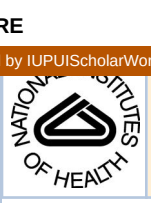

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Predictors of CNS Injury as Measured by Proton Magnetic Resonance Spectroscopy in the Setting of Chronic HIV infection and CART

J Harezlak[1], **R Cohen**[2], **A Gongvatana**[3], **M Taylor**[3], **S Buchthal**[4], **G Schifitto**[5], **J Zhong**[5], **ES Daar**[6], **J Alger**[7], **M Brown**[8], **E Singer**[7], **TB Campbell**[8], **D McMahon**[9], **YT So**[10], **CT Yiannoutsos**[1], **BA Navia**[11], and **the HIV Neuroimaging Consortium**

[1]Indiana University Fairbanks School of Public Health, Indianapolis, IN, USA

[2]University of Florida College of Medicine, Gainesville, FL, USA

[3]University of California-San Diego Medicine Center, La Jolla, CA, USA

[4]University of Hawaii, Honolulu, HI, USA

[5]University of Rochester School of Medicine, Rochester, NY, USA

[6]Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, University of California, Los Angeles, CA, USA

[7]David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

[8]University of Colorado Denver, Aurora, CO, USA

[9]University of Pittsburgh, Pittsburgh, PA, USA

[10]Stanford University Medical Center, Palo Alto, CA, USA

[11]Tufts University School of Medicine, Boston, MA, USA

Abstract

The reasons for persistent brain dysfunction in chronically HIV-infected persons on stable combined antiretroviral therapies (CART) remain unclear. Host and viral factors along with their interactions were examined in 260 HIV-infected subjects who underwent magnetic resonance spectroscopy (MRS) Metabolite concentrations (NAA/Cr, Cho/Cr, MI/Cr and Glx/Cr) were measured in the basal ganglia, the frontal white matter and grey matter and the best predictive models were selected using a bootstrap-enhanced Akaike Information Criterion (AIC). Depending on the metabolite and brain region, age, race, HIV RNA concentration, ADC stage, duration of HIV infection, nadir CD4, and/or their interactions were predictive of metabolite concentrations, particularly the basal ganglia NAA/Cr and the mid-frontal NAA/Cr and Glx/Cr whereas current CD4 and the CPE index rarely or did not predict these changes. These results show for the first time that host and viral factors related to both current and past HIV status contribute to persisting

Address Correspondence to: Jaroslaw Harezlak, PhD Department of Biostatistics Indiana University Fairbanks School of Public Health 410 W 10th St., Suite 3000 Indianapolis, IN 46202 USA harezlak@iupui.edu.

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cerebral metabolite abnormalities and provide a framework for further understanding neurological injury in the setting of chronic and stable disease.

Keywords

MRS; HIV dementia; Neuroimaging; Antiretroviral therapies; HIV RNA; Biomarkers

Background

HIV infection is associated with distinct pathological and cellular changes in the brain resulting in neurocognitive, motor, and behavioral disturbances, including dementia, collectively, referred to as the AIDS Dementia Complex (ADC) (Navia et al., 1986a, Navia et al. 1986b) or HIV-associated neurocognitive disorder (HAND) (Antinori et al., 2007). Since the introduction of combination antiretroviral therapy (CART), the incidence of HIV-associated dementia (HAD) has declined significantly (Palella et al., 1998, Hammer et al., 2006, Sacktor et al., 2002, Robertson et al., 2007, Tozzi et al., 2007). Nevertheless, an emerging body of evidence from neuroimaging, clinical, and pathological studies suggests that despite remarkable advances in treatment, Central Nervous System (CNS) dysfunction in the context of chronic and stable HIV persists and may continue to unfold despite successful systemic therapy (Heaton et al., 2010, Heaton et al., 2011, Harezlak et al., 2011, Cardenas et al., 2009, Cohen et al., 2010a, Cohen et al., 2010b, Tate et al., 2011).

The HIV Neuroimaging Consortium was formed to prospectively assess rates and patterns of brain injury and cognitive impairment among chronically infected subjects on CART. We recently examined the possibility that residual injury persists in these patients by proton magnetic resonance spectroscopy (MRS) (Harezlak et al., 2011, Cohen et al., 2010a). MRS provides a sensitive and useful *in vivo* method to detect changes in cellular metabolism across multiple brain regions in both HIV-infected subjects and the SIV-infected macaque at different stages of infection and neurocognitive impairment (Harezlak et al., 2011, Cohen et al., 2010a, Meyerhoff et al., 1994, Tracey et al., 1996, Lopez-Villegas et al., 1997, Salvan et al., 1997, Chang et al., 2002, Chang et al., 2004, Yiannoutsos et al., 2004, Paul et al., 2007, Mohamed et al., 2010). Abnormalities have included increases in choline (Cho)/Cr (creatine) or Cho, a marker of membrane integrity and myoinositol (MI)/Cr or MI, a marker of glial cell activity, along with decreases in N-acetyl aspartate (NAA/Cr) or NAA, a marker of neuronal integrity in deep white matter and gray matter structures, particularly the basal ganglia. Decreases in NAA/Cr or NAA and have been correlated with the severity of NCI, consistent with previously reported clinical-pathological findings (Navia et al., 1986). Both increases and decreases in Glx reflecting glutamate and glutamine levels have been reported, but the clinical significance of these findings remains unclear (Harezlak et al., 2011). MRS-derived metabolites were acquired from the basal ganglia, frontal white matter and midfrontal cortex in 240 HIV-infected subjects and revealed the persistence of inflammation (increases in Cho and MI) in both neuroasymptomatic and subjects with cognitive impairment (ADC) across all regions when compared to age-matched healthy controls despite stable antiretroviral treatment (Harezlak et al., 2011). Neuronal injury or loss (a decrease in NAA/Cr) was also observed but only in the ADC group, consistent with notion

that decreases in NAA provide a robust *in vivo* biomarker of HIV-associated cognitive impairment (Chang et al., 2002, Chang et al., 2004, Yiannoutsos et al., 2004).

The specific host and disease-related factors contributing to these patterns of injury in the setting of otherwise stable yet chronic disease are uncertain. In previous preCART studies, low current CD4 and detectable cerebrospinal fluid (CSF) HIV RNA levels were associated with HIV-related cognitive impairment (Ellis et al., 1997, McArthur et al., 1997). Recently we and others have shown significant associations between a history of advanced immunosuppression as reflected in the nadir CD4 count and HIV RNA concentrations with cognitive impairment and cortical atrophy (Heaton et al., 2010, Cohen et al., 2010, Tate et al., 2011, Valcour et al., 2006). In the present study, we hypothesized that patterns of brain injury as measured by MRS are associated with one or more host or disease related factors or their interactions.

Methods

Details for the clinical and imaging assessments in addition to the demographic characteristics of the cohort can be found in Harezlak et al. (2011).

Clinical Assessments

Subjects were recruited to participate in a HIVNC longitudinal study of brain injury and neurocognitive impairment at the following sites: UCSD, UCLA, Harbor-UCLA, Stanford University, University of Colorado at Denver, University of Pittsburgh, and University of Rochester. Inclusion criteria included: Nadir CD4 count ≤ 200 cells/mL, stable antiretroviral regimen with any FDA-approved antiretroviral therapy (i.e., nucleoside, non-nucleoside, and/or protease inhibitors) for at least 12 consecutive weeks prior to study screening. Exclusion criteria included unstable medical disorders (hepatic, renal, metabolic), pre-morbid or co-morbid psychiatric disorders (bipolar disorder and active depression) and focal or diffuse neurologic disorders, including chronic seizures, stroke, head trauma resulting in loss of consciousness > 30 min, infection (except for HIV-1). Subjects with active alcohol and drug abuse or related medical complications within 6 months of study entry were excluded (Harezlak et al., 2011).

Assessments included magnetic resonance imaging and MRS, neurological and neuropsychological evaluations, plasma and CSF assays for HIV RNA (Cohen et al., 2010a). Cognitive status was assessed by a trained clinician using the ADC Staging as previously described (Price and Brew, 1988). ADC stage 0 (asymptomatic) denotes neurocognitively normal status, stage 0.5 refers to subclinical neuropsychological impairment only, stage 1 is symptomatic functional impairment and stages 2 or greater indicate major impairment. Neuropsychological evaluation was performed in cognitive domains most implicated in HIV infection, including processing speed, verbal fluency, executive functioning, working memory, learning and memory, and psychomotor function (Heaton et al., 2004).

MRS Methods

Single-voxel proton MRS spectra were acquired using the GE PRESS sequence from voxels 6 cc in volume in 3 brain regions as previously described: mid-line frontal gray mid-frontal centrum semiovale (white matter), and right (or left) basal ganglia (deep gray matter) (Harezlak et al., 2011). Field homogeneity and water suppression were adjusted using automated algorithms from GE. Water suppressed spectra were collected with TE/TR = 35/3000ms, bandwidth = 2500 Hz, 128 averages, NEX=8. The metabolite ratios NAA/Cr, Cho/Cr, MI/Cr, and Glx(=Glu+Gln)/Cr were determined using LC Model spectral analysis software and an unsuppressed water FID at TE=35ms for eddy-current correction (Provencher, 2001). This automated processing method yields metabolite ratios with Cramer-Rao lower bound less than 20% (Lee et al., 2003).

Statistical Methods

The primary analysis consisted of determining predictive factors for each of four metabolite ratio levels (NAA/Cr, Cho/Cr, MI/Cr and Glx/Cr) in three brain regions (basal ganglia, frontal white matter and mid-frontal cortex grey matter). We considered both host and disease-related factors in linear regression models treating the brain metabolite ratios as outcomes. Statistical modeling was performed to assess both additive effects and two-way interactions. We attempted to fit the most parsimonious model (the model containing the fewest predictors) while at the same time maximizing the predictive ability of the model, measured by the proportion of variability of the outcome variable (MRS metabolite ratio levels) accounted for by the model (expressed as the R-squared in the regression model). Final additive and interaction linear regression models were selected by maximizing the Akaike Information Criterion (AIC), which balances model fit (maximum R-squared) and model complexity (minimum number of predictive factors simultaneously included in the model (Akaike, 1974). To mitigate the tendency of the AIC to select models containing predictors which are not statistically significant, we used a bootstrap procedure starting with a model preselected as the best fit in the original dataset, by selecting bootstrap samples (Austin and Tu, 2004) from the original dataset and repeating the fitting procedure. The bootstrap sample selection and fitting was repeated 500 times. A predictor (either additive or an interaction effect) was chosen for the final model if it were selected in at least 70% of the bootstrapped samples. As the primary goal for this study was to identify predictive models and the associated predictors, conclusions were not based on separately testing each covariate's association with the brain metabolite level and thus no adjustments for multiple comparisons were made.

The choice of the candidate predictive factors was based on both the prior findings (Yiannoutsos et al., 2004, Cohen et al., 2010b, Harezlak et al., 2011) and their availability in the database. We included demographic factors (age, sex, race, education), disease-specific factors (ADC stage, duration of HIV infection, nadir and current CD4 count, plasma RNA), and treatment-specific factors (CNS penetration effectiveness, or CPE score derived as previously described from an algorithm that considers the effectiveness of each agent in the antiretroviral regimen to cross the blood brain barrier (Letendre et al., 2008). In a separate analysis we included CSF HIV RNA concentrations, available in 117 subjects.

Results

Subject characteristics

Summary statistics are provided for the 260 subjects who had at least one brain metabolite-ratio out of 12 measured and an ADC assessment available. Age was the only demographic factor which differed significantly between ADC groups (Table 1). However, there was no clear pattern of older age associated with advanced ADC stage. The median age of the cohort was 46 years with a median duration of infection of 12 years. All subjects had a history of advanced disease with a median nadir CD4 count of 35 cells/ L but the median CD4 count at baseline was 307 cells/ L, consistent with an immunological response to CART. Most subjects received antiretroviral treatment for at least 1 year and had undetectable plasma HIV RNA levels (Table 1).

The availability of data for each metabolite varied depending on the metabolite and brain region. Most metabolites (excluding Glx) were missing less than 5% of the values with the exception of basal ganglia metabolites where between 9% and 10% of the values were missing. The remainder of the missing values included Glx/Cr in FWM-13%, Glx/Cr in MFC 15% and Glx/Cr in BG 27%. The differences in missing patterns were not found to be associated with the ADC stage.

Modeling MRS metabolite ratios as functions of predictive variables

From the four metabolite ratios modeled in three brain regions (12 models), seven models were found to contain both additive and interaction effects: NAA/Cr and MI/Cr in the frontal white matter (FWM), Cho/Cr, MI/Cr and Glx/Cr in the basal ganglia (BG) and Cho/Cr and Glx/Cr in the mid-frontal cortex (MFC) (Tables 2, 3). In the five remaining models (Cho/Cr and Glx/Cr in the FWM; NAA/Cr in BG; NAA/Cr and MI/Cr in MFC), only additive effects were observed (Table 2). The most frequent demographic variables selected were age (8/12 models) and race – coded as white versus non-white race (4/12 models) (Table 3). Among disease-related variables, ADC stage – coded as ADC stage 1-3 versus ADC stage 0 or 0.5 – was selected in 5/12 models; detectable plasma RNA level – coded as undetectable versus detectable – was chosen in 5/12 models; duration of HIV infection in 4/12 models and low nadir CD4 count – coded as high (greater than 50 cells/ml) versus low (50 cells/ml or less) in 3/12 (Table 3). In contrast current CD4 count was found in only in 1/12 models and the CPE index was not significantly associated with any of the12 metabolite ratios. The interaction between age and ADC stage was present in 4/12 models, and the interaction between detectable plasma RNA level and low nadir CD4 count was found in 2/12 models. As shown in Table 2, the best predictive model (adjusted R^2=0.183, p-value<0.001) was found for the NAA/Cr in the basal ganglia and the next most strongly predictive model (adjusted R^2= 0.111, p-value<0.001) involved NAA/Cr in the mid-frontal cortex. Details for these models are provided in the next Section.

Models of metabolite change and their predictors within each brain region

N-Acetyl Aspartate: a marker of neuronal integrity—Lower NAA/Cr levels in FWM (adj. R^2 = 0.050, p-value=0.002) were associated with older age, and the interaction of detectable plasma RNA level and low nadir CD4 count. Thus older subjects with low

nadir CD4 count and detectable plasma HIV RNA levels had the lowest NAA/Cr levels in the frontal white matter.

Decreases in NAA/Cr levels in the MFC (adj. $R^2 = 0.111$, p-value<0.0001) were also associated with older age in addition to longer duration of HIV infection, detectable plasma RNA levels and a less than high school education level.

As described above, the strongest predictive model among the 12 considered was found for NAA/Cr in the BG. Independent predictive factors included ADC stage 1-3, detectable plasma RNA levels, less than high school education and male sex.

Choline: a marker of membrane integrity—Cho/Cr in the FWM was weakly associated with gender (adj. $R^2 = 0.019$, p-value=0.016) such that slightly higher levels were found in males.

Elevated levels of Cho/Cr in the mid-frontal cortex (adj. $R^2 = 0.072$, p-value=0.001) were associated with ADC stage 1-3, non-white race, duration of HIV infection, and complex interactions involving age and ADC stage; age and duration of HIV infection as well as age and non-white race. Thus Cho/Cr levels were higher among young individuals with clinical ADC (ADC stage1-3), increased slightly in aging individuals with longer duration of HIV infection but decreased among aging white individuals.

Higher Cho/Cr levels in the basal ganglia (adj. $R^2 = 0.054$, p-value=0.003) were associated with ADC stage 1-3, non-white race, shorter duration of HIV infection, and the interaction of age and ADC stage. Cho/Cr levels were higher among younger individuals with clinical ADC while levels decreased in older, neuroasymptomatic and chronically infected individuals.

Myoinositol: a marker of glial cell activity—MI/Cr levels in the MFC (adj. $R^2 = 0.018$, p-value=0.021) were associated only with race such that slightly higher levels would be expected among non-white study participants.

Levels in the BG were weakly associated (adj. $R^2 = 0.023$, p-value=0.041) with the interaction of age and ADC stage. MI/Cr levels were higher in younger subjects with ADC stage 1-3 but as observed with Cho/Cr were lower in older asymptomatic subjects, suggesting that increasing age may be associated with a less of an inflammatory response in the brain.

Increased MI/Cr levels in the frontal white matter (adj. $R^2 = 0.060$, p- value<0.001) were associated with detectable plasma RNA level, ADC stage 1-3 and the interaction of age and ADC stage such that MI/Cr levels were higher in younger subjects with clinical ADC and, levels in the BG and again lower in older asymptomatic subjects. .

Glutamine+Glutamate: a marker of neuronal-glia cell activity—Glx/Cr in the FWM was associated with age and race such that higher levels would be found in younger white subjects (adj. $R^2 = 0.058$, p-value=0.001).

In contrast, Glx/Cr levels in the BG were strongly associated (adj. $R^2 = 0.101$, p-value<0.001) with lower nadir CD4 count and an interaction between current CD4 count and duration of HIV infection, so that subjects with longer duration of HIV infection and lower baseline CD4 count had higher Glx/Cr levels.

The strongest predictive model of Glx/Cr was in the mid-frontal cortex (adj. $R^2 = 0.117$, p-value<0.001). Older age, and the interaction of detectable plasma RNA concentration and low nadir CD4 count were the factors most strongly associated with lower Glx/Cr levels. Thus older subjects with low nadir CD4 count and detectable plasma HIV RNA levels showed the lowest Glx/Cr levels in this region.

Analysis including CSF HIV RNA

The same modeling strategy for the twelve metabolite ratios was applied to data obtained from 117 subjects with CSF HIV RNA assays with data in at least one of 12 metabolite-by-brain-region combinations. For the majority of the participants the plasma and CSF HIV RNA detectable and undetectable status coincided. However, there were 12 patients with detectable plasma HIV RNA and undetectable CSF HIV RNA and 4 with undetectable plasma and detectable CSF HIV RNA. CSF HIV RNA levels were included in the model along with all the previously listed demographic and disease-specific factors, including plasma HIV RNA levels. The findings are similar to those described above with the notable exception that CSF HIV RNA replaced plasma levels as the predictive factor in the majority of models. The two exceptions involved MI/Cr in the frontal white matter and Glx/Cr in the mid-frontal cortex.

Discussion

A number of in vivo studies prior to the advent of CART have shown significant cellular changes in the HIV- infected brain as measured by MRS, including inflammation primarily in the white matter with evidence of neuronal injury in the basal ganglia and cortex (Meyerhoff et al., 1994, Tracey et al., 1996, Lopez-Villegas et al., 1997, Salvan et al., 1997, Chang et al., 2002, Chang et al., 2004, Yiannoutsos et al., 2004). More recently, we have shown that these changes persist in chronically infected patients despite restoration of immunological status and effective viral suppression in response to antiretroviral therapies (Harezlak et al., 2011, Cohen et al., 2010a, Cohen et al., 2010b, Tate et al., 2011). The current study was directed at determining the demographic and clinical factors associated with the persistence of brain injury as measured by these metabolite abnormalities. The results show for the first time that events associated with these changes are likely to be multifactorial, driven by or one or more host and disease-related factors as well as their interactions in a metabolite and region dependent manner. As the majority of subjects were neuroasymptomatic at the time of the MRS assessment, these results further suggest that specific patterns of brain abnormalities may be amplified in the context of antiretroviral treatment and chronic HIV infection, independent of cognitive status.

Age was the most consistent factor, present in 66% of the models. Several studies have suggested relationships between aging and the risk for specific neurological outcomes associated with HIV infection (Valcour et al., 2004, Ances et al., 2010, Becker et al., 2010).

However, the relationship between age and specific brain patterns was more complex than might have been predicted and was modified by a number of factors, with ADC stage principal among them. Of note was the commonly observed interaction between ADC stage and age with regard to both inflammatory factors, Cho and MI, such that HIV-infected older patients showed lower levels of these metabolites compared to younger, cognitively impaired subjects. This is consistent with recent findings from a prospective study of the HIVNC cohort which showed significant decreases in choline (Navia et al., 2011). Such changes may in part reflect attenuated immune-mediated responses with age or the effects of chronic treatment. Additional studies are needed to unravel the mechanisms contributing to these age-related changes.

ADC stage, a robust clinical measure of cognitive impairment, which has been strongly correlated with other global measures including the global deficit score and the NPZ-8 (Paul et al., 2007) was the clinical factor most predictive of metabolite levels, notably decreases in NAA/Cr in the BG and MFC and increases in Cho or MI in all three regions but particularly in the BG. These findings reinforce the notion that functional disturbances in the basal ganglia are related to the extent of neurocognitive impairment (Chang, Lee et al. 2004; Yiannoutsos, Ernst et al. 2004) but also suggest that cortical involvement may contribute to clinical symptoms in the setting of chronic infection (Cardenas et al., 2009).

Of interest, ethnicity, and less commonly, education and gender were also associated with changes in specific metabolites. Non-white race was selected in models involving increases in Cho or MI while less than a high school education was associated with NAA decreases in the BG and MFC. The relationship between level of education and NAA decreases is noteworthy as education attainment is a determinant of cognitive reserve; i.e., a person's capacity to resist the effects of brain disease or injury with preservation of cognitive functioning (Katzman, 1993, Stern, 2011, Satz et al.,1993). High levels of education were found to be associated with less sensitivity to the effects of HIV in one of the early studies of cognitive reserve (Satz, Morgenstern et al. 1993; Stern, Silva et al. 1996), with subsequent studies providing empirical support for its protective effects in HIV (Ernst, Chang et al. 2002; Foley, Ettenhofer et al. 2012; Morgan, Woods et al. 2012; Shapiro, Mahoney et al. 2014). Education level has also been linked to increased risk for neurodegenerative disorders such as Alzheimer's disease (Stern, Silva et al. 1996; Stern, Albert et al. 1999; Qiu, Backman et al. 2001; Manly, Touradji et al. 2003; Karp, Kareholt et al. 2004; Stern 2006; Tucker and Stern 2011). Cognitive reserve has also been linked to brain reserve; the brain's capacity to maintain functioning when damage has occurred. Conversely, cognitive frailty occurs in the context of chronic medical illnesses such as HIV infection which increase vulnerability to functional decline in susceptible people due to aging or early neurodegenerative disease (Woods, Cohen et al. 2013). In this regard, chronically HIV–infected patients exhibit reduced dynamic range of their functional brain response on fMRI, which seems to correspond with reduced cognitive reserve and neural efficiency (Ernst, Yakupov et al. 2009; Chang, Holt et al. 2013; Caldwell in press). The finding in this study extends previous reports as it suggests that lower cognitive reserve in chronically HIV infected patients may be a risk factor for neuronal loss in brain regions linked to cognitive impairment in these patients.

Of the three clinical factors selected, one is directly associated with current disease status (detectable plasma RNA), while the other two, nadir CD4 and duration of infection, are related to disease history. The relationship of plasma RNA levels to changes NAA/Cr in all three regions, especially when compared to its effects on other metabolites, underscores the notion that current viral replication contributes to neuronal injury. It is noteworthy that metabolite changes in the frontal cortex were also strongly associated with age, duration of infection and nadir CD4, which mirror findings from an analysis of cerebral atrophy in these subjects showing that factors associated with history rather current viral activity had a greater effect on cortical volume loss.

Recent reports have shown that nadir CD4 is an important determinant for the persistence of cerebral atrophy and cognitive impairment among chronically infected subjects (Cardenas et al., 2009, Hammer et al., 2006, Valcour et al., 2006). In the present study, nadir CD4 was an important predictor in three models (NAA/Cr in FWM, Glx/Cr in MFC and BG), particularly in combination with HIV RNA levels. Together these findings suggest that the degree of immunosuppression prior to initiating CART is a critical determinant of subsequent neurological injury and that early intervention (i.e. at higher levels of CD4) may be necessary to prevent such compromise, including the possibility of irreversible damage. The fact that duration of infection emerged as a predictor independent of age, indicates that chronic infection may be detrimental among both young and older patients, even when current viral load is suppressed and CD4 count is increased.

Recent studies suggest that antiretroviral regimens that contain agents with superior CNS penetration as measured by the CPE or Central nervous system effectiveness score may be associated with lower rates of cognitive impairment (Smurzynski et al., 2011, Casado et al., 2014). It is noteworthy, however that neither the CPE score nor the current CD4 count were significant predictors of metabolite levels, further supporting the observation that HIV-associated brain injury may persist, even unfold, in the setting of otherwise stable HIV disease. Current CD4 levels were selected in only one model (Glx/Cr levels in the basal ganglia), suggesting that current immunological status is probably a less important factor associated with cellular injury than current viral RNA levels or some aspects of disease history (most notably nadir CD4 count). While differences in CNS penetration exist across different antiretroviral regimens(Letendre, Marquie-Beck et al. 2008), the findings from this study suggest that, contrary to what might have been anticipated, regimens that contain agents with better CNS penetration have little effect on current HIV-associated brain injury once neurological damage has occurred in the setting of advanced immune suppression (see above).

The additional influence of adding CSF RNA into the models was assessed in secondary analyses and in no models were both CSF and plasma levels selected simultaneously. The fact that CSF RNA replaced plasma RNA as the predictor in all, but two models, extends an existing body of evidence which shows that CSF RNA is more strongly associated with neurological outcomes than plasma levels and suggests CSF HIV RNA concentration may provide a better biomarker of CNS dysfunction in these patients (Ellis et al., 1997, McArthur et al., 1997). Although routine CSF monitoring may be difficult to implement in practice, results from large prospective multicenter studies such as the Alzheimer’s Disease

Neuroimaging Initiative suggest that this may be feasible with proper education and training (Siuciak et al., 2012).

Results from the models of the individual metabolites suggest a number of interesting conclusions: The strength of the association of NAA/Cr, a marker of neuronal integrity, in the basal ganglia with ADC stage fits with the prevailing understanding of the impact of HIV infection on the brain, which points to the basal ganglia as a critical locus of HIV activity and emerging cognitive impairment (Rostasy et al., 1999, Brew et al., 1995, Navia and Rostasy, 2005). In recent studies, based on a principal component analysis, we have shown that a neuronal factor derived from NAA/Cr levels measured in the basal ganglia are highly associated with risk for HIV-related cognitive impairment (Yiannoutsos et al., 2004). Further, in a recent prospective MRS study of the HIVNC cohort, reduced NAA/Cr in the basal ganglia was the strongest predictor for the development of neurocognitive impairment among asymptomatic subjects followed over two years.

The strength of the association of Glx/Cr with the same clinical factors in various models, including HIV RNA and nadir CD4, which is similar to the case of NAA, points to the potential importance of glutamate and glutamine in HIV-related neuropathogenesis. This observation has received further support from recent studies which have reported reduced levels of Glx in the frontal white matter or cortex in NA subjects as well as significant decreases in Glx over time in these subjects, suggesting a change in this metabolite may represent an early event associated with HIV involvement of the brain (Harezlak et al., 2011, Navia et al., 2011).

The findings from this study illustrate how varying metabolic patterns across the HIV-infected brain can be examined in relationship to clinical and laboratory measures. The study demonstrates that host and viral factors, particularly those linked to current disease status and HIV disease history along with their interactions are predictive of specific patterns of metabolite abnormalities that reflect pathological processes occurring in the chronically infected and treated brain. However, other factors not examined in these models may also be contributing to these changes, including the effects of various chemokines (Navia and Rostasy, 2005, Letendre et al. 2011, Kaul and Lipton, 1999) and co-morbid disorders such as hepatitis C and cardiovascular risk factors (Letendre et al., 2004, Valcour et al., 2005, van Gorp and Hinkin, 2005, Perry et al., 2008, Ances et al., 2009) which have been associated with cognitive impairment or brain injury. Future studies will address the relative contributions of these factors to patterns of brain injury and cognitive impairment.

The predictive modeling employed in this study provides a sensitive methodological approach for determining the specific clinical factors affecting this pathology, which ultimately may have considerable clinical value with additional validation of these models. The application of these models to identify clinical, immunological and viral predictors of longitudinal change in cerebral and cognitive function will be an important next step in determining which factors are most predictive of evolving brain disturbances in the setting of chronic HIV disease and CART.

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Table 1

Demographic and clinical characteristics of the study sample.

	ADC stage				
	0	**0.5**	**1+**	**Total**	**p-value**
Gender (% male)	118 (83%)	55 (85%)	46 (88%)	219 (84%)	0.599
Age (years)	45.0	48.0	47.0	46.0	
Median (IQR)	(40.0-52.0)	(43.0-53.0)	(43.0-57.2)	(42.0-53.0)	0.025
Race/ethnicity					
White	102	40	37	179	0.340
Non-white	41	25	15	81	
Education level					
High School or less	91	31	31	153	0.095
Some College or more	52	34	21	107	
IV drug use (%)	19 (13%)	20 (31%)	22 (42%)	61 (24%)	<0.001
HIV infection (years)					
Median (IQR)	12.0 (5-17)	11.0 (7-16)	13.5 (10-17)	12.0 (7-17)	0.074
CD4 count (cells/μL)					
Median(IQR)	320 (188-466)	330 (215-526)	269 (139-458)	309 (188-472)	0.308
Nadir CD4 (cells/μL)					
Median(IQR)	34 (12-88)	48 (19-80)	24 (7-100)	35 (12-88)	0.472
Plasma viral load					
Undetectable (%)	113 (79%)	46 (71%)	35 (67%)	194 (75%)	0.278
CSF viral load **					
Undetectable (%)	26 (59%)	19 (73%)	25 (81%)	70 (69%)	0.122
On HAART at baseline ***	125 (93%)	51 (91%)	45 (88%)	221 (92%)	0.529
ART drug classes					
HAART naïve	9	5	6	20	
HAART plus PI	87	38	37	162	0.411
HAART no PI	38	13	8	59	
CPE score Median(IQR)	1.5 (1.0-2.4)	1.5 (1.0-2.5)	1.5 (1.5-2.5)	1.5 (1.0-2.5)	0.519

** - CSF viral load measured in N=101 subjects

*** - N=241 out of 260 subjects had information on HAART

Table 2

Metabolite ratios by brain region and their predictors.

Metabolite[1]	Selected factors	Direction	Adjusted R^2	p-value	Comment
Frontal white matter					
NAA/Cr	age nadir CD4 plasma HV RNA nadir CD4×PVL	↓ ↑ ↑ ↓	0.050	0.002	Factors predicting lower NAA/Cr levels were older age and a combination of low nadir CD4 and detectable plasma HIV RN
Cho/Cr	sex	↑	0.019	0.016	Males have higher Cho/Cr levels
Mi/Cr	age ADC stage plasma HIV RNA age×ADC stage	↑ ↑ ↓ ↓	0.060	<0.001	Detectable plasma HIV RNA and age-by-ADC stage interaction were the strongest predictors of higher MI/Cr levels The highest MI/CR levels were predicted in younger ADC subjects
Glx/Cr	age race	↓ ↑	0.058	0.001	Younger white subjects had higher Glx/Cr levels.
Basal Ganglia					
NAA/Cr	ADC stage plasma HIV RNA education sex	↓ ↑ ↑ ↓	0.183	<0.001	Patients with advanced ADC, detectable plasma HIV RNA, lower education and male sex have lower NAA/Cr levels
Cho/Cr	age ADC stage race duration of HIV age×ADC stage	↑ ↑ ↑ ↓ ↓	0.054	0.003	White patients, patients with shorter duration of infection and younger patients with advanced ADC have higher Cho/Cr levels
Mi/Cr	age ADC stage age×ADC stage	↑ ↑ ↓	0.023	0.041	Younger subjects with advanced ADC have higher Mi/Cr levels
Glx/Cr	nadir CD4 duration of HIV CD4 duration of HIV×CD4	↓ ↑ ↓ ↑	0.101	<0.001	Patients with low nadir CD4 , longer duration of HIV and low current CD4 count have higher Glx/Cr levels
Mid-frontal cortex					
NAA/Cr	age duration of HIV plasma HIV RNA education	↓ ↓ ↑ ↑	0.111	<0.001	Older patients, patients with longer duration of HIV, detectable plasma HIV RNA and lower education have lower NAA/Cr levels
Cho/Cr	age ADC stage race duration of HIV age×ADC stage age×duration of HIV age×race	↑ ↑ ↑ ↓ ↓ ↑ ↓	0.072	0.001	Younger subjects with advanced ADC, older subjects with shorter duration of HIV and younger white subjects have higher Cho/Cr levels
Mi/Cr	race	↓	0.018	0.021	Non-white subjects have higher MI/Cr levels
Glx/Cr	age nadir CD4 plasma HIV RNA nadir CD4×PVL	↓ ↑ ↑ ↓	0.117	<0.001	Younger patients and those with an undetectable current plasma HIV RNA levels have higher Glx/Cr levels

[1] Age and duration of HIV infection are continuous factors; education is coded as some college or above versus high school or below; nadir CD4 count is coded as high (>50) versus low (≤ 50 cells/μL), current CD4 is coded as high (>350) versus low (≤ 350 cells/μL); plasma RNA is coded as undetectable versus detectable; gender as male versus female; race as White versus non-White. A positive relationship (↑) increases the slope of a continuous factor or the effect of the primary value of a categorical factor (e.g. male gender or White race). Positive interactions mean synergistic effects, negative interactions mean diminishing effects.

Table 3

Summary of the predictive factors of the metabolite ratios

Frontal white matter	**Age**	**Sex**	**Race**	**Education**	**Nadir CD4**	**Current CD4**	**Plasma HIV RNA**	**ADC stage**	**Duration of HIV**
NAA/Cr	+				*		*		
Cho/Cr		+							
Mi/Cr	*						+	*	
Glx/Cr	+		+						
Basal Ganglia									
NAA/Cr		+		+			+	+	
Cho/Cr	*		+					*	+
Mi/Cr	*							*	
Glx/Cr					+	*			*
Mid-frontal cortex									
NAA/Cr	+			+			+		+
Cho/Cr	*		*					*	*
Mi/Cr			+						
Glx/Cr	+				*		*		

Factors associated with the metabolite ratios in the 3 brain regions (FWM, BG and MFC). **“+”** denotes an additive effect and **“*”** denotes an interaction effect.