’s Diffusion Toolbox, part of FSL). Then brain masks were extracted by running BET (Brain Extraction Tool, part of FSL) on one of the no diffusion weighting (b = 0) images.26 After eddy current correction and brain extraction, the diffusion tensor model at each voxel was fit using DTI-FIT within FDT. With this, several DTI measures including FA, MD, AD, and RD can be derived from the tensor. The next stage is TBSS processing. As FA is a normalized measure of eigenvalue standard deviation and represents the degree of diffusion directionality,27 we analyzed the FA data using TBSS with the following four steps: first, a common registration target was identified and all subjects’ FA images were aligned to this target using FNIRT (FMRIB’s Nonlinear Image Registration Tool, part of FSL), which
uses a $b$-spline representation of the registration warp field; second, the mean of all aligned FA images were created and applied thinning to create a mean FA skeleton image (threshold $= 0.2$); third, each subject’s aligned FA image was projected onto the mean FA skeleton by filling the skeleton with FA values from the nearest relevant tract center; fourth, voxel-wise cross-subject statistical analyses of FA were carried out on the skeleton space. Furthermore, we also performed TBSS analysis on non-FA parameters, including MD, AD, and RD.

### Statistical Analysis
For VBM, a two-sample $t$-test based on the random effect model was performed voxel-wise to determine the differences in GM volume between HIV+ patients and healthy controls, with age and gender as covariates of no interest to exclude the possible effect of these variables on regional GM volume. Significance was set at $P < 0.05$, corrected for false discovery rate (FDR). A contiguous cluster of at least 20 voxels was accepted as significant. Significant clusters with a volume at least $100 \text{ mm}^3$ were superimposed on an MNI template.

For TBSS, a voxel-wise permutation-based ($n = 10,000$) nonparametric test was applied to compare the FA maps of HIV+ participants and controls using the Randomize tool in FSL (http://www.fmrib.ox.ac.uk/fsl/randomise/). Significance was set at $P < 0.05$, corrected for family-wise error (FWE) using threshold-free cluster enhancement. The preprocessing and groups analyses were also performed on non-FA maps, including MD, AD, and RD. The Johns Hopkins University ICBM-
DTI-81 White Matter Atlas was used to label significant tracts. To investigate the interactions between GM changes and WM alterations, a correlation analysis was performed between abnormal GM volumes and WM FA values in the HIV-infected group.

**Results**

There was no significant difference for age and gender between the HIV+ patient and healthy control groups (all $P > 0.05$) (Table 2).

**VBM Results**

Compared with healthy controls, significantly decreased GM in HIV-infected patients was present in the bilateral frontal cortices (ie, the bilateral superior frontal gyrus, the left middle frontal gyrus, and the left inferior frontal gyrus), the bilateral anterior cingulate cortex (ACC), and the left supplementary motor area (SMA) ($P < 0.05$, FDR-corrected). In addition, GM volumes of the frontal cortices, the ACC, and the left SMA showed a positive correlation with CD4 counts, but no significance ($r_1 = 0.1229$, $P_1 > 0.05$; $r_2 = 0.2478$, $P_2 > 0.05$; $r_3 = 0.1170$, $P_3 > 0.05$). The results are illustrated in Fig. 1 and Table 3.

**TBSS Results**

Compared with healthy controls, the HIV-infected patients showed decreased FA and increased MD, RD, and AD in

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**TABLE 2. Clinical and Demographic Characteristics of Study Participants**

<table>
<thead>
<tr>
<th>Items</th>
<th>HIV + patients</th>
<th>Healthy controls</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N$</td>
<td>26</td>
<td>26</td>
<td>N/A</td>
</tr>
<tr>
<td>age</td>
<td>$38.0 \pm 11.3$</td>
<td>$34.0 \pm 9.4$</td>
<td>0.18*</td>
</tr>
<tr>
<td>sex (M / F)</td>
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<td>19/7</td>
<td>0.17*</td>
</tr>
<tr>
<td>CD4 count</td>
<td>255 $\pm$ 220</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>CD4 $&gt; 200$ (%)</td>
<td>50 ($N = 13$)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

$N$ number of subjects; *$P > 0.05$; M, male; F, female; N/A not applicable or available.

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FIGURE 1: Voxel-based morphometry (VBM) results of the healthy controls vs. HIV infection group comparison.
multiple brain regions ($P < 0.05$, FEW-corrected). No region showed increased FA or decreased MD, RD, or AD in the patients ($P < 0.05$, FEW-corrected). In addition, FA values of the genu of capsule callosum (GCC), the bilateral anterior corona radiate (ACR) showed a positive correlation with CD4 counts ($r_4 = 0.2251$, $P > 0.05$; $r_6 = 0.2538$, $P > 0.05$; $r_7 = 0.3471$, $P > 0.05$), whereas negative correlation between FA of body of capsule callosum (BCC) and CD4 counts ($r_5 = −0.3376$, $P > 0.05$). However, none of those correlations were significant (all $P > 0.05$).

The results are illustrated in Fig. 2.

The location and cluster size (clusters $>100$ voxels) of the WM tracts where FA, MD, RD, and AD showed significant between-group differences are summarized in Table 4. In detail, the HIV-infected patients showed decreased FA in the GCC, BCC, and bilateral ACR when compared to the control group ($P < 0.05$, FEW-corrected) (Fig. 2, Table 4). In contrast, increased MD were found in the GCC, BCC, bilateral ACR, splenium of corpus callosum (SCC), bilateral anterior limb of internal capsule (ALIC), external capsule (EC), posterior thalamic radiation (PTR), superior longitudinal fasciculus (SLF), posterior corona radiate (PCR), superior corona radiate (SCR), bilateral section of cingulum around cingulate gyrus, right retrolenticular part of internal capsule (RIC), right superior fronto-occipital fasciculus (SOF), and right tapetum ($P < 0.05$, FEW-corrected) (Fig. 2, Table 4). In addition, an elevated RD value was observed in most skeleton locations that exhibited significantly increased MD within HIV-infected group ($P < 0.05$, FEW-corrected). However, areas of increased AD were much less prevalent when compared with either MD or RD (Fig. 2, Table 4).

**Interaction Between GM and WM Abnormalities**

The interaction analysis between GM and WM abnormalities in HIV-infected group revealed that there were no significant correlations between these two measures ($P > 0.05$) (Fig. 3).

**Discussion**

In this study we evaluated both the GM and WM changes of HIV-infected patients during the early stage by combining the VBM and TBSS. The results showed decreased GM accompanied by WM microstructural abnormalities in HIV-infected patients when compared to the healthy controls.

For GM changes in HIV infection, our study demonstrated that decreased GM volume occurred in several brain areas of HIV-infected patients in the early stage, which suggested that cortical atrophy occurs in HIV infection during this period. This finding is consistent with most previous studies with MRI, while it does not agree with several previous studies in perinatally HIV-infected adolescents. Since all perinatally HIV-infected adolescents were receiving ART medication at the time of the MRI scan, the average age for initiation of any antiretroviral therapy was too long and considerable cerebral damage may have already occurred. Anatomically, our results showed that decreased GM volume in the HIV infection group was mostly present in left frontal cortices. This finding was extremely consistent with the findings reported by Kuper.
et al.21 Changes in bilateral frontal GM in HIV infection have been reported in a previous morphometric study by Thompson et al.32 Conversely, a functional (f)MRI study demonstrated that left temporal blood oxygenation-dependent (BOLD) signal increases occurred over the time course of 1 year during a visual attention task in neurocognitive-stable HIV patients.33 It is well known that the frontal lobe is responsible for extensive cognitive function, such as long-term and working memory, awareness, and language sequencing, all of which may contribute to the impaired neuropsychological profile.34 Although it is still unknown whether the brain can be protected or recover from early HIV-infection, our finding of decreased frontal cortices in this period may partly explain the failure of neuroprotection strategies initiated later in infection.21

Meanwhile, decreased GM volume in bilateral ACR in the HIV infection group was also found in our study when compared to the healthy controls. The VBM study by

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**FIGURE 2:** Tract-based spatial statistics (TBSS) analysis of voxel-wise group comparisons between the HIV infection and healthy control groups.

![TBSS analysis](image_url)
Küper et al demonstrated GM tissue reduction in the ACR bilaterally, correlating with cognitive decline. Similar alterations related to cognitive dysfunction were also reported in another study by Chiang et al. Although strong evidence for the relationship between alteration in ACR and cognitive profile is lacking, our finding suggested that cognitive decline may have occurred in early HIV infection, even though symptoms are usually not present.

For WM abnormalities in HIV infection, our TBSS results showed decreased FA in several WM regions, and more extensive WM regions with increased MD, RD, and AD in HIV-infected patients. These findings were generally consistent with previous HIV infection studies. Importantly, using TBSS analysis our study provided evidence that the WM alterations occur in early HIV infection.

Most previous DTI studies in HIV infection have reported WM alterations in multiple brain regions, notably located in the corpus callosum, frontal WM, internal capsule, and corona radiate. In our study, we only identified decreased FA in the GCC, BCC, and bilateral ACR,
which was consistent with a prior study. Our findings may provide evidence that cognitive decline may occur in early HIV infection, even before symptoms appear.

Among four DTI parameters investigated in this study, simultaneous increases of MD, RD, and AD were present in more regions, similar to previous studies. Moreover, it appeared that changes in MD and RD were more prominent than AD, which is extremely consistent with the findings reported by Zhu et al. Previous animal model-based studies suggested that abnormalities in RD is a marker for demyelination. Thus, our findings would imply that the inflammatory insult to the WM is predominantly demyelinating, while axonal injury co-occurs but to a lesser degree in the early HIV infection.

In our study we also investigated the relationship between GM changes and WM alterations. Unfortunately, there were no significant correlations between these two measures. This phenomenon suggested that the morphological changes in the early stage of HIV infection on the brain’s GM and WM were not significantly linearly correlated, although abnormalities coexisted in both GM and WM.

Finally, there are several limitations of the current study that need to be considered. First of all, our results were limited to a small participant cohort, which may have an effect on the power of the statistical analysis in our study. Thus, more subjects are needed in further studies. Second, there was no neuropsychological testing in our study, so we cannot well analyze the association between cerebral cortex dysfunction and behavior patterns. The last inevitable limitation suffers from DTI’s inherent artifacts and limitations. The partial volume effect and the inability of the model to cope with non-Gaussian diffusion are the two main drawbacks of DTI, which results in local registration errors during the TBSS’s skeleton projection. Thus, a more accurate registration method should be considered to reduce the magnitude of such errors.

In conclusion, our cross-sectional study showed that HIV infection leads to structural regional brain abnormalities in the early period by using VBM and TBSS analysis. These changes included both GM and WM, which may contribute to the clinical picture of cognitive deterioration.
References


