Open Forum Infectious Diseases

MAJOR ARTICLE

Distinguishing Brain Impact of Aging and HIV Severity in Chronic HIV Using Multiparametric MR Imaging and MR Spectroscopy

Karen Chu,[1] Thao Tran,[1] Ke Wei,[1] Jeanne C. Lammering,[1] Anders Sondergaard,[2] Emad Mogadam,[2] Kimberly Shriner,[2] and Kevin S. King[1]

[1]Advanced Imaging and Spectroscopy Center, Huntington Medical Research Institutes, Pasadena, California; [2]Phil Simon Clinic, Huntington Hospital, Pasadena, California

Background. Combination antiretroviral therapy (cART) has transformed HIV into a manageable but complex chronic disease, in which it is uncertain which brain insults may relate to age vs initial disease severity. We evaluate N-acetyl-aspartate/creatine (NAA/Cr), white matter hyperintensities (WMH), and mean cortical thickness to identify which subclinical markers of brain insult best relate to CD4 nadir and aging. This is a prospective study of the association between brain markers with age and initial infection severity, based on CD4 nadir, in chronic HIV patients.

Methods. Thirty-seven chronic HIV patients (age 25–77 years) with successful viral suppression were scanned on a GE 3T magnetic resonance imaging scanner to obtain NAA/Cr (standardized and averaged over 5 brain regions), log-transformed WMH volume, and mean cortical thickness. The brain measures were fitted with both CD4 nadir and age to evaluate the significance of their relationship.

Results. NAA/Cr, WMH, and cortical thickness were all correlated with age and CD4 nadir in unadjusted associations. Stepwise regression models showed that NAA/Cr alone best predicted CD4 nadir ($\beta = 40.1 \pm 13.3$; $P = .005$), whereas WMH ($\beta = 2.3 \pm .9$; $P = .02$) and mean cortical thickness ($\beta = -2.7 \pm 6.6$; $P < .0001$) together produced the best model fit with age. NAA/Cr was higher for HIV stage 1 (CD4 nadir $\geq$ 500 cells/ μL; n = 15) compared with stage 2 (200 $\geq$ CD4 nadir < 500; n = 13) and stage 3 (CD4 nadir < 200; n = 9; $P < .01$ for both).

Conclusions. In patients with effectively suppressed HIV, NAA reflects the subclinical brain impact of initial disease severity related to development of even mild immune compromise, whereas cortical thickness and WMH volume are useful to evaluate age-related changes.

Keywords. brain markers; CD4 nadir; cortical thickness; magnetic resonance imaging; magnetic resonance spectroscopy; N-acetyl-aspartate; white matter hyperintensities.

In this modern era, HIV infection can be effectively suppressed with combined antiretroviral therapy (cART) before the development of AIDS [1]. Effective cART has made a significantly positive impact on the mortality, morbidity, and infectivity of healthy persons with HIV infection, allowing those with chronic HIV to live comparable lifespans to those of people not infected with HIV. Nonetheless, people living with chronic HIV still face many health challenges; HIV-related cardiovascular disease, non-HIV-related malignancies, chronic inflammation, accelerated cell death, and neurocognitive changes have been extensively documented even in the most well-controlled individuals. HIV-associated neurocognitive disorders (HANDs) remain prevalent [2], and the underlying brain changes may portend risk for development of cognitive decline as patients reach older age.

In many prior studies, comorbid conditions such as hepatitis, alcohol and drug abuse, and opportunistic infections may have all contributed to development of brain insults in chronic HIV [3, 4]. In this study, we rigorously selected HIV patients who were otherwise healthy (lacking the above risk factors commonly present in prior HIV publications) from the Huntington Hospital Phil Simon Clinic to better understand the effect of HIV disease in the current era of early diagnosis and effective cART therapy. Early treatment with cART and preventing low CD4 nadir may impact the risk of these comorbidities. Although many imaging studies on brain health have been done in persons with longstanding HIV infection and severe immune compromise, homogeneous cohorts of healthy HIV persons with longstanding viral suppression (including many with high CD4 counts due to early initiation of cART) are not as well studied [4].

HIV and aging have been shown to independently impact brain health [5], but it is difficult to distinguish their effects for individual patients. Though AIDS-defining conditions have decreased, HIV strongly influences expression of age-related disease [6]; HIV-associated comorbidities often resemble

Received 13 August 2018; editorial decision 13 September 2018; accepted 18 September 2018.
Correspondence: K. S. King, MD, MSCS, 10 Pico Street, Pasadena, CA 91105 (kevin.king@hmri.org).

Open Forum Infectious Diseases®

DOI: 10.1093/ofid/ofy243

accelerated aging with oxidative damage, cardiovascular disease, end-organ failure, and reduced cognitive functions [6]. The prevalence of multiple age-related diseases among HIV-seropositive patients has been shown to be equivalent to that among members of the general population who are 10–15 years older [7–9]. In this study, we hypothesized that lower CD4 nadir would be associated with more severe brain insults, and that there may be a nonlinear threshold effect whereby less severe stage of infections with lower immune reprogramming and shorter duration of initial infection before cART initiation may relatively preserve brain integrity. We further hypothesized that the trend of brain insults would distinctly differ for the severity of initial infection and age in chronic well-controlled HIV, which may help us to distinguish between their effects. To assess our hypotheses, we evaluated 3 distinct measures of brain insult previously associated with HIV (cortical thickness, n-acetyl-aspartate [NAA], and white matter hyperintensity [WMH] volume) to determine their associations with CD4 nadir and age among a well-controlled HIV cohort, in which most have lower stages of HIV and moderate to no prior depletion of CD4 cells.

METHODS

Subject Population

In this institutional review board–approved study with written informed consent, 37 HIV-positive research volunteers (median age [interquartile range {IQR}, min–max], 50.4 [40.5–60, 25–77] years) were recruited from the Phil Simon Clinic (Pasadena, CA) where historical and clinical data were gathered. Participants underwent magnetic resonance imaging (MRI) and spectroscopy at the Huntington Medical Research Institutes Advanced Imaging and Spectroscopy Center on a General Electric (GE, Waukesha, WI) 3T MRI clinical scanner with a high-resolution 8-channel head coil. All patients included in this study were adherent to their cART medication plans and had had effective suppression of viral loads for at least 1 year. HIV-seropositive volunteers were excluded for ongoing drug or alcohol abuse, including any injection drug use, history of heart attack or stroke, or serious neurologic disease such as epilepsy, multiple sclerosis, or clinical central nervous system (CNS) manifestations of HIV by historical record or evidence on brain MRI read by an experienced, board-certified neuroradiologist. Patients' initial HIV infection severity was staged according to Centers for Disease Control (CDC) criteria [3]. Stage 1 refers to relatively healthy patients with the lowest initial infection severity (CD4 nadir ≥ 500 cells/ μL; n = 15), whereas stage 3 patients were at great risk of opportunistic infections and AIDS (CD4 nadir < 200; n = 9). Stage 2 patients encompassed those in the intermediate range (200 ≤ CD4 nadir < 500; n = 13).

Cortical Thickness

The structural T1-weighted images were acquired using a 3D fast-spoiled gradient-recalled-echo (FSPGR) pulse sequence (repetition time [TR]/echo time [TE]/inversion time [TI] = 6.7/2.4/600 milliseconds; field of view [FOV] = 24 × 24 cm; flip angle = 8 degrees). T1-weighted images underwent cortical thickness analysis using the freely available Freesurfer image analysis suite 6.0 (http://surfer.nmr.mgh.harvard.edu/). Global cortical thickness was calculated by summing the left and right mean cortical thicknesses.

White Matter Hyperintensities

A 3D T2-weighted fluid-attenuated inversion recovery (FLAIR) sequence (TR/TE/TI = 6000/124.8/1865 milliseconds; FOV = 24 × 24 cm; flip angle = 90 degrees) was used for white matter hyperintensity evaluation. WMH were quantified by the Lesion Growth Algorithm (LGA) in the Lesion Segmentation Toolbox 2.0.15 (LST http://www.statistical-modelling.de/lst.html) using the corresponding T1-weighted images for reference with an optimal threshold of 0.3 through the Statistical Parametric Mapping (SPM) software package in Matlab [10]. The LGA algorithm produces a bias-corrected version of the FLAIR image that is co-registered to the T1 image and a lesion probability map. The total white matter hyperintensity volume (mL) from the T2 FLAIR lesion probability map was then calculated in an SPM script, and a log-transform was applied over the data to generate a more normal distribution.

N-Acetyl-Aspartate

Magnetic resonance spectroscopy was obtained using short echo time point-resolved spectroscopy (PRESS; TR/TE = 1500/35; flip angle = 90 degrees; FOV = 24 × 24 cm; voxel size = 8 cm [3]; number of averages = 128) in 5 brain regions: posterior gray matter (average line width [aLW]/average signal-to-noise ratio [aSNR] = 0.054 ppm/22.3), frontal gray matter (aLW/aSNR = 0.05 ppm/18.3), parietal white matter (aLW/aSNR = 0.05 ppm/20.9), frontal white matter (aLW/aSNR = 0.044 ppm/21.3), and basal ganglia (aLW/aSNR = 0.09 ppm/10.9) (Figure 1) [11, 12]. Spectroscopy analysis was completed using the Linear Combination Model (LCM; http://s-provencher.com/lcmodel.shtml) for automatic quantification of metabolites to obtain NAA over creatine ratio (NAA/Cr) for each brain region. NAA/Cr for each of the 5 brain regions were then standardized as the deviation from the mean (to account for differences in values for metabolites between brain regions) and then averaged over the 5 brain areas for a composite measure of NAA/Cr.

Statistical Analysis

Analysis was performed using JMP Pro, version 13.0.0 (SAS Institute, Inc., Cary, NC). We first tested the hypothesis that a lower CD4 nadir would be associated with more severe brain insults by evaluating the associations for CD4 nadir as the independent variable with cortical thickness, WMH volume, and NAA/Cr as dependent variables in simple linear regression. Some prior HIV studies have shown Cr to be impacted by the disease process, compared with healthy controls [13, 14]. To determine if we could still use Cr as an adjustment factor, we also evaluated

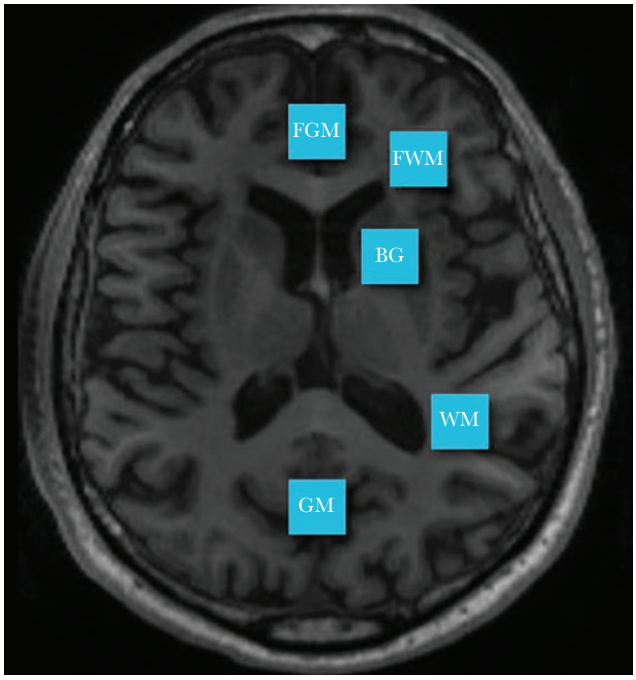

Figure 1. Locations of magnetic resonance spectroscopy voxels and the respective spectra acquired in the brain: frontal (FGM) and posterior (GM) gray matter, frontal (FWM) and parietal (WM) white matter, and basal ganglia (BG).

whether it correlated with the predictive factors we were studying. Of note, in this study, we were evaluating for factors within an HIV cohort that may impact brain health and were not including a non-HIV group for comparison where Cr may differ.

Statistical significance was set at $P = .05$ with adjustment for false discovery rate (FDR). Exploratory analysis included evaluation of age as an independent predictor for comparison. We then tested the hypothesis that the pattern of brain insults would differ between risk factors by performing a stepwise multivariable regression analysis minimizing Bayesian Information Criterion to determine the optimal model of brain imaging biomarkers as predictors, with age and CD4 nadir as dependent variables. Further analysis was done to confirm whether there were CD4 nadir thresholds for associations between selected imaging markers that best fit with the CD4 nadir. Analysis of variance (ANOVA) was used to identify between-group differences related to the CDC stage of HIV infection, and between-group comparisons were further conducted for significant ANOVA using the Tukey-Kramer honest significant difference test. We then explored nonlinear trends in the association for imaging markers producing the best linear model fit with the CD4 nadir without a priori thresholds using a cubic spline with varying smoothness and evaluated the resultant effect on model fit.

RESULTS

Subject Population

Among 37 patients recruited for this study, the median CD4 nadir (IQR, min–max) was 357 (179–566.5, 10–922) cells/μL. Patients' initial HIV infection severity according to CDC criteria: 15 had stage 1, 13 had stage 2, and 9 had stage 3 (AIDS).

Brain Markers Fitted With Premature Aging and HIV Severity

In preliminary unadjusted analysis, all brain markers significantly correlated with both age and CD4 nadir, respectively (composite NAA/Cr: -1.9 ± 0.6, $P = .006$; 40.1 ± 13.3, $P = .006$; WMH: 4.7 ± 0.9, $P < .0001$; -58.4 ± 24.9, $P = .02$; mean cortical thickness: -43.8 ± 5.6, $P < .0001$; 545.9 ± 170.8, $P = .006$). As shown in Table 1, CD4 nadir was significantly associated with composite NAA/Cr when adjusted for age, whereas WMH and mean cortical were not. To assess whether creatine influenced study results, we found that creatine did not correlate with age (0.03 ± 0.04, $P = .4$) or CD4 nadir (-0.0002 ± 0.0004, $P = .6$). NAA concentration alone yielded significance with only CD4 nadir (0.005 ± 0.002, $P = .02$) when adjusted for age. In Table 2, analysis of associations for specific magnetic resonance spectroscopy (MRS) NAA/Cr brain regions revealed that FGM and GM mainly drove the association with CD4 nadir, whereas FWM and BG regions showed more significant associations with age. Parameter estimates and significance for selective regression models are shown in Table 3. Only NAA/Cr predicted CD4 nadir, with an adjusted R^2 of .2 and an intercept of 402.1 ± 41.7.

Table 1. Associations Between Age and CD4 Nadir With Brain Imaging Biomarkers

	Multivariable Correlations With CD4 Nadir Adjusted for Age			
	Age		CD4 Nadir	
	β	*P*	β	*P*
Composite NAA/Cr, standardized units[a]	–0.05 ± 0.03	.2	0.004 ± 0.002	.03
Log WM hyperintensities[b]	0.07 ± 0.02	.0002	–0.0009 ± 0.0009	.3
Mean cortical thickness, mm[c]	–0.01 ± 0.002	<.0001	0.0002 ± 0.0001	.07

All *P* values were adjusted for false discovery rate. Unadjusted associations are shown in the results.

Abbreviations: NAA/Cr, N-acetyl-aspartate/creatine; WM, white matter.

[a]NAA/Cr are composite measures taken as the average of the standard deviation from the mean across 5 brain regions: frontal and posterior gray matter, frontal and parietal white matter, and basal ganglia.

[b]WM hyperintensity volume was log-transformed.

[c]Mean cortical thickness was averaged over right and left mean cortical thickness.

Table 2. Associations Between Age and CD4 Nadir With NAA/Cr in 5 Brain Regions of Interest: Frontal and Posterior Gray Matter, Frontal and Parietal White Matter, and Basal Ganglia

	Multivariable Correlations With CD4 Nadir Adjusted for Age			
	Age		CD4 Nadir	
	β	*P*	β	*P*
Posterior GM NAA/Cr, mM	0.0007 ± 0.001	.7	0.0002 ± 0.00007	.004
Frontal GM NAA/Cr, mM	–0.00001 ± 0.001	.9	0.0002 ± 0.00005	.006
Parietal WM NAA/Cr, mM	–0.0007 ± 0.002	.7	0.0001 ± 0.00007	.06
Frontal WM NAA/Cr, mM	–0.004 ± 0.002	.04	0.0001 ± 0.00008	.2
Basal ganglia NAA/Cr, mM	–0.005 ± 0.002	.03	–0.00003 ± 0.0001	.7

Abbreviations: NAA/Cr, N-acetyl-aspartate/creatine; WM, white matter.

WMH and cortical thickness independently predicted age, with an adjusted R^2 of .7 and an intercept of 215.1 ± 32.1.

Brain Markers Correlation With CDC-Defined HIV Infection Stages

ANOVA showed significant differences in distribution of NAA/Cr between HIV stages ($P = .004$). Group-to-group comparison showed lower NAA/Cr among those with stage 2 ($P = .008$) and stage 3 ($P = .009$) compared with those with stage 1, as shown in Figure 2A. However, there was no significant difference between stages 2 and 3 ($P = .9$). A cubic spline showed a steeper decline in NAA/Cr for a given decrease in CD4 nadir around 500 cells/μL across the cohort, as shown in Figure 2B.

DISCUSSION

Main Findings

In a healthy HIV cohort without neurologic complaints and with successful chronic viral suppression, we found significant associations for age and CD4 nadir with cortical thickness, NAA/Cr, and WMH volume. When CD4 nadir and age effects were evaluated together in a joint model, only CD4 nadir was significantly associated with NAA/C, whereas age was the only significant predictor of cortical thickness and WMH. This suggests CD4 nadir to be a more specific indicator of brain impact due to the severity of the initial infection, whereas WMH volume and cortical thickness are more reflective of age-related differences. Despite the lack of association with cortical thickness with NAA/Cr, in regional analysis we found significant correlations between CD4 nadir and NAA/Cr in both the frontal and posterior cortical gray matter, suggesting that occult insult is occurring though it has not yet led to detectable atrophy. Exploration revealed a threshold of brain insult related to development of even mild immune compromise (CD4 nadir at or above 200 but below 500) compared with those without immune compromise (CD4 nadir ≥ 500), which was equivalent to changes seen among those within AIDS territory (CD4 nadir ≤ 200) without evidence of CNS infection. This supports the importance of vigilant surveillance and a potential need for interventions to promote brain health among long-term survivors of HIV as decreased NAA/Cr is indicative of neurodegeneration that may have relevance for risk of dementia [15].

Table 3. Stepwise Regression Used to Evaluate Which Brain Markers Best Fit With Age and CD4 Nadir

	NAA/Cr, Standardized Units	Log WMH	Mean Cortical Thickness, mm
Age	*P* = .7[a]	2.3 ± 0.9, *P* = .02	–32.7 ± 6.6, *P* < .0001
CD4 nadir	40.1 ± 13.3, *P* = .005	*P* = .7[a]	*P* = .2[a]

Abbreviations: NAA/Cr, N-acetyl-aspartate/creatine; WMH, white matter hyperintensities.
[a]Did not contribute to model fit. *P* value given for association with dependent variable adjusted for independent variables selected for the model.

In MRS, values for metabolites are traditionally adjusted for creatine levels. Creatine is stable across most conditions and is used as a reference metabolite. Some studies have shown that creatine may itself be altered in HIV. We therefore first confirmed that creatine did not correlate with CD4 nadir, which indicated that it was not affected by the severity of the initial HIV infection. Prior studies showing alterations in creatine were largely performed on HIV+ individuals with active disease. In a study by Chang et al., all their cohort had active infection and had not yet even initiated treatment [13]. In work by Mohamed et al., HIV+ participants were treated but had persistent viremia [14] and nearly half had HIV-associated dementia. In more recent studies, creatine alterations have been seen in individuals who have recently started therapy [16]. In contrast, our population had had effective viral suppression for a year or more. Further work is needed to characterize MRS metabolite profiles among individuals today with long-term effective viral suppression to determine which metabolites may return to normal levels compared with non-HIV controls.

Implications of Initial HIV Severity

NAA is an abundant neuron-specific metabolite that has prominent roles in energetic and synthetic functions in the CNS; therefore, reductions are often attributed to neuronal loss. In our study, however, the differences we observed in NAA/Cr

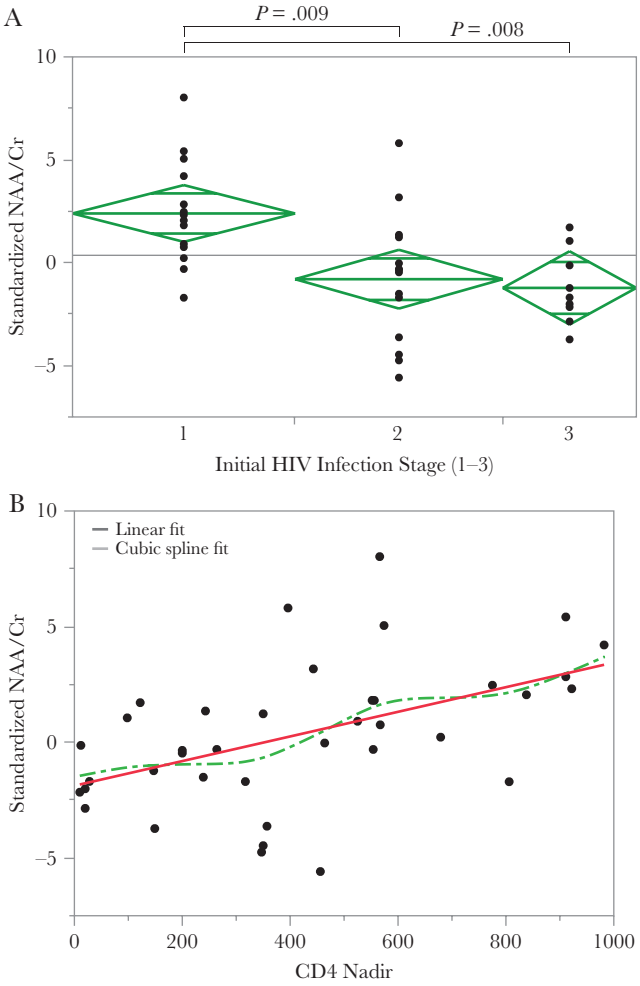

Figure 2. A, Those with HIV infection without significant prior immune compromise (Centers for Disease Control stage 1) had higher N-acetyl-aspartate/creatine (NAA/Cr) compared with those with mild (stage 2) or severe (stage 3) immune compromise, with no significant difference in brain impact between stages 2 and 3. B, Cubic spline fit (lambda = 6×10^6, cubic spline R^2 = .26, linear R^2 = .17) shows a steeper decline in NAA/Cr between patients with CD4 nadir below and above 500 cells/μL.

may instead reflect impaired neuronal metabolism. We saw a strong association between CD4 nadir and NAA/Cr and cortical thinning after considering the effects of age, but cortical thickness was more significantly correlated with age than CD4 nadir. This is in agreement with prior pathology studies conducted before cART that showed only microscopic neuronal injury about the synapse rather than frank cell death among those with mild cognitive symptoms [17] and in the absence of HIV-associated encephalitis [18]. Impaired NAA metabolic functions may have significant detrimental effects on brain health. Levels of NAA are closely linked with mitochondrial efficiency within neurons [19]. NAA exported from neurons is also an essential building block in the synthesis of fatty acids and steroids essential for maintaining the integrity of the myelin sheath lining axonal processes [19].

The association between low NAA/Cr and CD4 nadir we observed differs from observations from prior studies. Low NAA/Cr had previously been linked to development of HIV-associated encephalopathies [20]. In a large study in the cART era (n = 268) published in 2011, a significant reduction in NAA/Cr was identified among 50 participants with AIDS dementia complex, and there was only an insignificant trend for decrease in the basal ganglia among 124 asymptomatic HIV patients [21]. In contrast to this, we observed significant associations for NAA/Cr with both CD4 nadir and CDC stage. Several factors may account for this difference. Prior studies likely had greater variations in brain health due to major insults such as encephalitis and may not have been optimal to identify subtle subclinical changes. We further attempted to reduce confounding factors related to comorbid conditions by excluding those with active intravenous drug use or uncontrolled vascular risk factors. Technical differences from our work also may contribute to an increased ability to better detect smaller differences. We used a 3T MRI system, which increases the signal strength from metabolites and causes spectral dispersion for more accurate delineation of individual metabolites [22]. Aggregated standard deviations across multiple brain regions of interest may give better signal to noise and less reduction in power related to multiple comparison testing.

Implications of Aging With HIV

Although our primary aim was to identify markers of brain insult related to initial severity of HIV, there is a significant need to follow the impacts of aging among those with HIV. With the improvements in the effectiveness of cART, individuals living with long-term HIV infection exhibit many clinical characteristics commonly observed in aging, confirmed by our data showing increased WMH volume and decreased cortical thickness best fit with age. This finding may be better understood in relation to the recent findings of the Comorbidity in Relation to AIDS (COBRA) study. COBRA found brain atrophy and white matter hyperintensity volumes to be increased among those with HIV, but they did not identify any accelerated progression during 2 years of follow-up [23]. Two years is not a long time to observe the effects of more subtle chronic HIV effects, particularly as its effects must be apparent above the influence of aging itself. The predictive nature of NAA in our study may be in large part due to its levels being maintained in healthy aging. In the cART era, the effects of HIV on WMH and brain structure may mimic those of other vascular risk factors whose impact on the brain may only manifest after many years and with older age. In a population study including individuals across the adult lifespan, we found that WMH becomes prevalent after about age 50–60 years, at which time the associations between vascular risk and WMH first become evident [24]. Similar to what we observed for WMH, we also found that some associations

between vascular risk factors and brain volumes are present after age 50 years, but not before [25]. There is evidence for a similar interaction between HIV and age effects on the brain. Aging may contribute to susceptibility to neurologic diseases in HIV, as prevalence of HANDs increases with advancing age [26, 27].

As many of our HIV cohort in this study are relatively young, younger than age 50 years, an increased risk of WMH accumulation and cortical thinning related to CD4 nadir may yet become apparent as our group ages. In a recent 2016 study in the cART era among chronic HIV patients (n = 103; CD4 nadir: mean [IQR], 170 [60–250]) with successful viral suppression, those who spent a longer time with a CD4 count below 500 cells/mm^3 were found to have more WMH volume in older age [28]. Our findings agree with a longitudinal study that found that more brain atrophy correlated with patients who spent a longer duration with untreated HIV [29]. More importantly, the study also found that structural loss did not continue after cART initiation [29], suggesting that structural changes in patients with effectively suppressed HIV may be evidence of only natural aging processes.

We expect that a more lengthy longitudinal study is needed to understand the interaction between aging and HIV in relation to brain health, particularly among those with less severe disease. Older studies have shown cortical thinning among those with AIDS that was more pronounced with lower CD4 cell counts [30], but this association has not persisted in recent studies in the cART era, which have included many with less severe immune compromise [31, 32].

Limitations

There are some important limitations to our study. As we are evaluating a chronic disease cohort at one point in time, it is not possible to ascertain whether the changes we identified occurred during initial infection and persisted, or if further accelerated brain injury continues to occur despite cART. Without a well-matched control group, we cannot say whether the progression of age-related changes we observed is more severe than typical aging without HIV. As such, it is uncertain whether those with higher CD4 nadirs also possess an increased burden of brain insults compared with age-matched controls. Additionally, due to limited numbers of females in our HIV cohort, we did not have the power to evaluate for potential sex differences.

CONCLUSIONS

Our study provides important evidence of decreased NAA/Cr, reflecting neuronal insult, in a healthy HIV cohort without neurologic complaints related to mild to severe immune compromise. NAA/Cr may be a useful screening measure to prompt neuropsychological testing for early identification of HAND. As a quantitative measure of neuronal health, NAA/Cr may also have utility for monitoring disease progression and response to therapy.

Acknowledgments

Financial support. This study was funded by the Huntington Medical Research Institutes.

Potential conflicts of interest. All authors: no reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. The Antiretroviral Therapy Cohort Collaboration. Life expectancy of individuals on combination antiretroviral therapy in high-income countries: a collaborative analysis of 14 cohort studies. Lancet **2008**; 372:293–9.
2. Heaton RK, Franklin DR, Ellis RJ, et al; CHARTER Group; HNRC Group. HIV-associated neurocognitive disorders before and during the era of combination antiretroviral therapy: differences in rates, nature, and predictors. J Neurovirol **2011**; 17:3–16.
3. Centers for Disease Control and Prevention. Revised surveillance case definition for HIV infection—United States, 2014. MMWR Recomm Rep **2014**; 63:1–10.
4. Chan P, Hellmuth J, Spudich S, Valcour V. Cognitive impairment and persistent CNS injury in treated HIV. Curr HIV/AIDS Rep **2016**; 13:209–17.
5. Ances BM, Vaida F, Yeh MJ, et al. HIV infection and aging independently affect brain function as measured by functional magnetic resonance imaging. J Infect Dis **2010**; 201:336–40.
6. Justice AC. HIV and aging: time for a new paradigm. Curr HIV/AIDS Rep **2010**; 7:69–76.
7. Deeks SG. HIV infection, inflammation, immunosenescence, and aging. Annu Rev Med **2011**; 62:141–55.
8. Guaraldi G, Zona S, Alexopoulos N, et al. Coronary aging in HIV-infected patients. Clin Infect Dis **2009**; 49:1756–62.
9. Caron-Debarle M, Lagathu C, Boccara F, et al. HIV-associated lipodystrophy: from fat injury to premature aging. Trends Mol Med **2010**; 16:218–29.
10. Schmidt P, Gaser C, Arsic M, et al. An automated tool for detection of FLAIR-hyperintense white-matter lesions in multiple sclerosis. Neuroimage **2012**; 59:3774–83.
11. Mohamed MA, Barker PB, Skolasky RL, et al. Brain metabolism and cognitive impairment in HIV infection: a 3-T magnetic resonance spectroscopy study. Magn Reson Imaging **2010**; 28:1251–7.
12. Paul RH, Yiannoutsos CT, Miller EN, et al. Proton MRS and neuropsychological correlates in AIDS dementia complex: evidence of subcortical specificity. J Neuropsychiatry Clin Neurosci **2007**; 19:283–92.
13. Chang L, Ernst T, Witt MD, et al. Relationships among brain metabolites, cognitive function, and viral loads in antiretroviral-naïve HIV patients. Neuroimage **2002**; 17:1638–48.
14. Mohamed MA, Lentz MR, Lee V, et al. Factor analysis of proton MR spectroscopic imaging data in HIV infection: metabolite-derived factors help identify infection and dementia. Radiology **2010**; 254:577–86.
15. Wang H, Tan L, Wang HF, et al. Magnetic resonance spectroscopy in Alzheimer's disease: systematic review and meta-analysis. J Alzheimers Dis **2015**; 46:1049–70.
16. Mohamed M, Barker PB, Skolasky RL, Sacktor N. 7T brain MRS in HIV infection: correlation with cognitive impairment and performance on neuropsychological tests. Am J Neuroradiol **2018**; 39:704–12.
17. Masliah E, Heaton RK, Marcotte TD, et al. Dendritic injury is a pathological substrate for human immunodeficiency virus-related cognitive disorders. HNRC Group. The HIV Neurobehavioral Research Center. Ann Neurol **1997**; 42:963–72.
18. Masliah E, Achim CL, Ge N, et al. Spectrum of human immunodeficiency virus-associated neocortical damage. Ann Neurol **1992**; 32:321–9.
19. Moffett JR, Ross B, Arun P, et al. N-Acetylaspartate in the CNS: from neurodiagnostics to neurobiology. Prog Neurobiol **2007**; 81:89–131.
20. Paley M, Cozzone PJ, Alonso J, et al. A multicenter proton magnetic resonance spectroscopy study of neurological complications of AIDS. AIDS Res Hum Retroviruses **1996**; 12:213–22.
21. Harezlak J, Buchthal S, Taylor M, et al; HIV Neuroimaging Consortium. Persistence of HIV-associated cognitive impairment, inflammation, and neuronal injury in era of highly active antiretroviral treatment. AIDS **2011**; 25:625–33.
22. Otazo R, Mueller B, Ugurbil K, et al. Signal-to-noise ratio and spectral linewidth improvements between 1.5 and 7 Tesla in proton echo-planar spectroscopic imaging. Magn Reson Med **2006**; 56:1200–10.
23. Cole JH, Caan MWA, Underwood J, et al. No evidence for accelerated ageing-related brain pathology in treated HIV: longitudinal neuroimaging results from the comorbidity in relation to AIDS (COBRA) project. Clin Infect Dis **2018**; 66:1899–909.

24. King KS, Peshock RM, Rossetti HC, et al. Effect of normal aging versus hypertension, abnormal body mass index, and diabetes mellitus on white matter hyperintensity volume. Stroke **2014**; 45:255–7.
25. Srinivasa RN, Rossetti HC, Gupta MK, et al. Cardiovascular risk factors associated with smaller brain volumes in regions identified as early predictors of cognitive decline. Radiology **2016**; 278:198–204.
26. Holt JL, Kraft-Terry SD, Chang L. Neuroimaging studies of the aging HIV-1-infected brain. J Neurovirol **2012**; 18:291–302.
27. Ances BM, Hammoud DA. Neuroimaging of HIV-associated neurocognitive disorders (HAND). Curr Opin HIV AIDS **2014**; 9:545–51.
28. Su T, Wit FW, Caan MW, et al; AGEhIV Cohort Study. White matter hyperintensities in relation to cognition in HIV-infected men with sustained suppressed viral load on combination antiretroviral therapy. AIDS **2016**; 30:2329–39.
29. Sanford R, Ances BM, Meyerhoff DJ, et al. Longitudinal trajectories of brain volume and cortical thickness in treated and untreated primary human immunodeficiency virus infection. Clin Infect Dis. **In press**.
30. Thompson PM, Dutton RA, Hayashi KM, et al. Thinning of the cerebral cortex visualized in HIV/AIDS reflects CD4+ T lymphocyte decline. Proc Natl Acad Sci U S A **2005**; 102:15647–52.
31. Corrêa DG, Zimmermann N, Tukamoto G, et al. Longitudinal assessment of subcortical gray matter volume, cortical thickness, and white matter integrity in HIV-positive patients. J Magn Reson Imaging **2016**; 44:1262–9.
32. Sanford R, Fernandez Cruz AL, Scott SC, et al. regionally specific brain volumetric and cortical thickness changes in HIV-infected patients in the HAART era. J Acquir Immune Defic Syndr **2017**; 74:563–70.