

*Invited Review***CROI 2017: Neurologic Complications of HIV Infection****Serena S. Spudich, MD; Beau M. Ances, MD**

*The brain is a major target for HIV infection and is a potential viral reservoir even in virologically well-controlled HIV-infected individuals. Data presented at the 2017 Conference on Retroviruses and Opportunistic Infections (CROI) suggested that during early HIV infection, CD4+ T cells in the meninges and choroid plexus serve as an important early site of HIV infection in the central nervous system (CNS), with brain macrophages and microglial cells becoming an important source of viral replication with advancing disease. Longitudinal evaluations of HIV-associated neurocognitive disorder (HAND) demonstrated that cognitive changes occur during early HIV infection and may remain during chronic infection despite virologic control by antiretroviral therapy. Cerebrospinal fluid escape during treatment was noted in numerous cohorts and pathogenetically evaluated as a state of persistent CNS HIV infection despite antiretroviral therapy. Non-HIV risk factors identified for cognitive impairment were depression and frailty. Questions remain concerning appropriate cognitive screening tests to evaluate for HAND. Additional studies highlighted the increasing role of neuroimaging to longitudinally assess potential changes in brain integrity in individuals on systemically suppressive therapy, and provided new CNS considerations in antiretroviral regimens.*

**Keywords:** CROI, 2017, HIV, central nervous system, HAND, neurocognitive disorder, neuroimaging, cognition, neuropathogenesis, reservoir, stroke, cerebrospinal fluid

The effect of HIV in the central nervous system (CNS) was a central theme of several sessions at the 2017 Conference on Retroviruses and Opportunistic Infections (CROI). Neurologic presentations continued to focus on pathogenesis of HIV in the CNS and persistent neurologic dysfunction (as assessed by neuropsychological performance, neuroimaging, and cerebrospinal fluid [CSF]) in virologically well-controlled HIV-infected individuals.

**HIV Neuropathogenesis Using Animal Models**

Animal models of CNS HIV infections potentially afford insight into processes that occur in inaccessible brain tissues during human HIV infection. Mallard and colleagues (Abstract 69) quantitated T lymphocytes and monocytes, and examined viral RNA and phylogenetic relationships between

sequences of simian immunodeficiency virus (SIV) in brain parenchyma, CSF, and choroid plexus of 16 SIVmac251-infected and 2 uninfected rhesus macaques (half of these animals were also CD8+ lymphocyte depleted). SIV RNA was detected in CD4+ T lymphocytes and in monocytes/macrophages in the choroid plexus. In animals that developed SIV encephalitis, numbers of monocytes/macrophages were increased in the choroid plexus, and highly compartmentalized SIV was detected in the choroid plexus and brain. The phylogeny of CSF viral sequences included sequences consistent with those from peripheral blood, brain parenchyma, and choroid plexus, indicating mixed systemic and CNS sources of CSF SIV.

Vasan and colleagues (Abstract 70) examined early CNS inflammation and infection in a simian human immunodeficiency virus (SHIV) rhesus macaque model of HIV infection that closely recapitulates human acute and early HIV infection. After 12 weeks of SHIV infection, T cells infiltrated the brain parenchyma, CD4+ T-cell collections surrounded the meninges, and rare SHIV RNA-positive cells were seen in the meninges and brain parenchyma. Demonstration of these processes in a nonaccelerated SHIV infection helps to elucidate the processes that might be occurring in the brains

*Studies using animal models revealed that HIV DNA and RNA may be detected in brain, choroid plexus, and meninges of rhesus macaques in the early stages of infection, and that the hippocampus might be susceptible to injury in pediatric infection.*

of humans during the natural course of HIV infection, and provides a potential model for understanding CNS reservoirs, pathogenesis, and impact of treatment or other interventions at a histological level.

Carryl and colleagues conducted a study of pediatric neurodevelopment in a perinatal SIV infection model (Abstract 373). Investigators infected infant rhesus macaques with SIVmac251 intravenously (IV) on postnatal day 3 (n = 3) or orally at week 9 (n = 15) and compared postmortem (week 6 for IV infection and week 10 for oral infection) pathology with that seen in uninfected control groups at their demise at 22 weeks. Hippocampal neuron loss was found in CA 1 and CA 2 neuronal layers in both groups of SIV-infected macaques compared with the control group, independent of plasma

viral load. These studies may provide histologic correlates of the cognitive impairment associated with untreated perinatal HIV infection.

### HIV Compartmentalization and Reservoirs in the CNS

Studies of HIV replication and compartmentalization in the CNS in untreated HIV-infected individuals have implications for understanding pathogenesis of HIV-related CNS injury and potential establishment of tissue reservoirs for HIV. Although HIV replication in the CNS, reflected as detectable CSF HIV RNA, is a ubiquitous feature of untreated HIV infection, CSF HIV RNA is typically 1 log lower than that in plasma in chronic infection. In an examination of paired CSF and plasma samples from 155 prospectively enrolled, antiretroviral-naïve study participants in Milan, Italy, Bai and colleagues (Abstract 357) detected greater HIV RNA level in CSF than plasma in 24 (15%) participants. In a multivariate analysis, a CSF:plasma HIV RNA ratio greater than 1 was independently associated with a 3-fold higher risk of HIV-associated neurocognitive disorder. This finding supports a pathogenetic relationship between enhanced viral replication in the CNS and CNS injury resulting in clinical neurologic disease, although the causes of heightened CSF HIV RNA in these individuals are unclear. It remains unknown if some individuals have a virus that is more tropic for the brain.

Genetic compartmentalization of CSF HIV also reflects enhanced local CNS replication of HIV with emergence of unique genetic variants within the CNS and a restriction of viral migration between compartments. Deep sequencing, which can detect less frequent (minor) variants within a diverse viral population, was employed in several studies to dissect compartmentalization comparing HIV RNA obtained from the CNS with that from blood. CNS compartmentalization has been previously documented in chronic subtype B and C HIV infection. Adewumi and colleagues (Abstract 369) used traditional single genome amplification with phylogenetic analysis and deep sequencing of paired CSF and blood HIV RNA to demonstrate CNS viral compartmentalization in subtype G and CRF02\_AG. These findings suggest that neuropathogenesis may involve local HIV replication with compartmentalized viral evolution occurring in common HIV subtypes in sub-Saharan Africa.

Targeted deep sequencing methods (Sirijatupat and colleagues, Abstract 71) were also used to examine CSF for compartmentalization of subtype CRF01\_AE HIV in acute infection in humans. Initial HIV disseminating to the CNS was examined with targeted deep sequencing of protease and reverse transcriptase (RT) genes in HIV derived from paired CSF and plasma samples in 13 participants. In 1 participant at an estimated 19 days postinfection, HIV variants that were present as minor variants (15%-23%) in the blood plasma were present at frequencies more than twice as high in CSF (42%-50%). This is the first indication that enrichment or a sequestration of HIV variants may occur with initial dissemination of transmitted founder viruses to the CNS in acute

infection, although its relevance for subsequent development of compartmentalized CNS reservoirs is uncertain.

To assess the clinical relevance of compartmentalization, Price and colleagues (Abstract 364) used deep sequencing of *env* to investigate the relationship between viral compartmentalization and neurologic injury. They generated sequences from 33 paired CSF and blood plasma samples from individuals with untreated chronic HIV infection, determining the presence and phylogenetic patterns of CNS compartmentalization and relating these to levels of CSF neurofilament light chain (NFL), a cytoskeletal protein marker released in response to damage to myelinated axons. Although HIV-associated dementia (HAD) was associated with elevated NFL level and major (> 30%) CSF *env* compartmentalization, major compartmentalization was also observed in individuals with CD4+ cell counts 200/μL or less and normal NFL levels (25%) and even more frequently in individuals with CD4+ cell counts greater than 200/μL (62.5%). These findings support prior reports that extensive compartmentalized CNS evolution of HIV is associated with severe neurologic injury. They also add to the literature indicating that measurable compartmentalization can predate evidence of clinical neurologic disease and advanced immunosuppression.

Although CSF is the most readily sampled CNS tissue in humans, a few presentations focused on findings from other CNS tissues to investigate HIV neuropathogenesis and compartmentalization. Gelman and colleagues (Abstract 68) examined autopsy tissues from 29 decedents whose samples were collected in the National NeuroAIDS Tissue Bank. One-third of participants had complete viral suppression on antiretroviral therapy, one-third had partial viral suppression on antiretroviral therapy, and one-third had no viral suppression.

***Compartmentalized HIV infection develops during asymptomatic infection and may be macrophage tropic. Compartmentalization of antibody responses between CSF and blood may reflect distinct levels of HIV antigen in the 2 compartments during therapy.***

The amount of HIV DNA in the brain was very small compared with other tissue compartments in individuals overall, but detection of brain white matter DNA was not reduced in individuals on suppressive antiretroviral therapy and was not different according to HIV RNA level in the periphery. The researchers also found variable concentrations of HIV DNA in white versus grey matter in the brain in distinct decedents.

Gonzalez-Perez and colleagues (Abstract 365) described macrophage-tropic HIV Env in autopsy brain tissue in HIV-infected individuals without neurologic signs or with only minor complications during life. Macrophage-tropic variants were detected in the brain at a much higher frequency than in the spleen or bone marrow, and HIV DNA sequences from the brain demonstrated phylogenetic compartmentalization with respect to immune tissues. These findings complement the findings of Price and colleagues to suggest that even in

neuroasymptomatic HIV infection, CNS infection can be compartmentalized and Env may adapt to facilitate replication in macrophages and microglia. De Oliveira and colleagues (Abstract 367) examined brain tissue from 63 individuals who died after a median of 5.6 years of suppressive antiretroviral therapy, comparing the HIV DNA quantitation (copies/million cells) in 3 brain tissue regions and lymph nodes between women (19% of the cohort) and men. They found that HIV DNA levels in the entire group were inversely associated with speed of information processing tested during life. They also found higher quantities of HIV DNA in the brain, but not lymphoid tissue, in women than men.

This association between sex and proviral DNA in the brain warrants further investigation in concert with the study of sex differences in the HIV reservoir distribution in other tissues. Additionally, methods for assessing brain tissue in living humans are actively being sought for studies of CNS reservoirs. The olfactory mucosa has recently been recognized as a site to directly sample the axons of neurons that pass through the cribiform plate into the brain. Calcagno and colleagues (Abstract 379) used the noninvasive technique of nasal brushing in 19 participants to sample the olfactory mucosa as a source of tissue that might provide insight into HIV reservoir in the brain. They were able to quantify HIV RNA in olfactory mucosa as well as plasma and CSF in 10 HIV-infected participants (in a mixed group of some individuals on and some off antiretroviral therapy), finding levels of HIV RNA in olfactory mucosa that correlated with plasma but not CSF levels. Further studies that examine immunologic, histologic, and viral genetic features of this olfactory mucosa are warranted to evaluate this potentially accessible window into brain tissue.

New biologic measures that detect HIV persistence in disparate tissues are needed to better understand potential HIV reservoirs. Gisslen and colleagues measured antibody responses to 7 different HIV antigens in CSF and blood using luciferase immunoprecipitation systems (LIPS) in different stages of untreated and treated HIV infection (Abstract 391) as a means of examining persistence of HIV antigen in the CNS and in blood. In paired samples across the spectrum of untreated HIV infection, CSF HIV antibody levels were detected at high levels that paralleled those found in blood, and with slightly lower levels following the same pattern in individuals on antiretroviral therapy started during chronic HIV infection. However, treatment initiated during primary infection was associated with a distinct pattern, wherein some individuals had antibody levels in CSF similar to HIV seronegative controls, despite elevated levels in blood. This compartmentalization of antibody levels in the CSF suggests that detection of these responses in the CNS is not based simply on passive transfer of antibodies from the blood, but instead may have importance in reflecting local HIV reservoirs during antiretroviral therapy.

Strategies to assess HIV reservoir persistence often involve interruption of antiretroviral therapy to assess the timing of viral rebound in the plasma and in other tissues. Chan and colleagues (Abstract 363) evaluated the impact of

analytic treatment interruption on the CNS in 8 individuals who initially started antiretroviral therapy during Fiebig I (HIV RNA+, p24-, and IgM-) acute HIV infection. No adverse outcomes during treatment interruption or after antiretroviral therapy resumption (triggered by rise in plasma HIV RNA level to > 1000 copies/mL) were noted while monitoring CSF for viral rebound and inflammation (n = 4), magnetic resonance spectroscopy (MRS) assessment for changes in metabolites suggesting inflammation or neuronal injury (n = 5), and neuropsychologic testing with the Flanker Task (a tablet-based measure of attention and inhibitory control from the National Institutes of Health [NIH]-Toolbox; n = 8). This study demonstrates the feasibility of intensive CNS monitoring during “cure” studies and treatment interruption; further similar studies should be completed to ensure the CNS safety of HIV remission strategies and evaluations.

### CSF HIV Escape and Neurologic Disorders During Antiretroviral Therapy

Several presentations focused on variants of CSF HIV escape, a virologically defined state in which HIV is either detectable in the CSF but undetectable in the plasma, or elevated in the CSF compared with levels in blood, despite antiretroviral therapy.<sup>1</sup> Joseph and colleagues (Abstract 73) reported cases of virologic escape in the CSF in a prospective cohort of study participants without neurologic symptoms assessed during systemically suppressive antiretroviral therapy. They observed 2 virologic states of CSF escape: an example of nonpersistent escape, occurring at a single time interval associated with clonal amplification of a viral variant exhibiting X4 coreceptor tropism, and an example of CSF escape persisting over 2 time points associated with a diverse viral population and a heightened ability to infect cells with low-CD4 receptor density, presumably macrophages. In a separate study examining viral characteristics of CSF escape, HIV sampled from the CSF in 9 out of 62 participants in an antiretroviral therapy—suppressed cohort had CSF escape. In 2 participants, CSF escape was characterized by X4 coreceptor tropism, and in a third participant, sequences were associated with defective viruses (Smith and colleagues, Abstract 371). Johnson and colleagues (Abstract 370) observed compartmentalized viral variants and drug resistance mutations in 6 individuals with elevated CSF compared with plasma HIV RNA in the setting of incompletely suppressive antiretroviral therapy. They also used an antibody capture method to investigate cell sources of CSF HIV by targeting host cell proteins incorporated in the HIV envelope detected in CSF, and identified markers for classical monocytes/macrophages, homing monocytes, natural killer cells, and T-helper cells. These experiments suggest that compartmentalized viral populations in the CNS may be sustained by numerous cell types in the setting of partially effective antiretroviral therapy.

Additionally, Pinnetti and colleagues studied the clinical and laboratory factors associated with the risk of CSF escape during antiretroviral therapy in individuals undergoing a lumbar puncture for neurologic symptoms or evaluation of

systemic lymphoma (Abstract 366). They defined CSF escape as CSF HIV RNA greater than 50 copies/mL with concurrent plasma HIV RNA 50 copies/mL or less, or CSF HIV-RNA greater than  $1\text{-log}_{10}$  copy/mL, higher than that in concomitant plasma. An elevated CSF to plasma albumin ratio, a marker of blood brain barrier disruption, was associated with a substantially higher risk of concurrent CSF escape, with an adjusted odds ratio of 5.75 (95% confidence interval [CI], 1.10–30.09;  $P = .038$ ). Questions still remain concerning whether escape occurs as a result of increased permeability in the blood brain barrier. Current regimen type (protease inhibitor-based versus nonnucleoside reverse transcriptase inhibitor [NNRTI]—based versus other) and presumed drug efficacy in the CNS (95% inhibitory quotient of the third drug corrected for CSF drug concentration) were not associated with risk for CSF escape.

In related studies, Soulie and colleagues (Abstract 349) evaluated 227 HIV-infected individuals who on antiretroviral therapy had CSF HIV RNA concentrations greater than 50 copies/mL noted during evaluation for neurologic disorders. The group was divided into 195 individuals who had a concomitant plasma HIV RNA greater than 50 copies/mL and 32 individuals with CSF escape (defined as  $< 50$  copies/mL HIV in plasma despite the detection in CSF). CSF escape was seen in 14% of neurologically symptomatic individuals with high CD4+ cell counts (median  $476/\mu\text{L}$ ), of whom 78% had genotypic evidence of drug resistance in the CSF, thus providing a rationale for testing CSF for HIV RNA in HIV-infected individuals who have neurologic findings. The long-term clinical significance of CSF escape in this context is still unknown. In individuals on antiretroviral therapy in the CHARTER (CNS HIV Antiretroviral Therapy Effects Research) study, better executive function on neuropsychological testing was strongly associated with greater odds of having undetectable plasma viremia (Anderson and colleagues, Abstract 353). The cause and effect in the association between cognitive function and systemically successive therapy is still unknown, but it is likely a bidirectional relationship.

### Clinical and Biomarker Associations With CNS Immune Activation in HIV Infection

Abnormally elevated immune activation in treated HIV infection is a universal facet of systemic HIV and likely underlies numerous end-organ complications observed with antiretroviral therapy–treated HIV infection.<sup>2</sup> Inflammation is also frequently detected in the CNS despite virologic suppression. Prior studies have found that abnormal inflammation in the CNS is associated with the presence of cognitive dysfunction in HIV infection despite treatment.<sup>3</sup> One presentation at CROI 2017 supported prior work documenting residual CNS inflammation despite long-term virologic suppression on antiretroviral therapy of 10 years or more (Hammarlund and colleagues, Abstract 340). CSF neopterin, a marker of CNS macrophage activation, was elevated in 55% of 22 virally suppressed participants. However, this study did not detect associations of CSF neopterin levels with either CSF NFL level

or neuropsychologic performance on the Cogstate Brief Battery of 4 neuropsychological tests.

Several studies examined novel measures of immune responses in the CNS to investigate neuropathogenesis and to provide new biomarkers of disease. Hermansson and colleagues investigated levels of YKL-40, a chitin-binding glycoprotein, in the CSF of HIV-infected individuals as a putative biomarker of microglial activation during different stages of HIV infection and HAD (Abstract 383). YKL-40 was elevated compared with controls in untreated neuroasymptomatic individuals with a CD4+ cell count of  $350/\mu\text{L}$  or less, and very highly elevated in individuals with HAD, suggesting its utility as a biomarker for HIV-associated neurocognitive disorder (HAND). Furthermore, CSF YKL-40 levels indepen-

***Biomarkers of immune activation, including soluble CSF markers of microglial activation such as YKL-40, may reflect the activation of microglial cells and macrophages that underlies neuropsychological impairment in untreated and treated HIV infection.***

dently strongly correlated with NFL levels in a multivariable analysis across the spectrum of HIV disease, indicating a role for this protein in the pathogenetic dissection of HAND.

Additional studies revealed relationships between systemic immune activation and neurologic and cognitive function. Imp and colleagues (Abstract 341) evaluated 253 participants in the WHIS (Women's Interagency HIV Study). Systemic monocyte activation, measured by sCD163 and sCD14 in plasma, was higher in women with impaired neuropsychologic performance. These differences remained statistically significant when the analysis was limited to the 50% of participants with undetectable plasma HIV RNA level. Thus, potential sex-specific monocyte activation and response to antiretroviral therapy warrant further investigation in order to optimize the neurocognitive status of HIV infected-women.

In ACTG (AIDS Clinical Trials Group) 5303, a study of initial antiretroviral therapy, Robertson and colleagues (Abstract 342) also identified a role for monocytes in cognitive functioning during treatment. They identified modest inverse correlations between neuropsychologic performance and frequency of classical (CD14++CD16–) and nonclassical monocytes (CD14+CD16++) after 48 weeks of antiretroviral treatment. Mitchell and colleagues (Abstract 385) described the percentage of blood CD4+ T lymphocytes positive for the programmed cell death protein 1 (PD-1+), indicating an exhausted phenotype associated with smaller regional brain volumes in individuals on at least 3 months of stable antiretroviral therapy. These associations between monocyte activation or T-cell exhaustion and evidence of neurologic impairment or injury during treatment support the concept that ongoing systemic immune perturbation despite antiretroviral therapy may contribute to CNS pathology in HIV infection. Furthermore, a study relating systemic and CNS immune activation and concurrent neuropsychologic performance

during acute HIV infection with a history of past or active syphilis suggests that coinfections may contribute to immune activation and associated neurologic consequences in HIV infection (Chan and colleagues, Abstract 339).

### **HAND Persists in the Antiretroviral Therapy Era**

HAND continues to occur in HIV-infected individuals despite the introduction of antiretroviral therapy. This condition impacts the overall daily activities of HIV-infected individuals. Numerous studies at CROI 2017 focused on longitudinal persistence of HAND. Questions remain as to when cognitive changes occur in HIV-infected individuals. Robertson and colleagues (Abstract 380) studied individuals with acute or recent HIV infection within a subgroup of the Sabes Study in Peru who either immediately started antiretroviral therapy after diagnosis ( $n = 42$ ), or deferred treatment for 24 weeks ( $n = 45$ ). Neuropsychologic performance was evaluated at baseline, 12, 24, and 48 weeks. Performance improved over time for both groups. At 48 weeks, mean change in neuropsychologic performance was statistically significantly better for HIV-infected individuals who immediately started antiretroviral therapy than for those who deferred treatment. This potential protection of brain function associated with immediate therapy in individuals recently infected with HIV support current guidelines recommending that clinicians should not wait to initiate antiretroviral therapy. The cognitive trajectories of individuals with chronic HIV infection were also studied by several groups in adults (Abstracts 350, 351, 352LB, and 397) and children (Abstract 826). Rubin and colleagues (Abstract 350) performed repeated neuropsychologic performance testing at baseline, at 2 years, and at 4 years in a large sample of HIV-infected women who were followed in the WIHS. Cognitive trajectories were compared for women not infected with HIV (control group;  $n = 301$ ), women infected with HIV on antiretroviral therapy who were virologically well controlled ( $n = 239$ ), and women with HIV infection on antiretroviral therapy but not virologically well controlled ( $n = 302$ ). HIV-infected women performed worse than the control group in learning, memory, and attention domains. The HIV-infected and uninfected groups had similar overall trajectories (rates of change). Within the group of HIV-infected women, there was substantial heterogeneity. HIV-infected women who were virologically well controlled had better performance in learning and memory, but worse performance in fluency, attention, and speed of processing than HIV-infected individuals without well-controlled suppression. The etiology of these differences is not known, but may reflect differences in viral burden or greater sensitivity to the virus in certain brain areas.

Cole and colleagues (Abstract 352LB) and Sanford and colleagues (Abstract 397) also demonstrated that virologically well-controlled HIV-infected individuals performed worse than a control group of women not infected with HIV in terms of attention, processing speed, and executive function. For both of these studies, the rate of cognitive change was similar

between the group with HIV infection and the group without HIV infection. In contrast, Gates and colleagues (Abstract 351) studied 96 men infected with HIV who were virologically well controlled and a control group of 44 men uninfected with HIV longitudinally at a baseline assessment and 18 months later. A greater proportion of the HIV-infected individuals had a subtle subclinical worsening of cognitive impairment (speed and executive function) compared with the control group. Finally, neuropsychologic performance was evaluated at baseline and at 48 weeks in a cohort of children from 4 African countries who were uninfected with HIV, uninfected with HIV but perinatally exposed, and infected with HIV on antiretroviral therapy (Bolvin and colleagues, Abstract 826). HIV-infected children performed worse than children not in-

*Cognitive performance is lower in HIV-infected individuals on suppressive antiretroviral therapy than in HIV-uninfected controls, but rates of change over time between the 2 groups are similar in studies of children, women, and men.*

fectured with HIV in numerous cognitive domains. Similar to many of the results in adults, children infected with HIV had no substantial changes detected in cognition between week 0 and week 48, with cognition remaining reduced compared with a control group of children without HIV infection.

Neuropsychologic performance results from several studies indicate that persistent cognitive impairment occurs despite virologic control. These changes in cognition may originate during early infection. Data presented at CROI 2017 suggest that overall neuropsychologic performance trajectory of HIV-infected individuals who are virologically well controlled is similar to individuals without HIV infection. These results suggest that it is not only if but when antiretroviral therapy is administered because persistent impairment may remain even after virologic control is established.

### **Screening for HAND Remains Difficult**

Questions still abound as to how to diagnose HAND in the clinical setting. The optimal set of screening tests for HAND is actively being pursued by several groups. Milanini and colleagues (Abstract 355) compared the International HIV Dementia Scale (IHDS) with a standard neuropsychologic performance test battery to evaluate for cognitive impairment within a large group of young individuals with HIV infection ( $n = 2009$ ) and without HIV infection ( $n = 405$ ) from a number of African countries. Approximately 25% of the individuals without HIV infection and 40% of the individuals with HIV infection were cognitively impaired in this relatively young cohort. Using various cut points, the authors demonstrated that the IHDS had relatively poor sensitivity and specificity in identifying HAND.

In another series, Trunfio and colleagues (Abstract 360) studied a sample of 279 HIV-infected individuals in Italy using a series of screening tests (3 questions, IHDS, clock

drawing, and frontal assessment battery) and a longer neuropsychologic performance battery. Each of the screening tests had poor sensitivity and specificity in distinguishing HAND compared with the “gold” standard, which was a neuropsychologic performance battery that covers numerous domains. Results from these relatively large HIV population studies suggest that cognitive screening tests are not reliable for successfully identifying HAND in the clinical setting. Additional biomarkers may instead be needed for assisting in the diagnosis of HAND.

### **Risk Factors for HAND and Stroke**

Risk factors for HAND were also investigated at the conference. Deiss and colleagues (Abstract 358) studied 189 HIV-infected US military personnel. HIV-infected individuals with a previous history of posttraumatic stress disorder (PTSD) were more than 6 times more likely to develop HAND than HIV-infected individuals without a diagnosis of PTSD. Many of the HIV-infected individuals with PTSD also had residual mood disorders that could affect neuropsychologic performance results. Erlandson and colleagues (Abstract 665) studied the relationship between frailty and cognitive impairment in 954 HIV-infected individuals in ACTG 5322, the HAILO (HIV Arterial Dysfunction, Lipids, and Lovaza) study. Frailty was assessed using the Fried criteria,<sup>4</sup> and cognition was evaluated using 3 neuropsychologic tests. Participants were classified into 1 of 4 possible categories: nonfrail and cognitively normal (80%), nonfrail and cognitively impaired (18%), frail but cognitively normal (4%), and frail and cognitively impaired (2%). Frailty was associated with a greater risk of falls regardless of the degree of cognitive impairment. These results suggest that frailty and cognition reflect different spectrums of an HIV-infected individual. Interventions that focus on frail individuals rather than cognitively impaired individuals may have a greater impact on fall risk.

Le and colleagues investigated whether the standard clinical laboratory information of CD4+:CD8+ cell ratio was a useful blood biomarker associated with HAND in a longitudinal observational cohort of 109 individuals with early HIV infection over 550 visits of follow-up (Abstract 72). When taking into account clinical factors such as plasma HIV RNA level, treatment status, and age at baseline, CD4+:CD8+ cell ratio emerged as independently associated with neuropsychologic performance in a multivariable analysis. These findings support prior associations observed in chronic infection between lower CD4+:CD8+ cell ratio and poorer cognitive performance, cross-sectionally as well as longitudinally, and suggest that this clinically available test may be useful in assessing the risk of HAND.

Gutierrez and colleagues (Abstract 346) performed a retrospective study that looked at the relationship between immune status and risk of developing stroke in 115 HIV-infected individuals at a single tertiary center. Etiology of strokes were identified and classified as small artery disease; due to an embolus from the heart (cardioembolic); infectious; cryptogenic; or other mechanism. Unlike in other studies,

the most common stroke mechanism was cryptogenic (31%) followed by intracranial large artery disease (26%), and infectious etiologies (14%). A decrease in CD4+ cell count was associated with increased risk of infectious etiology for stroke, and individuals who had large gains in CD4+ cell counts after a lower CD4+ cell count nadir were more likely to have intracranial artery strokes. Individuals with immune reconstitution inflammatory syndrome (IRIS) may be at increased risk for stroke. Crane and colleagues (Abstract 347) investigated the types of strokes seen in HIV-infected individuals at 5 institutions. From a total of 23,189 HIV-infected individuals studied, 238 had a stroke. Within this adjudicated sample, ischemic strokes were most common (75%) followed by unknown (13%) and hemorrhagic (12%). Within the ischemic group the etiologies were equally distributed among small vessel (31%), cardioembolic (30%), and atheroembolic (23%). A high proportion of the cardioembolic strokes resulted from illicit drug use. These results suggest strokes occur because of a variety of distinct causes in HIV-infected individuals. Stroke may prove to be an increasing problem in HIV-infected individuals, especially as this population lives longer as a result of antiretroviral therapy.

### **Neuroimaging of HIV Infection in the Brain**

Quantification of changes in cortical and subcortical volumetrics was used to assess the effects of HIV infection in the brain. Van Zoerst and colleagues (Abstract 352LB) studied 134 HIV-infected individuals who were virologically well controlled and 79 HIV-well-matched controls using a variety of neuroimaging methods. Both groups of participants were studied at baseline and at a 2-year follow-up. At baseline, HIV-infected individuals had smaller brain volumes than those without HIV infection. Age-related changes were seen in both groups but no interaction was observed between age and HIV serostatus. For many of the neuroimaging measures the rates of change were comparable for both groups. Similar results were also seen by Sanford and colleagues (Abstract 397). A cohort of 46 HIV-infected individuals who were virologically suppressed and 31 well-matched HIV-seronegative individuals in a control group were studied over a 2-year interval. Statistically significant reductions in volume within the thalamus, caudate, putamen, and globus pallidus were seen in HIV-seropositive individuals compared with HIV-seronegative individuals at baseline. The overall rate of change in volume was similar in the HIV-seropositive and HIV-seronegative groups. These findings support the hypothesis that structural brain changes occur early in HIV-infected individuals during the period of untreated infection. Early initiation of antiretroviral therapy may reduce volume loss associated with HIV infection.

As previously noted, Mitchell and colleagues (Abstract 385) studied the association between blood immune activation and inflammation (in particular, T-cell and monocyte subpopulations) and brain volumetrics in 33 HIV-infected individuals on stable antiretroviral therapy. Using flow cytometry, peripheral blood mononuclear cells (PBMCs) were

evaluated for markers of T-cell activation (CD38+HLA-DR+), senescence (CD57+CD28-), and exhaustion (PD-1, TIM-3, TIGIT). The presence of exhausted CD8+ T cells and CD16+ monocytes was primarily associated with subcortical brain atrophy (putamen and nucleus accumbens) and cerebellar reduction. These results suggest that certain cell types may be

***Structural measurements of regional brain volumes, assessments of cellular metabolites by MRS, and functional connectivity of brain regions also applied in longitudinal studies generally found no accelerated injury in HIV-infected individuals on suppressive antiretroviral therapy compared with controls.***

responsible for continued volume loss seen in virologically well-controlled HIV-infected individuals. More recently, a fluorodeoxyglucose positron emission tomography (FDG-PET) approach was developed to image CD206+ macrophages. Zanni and colleagues (Abstract 635LB) demonstrated that macrophage-specific immune mechanisms can lead to end-organ damage in HIV-infected individuals who are virologically well controlled. Application of this technique and others that can examine microglial activity in the brain are needed. The continued development of novel imaging methods for inflammatory changes in HIV-infected individuals on stable therapy hold great promise for potentially identifying remaining viral reservoirs.

Other imaging studies used MRS to evaluate metabolite changes in HIV-infected individuals. Hellmuth and colleagues (Abstract 394) performed MRS in 297 acute HIV-infected individuals before the initiation of antiretroviral therapy. Individuals with acute HIV infection who had cognitive impairment had increased glutamine (a marker of excitotoxicity) in frontal grey matter, decreased n-acetyl aspartate (a marker of neuronal function) in frontal white matter, and increased choline (a marker of inflammation) in parietal grey matter, compared with individuals with acute HIV infection and no cognitive impairment. These results suggest that frontal brain regions may be susceptible to HIV infection soon after seroconversion.

Gates and colleagues (Abstract 398) studied 26 men with chronic HIV infection who were virologically well controlled on antiretroviral therapy. Changes in creatinine (brain energy metabolism) and choline (inflammation) were associated with levels of the HIV Tat protein in the CSF. Perez-Valero and colleagues (Abstract 400) evaluated HIV-infected individuals who were randomly assigned to a high CNS penetration but also potentially neurotoxic regimen (abacavir/lamivudine plus efavirenz; n = 11) or a low CNS penetration and less neurotoxic regimen (tenofovir/emtricitabine plus ritonavir-boosted atazanavir; n = 11) at baseline and 48 weeks after therapy began. No statistically significant differences were seen between the 2 regimens with regard to neuropsychologic performance or MRS markers. However, for many of these MRS studies a matched control group of individuals without HIV infection

was not included. Additional longitudinal studies using MRS are required comparing HIV-seronegative individuals and HIV-seropositive individuals who are virologically well controlled.

Changes in brain function were assessed in HIV-infected individuals. Kallianpur and colleagues (Abstract 395) studied the relationship between functional connectivity and PBMCs (CD14+ and CD14- cells) in 38 HIV-infected individuals and 46 individuals without HIV infection. HIV-infected individuals had lower resting state functional connectivity (rs-fc) (especially between the insula and prefrontal cortex) than individuals without HIV infection. However, increases in rs-fc were seen between the caudate and superior parietal cortex in individuals with HIV infection compared with those without HIV infection. Among HIV-infected individuals, increases in CD14+ monocytes were associated with decreased rs-fc. Smith and colleagues (Abstract 401) studied the relationship between resting cerebral blood flow (CBF) and performance on the Iowa Gambling Task (IGT), a measure of riskiness. Risky decision-making was supported by affective systems in HIV-infected individuals but by attention systems in individuals without HIV infection. HIV-infected individuals often recruit attention and emotional processing areas to meet increasing cognitive demands.

Finally, Nichols and colleagues (Abstract 403) studied 11 youth with HIV infection and 13 uninfected youth using magnetoencephalography (MEG). HIV-infected individuals had hypoactivation within deeper structures (eg, basal ganglia) and increases in slow-wave (delta/theta) activity were seen in other subcortical areas. Widespread superficial hyperactivation was seen in higher frequency bands, possibly reflecting a compensatory increase in activation within cortical areas. MEG may be a sensitive marker of early changes in the brain seen in young, asymptomatic HIV-infected individuals.

### Treatment Considerations for the CNS

The question of how best to ameliorate the clinical signs and symptoms of HAND remains of central importance to HIV-infected individuals and HIV practitioners. Ndhlovu and colleagues (Abstract 381) investigated whether antiretroviral intensification with cenicriviroc, an investigational dual CCR2 and CCR5 antagonist, might improve neurocognitive function in individuals on suppressive treatment. In an open-label, single-arm pilot study, 17 participants on stable antiretroviral therapy for 1 year with plasma HIV RNA level of 50 copies/mL or less received once-daily cenicriviroc for 24 weeks. Cenicriviroc treatment was associated with an improvement across several cognitive domains and in global neuropsychologic performance, as well as with improvement in 3 plasma soluble biomarkers of macrophage activation (soluble CD14, soluble CD163, and neopterin). Improvements in some domains of neuropsychologic performance and systemic macrophage activation were correlated. Although the learning effect in a nonrandomized trial with repeated testing cannot be excluded, these findings provide rationale for a randomized clinical trial of cenicriviroc intensification for numerous complications of monocyte activation, including HAND.

Although standard antiretroviral therapy is almost universally associated with improvement in neurologic function, including in limited resource settings and across HIV clades (Sacktor and colleagues, Abstract 359), potential complications of treatment in the CNS require consideration. Viswanathan and colleagues (Abstract 372) reported on a meta-analysis of clinical trial-derived data and suggested that the highly effective integrase strand transfer inhibitor (InSTI) class of antiretrovirals is associated with a risk of neuropsychiatric adverse effects, primarily depression, similar to that of efavirenz and protease inhibitors. The absolute risk of depression in the InSTI group was 7.1%, and the absolute risk of memory loss was 0.5%. It is uncertain whether these symptoms could have been premorbid and not associated specifically with treatment. However, the favorable risk profile of InSTIs should be carefully assessed with further investigation of possible CNS side effects in future studies, potentially including a dedicated prospective study or assessment of neurologic and mental health complications in a randomized controlled trial. 

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