Review

Neurologic Complications of HIV Infection

Serena S. Spudich, MD; Beau M. Ances, MD, PhD

More than 30 years into the HIV epidemic, research efforts are focusing on better understanding how the central nervous system (CNS) is adversely affected by HIV and on improving the quality of life of HIV-infected individuals. At the 2015 Conference on Retroviruses and Opportunistic Infections, neurologic presentations concentrated on characterization of potential CNS reservoirs of HIV, the pathogenesis of HIV-associated neurocognitive disorders (HAND), diagnosis of cognitive dysfunction caused by HIV, neuroimaging biomarkers of HAND, and treatment of modifiable risk factors of HAND. Studies presented also highlighted research on CNS disorders in international, resource-limited settings, setting the stage for a growing collection of collaborative studies that will directly impact the largest concentrations of people living with HIV worldwide.

Keywords: CROI 2015, HIV, central nervous system, HIV-associated neurocognitive disorder, neuroimaging, neuropathogenesis, cerebrospinal fluid

A potentially deleterious impact of HIV on the nervous system in otherwise stable-appearing individuals has garnered growing concern. HIV may establish infection and immune activation in the nervous system early during infection, and recognition of these early events has implications for potential persistent sites of HIV infection and for early and long-term neuropathogenesis.[1] Although the incidence of more advanced forms of HIV-associated neurocognitive disorders (HAND) has declined with the use of potent antiretroviral therapy, milder forms of HAND remain relatively common.[2,3] Further, HIV infection and associated inflammation may persist in the central nervous system (CNS) in some individuals during antiretroviral treatment. Studies presented at the 2015 Conference on Retroviruses and Opportunistic Infections (CROI), held from February 22 to 26, aimed to unravel the biologic underpinnings of HIV effects in the CNS and to develop therapeutic strategies to address ongoing abnormalities related to these in HIV-infected individuals.

Central Nervous System HIV Persistence and Latency: Evidence, Measurements, and Mechanisms

Several studies at CROI 2015 focused on characterizing and interrogating HIV in CNS tissues in order to shed light on the possibility and nature of a CNS reservoir. Gianella and colleagues (Abstract 58) examined genetic attributes of paired cerebrospinal fluid (CSF) and plasma samples from 14 HIV-infected men after interruption of antiretroviral therapy. This study investigated the patterns of genetic compartmentalization between CSF and blood that might reveal the source of HIV rebound after interruption of therapy.

Study participants had been on antiretroviral therapy for more than 4 years, although whether they were all virally suppressed during this period was unknown. Next-generation sequencing using the 454 platform was employed to examine viral sequences (including *env, gag,* and *pol*) in CSF and blood after treatment interruption. HIV rebounded first in blood and then in CSF. Rebounding HIV was occasionally equilibrated, meaning that the HIV found in CSF was identical to that found in blood. However, in many participants (10/14), HIV rebounding in CSF was distinct from that in blood in at least 1 gene, most typically *env*. Although this was a retrospective study with variable timing of sampling after treatment interruption, the 4 participants who had samples taken in the first weeks after treatment interruption all had compartmentalization of rebounding HIV. In 11 participants, serial sampling after treatment interruption provided a window to study if compartmentalization in the CSF was sustained; in a majority of these, the compartmentalization persisted. These studies suggest a source of HIV in CNS independent from blood after treatment interruption. Methods such as these, including sampling after treatment interruption and the use of next-generation sequencing, are likely to be powerful tools for dissecting compartmentalized sources of HIV and their characteristics in future studies.

A key challenge to assessing HIV persistence in the CNS compartment is the need to quantify HIV in the CNS in HIV-infected participants, using current tissue sampling strategies. To date, most studies have relied on measuring cell-free HIV RNA from CSF supernatant to provide an assessment of HIV burden in the CNS. Hellmuth and colleagues (Abstract 438) compared levels of HIV RNA in CSF and blood, using standard viral load assays (lower limit of detection of 50 copies/mL in blood and 100 copies/mL in CSF) in HIV-infected individuals during acute and chronic infection, with a

Dr Spudich is Associate Professor of Neurology at Yale University School of Medicine in New Haven, Connecticut. Dr Ances is Associate Professor of Neurology at Washington University in St Louis, Missouri. Send correspondence to Serena S. Spudich, MD, Yale University Department of Neurology, PO Box 208018, New Haven, CT 06520. Received on March 25, 2015; accepted on March 30, 2015.

higher ratio of plasma to CSF HIV RNA observed during early than in chronic infection. Further, 10 of 42 individuals assessed during acute HIV infection had levels of HIV RNA in CSF that were below the limit of detection and were associated with lower levels of biomarkers of immune activation in plasma and CSF. This study raises the question of whether initiation of antiretroviral therapy during acute HIV infection may reduce the burden of persistent CNS infection or prevent dissemination to the CNS, given the lack of detectable HIV RNA in the CNS in 24% of individuals during this period.

Two studies that also focused on HIV RNA recovered from the CSF suggested a relationship between the extent of local HIV replication within the CNS compartment and neuropathogenesis. Joseph and colleagues (Abstract 440) examined the genetic attributes and phylogenetic relationships of HIV in paired CSF and blood samples from 40 individuals with neuropsychologic testing–based diagnoses of distinct categories of HAND. In some individuals, partial *env* genes were amplified using deep sequencing with a primer ID, and in others, full-length *env* was amplified by single genome amplification. The investigators found increasing proportions of CNS compartmentalization with increasing severity of HAND: 29% in neurologically normal participants, 40% in participants with asymptomatic neurocognitive impairment (ANI) or mild neurocognitive disorder (MND), and 70% in participants with HIV-associated dementia (HAD). Using an Affinofile assay to determine putative macrophage infectivity of HIV based on entry into cells with varying surface levels of CD4 receptors, the investigators determined that 71% of compartmentalized viruses derived from CSF appeared to be adapted to replication within macrophages.

Robertson and colleagues (Abstract 439) also used CSF HIV RNA as a measure of CNS HIV infection, using the HIV RNA level as a proxy for extent of CNS infection in a group of approximately 30 individuals evaluated with CSF collection and neuropsychologic testing before and after antiretroviral therapy (regimens were determined separately from the study). A lumbar puncture and neuropsychologic testing (11 tests, normed and averaged to yield a total *z* score) were obtained prior to treatment, with repeat testing at intervals following antiretroviral therapy initiation: lumbar puncture at 2 weeks to 4 weeks and neuropsychologic testing at 24 weeks. The median CSF HIV RNA level of 3.14 $\log_{10}$ copies/mL at baseline was reduced to 1.60 $\log_{10}$ copies/mL after 2 weeks of antiretroviral therapy, and the baseline summarized total *z* score of -0.91 had improved to -0.71 at the time of follow-up.

Analysis demonstrated relationships between the degree and rapidity of HIV RNA reduction in the first 2 weeks of antiretroviral treatment and the extent of improvement in neurocognitive functioning during the first 6 months after initiating therapy, suggesting that early attenuation of HIV RNA replication in the CNS may lead to enhanced neurocognitive responses to treatment. Alternatively, these data could suggest that individuals with a slower or less pronounced reduction in HIV RNA initially have a more severe local CNS HIV infection, which is associated with poorer long-term reversal in response to standard antiretroviral treatment.

CSF HIV RNA is typically reduced to below levels of standard detection in individuals taking antiretroviral treatment. Attention has recently turned to whether HIV nucleic acid detected in cells recovered from the CSF may be a useful measure of HIV persistence in the CNS. de Oliviera and colleagues (Abstract 435) measured *pol* using a droplet digital polymerase chain reaction (ddPCR) assay, to quantitate HIV DNA in peripheral blood mononuclear cells (PBMCs) and CSF cell pellets obtained from 29 HIV-infected individuals. Twenty of these individuals were taking antiretroviral therapy for a median of 2.3 years and were virologically suppressed, with HIV RNA levels in blood and CSF of less than 50 copies/mL. ddPCR yielded detectable HIV DNA in 66% of CSF cell pellet samples from the entire group, and in 50% of CSF samples from individuals with undetectable HIV RNA in both compartments. Although a higher proportion of PBMC samples had detectable HIV DNA (100% of the overall group), median levels of HIV DNA were similar or higher in CSF (3.4 $\log_{10}$ copies/million cells) than in PBMCs (2.2 $\log_{10}$ copies/million cells) in individuals taking suppressive antiretroviral therapy.

In the group overall, higher levels of HIV DNA in CSF correlated with higher levels measured in PBMCs and with higher levels of HIV RNA in CSF. More than 4.0 $\log_{10}$ copies/million CSF cells were detected in some individuals with undetectable HIV RNA levels, indicating persistence of HIV in CNS cells during suppressive antiretroviral therapy. These data provide rationale for future studies focused on characterization of HIV detected in CNS cells during suppressive antiretroviral therapy, to potentially yield further insight into the sites and mechanisms of HIV persistence.

In further studies examining HIV persistence and latency in the CNS, Gelman and colleagues (Abstract 61) studied brain specimens from 40 individuals who died with HIV infection and from 20 HIV-uninfected individuals, using autopsy materials obtained through the National NeuroAIDS Tissue Consortium. In order to determine tissue biomarkers and mechanisms associated with HIV DNA in the brain, the investigators examined the relationship between macrophage markers associated with and levels of HIV DNA relative to HIV RNA measured in the dorsal prefrontal cortex, an area often affected by HIV. Although a number of the markers considered standard indicators of macrophages and microglia (including CD16, CD14, and CD163) did not differentiate between those with proportionally higher HIV DNA, other markers (including interferon regulatory factor 4 [IRF-4]; C-type lectin domain family 4, member A [CLEC4A, also termed DCIR]; and interleukin 10 [IL-10]) were higher in this group.

These results are of interest because IRF-4 is a transcription factor that could

potentially mediate the relationship between integrated HIV DNA and expressed HIV RNA. IRF-4 is also regulated by a polycomb repressive complex such that the methyltransferase enhancer of zeste homolog 2 (EZH2) suppresses the expression of IRF-4; thus, if important as a regulator, EZH2 might lower HIV DNA relative to HIV RNA. Dual staining of macrophages in the leptomeninges of the brains of these individuals showed that the presence of EZH2 was associated with increased expression of HIV RNA, suggesting that this pathway might be related to control of viral expression versus maintenance of HIV DNA in a latent state. Although these analyses were performed in autopsy studies of HIV-infected individuals who were not necessarily taking suppressive antiretroviral therapy at the time of death, these results reveal potentially important mechanisms of endogenous regulation of HIV DNA transcription in the human brain.

Another study presented at CROI 2015 that was focused on brain autopsy tissue explored an issue of relevance to control of HIV replication in the setting of antiretroviral treatment. In order to assess how antiretroviral drugs may access brain tissue and potentially impact levels of HIV infection in this compartment, Bumpus and colleagues (Abstract 436) examined samples from 3 brain regions (globus pallidus, cortical grey matter, and white matter) obtained from autopsy studies from the California NeuroAIDS Tissue Consortium. Concentrations of atazanavir, efavirenz, emtricitabine, and lamivudine in the brain were similar to those reported in CSF. However, tenofovir, which is considered to have potentially poor CNS efficacy owing to low measured concentrations in the CSF, had concentrations that were notably higher in all brain regions than in the CSF. Lopinavir concentrations were also higher in the brain in the frontal white matter than in the CSF. This study reveals that although concentrations of certain drugs reaching the brain can be extrapolated from levels found in the CSF, in some cases CSF may underestimate concentrations in the brain tissue compartment. Further studies focused on measuring not only whole drug concentrations but also concentrations of phosphorylated nucleotide analogue reverse transcriptase inhibitor anabolites may further enhance precision of antiviral activity estimates in the CNS.

New approaches for assessing HIV persistence or replication in the CNS include comparison of HIV variants in compartments after treatment interruption, ddPCR measurement of CSF fluid cell-associated DNA, and next-generation sequencing to genetically characterize HIV in the CNS compartment.

Gama and colleagues (Abstract 416) examined issues of latency and explored strategies for reducing persistent lentiviral integration in the CNS by employing latency-reactivating agents in neurologic studies of rhesus macaques. Using an accelerated macaque model of neuroAIDS created by dual infection with 2 neurovirulent strains of simian immunodeficiency virus (SIV; SIVDeltaB670 and SIV/17-Fr), these investigators treated 3 animals with antiretroviral therapy such that they were virally suppressed to less than 100 copies/mL of SIV RNA in plasma for 500 days. They then exposed 2 of the animals to the protein kinase C activator ingenol-3-hexanoate (Ing-B, a putative latency-reactivating agent) for 40 days, allowed a 2-week washout period, and then exposed these animals to Ing-B plus vorinostat (total 6 mg/kg) for 15 days. One control animal received antiretroviral therapy alone without Ing-B or vorinostat.

CSF assessments were obtained throughout the study, and the animals were euthanized for examination of brain tissue after the interventions, with measurement of CSF and plasma SIV RNA by quantitative PCR (qPCR) and ddPCR, measurement of SIV DNA in brain by qPCR, and measurements of SIV RNA in brain by in situ hybridization. One animal treated with latency-reactivating agents had a marked rise in plasma and CSF SIV RNA after treatment with the combination of agents, with a 10-fold higher SIV RNA level in CSF than in plasma and development of SIV encephalitis despite continued treatment with antiretroviral therapy. This animal also had increases in levels of CSF neopterin (a marker of macrophage activation), chemokine (CC motif) ligand 2 (CCL2; a marker of monocyte chemoattraction), and neurofilament light chain (NFL; a marker of neuronal injury) after intervention. Moreover, SIV RNA was elevated in the occipital cortex in this animal at sacrifice, and a quantitative outgrowth assay to determine absolute number of infected resting CD4+ T cells harvested from PBMCs revealed that in both treated animals, there was a decline in infected resting CD4+ T cells after use of latency-reactivating agents. Although a small study in a simian model developed for study of encephalitis and thus of accelerated disease with uncertain generalizability to human CNS HIV infection, this is the first study to demonstrate the activity of latency-reactivating agents in the CNS and the potential deleterious effects of this strategy in the CNS compartment.

Mechanisms of Neuropathogenesis in HIV: Immune Activation, Mitochondrial Dysfunction, and Toxicity

That activation of the host immune system resulting in inflammatory-mediated damage in the CNS is a key substrate of HIV-related neurologic injuries is well established. However, the details of the processes and pathways involved in this immune response are still not well understood, and must be determined in order to develop effective therapies for injuries to the nervous system in individuals infected with HIV. As a parallel process to cellular infiltration and soluble inflammatory mediator activation in the CNS, perturbation of blood-CNS barriers may serve an important role in neuropathogenesis of HIV, by allowing increased influx of infected and activated cells and of toxic soluble products into the CNS.

Two oral presentations at CROI 2015 examined the status of the blood-CNS barrier in HIV-infected individuals, using a measure of albumin concentration in CSF compared with blood (CSF-to–plasma albumin ratio). Anesten and colleagues (Abstract 59) measured CSF-to–plasma albumin ratio in 657 HIV-infected individuals categorized by HIV treatment status, and by clinically based diagnosis of HAD versus lack of neurologic symptoms (neuroasymptomatic) in individuals not taking antiretroviral therapy. Neuroasymptomatic individuals were further divided based on CD4+ cell count range. When albumin ratio in each HIV-infected group was compared with that in a group of 53 HIV-uninfected controls who had CSF samples collected for research purposes, statistically significant elevations were only found in the group with HAD. No differences were noted across the CD4+ cell count spectrum between HIV-uninfected controls and HIV-infected individuals whether they were or were not taking suppressive antiretroviral therapy.

When compared with age-determined published cutoffs of upper limit of normal albumin ratio, abnormally elevated levels were detected in 16% of participants in the neuroasymptomatic group that was not taking antiretroviral therapy and in 68% of individuals with HAD. Despite albumin ratios within the normal range in most individuals in the study, NFL (as noted above, a marker of active neurologic injury) level correlated with albumin ratio in the HIV-infected groups, and in the antiretroviral treated, virally suppressed group. In a multivariate model, albumin ratio was a predictor of NFL level independent of age. These results indicate that blood-CNS barrier disruption is associated with neurologic injury in HIV infection, and suggest that this process is a late-stage complication of HIV that is specifically related to severe encephalitis and dementia.

In a longitudinal study of primary HIV infection—defined as within the first year of HIV acquisition—Rahimy and colleagues (Abstract 62) examined the CSF-to–plasma albumin ratio. Albumin ratio measured at baseline (a median 3 months postinfection) in 108 individuals with primary HIV infection was elevated compared with an age-matched group of HIV-uninfected individuals. Over a median 1 and one-half years of longitudinal follow-up prior to initiation of antiretroviral therapy, CSF-to–plasma albumin ratios did not change, suggesting a lack of resolution of blood-CNS barrier disruption during this early period. Moreover, in a smaller cohort (n = 57), the CSF-to–plasma albumin ratio did not statistically change 1 year after initiating antiretroviral therapy. The CSF-to–plasma albumin ratio correlated with CSF NFL level in individuals with primary HIV infection at baseline and in longitudinal follow-up, and inversely correlated with N-acetylaspartate–to-creatine ratio, a neuroimaging measure of neuronal integrity in the parietal grey matter.

Differing results between this study and those noted in Abstract 59 may relate to a lack of age matching in HIV-uninfected comparison participants in the larger study, or to a distinct and dynamic pattern of blood-CNS perturbation that is specific to early HIV infection. In this same cohort of primary HIV infection, Wright and colleagues (Abstract 60) demonstrated that a higher CSF-to–plasma albumin ratio correlated with reduced putaminal volume. These data underscore the potential pathogenic significance of blood-CNS barrier perturbation in CNS and emphasize that processes in HIV infection associated with neurologic damage and neurocognitive impairment are initiated during the early stages of infection.

Edén and colleagues (Abstract 474) focused on the concept that progressive neurologic injury in the CNS in individuals taking suppressive antiretroviral therapy may relate to underlying mechanisms of persistent immune activation. They explored measures of intrathecal macrophage activation (CSF neopterin) and neuronal injury (CSF NFL) in 100 individuals taking antiretroviral therapy who had successful plasma viral suppression (HIV RNA level < 50 copies/mL) and baseline and follow-up CSF and neuropsychologic testing through the CHARTER (CNS HIV Anti-Retroviral Therapy Effects Research) study or the HIV Neurobehavioral Research Center (HNRC). Participants were classified as neurologically normal or as having neurocognitive impairment (ANI or MND) and were assessed for changes in neuropsychologic testing performance between 2 time points. CSF NFL levels were not elevated in the group of individuals with neurocognitive impairment at baseline (n = 70) or in those who had neurocognitive decline over the course of follow-up (n = 32). However, CSF neopterin was elevated in those with impairment at baseline compared with neurocognitively normal individuals, and was also elevated in the group that experienced neurocognitive decline. These data indicate that heightened intrathecal macrophage activation is associated with the presence and progression of impairment in participants on systemically successful treatment. These are the first data to tie a mechanism of neurologic injury to progressive clinical signs in well-treated individuals with HIV infection, and have important implications for strategies to reverse or ameliorate HAND.

Peluso and colleagues (Abstract 473) examined a novel immune activation marker assessed longitudinally in CSF before and after initiation of antiretroviral therapy in study participants with acute or chronic HIV infection in Thailand. CSF YKL-40, a systemic biomarker of inflammation and cancer that localizes to activated microglial cells and reactive astrocytes in the CNS, was measured in 33 individuals with acute HIV infection (median 18 estimated days of infection) compared with chronic infection (n = 34), owing to its predictive value in development of neurodegeneration (including SIV encephalitis). In individuals with acute HIV infection prior to intitiation of antiretroviral therapy, CSF YKL-40 was lower than in participants with chronic HIV infection and was not different than in HIV-uninfected Thai volunteers (n = 18). After initation of antiretroviral therapy during acute HIV

infection (6-month treatment duration), the median level of CSF YKL-40 remained stable and was statistically significantly lower than in those starting treatment during chronic HIV infection (12-month treatment duration). These findings suggest that microglial and perhaps astrocyte activation may be prevented or ameliorated by very early antiretroviral therapy.

Numerous studies presented at CROI 2015 examined how altered cellular bioenergetics or metabolism may contribute to neuropathogenesis of HAND. Two studies employed metabolomic profiling to assess processes associated with neurocognitive dysfunction. Haughey and colleagues (Abstract 497) explored how the composition of energy metabolites in the CSF, as measured by hydrogen nuclear magnetic resonance (^{1}H-NMR) spectroscopy, may relate to neurocognitive status over time in HIV-infected individuals. Using partial least squares regression, which allowed them to identify predictors associated with outcomes from a very large number of potentially contributing variables, these investigators found that the metabolites in CSF involved with aerobic glycolysis along with other clinical factors were predictive of neurocognitive decline, whereas those involved with anaerobic glycolysis were predictive of neurocognitive improvement. This pattern suggests potential novel approaches for predicting response to therapy in HIV-infected individuals. In a related study, Cassol and colleagues (Abstract 498) analyzed cellular metabolites in blood plasma using liquid or gas chromatography followed by mass spectrometry in 68 HIV-infected and 36 HIV-uninfected study participants. Participants were further classified by depression status based on a self-administered depression inventory. Depressed individuals had lower levels of metabolites of phenylalanine-tyrosine catabolism and acylcarnitine than individuals without depression. Interestingly, these results were found both in the HIV-infected and HIV-uninfected groups, suggesting that this pathway might be an important target for the treatment of depression regardless of HIV infection.

Alteration in mitochondrial function is postulated to potentially result from HIV infection itself and the toxic effects of antiretroviral medications. Funes and colleagues presented data from a study that investigated nitric oxide as a potentially crucial molecule in the mechanism of efavirenz-induced neuronal toxicity (Abstract 500). Levels of nitric oxide synthase and nitric oxide (a free radical associated with mitochondrial dysfunction and inflammation) were measured in human brain tumor cell lines and cultured rat cortical neurons after brief exposure to efavirenz. In this system, efavirenz provoked inducible nitric oxide synthase in glial cells, and this increase in nitric oxide impaired mitochondrial function. These effects were not seen in neurons, suggesting that bioenergetic toxicity of efavirenz may occur through glial dysfunction rather than neuronal injury. Therapies aimed at reducing nitric oxide or its precursors may reduce the potential adverse effects associated with efavirenz.

Another molecule that has been implicated in the etiology of HAND through induction of cell death and CNS inflammation is HIV transactivator of transcription (Tat). Brew and colleagues (Abstract 505) explored whether HIV Tat levels in CSF might remain persistently abnormal in individuals taking suppressive antiretroviral therapy, as Tat can be secreted by infected cells even during therapy. HIV Tat remained detectable in the CSF in 5 (13.5%) HIV-infected individuals taking antiretroviral therapy who had HIV RNA levels below 50 copies/mL in blood or CSF (n = 37). Detectable Tat was not associated with current HAND status or B-cell CLL/lymphoma 11B (zinc finger protein) (BCL11B), a putative measure of viral latency. However, further studies are needed to investigate whether levels of CSF Tat detected in individuals on successful systemic treatment may be associated with other highly sensitive markers of neuronal injury, such as CSF NFL or imaging markers of inflammation or neuronal injury.

Several studies focused on heme oxygenase-1 (HO-1) as a potentially neuroprotective factor in HIV-related brain injury. HO-1 has been previously shown to be reduced in the frontal cortex and striatum of HIV-infected individuals with HAD, and inversely related to HIV RNA level and neuroinflammation.[4] Gill and colleagues (Abstract 501) focused on the relationship between HIV strain and degree of macrophage HO-1 down regulation by studying an in vitro model of HIV-infected monocyte-derived macrophages (MDMs) isolated from noninfected donors and infected the cells with 15 HIV strains. In this study, replication of HIV strains consistently reduced HO-1 in MDMs. Levels of HO-1 were inversely associated with levels of viral replication and extracellular glutamate measured in supernatant in this MDM model, suggesting that enhancing production of HO-1 in MDMs may benefit the CNS in HIV.

HIV neuropathogenesis appears to relate to processes such as blood-CNS barrier disruption and intrathecal immune activation, which may persist despite initiation of antiretroviral therapy. Alteration of intracellular energy metabolism may be associated with neurologic or psychiatric morbidity and may be worsened by neurotoxic medications.

Duncan and colleagues (Abstract 502) presented a study that identified atorvastatin as a medication with potential benefit for the HO-1 deficiency noted in HIV infection. Atorvastatin belongs to a class of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors that has numerous putative immunomodulatory effects, including reduction of monocyte or macrophage activation. Thus, the investigators used an in vitro model of primary MDMs infected with a macrophage-tropic HIV strain to assess the impact of atorvastatin treatment on HO-1. They found that although HIV infection of MDMs reduced HO-1, atorvastatin added to MDMs at 8 days postinfection increased HO-1 protein expression. This study not only

provides rationale for a targeted treatment study based on these preliminary findings, it also suggests a potential beneficial mechanism of broad statin use in ameliorating the neurologic processes associated with HAND.

Diagnosis of HAND

Key questions remain concerning how to identify HIV-infected individuals at increased risk for HAND. Rourke and colleagues (Abstract 465) assessed 575 HIV-infected adults taking antiretroviral therapy in the OCS (Ontario HIV Treatment Network Cohort Study). At baseline, participants had neuropsychologic performance testing and were categorized as normal (n = 299) or as having ANI (n = 276). At 52 weeks of follow-up a greater percentage of individuals with ANI progressed to more severe forms of HAND (MND or HAD) compared with the cognitively normal HIV-infected individuals. Factors associated with faster progression included depression and history of smoking.

Similar results were observed by Brouillette and colleagues (Abstract 469) in a subset of the CHARTER cohort (n = 191). Factors associated with cognitive decline included atherosclerotic vascular disease, duration of HIV infection, and education level. The investigators noted that a large proportion of the CHARTER cohort (approximately 80%) had modifiable risk factors, including smoking and a body mass index of 25 or higher. These risk factors could be targeted by primary care physicians.

The effects of potentially modifiable risk factors were further confirmed by several groups, using the VACS (Veterans Aging Cohort Study) Index score (risk points assigned for age, CD4+ cell count, plasma HIV RNA level, hemoglobin value, fibrosis stage, renal glomerular filtration rate, and presence of hepatitis C virus infection). Calcagno and colleagues (Abstract 487) demonstrated that HIV-infected participants with HAND (n = 441) were at higher cardiovascular risk based on the VACS Index. Rourke and colleagues (Abstract 467) showed that a higher VACS Index score at baseline was associated with greater cognitive decline (as assessed by neuropsychologic performance testing) at subsequent follow-up. Collectively, these results suggest that screening of higher-risk HIV-infected participants based on cardio- and cerebrovascular abnormalities (including smoking) may assist in the early diagnosis of HAND. Treating these modifiable risk factors could lead to a reduction in HAND.

Identification and treatment of modifiable risk factors (eg, smoking, obesity, or depression) could reduce HIV-associated neurocognitive disorders.

Neuroimaging

A variety of neuroimaging techniques were used to assess the effects of HIV in the CNS. Becker and colleagues (Abstract 494) used a trajectory model based on volumetric data from 3892 HIV-infected individuals followed by the MACS (Multicenter AIDS Cohort Study) to identify 3 possible trajectories of disease progression: 1) normal aging, a profile with relatively low probability of even mild impairment until middle age; 2) premature aging, a profile with the probability of mild impairment occurring at age 45 years to 50 years; and 3) unhealthy, a profile with a high probability of impairment at a young age. Changes in the posterior cingulate–precuneus cortex, the hippocampus, and the inferior frontal cortex were associated with the unhealthy profile, and changes in the cingulate gyrus, the insula, and the basal ganglia were associated with the premature aging profile.

Observed neuroimaging differences may assist in differentiating between the effects of HIV and those with aging. Sacktor and colleagues (Abstract 482) obtained ^{18}F-AV-45 positron emission tomography (PET) scanning to assess amyloid deposition in HIV-infected participants (n = 25) and HIV-uninfected controls (n = 6). HIV-infected individuals with symptomatic HAND (MND and HAD) had mildly increased amyloid depositions in the hippocampus and basal ganglia compared with HIV-infected individuals with ANI or normal cognition. Observed areas of increased amyloid deposition were different than those typically seen with Alzheimer's disease. However, amyloid depositions were not different for HIV-infected individuals compared with HIV-uninfected individuals.

Using another PET ligand, Vera and colleagues (Abstract 477) examined the relationship between microbial translocation (measured by 16S ribosomal [r] DNA) and brain inflammation ([^{11}C]–peripheral benzodiazepine receptor [PBR] 28) and structure (by diffusion tensor imaging [DTI]) in HIV-infected individuals (n = 12). An association existed between plasma 16S rDNA and brain biomarkers (^{11}C-PBR28 and DTI metrics). In addition, Smith and colleagues (Abstract 485) observed increased inflammation using a 3D postcontrast T2-weighted fluid-attenuated inversion recovery (FLAIR) imaging technique. A greater proportion of HIV-infected participants had focal leptomeningeal contrast enhancement than did HIV-uninfected participants. Observed enhancement was not correlated with CD4+ cell count, CD4+ cell nadir, duration of HIV infection, or history of neurologic disorders. Finally, Cohen and colleagues (Abstract 935) demonstrated that perinatally HIV-infected children (n = 35) had statistically significant structural imaging (DTI and volumetric) and neuropsychologic performance differences compared with healthy, HIV-uninfected children (n = 37). These neuroimaging studies hint at the possibility of including imaging biomarkers in diagnostic criteria for HAND.

Novel neuroimaging markers may noninvasively assess structural and inflammatory changes seen in asymptomatic HIV-infected individuals, and thus may provide preclinical markers of and insight into pathogenesis of HIV-associated neurocognitive disorders.

Treatment of HAND

A number of different treatment options are now available and should

be considered for HIV-infected patients, especially those with HAND. Bowman and colleagues (Abstract 445) assessed HIV-infected, antiretroviral treatment–naive participants (n = 40) before and 2 weeks after initiating therapy. Viral load in blood decayed faster than in CSF after initiation of antiretroviral therapy. In general, HIV protease inhibitors were associated with faster CSF viral suppression, and integrase strand transfer inhibitors were associated with slower suppression.

There is increasing concern regarding the potential adverse effects of efavirenz on cognition. In a large Canadian cohort (n = 831), Rourke and colleagues (Abstract 448) observed no differences among HIV-infected participants who were currently taking efavirenz, those who had previously received efavirenz, or those who had never taken efavirenz. Although these results complement previous published studies nicely,[5,6] they conflict with other reports, including studies presented at CROI 2015 that suggest the potential neurotoxicity of efavirenz.

Ma and colleagues (Abstract 444) assessed the incidence of neurocognitive impairment in HIV-infected participants (n = 23) in China assigned an antiretroviral regimen of tenofovir, lamivudine, and efavirenz compared with a regimen of zidovudine, lamivudine, and nevirapine. A higher incidence of cognitive impairment was seen in HIV-infected participants receiving the regimen that included tenofovir than in those receiving the efavirenz-containing regimen. As part of the same randomized study, Letendre and colleagues (Abstract 56) presented results of these regimens used in antiretroviral treatment–naive, HIV-infected adults in China. Participants were randomly assigned to receive an open-label antiretroviral regimen of zidovudine, lamivudine, and nevirapine or tenofovir, lamivudine, and efavirenz, and were assessed longitudinally by neuropsychologic performance testing at 48 weeks and 96 weeks. The group taking the efavirenz-containing regimen had a greater risk of incident neurocognitive impairment than the group taking the nevirapine-containing regimen. However, those receiving the nevirapine-containing regimen had more adverse events than those receiving treatment containing efavirenz. For each of these studies, the investigators hypothesized that observed differences may reflect differences in drug distribution into the CNS or neurotoxicity.

With regard to other therapies currently being used to treat HIV-infected individuals with HAND, Caramatti and colleagues (Abstract 442) observed no differences in neuropsychologic performance in participants (n = 37) who received monotherapy with ritonavir-boosted (/r) atazanavir compared with those who received triple therapy that included atazanvir/r. For both groups, a substantial decrease in HAND was seen at 96 weeks. These results complement work by Ferretti and colleagues (Abstract 443) in a smaller nested cohort (n = 23), which showed that CSF viral escape was similar between HIV-infected participants receiving long-term, successful monotherapy with atazanvir/r and those receiving triple therapy that included atazanvir/r. Baker and colleagues (Abstract 447) demonstrated that the CNS penetration effectiveness (CPE) of antiretroviral therapy did not affect neuropsychologic performance and brain volumetrics in a cohort of HIV-infected participants (n = 64). No differences in neuropsychologic performance testing results or brain volumetrics existed between groups with low versus high CPE scores.

Gates and colleagues (Abstract 441) conducted a small randomized controlled trial among virologically suppressed HIV-infected participants with HAND (n = 19) who were assigned to receive antiretroviral therapy or antiretroviral therapy enhanced with maraviroc. Maraviroc was chosen because of its high level of CNS penetration and dual antiretroviral and anti-inflammatory activity. At 52 weeks, neuropsychologic performance had improved more in the maraviroc-containing arm than in the control arm, and neuroimaging measures of glutamate concentrations (a possible measure of excitotoxicity) were higher in the control arm than in the maraviroc-containing arm. Evering and colleagues (Abstract 446) studied individuals taking prolonged (median, 5.7 years) antiretroviral therapy that had been initiated at a median 1.6 months after infection, who did not have comorbidities such as depression or substance abuse. Cognitive impairment was observed in only 4% (1/26) of this group, suggesting the potential benefit of early treatment. Although many of these preliminary studies are promising, larger studies with longer follow-up are needed for HIV-infected participants with varying degrees of cognitive impairment.

Antiretroviral therapy enhancement with maraviroc may lead to reduced inflammation and better neurocognition. However, larger longitudinal studies are needed.

Adjunctive measures (eg, exercise and engagement in mental exercises) were also considered for HIV-infected participants at risk for cognitive disorders. Basco and colleagues (Abstract 488) studied neuropsychologic performance, neuroimaging, and self-reported aerobic exercise in HIV-infected individuals categorized as physically active (n = 22) or sedentary (n = 48). Physically active participants performed statistically significantly better than sedentary HIV-infected participants on neuropsychologic performance tests of executive function but not of motor function. Monroe and colleagues (Abstract 489) found similar results in the large MACS cohort of HIV-infected men (n = 622). High physical activity was associated with better neuropsychologic performance on executive and psychomotor tests than was low physical activity. Whether exercise leads to cognitive improvement or whether cognitive improvement leads to an increased ability to participate in exercise remains in question.

Pinnetti and colleagues (Abstract 63) demonstrated that in a single-site cohort (n = 569), better virologic control (higher current CD4+ cell count and lower viral load) and higher education level were associated with reduced

risk of developing HAND in the current antiretroviral therapy era. Results from Milanini and colleagues (Abstract 495) showed that in a cohort of 50 HIV-infected participants, higher cognitive reserve (assessed by IQ) was associated with lower risk of ANI. Overall, these studies suggest that engagement in physical, intellectual, and social activities may independently protect against cognitive impairment in HIV-infected individuals, but larger studies with more detailed measurements of physical function and cognitive reserve before and after an intervention are needed.

Adjunctive measures (including engagement in physical and mental exercises) may help reduce HIV-associated neurocognitive disorders. However, larger longitudinal studies are needed.

New Frontiers for Understanding HAND and HIV Neuropathogenesis: Resource-Limited Settings

At CROI 2015, neurologic studies conducted in resource-limited settings, led by or in collaboration with local investigators were emphasized. Kambugu and colleagues (Abstract 57) presented data from the EARNEST (Europe-Africa Research Network for Evaluation of Second-Line Therapy) study examining the magnitude of and factors associated with neurocognitive function at the time of failure of initial antiretroviral therapy, and assessing any changes in neurocognitive function that may occur after switching antiretroviral treatment. The EARNEST trial enrolled 1277 participants whose initial antiretroviral therapy had failed according to World Health Organization clinical and immunologic criteria at the time of the study design. Individuals were randomly assigned to receive lopinavir/r plus 2 or 3 nucleotide analogue reverse transcriptase inhibitors; a protease inhibitor plus raltegravir; or protease inhibitor mono therapy (with a 12-week raltegravir induction period). Three domains were examined by neurologic testing at baseline, week 48, and week 96: the Color Trails Test 1 (measuring attention and concentration), the Color Trails Test 2 (measuring cognitive flexibility), and the Grooved Pegboard test (measuring psychomotor speed/fine motor skills). Test scores were standardized to a *z* score based on demographically adjusted, US-derived norms and were then averaged into an overall score (NPZ-3 score). Mean composite *z* score at baseline was -2.96, suggesting that individuals in this study had performance levels approximately 3 standard deviations below the norm at baseline. In multivariable analyses, *z* scores were independently lower with a number of factors, including older age, lower body weight, higher viral load, lower hemoglobin value, and fewer years of education. Scores improved substantially after starting antiretroviral treatment, with equal improvement between regimen arms. The dramatically low *z* scores of individuals in the EARNEST study at baseline may reflect true substantial cognitive impairment or may indicate that norms derived from the United States may not yield accurate results when assessing individuals in resource-limited settings.

A number of other studies detailed the prevalence or incidence of HAND, or response to treatment in resource-limited settings. Robertson and colleagues (Abstract 451) presented the results of the AIDS Clinical Trials Group (ACTG) 5199 (International Neurological Study) and the ACTG 5271 (International Neurocognitive Normative Study) studies. These large studies accrued more than 3200 participants over 12 years in various countries (Malawi, Thailand, Brazil, India, Peru, Zimbabwe, and South Africa), specifically included site-specific HIV-uninfected individuals appropriately matched to HIV-infected articipants, and performed extensive coordinated oversight of the sites involved in the study for quality control. These studies demonstrated that the prevalence of neurocognitive impairment compared with well-matched HIV-uninfected controls is similar to what is seen in the United States: 25% with mild impairment, 17% with moderate impairment, 3% with severe impairment, and 54% falling in the normal range. Effective antiretroviral therapy reduced neurocognitive impairment substantially over time, from 46% at baseline to 28% at week 168 of treatment.

Sacktor and colleagues (Abstract 452) conducted a large study of HIV-infected (n = 299) and HIV-uninfected indiviuals (n = 210) living in rural Rakai, Uganda, and found a very high rate of HAD in antiretroviral treatment–naive individuals compared with normative data from HIV-uninfected individuals in Kampala, Uganda. Twenty-seven percent of individuals with HIV infection met criteria for HAD compared with 7% of HIV-uninfected individuals. Most HIV-infected individuals in this region harbor HIV subtype (or clade) A or D, allowing for important future analyses regarding possible associations between HIV subtype and risk for HAND. Finally, Valcour and colleagues (Abstract 459) studied more than 900 participants from East Africa in order to investigate determinants of neuropsychologic performance in the AFRICOS (The African Cohort Study) study. Cognitive impairment, as measured by all testing methods, was associated with HIV infection status, age, and level of education. Nadir CD4+ cell count was also associated with performance on 2 specific tests. However, cognitive performance was not associated with number of infectious and noninfectious comorbidities, a finding distinct from studies in resource-endowed settings. All of these studies highlight the need for appropriate norms to study correlates to neurocognitive disorder that are relevant in resource-limited settings, and the need to collaborate with and foster investigations by local experts in order to implement highly rigorous and relevant research in these settings.

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