Resting cerebral blood flow

A potential biomarker of the effects of HIV in the brain

B.M. Ances, MD, PhD
D. Sisti, BA
F. Vaida, PhD
C.L. Liang, BA
O. Leontiev, MD
J.E. Perthen, PhD
R.B. Buxton, PhD
D. Benson, BA
D.M. Smith, MD
S.J. Little, MD
D.D. Richman, MD
D.J. Moore, PhD
R.J. Ellis, MD, PhD
On behalf of the HNRC group*

Address correspondence and reprint requests to Dr. Beau M. Ances, 660 South Euclid Avenue, Box 8111, St. Louis, MO 63110
bances@wustl.edu

ABSTRACT

Objective: HIV enters the brain soon after infection causing neuronal damage and microglial/astrocyte dysfunction leading to neuropsychological impairment. We examined the impact of HIV on resting cerebral blood flow (rCBF) within the lenticular nuclei (LN) and visual cortex (VC).

Methods: This cross-sectional study used arterial spin labeling MRI (ASL-MRI) to measure rCBF within 33 HIV+ and 26 HIV− subjects. Nonparametric Wilcoxon rank sum test assessed rCBF differences due to HIV serostatus. Classification and regression tree (CART) analysis determined optimal rCBF cutoffs for differentiating HIV serostatus. The effects of neuropsychological impairment and infection duration on rCBF were evaluated.

Results: rCBF within the LN and VC were significantly reduced for HIV+ compared to HIV− subjects. A 2-tiered CART approach using either LN rCBF ≤50.09 mL/100 mL/min or LN rCBF >50.09 mL/100 mL/min but VC rCBF ≤37.05 mL/100 mL/min yielded an 88% (29/33) sensitivity and an 88% (23/26) specificity for differentiating by HIV serostatus. HIV+ subjects, including neuropsychologically unimpaired, had reduced rCBF within the LN ($p = 0.02$) and VC ($p = 0.001$) compared to HIV− controls. A temporal progression of brain involvement occurred with LN rCBF significantly reduced for both acute/early (<1 year of seroconversion) and chronic HIV-infected subjects, whereas rCBF in the VC was diminished for only chronic HIV-infected subjects.

Conclusion: Resting cerebral blood flow (rCBF) using arterial spin labeling MRI has the potential to be a noninvasive neuroimaging biomarker for assessing HIV in the brain. rCBF reductions that occur soon after seroconversion possibly reflect neuronal or vascular injury among HIV+ individuals not yet expressing neuropsychological impairment. ***Neurology*® 2009;73:702–708**

GLOSSARY

AEH = acute/early HIV infection; **ANOVA** = analysis of variance; **ASL-MRI** = arterial spin labeling MRI; **CART** = classification and regression tree; **CBF** = cerebral blood flow; **CH** = chronic HIV infection; **FOV** = field of view; **GDS** = global deficit score; **HAART** = highly active antiretroviral therapy; **HAND** = HIV-associated neurocognitive disorders; **LN** = lenticular nuclei; **rCBF** = resting cerebral blood flow; **TE** = echo time; **TI** = inversion time; **TR** = repetition time; **VC** = visual cortex.

HIV crosses the blood–brain barrier through infected macrophages and microglia via a "Trojan horse" mechanism.[1] Once inside the brain, neuronal, astrocyte, and microglia dysfunction occurs due to viral envelope proteins, cytokines, and chemokines.[2]

Highly active antiretroviral therapy (HAART) has decreased the incidence but not prevalence of HIV-associated neurocognitive disorders (HAND).[3] HAND is diagnosed by impaired neurologic and neuropsychological performance.[3] However, subtle preclinical changes are not detected by these methods. Early sensitive biomarkers of HIV-associated brain injury prior to overt impairment could identify individuals at risk for progressing to HAND.

Neuroimaging, in particular resting cerebral blood flow (rCBF), could be a preclinical biomarker of HAND.[4,5] Hypoperfusion within cortical and subcortical structures was present in HIV-infected (HIV+) compared to HIV-uninfected (HIV−) subjects using single photon electron computed

Supplemental data at www.neurology.org

*Members of the San Diego HIV Neurobehavioral Research Center (HNRC) group are listed in the appendix.

From the Department of Neurology (B.M.A.), Washington University in St. Louis, MO; Departments of Medicine (D.S., O.L., D.M.S., S.J.L., D.D.R.), Family and Preventive Medicine (F.V., D.B.), Radiology (C.L.L., J.E.P., R.B.B.), Psychiatry (D.D.R., D.J.M.), and Neurosciences (R.J.E.), University of California San Diego; and Veterans Administration San Diego Healthcare System (D.M.S., D.D.R.), San Diego, CA.

The HNRC is supported by Center award MH 62512 from the National Institute of Mental Health.

Disclosure: Author disclosures are provided at the end of the article.

tomography[6,7] and perfusion imaging.[8] Decreased rCBF occurred prior to neurologic examination, neuropsychological performance testing, or structural imaging changes.[9] While rCBF may be a promising biomarker, these studies required IV injections and classified subjects by serostatus and not degree of neuropsychological impairment or infection duration.

Arterial spin labeling MRI (ASL-MRI) provides rapid noninvasive quantifiable rCBF measurements.[10] We evaluated rCBF within the lenticular nuclei (LN) and visual cortex (VC) of HIV− and HIV+ subjects. The effects of not only serostatus but also degree of neuropsychological impairment and infection duration on rCBF were studied. Decision rules from classification and regression tree (CART) analysis assessed the potential of rCBF as a biomarker.

METHODS

Subjects. A total of 33 HIV+ and 26 HIV− controls were recruited and studied. Each subject provided written consent that was approved by the University of California San Diego Institutional Review Board. Individuals with a history of other neurologic illness, major psychiatric disorders, or substance abuse within the past 3 months were excluded. Serologic status was confirmed by documented positive HIV enzyme-linked immunoassay and Western blot or detection of plasma HIV RNA by PCR. Laboratory evaluations were performed within 3 months of imaging and included hematocrit, CD4 cell counts, plasma viral load, and CSF viral load. All subjects were instructed to continue current medications.

Neuropsychological and neurologic examination. All HIV+ subjects received a clinical examination and neuropsychological performance testing.[3,11] Neuropsychological performance testing assessed verbal fluency, psychomotor skills, motor skills and praxis, learning and recall, speed of information processing, and executive functioning.[12] Raw test scores were converted to demographically corrected t scores minimizing the influence of age, education, sex, and ethnicity. An overall global deficit score (GDS) was determined. HIV+ subjects were classified as either neuropsychologically unimpaired (GDS <0.5) or impaired (GDS ≥ 0.5) using a cutoff that has excellent positive predictive value for differentiating impairment.[11,13]

HIV+ subjects were also classified as either acute/early (<1 year since seroconversion) or chronic (>1 year after seroconversion) HIV infection. Acute HIV infection was defined as a detectable plasma HIV RNA with a negative HIV enzyme immunoassay, or detectable enzyme immunoassay with a negative or indeterminate Western blot. Early HIV infection was defined as a positive HIV enzyme immunoassay with a less sensitive (detuned) enzyme immunoassay assay or a positive enzyme immunoassay and Western blot with a documented negative HIV enzyme immunoassay within the past 12 months.[14]

Imaging protocol. ASL-MRI was performed on a 3-Tesla whole body system (3T GE Excite, Milwaukee, WI) with an 8-channel receive head coil. Quantitative imaging of perfusion using a single subtraction pulse sequence (repetition time [TR] = 2.5 s, inversion time $[TI]_1$ = 700 msec, TI_2 = 1,500 msec, 20-cm tag width, and a 1-cm tag-slice gap) with a dual-echo gradient echo readout and spiral acquisition of k-space was performed (echo time $[TE]_1$ = 9.4 msec, TE_2 = 30 msec, flip angle = 90°, field of view [FOV] = 24 cm, 64 × 64 matrix).[15-17] ASL-MRI quantified rCBF using magnetically labeled arterial water as an endogenous tracer.[18,19] This method alternated between tag and control images with the difference between images providing rCBF units. Four 7-mm-thick axial slices were acquired in a linear fashion from inferior to superior. An inversion recovery prepared 3-dimensional fast spoiled pulse sequence (TI = 300 msec, TR = 7.9 msec, TE = 3.1 msec, flip angle = 15°, FOV = 25 × 25 × 16 cm, matrix 256 × 256 × 192) provided high-resolution structural images.

rCBF measures were converted into absolute values using CSF and minimum contrast scans. The CSF scan (TR = 2 s and TE = 2.8 msec) was acquired using the same in-plane parameters as the ASL-MRI scan, but the number of slices was increased to ensure coverage of the lateral ventricles. The minimum contrast scan (TR = 2 s and TE = 11 msec) consisted of two 8-interleave repetitions acquired at the same slice prescription as the CSF scan. Total scanning time for all images was less than 9 minutes.

Data analysis. Both subcortical (LN) and cortical (VC) areas within the same axial slices were chosen to assess if regional differences in progression existed. rCBF values in the LN and VC were determined for each subject. Possible subject movement was corrected by Analysis of Functional NeuroImage software.[20] Each region was manually delineated on high-resolution images. The LN consisted of the putamen and globus pallidus while the VC included the area within the parietal-occipital sulci.

rCBF in the LN and VC were corrected for coil sensitivity inhomogeneities using smoothed minimum contrast images.[15] rCBF was converted to physiologic units (mL/100 mL/min) using the CSF image as a reference signal for determining the fully relaxed magnetization of blood.[21] Mean rCBF values within the LN and VC for each subject were calculated by averaging the time series over all time points and voxels within each region. Correction for partial volume effects due to atrophy were performed using the assumption that CSF has zero flow.[22]

Statistics. To study the effect of the degree of neuropsychological impairment and duration of infection on rCBF, a one-way analysis of variance (ANOVA) was performed ($p < 0.05$) followed by pairwise comparisons using Bonferroni correction. The normality of the data distribution was assessed by visual inspection. A nonparametric CART analysis optimized the best covariates and classification cutoff points. rCBF values in the LN and VC were used as predictors and HIV serostatus was the outcome measure. CART trees were constructed using a 10-fold cross-validation, the Gini index of node homogeneity, and a minimum size of 20 observations per node for a split.[23]

RESULTS

Demographic characteristics of HIV− and HIV+ subjects were similar. HIV− and HIV+ subjects did not differ in demographic characteristics (table). Among HIV+ subjects, neuropsychologically unimpaired and impaired individuals did not differ demographically (table e-1 on the *Neurology*® Web site at www.neurology.org). In regards to duration of infection, acute/early HIV+ subjects were younger and less likely taking HAART medi-

Table Demographic, medical, and laboratory comparisons between HIV− and HIV+ subjects

	HIV− (n = 26)	HIV+ (n = 33)	*p* Value
Demographic data			
Mean age, y	40 ± 3	39 ± 2	0.89
% Male	71	82	0.23
% Caucasian	73	66	0.37
Medical and neuropsychological data			
Duration of infection, mo	—	64 ± 11	—
CDC stage			
Stage A (%)	—	47	—
Stage B (%)	—	16	—
Stage C (%)	—	37	—
Global Deficit Score	—	0.38 ± 0.07	—
% Taking highly active antiretroviral therapy	—	82	—
Laboratory data			
Median CD4, cells/μL	—	519 ± 45	—
Median CD4 nadir, cells/μL	—	303 ± 49	—
Median log plasma viral load (IQR)	—	3.04 (1.70–4.31)	—
Median log CSF viral load (IQR)	—	2.24 (1.70–2.93)	—
% Virologically detectable virus in plasma (>50 copies)	—	76	—
% Virologically detectable virus in CSF (>50 copies)	—	45	—
Hematocrit (%)	—	42.2 ± 0.6	—

All errors are reported as the standard error of the mean.
IQR = interquartile range.

cations compared to chronic infected HIV+ individuals (table e-2).

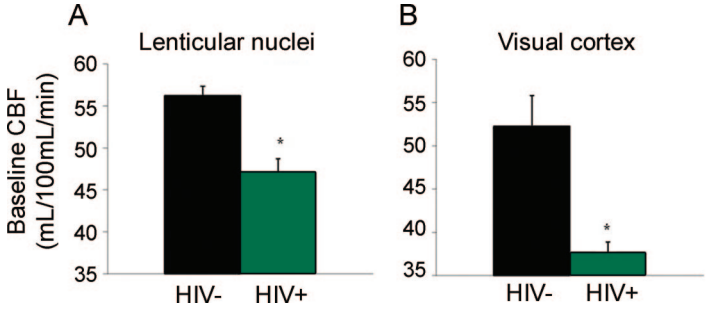
rCBF distinguished HIV+ subjects from HIV− subjects. rCBF was diminished for HIV+ compared to HIV− subjects for both the LN ($p < 0.0001$) and VC ($p = 0.0001$) (figure 1, A and B) and could not be explained by underlying structural differences as volumes were similar regardless of serostatus (data not shown). A 2-tiered nonparametric CART analysis selected thresholds for classifying subjects according to serostatus (figure 2). LN rCBF ≤50.09 mL/100 mL/min or LN rCBF >50.09 mL/100 mL/min and VC rCBF ≤37.05 mL/100 mL/min yielded a sensitivity of 88% (29/33) and specificity of 88% (23/26).

No correlation existed between LN rCBF and plasma ($r^2 = 0.01$) or CSF ($r^2 = 0.11$) viral loads. Similar results were observed for VC rCBF compared to plasma ($r^2 = 0.001$) or CSF ($r^2 = 0.003$) viral loads. For both regions no significant relationships existed between CD4 cell count and rCBF (data not shown).

rCBF was reduced prior to neuropsychological impairment. To determine if rCBF was associated with neuropsychological impairment, we classified HIV+ subjects as either neuropsychologically unimpaired (n = 22) or impaired (n = 11) (table e-1). An ANOVA followed by pairwise comparisons using Bonferroni correction showed a decrease in LN rCBF for both neuropsychologically unimpaired ($p = 0.001$) and impaired ($p = 0.0004$) HIV+ subjects compared to HIV− controls (figure 3A). LN rCBF was similar for the two HIV+ subgroups ($p = 0.68$). VC rCBF was reduced for neuropsychologically unimpaired ($p = 0.01$) and impaired ($p = 0.01$) HIV+ subjects compared to HIV− controls (figure 3B, table e-3). VC rCBF was similar for the 2 HIV+ subgroups ($p = 0.11$). These results suggest that rCBF is reduced even within neuropsychologically unimpaired HIV+ individuals and could potentially be a sensitive biomarker of neuronal dysfunction.

rCBF was reduced in acute/early HIV+ subjects within subcortical areas. To delineate if rCBF was affected by duration of infection, HIV+ subjects were classified as either acute/early (n = 10) or chronic (n = 23) HIV-infected (table e-2). An ANOVA followed by pairwise comparisons using Bonferroni correction showed a decrease in LN rCBF for both acute/early ($p = 0.0007$) and chronic ($p = 0.0002$) HIV+ individuals compared to HIV− controls (figure 4A). In contrast, VC rCBF was reduced for chronic HIV-infected ($p = 0.0002$) but not acute/early HIV-infected subjects ($p = 0.19$) when compared to HIV− controls (figure 4B, table e-3). These results suggest a possible temporal time course with subcortical brain areas affected early in disease followed by cortical involvement with chronic infection.

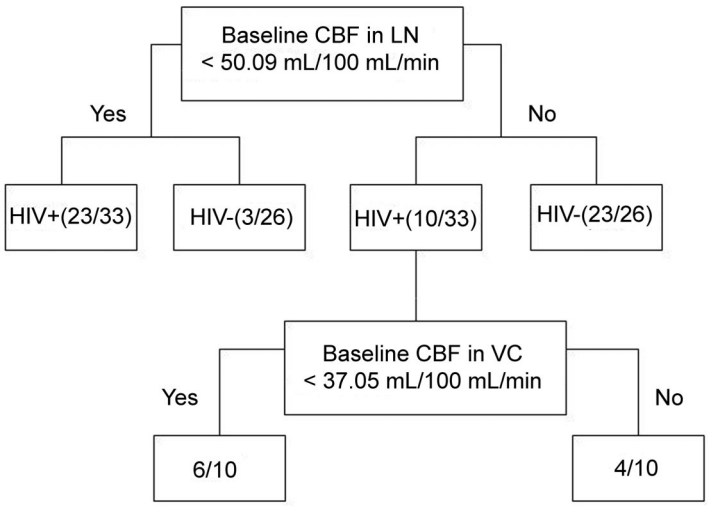
Figure 1 Resting cerebral blood flow (rCBF) within the lenticular nuclei (LN) and visual cortex (VC) for HIV− and HIV+ subjects



A significant reduction in resting cerebral blood flow (rCBF) was seen for HIV+ compared to HIV− subjects within both regions. *$p < 0.01$ compared to HIV− controls. All error bars are SEM.

DISCUSSION This study demonstrated significant reductions in rCBF for HIV+ compared to HIV−

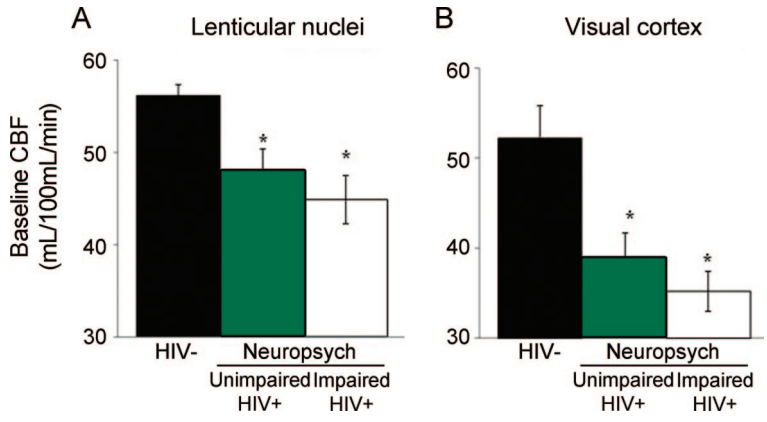
Figure 2 **A nonparametric classification and regression tree (CART) analysis determined thresholds for classification of subjects according to serostatus**



A 2-tiered approach using a resting cerebral blood flow (rCBF) ≤50.09 mL/100 mL/min in the lenticular nuclei (LN) or rCBF in the LN >50.09 mL/100 mL/min but rCBF ≤37.05 mL/100 mL/min in the visual cortex (VC) yielded a sensitivity of 88% (29/33) and specificity of 88% (23/26).

subjects. A 2-tiered CART approach using LN and VC rCBF distinguished subjects by HIV serostatus. In regards to degree of neuropsychological impairment, hypoperfusion deficits were observed for neuropsychologically unimpaired HIV+ subjects, suggesting a reduction in rCBF due to neuronal or vascular dysfunction occurs prior to neuropsychological impairment. Within the LN, rCBF was reduced in acute/early HIV+, possibly reflecting that brain injury occurs among individuals soon after infection. Overall, our results suggest that rCBF could be a potential quantifiable preclinical marker for detecting early HIV brain pathology.

Figure 3 **Effects of degree of neuropsychological impairment on rCBF within the LN and VC**



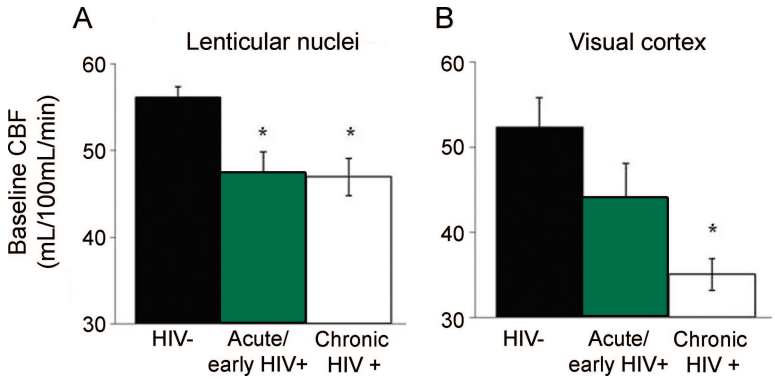
For both the lenticular nuclei (LN) and visual cortex (VC), a significant reduction in resting cerebral blood flow (rCBF) was seen for both neuropsychologically unimpaired and impaired HIV+ subjects compared to HIV− controls. *$p < 0.01$ compared to HIV− controls. All error bars are SEM.

Our ASL-MRI observations of decreased rCBF within the LN and VC of HIV+ subjects are consistent with previous neuroimaging studies such as single photon emission computed tomography.[6,7,9] However, these neuroimaging studies required the injection of a radioactive tracer, have relatively limited availability, and are time intensive. In contrast, ASL-MRI is noninvasive, can be performed on conventional MRI scanners if the correct sequences are available, provides reproducible detailed quantitative measures in mL/100 mL/min, and usually takes less than 10 minutes to perform.[15-17,19] This technique is becoming more accessible at high field magnets in both academic and nonacademic facilities. A unique aspect of rCBF studies compared to other functional MRI techniques, such as blood oxygen level dependent imaging, is that no task performance is required of subjects. This is particularly useful for neuropsychologically impaired HIV+ subjects, where performance can impact functional blood oxygen level dependent changes.[10]

Observed rCBF decreases in HIV+ patients could not be explained by underlying structural variations. Notably, while a reduction in volume is reflected by degree of tissue loss, perfusion measurements reflect rCBF within the residual volume rather than effects of volume loss per se. We do not believe that confounding substance abuse disorders could account for rCBF differences as subjects who actively abused within 3 months prior to scanning were excluded. Previous research has demonstrated that many measures of brain dysfunction related to substance abuse normalize after 3–6 months of abstinence.[24] These findings extend our previous study using ASL-MRI[13] by demonstrating that perturbations in rCBF were present in HIV+ subjects who had recently seroconverted, in addition to HIV+ subjects who were chronically infected. It is plausible that a preclinical state exists with early pathologic changes limited to distinct subcortical brain regions with subsequent progression leading to more widespread cerebral involvement.

A novel aspect of this study was the application of CART analysis within a neuroimaging study to demonstrate that rCBF could differentiate subjects according to HIV serostatus. While ASL-MRI is not an economical diagnostic method, it does allow for noninvasive in vivo monitoring of HIV brain pathology and could influence decisions concerning early initiation of neuroprotective therapies. Compared to other classification methods, CART analysis is ideally suited for the generation of decision rules concerning neuroimaging as a biomarker. First, it is inherently nonparametric with no assumptions made regarding the underlying distribution of values of

Figure 4 Effects of HIV disease duration on rCBF within the LN and VC



For the lenticular nuclei (LN), a significant reduction in resting cerebral blood flow (rCBF) was seen for both acute/early and chronic HIV-infected subjects. (A) Within the VC, only chronic HIV-infected subjects had a significant reduction compared to HIV− controls. *$p <$ 0.01 compared to HIV− controls. All error bars are SEM.

predictor variables. Second, CART uses an automatic "machine learning" method that requires few assumptions. Finally, clinical decision rules based on CART analyses are easy to interpret and apply to clinical scenarios.[23]

The mechanisms for observed decreases in rCBF within HIV+ subjects remain unknown. One possible mechanism could be the direct effects of HIV on platelet function but not quantity.[25] The proportion of circulating platelets in an activated state correlates with disease severity. Overall, HIV+ subjects have an increase in the proportion of activated platelets. These activated platelets may have increased susceptibility to adhere to not only each other but also blood vessel walls stimulating serotonin release and vasoconstriction[26] and subsequent reductions in rCBF. A second mechanism may occur due to the interaction between endothelial cell migration and HIV. Under normal conditions, endothelial cells respond to an increase in inflammatory cytokine release by migrating, proliferating, and forming new blood vessels, which turn off the process through negative feedback. In contrast, HIV in the brain can propagate an increase in cytokine and chemokine release but not migration, proliferation, or angiogenesis of blood vessels.[27] An increase in these mediators could cause deleterious consequences to neurons and subsequent rCBF decreases. Decreases in baseline CBF could also be due to increased atherosclerosis in HIV+ patients.[28] However, we think this is less likely as HIV+ patients did not have an increased presence of significant cerebrovascular risk factors compared to HIV− subjects.

Reductions in rCBF occurred in both acute/early and chronic HIV-infected subjects, but did not worsen in proportion to the presence of neuropsychological impairment. These findings could be interpreted in 2 general ways. First, rCBF decreases might reflect a generalized, systemic alteration in vascular physiology induced by HIV infection. Alternatively, they might represent a CNS-specific disturbance. Since we did not examine other tissues, this study could not distinguish between these possibilities. Whether greater or more persistent rCBF reductions might predict future progression to HAND is a question that can be answered only in the context of longitudinal studies. However, if this were the case, then treatments specifically designed to normalize CBF could potentially prevent or slow the progression of neuropsychological impairment. This could permit rCBF to be a proxy for monitoring the effectiveness of various therapies. Alternatively, rCBF could identify subjects requiring early HAART intervention despite relatively good immune status.

The observed temporal time course of regional involvement is in accordance with previous neuropsychological, neuropathologic, and neuroimaging studies of HIV+ subjects.[29] The first neuropsychological performance testing domains typically affected in HAND are subcortical areas resulting in psychomotor slowing, forgetfulness, and inattention.[30] Neuropathologic studies have confirmed these observations with the highest concentration of virus found in subcortical and frontal regions.[31] Structural neuroimaging studies using either volumetric measures[32] or magnetic resonance spectroscopy metabolite values[33,34] have confirmed this pattern. Our cross-sectional study extends these findings, suggesting regional progression of disease with subcortical rCBF affected during acute/early seroconversion followed by additional cortical involvement with chronic infection.

Our study has several limitations. First, only 4 axial brain slices were acquired in our protocol as a dual echo ASL-MRI technique was used. Other neuroimaging techniques, like single photon emission tomography, do not have this limitation. We chose only 2 specific regions to contrast possible differences between subcortical (LN) and cortical (VC) with ASL parameters optimized for analysis. Extension of our analysis to other areas within the acquired slices would not be appropriate without investigating the suitability of these specific parameters for alternative brain regions. Future studies measuring whole brain rCBF could be obtained using stand-alone background suppression techniques.[10,21] While ASL-MRI is a promising neuroimaging technique, the accuracy of rCBF is restricted by the low signal intensity-to-noise ratio, which is inherent in this method.[10] This limitation can be quite serious within certain brain structures such as deeper frontal lobe areas but is less problematic within posterior structures investigated in this study.[35] However, ASL-MRI can provide noninva-

sive quantifiable measures of rCBF. Second, limitations exist concerning the utilization of a cross-sectional design. It is often difficult to make a causal inference as only a snapshot of disease progression is obtained. Other confounding factors may not be equally distributed between the groups being compared and this unequal distribution may lead to bias and subsequent misinterpretation. Third, we could not assess the possible contribution of HAART on rCBF[36,37] as most of our subjects (>80%) were receiving medications. This study lacks the power to differentiate if HAART could affect rCBF. Larger longitudinal neuroimaging studies of HIV+ patients both prior to and after starting HAART are required. Fourth, it was surprising that despite 80% of the HIV+ subjects being on stable HAART regimens, many still had detectable plasma and CSF viral loads. This might imply that subjects could have been only partially adherent to therapy, had developed significant resistance to their therapeutic regimens, or may be on poor CNS penetration effective regimens.[38] Further neuroimaging studies stratifying patients according to degree of CNS penetration effectiveness are needed.

Our results highlight a potential role for rCBF in the evaluation of the effects of HIV in the brain. Although we are not advocating for ASL-MRI measurements of rCBF to be used as a screening tool for the presence of neuropsychological impairment due to its relatively limited use and expense, our finding of an early effect of HIV infection on rCBF could represent a purely vascular influence, prior to direct neuronal involvement, or could reflect early neuronal changes that may be missed with existing techniques such as neuropsychological testing, clinical neurologic examination, or laboratory studies. While recovery is the ultimate goal for HAART, the ability of existing neuropsychological performance testing to demonstrate this improvement may lag behind molecular and physiologic changes measured by neuroimaging.[4] Noninvasive quantifiable neuroimaging techniques such ASL-MRI could potentially provide a method to assess HAART regimens and neuroprotective therapy strategies for the brain in clinical trials.[5]

AUTHOR CONTRIBUTIONS

Beau Ances and Florin Vaida performed the statistical analysis.

DISCLOSURE

Dr. Ances has received research support from the NIH [NIMH 1K23MH081786 (PI)], the Foundation for AIDS Research (amfAR) [106729-40-RFRL (PI), and from the Dana Foundation [#DF3857-41880 (PI).] D. Sisti reports no disclosures. Dr. Vaida serves on a Data Safety and Monitoring Board for Ardea Biosciences, Inc.; serves as Research Methodology Section Editor for the *Californian Journal of Health Promotion;* has served as Associate Editor for *Statistica Sinica*; and receives research support from the NIH [MH22005HIV (co PI)]. C.L. Liang reports no disclosures. Dr. Leontiev has received an RSNA medical student departmental grant. Dr. Perthen reports no disclosures. Dr. Buxton receives royalties from publishing *Introduction to Functional Magnetic Resonance Imaging,* Cambridge University Press, 2002; has received speaker honoraria from the University of Washington, Seattle; and receives research support from Roche Pharmaceuticals and the NIH [PHS grant NS- 036722 (PI)]. D. Benson reports no disclosures. Dr. Smith served on a scientific advisory board for and has received research funding from Pfizer; serves on the editorial advisory board of *The Open AIDS Journal* and *The Open Infectious Diseases Journal*; serves as a consultant for and on the Board of Directors of Symmunity LLC; and receives research support from the NIH [MH083552 (coinvestigator), AI077304 (coinvestigator), AI69432 (coinvestigator), MH62512 (coinvestigator), AI27670 (coinvestigator), AI38858 (coinvestigator), AI43638 (coinvestigator), AI43752 (coinvestigator), AI047745 (coinvestigator)], from the UCSD Centers for AIDS Research Viral Pathogenesis Core [AI36214 (coinvestigator)], and from the HIV Infection of the San Diego Veterans Affairs Healthcare System [10-92-035 (coinvestigator)]. Dr. Little serves as an editorial board member of *Antiviral Therapy*; has served on a clinical advisory board for Monogram Biosciences; serves as a consultant for Koronis; and has received research support from Merck Laboratories. Dr. Richman has served on scientific advisory boards for Pfizer, Merck, Bristol Myers Squibb, Gilead, Idenix, Chimerix, Roche, Gen Probe, and Monogram Biosciences; serves as an editor for *Antiviral Therapy and Topics in HIV Medicine*; receives royalties from publishing from Clinical Virology; and has received research support from Merck and from the NIH AI69432 (ACTG) (coinvestigator), AI043638 (AIEDRP) (PI), MH62512 (HNRC) (coinvestigator), AI047745 (Dynamics) (PI), AI074621 (Transmission) (coinvestigator), AI07384 (AIDS Training Grant) (PI), AI36214 (Center for AIDS Research) (PI). Dr. Moore received honoraria for an article published in *Dialogues in Clinical Neuroscience* (Servier Medical Publishing Division); and receives research support from the NIH [R03 MH78785 (PI), R24 MH59745 (coinvestigator), U01 MH83506 (coinvestigator)] and from the California HIV/AIDS Research Program (CHRP) [ID06-SD-201 (PI)]. Dr. Ellis has consulted and received speaker fees from GlaxoSmithKline, serves on the speakers' bureau of GlaxoSmithKline, and receives research support from the NIH [R01 MH058076 (PI), P30 MH062512 (coinvestigator), N01 MH22005 (coinvestigator)].

APPENDIX

The San Diego HIV Neurobehavioral Research Center (HNRC) group includes the following: Director: Igor Grant, MD; Co-Directors: J. Hampton Atkinson, MD, Ronald J. Ellis, MD, PhD, and J. Allen McCutchan, MD; Center Manager: Thomas D. Marcotte, PhD, Jennifer Marquie Beck, Melanie Sherman; *Neuromedical Component:* Ronald J. Ellis, MD, PhD (PI), J. Allen McCutchan, MD, Scott Letendre, MD, Edmund Capparelli, PharmD, Rachel Schrier, PhD, Terry Alexander, RN; *Neurobehavioral Component:* Robert K. Heaton, PhD (PI), Mariana Cherner, PhD, Steven Paul Woods, PsyD, David J. Moore, PhD, Matthew Dawson; *Neuroimaging Component:* Terry Jernigan, PhD (PI), Christine Fennema-Notestine, PhD, Sarah L., Archibald, MA, John Hesselink, MD, Jacopo Annese, PhD, Michael J. Taylor, PhD; *Neurobiology Component:* Eliezer Masliah, MD (PI), Ian Everall, FRCPsych, FRCPath, PhD, Cristian Achim, MD, PhD; *Neurovirology Component:* Douglas Richman, MD (PI), David M. Smith, MD; *International Component:* J. Allen McCutchan, MD (PI); *Developmental Component:* Ian Everall, FRCPsych, FRCPath, PhD (PI), Stuart Lipton, MD, PhD; *Clinical Trials Component:* J. Allen McCutchan, MD, J. Hampton Atkinson, MD, Ronald J. Ellis, MD, PhD, Scott Letendre, MD; *Participant Accrual and Retention Unit:* J. Hampton Atkinson, MD (PI), Rodney von Jaeger, MPH; *Data Management Unit:* Anthony C. Gamst, PhD (PI), Clint Cushman, BA (Data Systems Manager), Daniel R. Masys, MD (Senior Consultant); *Statistics Unit:* Ian Abramson, PhD (PI), Christopher Ake, PhD, Florin Vaida, PhD.

Received December 31, 2008. Accepted in final form June 8, 2009.

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