CROI 2014: Neurologic Complications of HIV Infection

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A shift in focus in the field of neuroHIV was clearly manifest at the 2014 Conference on Retroviruses and Opportunistic Infections (CROI), where a major emphasis was on the milder forms of neurologic morbidity, including cognitive impairment, seen in well-treated patients. Mechanisms of this persistent abnormality were investigated, including extensive analysis of the prevalence and associations of persistent HIV detection in cerebrospinal fluid (CSF) and characterization of persistent CNS immune activation. Another key emphasis was the early establishment of HIV replication and inflammation within the central nervous system (CNS) and the potentially salutary effect of very early HIV diagnosis and treatment in protecting the CNS from HIV-related injury. Mitochondrial function was identified as a potential mediator of a number of aspects of HIV-associated CNS dysfunction, including neurotoxicity associated with efavirenz, host genetic determinants of HIV-associated neurocognitive disorders (HAND), associations with direct measures of mitochondria in CSF, and metabolomic screening of CSF in HIV-infected subjects and those with HAND. Many studies employed laboratory rather than neuropsychologic end points, with a major focus on CSF biomarkers. Overall, neuroHIV presentations at CROI 2014 provided new insights into pathogenesis and treatment of the CNS, raising new challenges for researchers and practitioners aiming to optimize the status of the brain in people living with HIV infection.

Keywords: biomarker, central nervous system, CROI 2014, dementia, HAND, HIV, HIV-associated neurocognitive disorder, neuroimaging, neurology, neuropathy, viral escape

Mechanisms of Persistent CNS Injury on Antiretroviral Therapy

As highlighted in a plenary talk on neuroHIV (Abstract 66), a major theme of the scientific presentations at the 2014 Conference on Retroviruses and Opportunistic Infections (CROI), held March 3 to 6, 2014, was the characterization and investigation of central nervous system (CNS) perturbation during antiretroviral therapy. A number of lines of evidence suggest that the CNS may exhibit persistent abnormalities in patients on antiretroviral therapy. For example, several studies report neuropsychologic testing performance in an impaired range in a higher proportion of HIV-infected persons on antiretroviral therapy than -uninfected persons,[1,2] with the proportions of impaired subjects in distinct studies ranging from 18% to 80% depending on the demographics of the subject populations and the -uninfected control groups employed for comparison. Additionally, brain imaging[3] and cerebrospinal fluid (CSF) biomarkers[4] suggest neuronal injury in a proportion of treated patients. Notably, in these investigations, many study volunteers with HIV infection manifest neuropsychologic performance, imaging, and CSF markers that are within the normal range, suggesting that not all patients with HIV infection are living with CNS injury. However, research is needed to explain, and address, the CNS injury detected in some individuals who appear to be optimally treated for HIV infection with antiretroviral therapy.

CSF HIV Escape or CSF/Plasma HIV Discordance in Patients on Antiretroviral Therapy

A variety of factors may underlie detection of abnormality in the CNS in some patients treated with antiretroviral therapy. One possibility is that viral replication may not be completely suppressed by antiretroviral therapy within the CNS compartment, or that HIV persistence within cells of the CNS contributes to intermittent release of virus and thus a cascade of events in the CNS that allows for ongoing damage despite antiretroviral therapy. Despite early findings that the CNS responds very favorably to antiretroviral therapy, as reflected by dramatic improvement of signs and symptoms of severe dementia in many patients and typical reduction of CSF HIV RNA levels, recent evidence suggests that with more sensitive testing, the CNS may be a site of continued viral release or replication in some patients even when the periphery is controlled. Detection of HIV in the CSF despite antiretroviral therapy that successfully suppresses plasma HIV RNA levels has been described in neurologically asymptomatic research subjects[5] and neurologically symptomatic patients.[6]

The phenomenon of CNS viral escape from antiretroviral therapy—defined in different studies as detectable CSF HIV RNA in the presence of undetectable plasma HIV RNA, a discordance of greater than 0.5 $\log_{10}$ copies/mL or more between CSF and plasma HIV RNA levels in fairly well-suppressed patients, or most strictly, greater than 50 copies/mL in CSF with less than 50 copies/mL in plasma—was described in numerous studies at CROI 2014 and was the focus of a themed discussion (Session 15). Nightingale

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and colleagues (Abstract 442) presented findings from the PARTITION (Penetration of Antiretroviral Therapy into the Nervous System) study in the United Kingdom. This study evaluated subjects with signs or symptoms of possible neurologic disease (typically headache or cognitive impairment) or with unexplained plasma HIV RNA levels greater than 50 copies/mL to assess whether either of these phenomena might associate with CSF/plasma discordance. In their study, discordance—defined as CSF HIV RNA levels more than 0.5 $\log_{10}$ copies/mL higher than plasma levels—was found in 13.1% of 145 subjects and associated with longer duration of HIV infection and lower nadir CD4+ cell count. Low-level detection of HIV RNA in CSF was more frequent in subjects with blood plasma residual viremia (detectable HIV RNA levels between 10 copies/mL and 49 copies/mL), though whether CSF HIV is a consequence or cause of residual viremia in the plasma is unclear.

Importantly, despite low median CSF HIV RNA levels in the discordant subjects, CSF from the 4 subjects who underwent HIV genotyping demonstrated resistance mutations, including subjects with levels of only 138 copies/mL and 162 copies/mL in CSF, suggesting that these may have been biologically relevant levels of CSF viral escape from antiretroviral therapy. Pinnetti and colleagues (Abstract 443) analyzed paired CSF and plasma samples from neuroasymptomatic subjects for evidence of CSF viral escape, which they defined as HIV RNA levels greater than 50 copies/mL in CSF and less than 50 copies/mL in plasma or a CSF/plasma HIV RNA discordance of greater than 1.0 $\log_{10}$ copies/mL; of 303 sample pairs analyzed, 10.6% met 1 of these criteria. The investigators found that CSF viral escape was more frequent in subjects with CD4+ cell counts less than 350/µL and with antiretroviral regimens containing atazanavir boosted with ritonavir versus regimens containing a different third drug.

To assess the question of whether HIV persistence in CSF is associated with active HIV-related neurologic damage, Eden and colleagues (Abstract 445) examined 75 subjects in a longitudinal cohort who had plasma HIV RNA levels less than 50 copies/mL at enrollment. Of these subjects, 23% had CSF HIV RNA levels greater than 50 copies/mL detected during at least 1 study visit. Only 3% had CSF viral escape by this definition in consecutive samples, perhaps indicating persistent replication within the CNS versus intermittent detection of HIV in this compartment, possibly equivalent to a plasma "blip." Although CSF neopterin, a pteridine marker of intrathecal immune activation, was higher in the subjects with detectable versus undetectable CSF HIV RNA, a biomarker of active neuronal injury, CSF neurofilament light chain (NFL), was no different between these 2 groups. These findings suggest that though viral detection and immune activation in the CNS during antiretroviral therapy are linked, CNS persistence, at least by this measure, may not be a cause of neuronal injury detected during antiretroviral therapy.

Overall, the clinical and biologic significance of asymptomatic CSF viral escape or discordance remains unclear. Hammond and colleagues (Abstract 33) presented an analysis of CSF and mood data from the CHARTER (CNS HIV Anti-Retroviral Therapy Effects Research) study to examine whether signs or symptoms of depression may associate with detectable CSF HIV RNA in treated subjects. Subjects were included in the current analysis if they did not have depression at baseline and also had a number of CSF samples available. Using the Beck Depression Inventory to assess mood and a cutoff of 17 points or greater on this evaluation as a marker of moderate to severe depression, these investigators found that 14.4% had CSF HIV RNA levels greater than 50 copies/mL at the baseline visit. Participants with a detectable CSF HIV RNA at any visit had a 4.7x greater risk than those with consistantly undectable CSF HIV RNA for developing new onset of moderate to severe depression during the course of the study. Although cause and effect cannot be clearly established in this study, which may be affected by potential effects of variable adherence and other issues, these findings are provocative in suggesting that depression in HIV-infected persons may have a neuroinflammatory or virologic basis and that CSF viral escape may have clinical consequences.

Persistent CNS Immune Activation During Antiretroviral Therapy

Viral persistence may be related to or independent from smoldering, low-grade immune activation detected within the CNS during antiretroviral therapy in some patients.[7,8] This immune activation, considered the substrate of HIV-associated neurocognitive disorder (HAND), may thus perpetuate progressive neuronal injury despite treatment. Several presentations at this year's CROI explored the nature of and mechanisms underlying persistent immune activation on antiretroviral therapy. Abstract 445, as noted, confirmed prior findings that low-level CSF HIV RNA in the setting of antiretroviral therapy associates with CSF neopterin.

Eden and colleagues (Abstract 490) presented a separate study of 100 subjects with plasma viral suppression on antiretroviral therapy identified through the multisite CHARTER study and the HIV Neurobehavioral Research Center in San Diego, California. CSF markers, including CSF neopterin and CSF NFL, were compared between subjects classified as neuropsychologically normal (NPN) and those identified as having neuropsychologic impairment—either asymptomatic neurocognitive impairment or mild neurocognitive disorder. CSF neopterin was associated with impairment, with median levels in the impaired group (7.3 nmol/L) statistically significantly elevated compared with median levels in the NPN group, and higher than the upper limit of normal reference value (5.8 nmol/L). This is one of the first studies to correlate persistent immune activation with neuropsychologic outcomes, suggesting that inflammation may indeed produce neurologic damage and measurable reduction in

performance. However, although CSF neopterin correlated with CSF NFL in the 100 subjects studied, there was no statistically significant difference in NFL levels between the NPN and impaired groups, suggesting that neuropsychologic performance impairment may not reflect active axonal injury in the setting of treated HIV infection.

Peterson and colleagues (Abstract 491) examined 76 paired plasma and CSF samples from 45 subjects on systemically suppressive (plasma HIV RNA level < 40 copies/mL) antiretroviral therapy using single-copy assay measurement of CSF and blood HIV RNA and a panel of 8 biomarkers of inflammation (not including neopterin) as well as NFL to assess axonal injury. CSF biomarkers were compared between the HIV-infected subjects and 21 -uninfected controls, and statistically significant elevations in tumor necrosis factor (TNF)-α and soluble CD163 were indicated. Single-copy assay measurement revealed detectable CSF HIV RNA in 17% of total CSF samples versus 57% of total plasma samples. In this study, unlike Abstract 445 and prior studies, no correlations were found between detection of HIV in the CSF by this highly sensitive assay and level of intrathecal immune activation.

In a complementary study, Peterson and colleagues (Abstract 484) demonstrated in a large cross-sectional investigation of HIV-infected subjects assessed at different stages of HIV infection that CSF and plasma biomarkers of immune activation diverged with progressive immune compromise and neurologic disease in HIV infection. In primary HIV infection, intrathecal immune activation markers tended to parallel those in the blood, whereas in more advanced HIV infection and HIV-associated dementia, biomarkers of immune activation tended to be higher in the CSF compartment than in the blood. These findings suggest that although blood might serve as a proxy for direct CNS sampling in the early stages of HIV infection, in more advanced disease, interrogation of CNS tissue via collection of CSF may add information beyond that available in the periphery.

Potentially novel biomarkers of immune activation, which may reveal information about not only viral persistence but mechanisms of ongoing inflammation, was another topic of presentation at CROI 2014. Perez-Santiago and colleagues (Abstract 446) presented a study of cell-free mitochondrial DNA (mtDNA) in CSF, measured by droplet digital polymerase chain reaction (PCR), from 28 HIV-infected individuals. These investigators found that in individuals with impairment, higher levels of mtDNA were associated with neurocognitive impairment and levels of inflammation. These investigators also demonstrated that mtDNA rose after treatment interruption in the CSF prior to increases in CSF white blood cell count, supporting the concept that mtDNA detected in CSF is not simply a proxy for pleocytosis in HIV infection. These investigators posit that mtDNA detected in the CSF in HIV-infected individuals might reflect the breakdown of neurons or might potentiate inflammation within the CNS during HIV infection.

Another potentially novel biomarker was presented by Vera and colleagues (Abstract 486LB), who demonstrated abnormal levels of brain uptake of [^{11}C]PBR28, a radioligand that recognizes a receptor for a translocator protein expressed on microglia, in 12 asymptomatic HIV-infected subjects on suppressive antiretroviral therapy versus 10 -uninfected controls. Greater volume of distribution (V_T) of the radioligand, interpreted as a measure of microglial activation, associated with plasma HIV RNA levels prior to antiretroviral therapy and inversely associated with performance in specific neuropsychologic domains. Longitudinal follow-up of subjects assessed with this positron emission tomography imaging marker should reveal whether elevated [^{11}C]PBR28 V_T observed in HIV-infected subjects has a predictive value in the development of neurocognitive impairment.

Vascular Pathology, Aging, and CNS Injury in HIV Infection

Excess cardiovascular disease has been noted in well-treated HIV-infected patients, leading investigators in the field of neuroHIV to explore whether vascular pathology, likely related to chronic low-grade systemic immune activation, may be a new mechanism underlying neurologic injury in the antiretroviral therapy era that is largely distinct from the classic mechanisms of HIV pathogenesis seen in the pre–antiretroviral therapy era. Several prior studies found markers of vascular disease or classic vascular risk factors to be greater predictors of HAND in subjects treated with antiretroviral therapy than HIV disease factors or even HIV serostatus.[9,10] Other recent research has highlighted an increased risk of overt ischemic stroke in persons with HIV infection, perhaps disproportionately affecting young people and women.[11,12]

Soontornniyomkij and colleagues (Abstract 35) examined the relationship between cerebral small vessel disease and HAND in subjects treated with antiretroviral therapy. These investigators examined brain tissue specimens from 144 deceased, mostly male, chronically HIV-infected tissue donors for research purposes. Most subjects had neuropsychologic testing prior to death, and 44% had been on what was considered combination antiretroviral therapy prior to death. Cerebral small vessel disease was assessed in white matter brain tissue and defined on histopathology as absent, mild (reflecting partial-thickness involvement of vessel walls), or moderate to severe (full-thickness involvement). Histopathology evidence of mild cerebral small vessel disease was associated with diagnosis of HAND during life. Mild and moderate to severe cerebral small vessel disease was associated with protease inhibitor (PI)-based combination antiretroviral therapy prior to death. These findings are compelling in that they suggest that cognitive impairment seen in HIV-infected subjects may be directly associated with vascular disease in the era of antiretroviral therapy and, further, that PI treatment may contribute to the presence of this vascular disease independent of the effects of classic risk factors such as diabetes and hypertension.

Urday and colleagues (Abstract 463) presented data from a small study investigating correlations between neuropsychologic testing performance and vascular measures during primary HIV infection. In 15 subjects enrolled at a median of 3 months postinfection, associations were examined between neuropsychologic testing performance and vascular measures available from corresponding time points, including brachial artery flow–mediated dilation, carotid intima-media thickness, and a putative blood biomarker of endothelial dysfunction, asymmetric dimethylarginine (ADMA). In this pilot study, higher ADMA levels tended to correlate with poorer neuropsychologic performance, a novel finding that might suggest a role of this inhibitor of nitric oxide synthase in HAND, either through vascular damage or loss of neuroprotection. Carotid intima-media thickness, considered a marker of vascular pathology, correlated with better performance on neuropsychologic testing in this study, contrary to the relationship observed in prior studies in chronic HIV infection. These findings warrant further evaluation in a larger group of subjects.

Several studies investigated the status of the CNS in aging HIV-infected populations on stable antiretroviral therapy. Magnus and colleagues (Abstract 449) employed functional magnetic resonance imaging (MRI) to assess the effects of suppressive antiretroviral therapy on brain structure and function among HIV-infected subjects aged 50 years and older. These investigators measured cerebral white matter volumes and brain activity in response to a task switch in 10 HIV-infected individuals, comparing these with measures in 5 age-matched -uninfected controls. In the HIV-infected subjects, although neuropsychologic testing performance was only mildly impaired, white matter brain volume was reduced, and task switching led to increased brain activity detected on functional MRI, implying that a greater impact was associated with the task switch in these subjects. Additionally, HIV-infected subjects demonstrated a higher number of detectable responses to irrelevant stimuli, interpreted as decreased inhibition in these networks. These findings confirm prior reports that functional MRI is a sensitive marker that may reveal pathology and also potential pathways of subtle neurologic injury in antiretroviral therapy–suppressed subjects with HIV infection.

Nowak and colleagues (Abstract 450) longitudinally assessed cortical brain volumes using structural MRI imaging in a group of 18 subjects with a baseline median age of 48.4 years, demonstrating statistically significant atrophy over a 1-year period in patients on stable antiretroviral therapy. Although no HIV-infected control subjects were available for comparison of cortical atrophy over this period, a strong correlation between nadir CD4+ cell count and cortical atrophy in numerous brain regions suggests that prior immunologic injury may be a determinant of subsequent atrophy in HIV-infected persons despite stabilization of virologic and immunologic parameters. Becker and colleagues (Abstract 448) also examined HIV- and age-related brain atrophy using structural MRI, comparing cross-sectional, whole-brain volumes in 186 HIV-infected and 142 -uninfected men enrolled at a median age of 57.5 years in MACS (Multicenter AIDS Cohort Study). In this study, HIV-infection and age were associated with reduced whole-brain volumes, though the effect of age was stronger than that of HIV serostatus. Importantly, no clear interaction was noted in whole-brain volumes between age and infection with HIV, implying that an accelerated aging phenotype was not revealed by this brain measure.

CSF hyperphosphorylated tau (p-tau) was examined as a potential biomarker for neurologic aging in subjects with HIV infection and comparison control subjects in a study by Krut and colleagues (Abstract 453). CSF p-tau, a neural marker used in the diagnosis of Alzheimer's disease and other neurodegenerative dementias, is a microtubule-associated protein that serves to stabilize axons. These investigators examined p-tau levels across the age spectrum in 291 HIV-uninfected controls, 172 HIV-infected neuroasymptomatic subjects off antiretroviral therapy, 68 HIV-infected subjects on antiretroviral therapy with plasma HIV RNA levels less than 50 copies/mL, and 33 subjects with HIV-associated dementia. In the HIV-infected subjects, p-tau levels correlated statistically significantly with age only in those on suppressive antiretroviral therapy, perhaps suggesting that p-tau increases in untreated HIV-infected subjects are a consequence of neuropathologic mechanisms distinct from aging. Further examination of CSF p-tau as a possible predictive measure of neurologic status or HAND in HIV-infected individuals may reveal a utility for this biomarker in neurologic assessment in this population.

CNS Consequences of Antiretroviral Therapy Exposure and Toxicity

A key question in the field of neuro-HIV remains whether antiretroviral therapy exposure within the CNS contributes importantly to the abnormalities detected in HIV-infected subjects who appear to be successfully treated from a systemic standpoint. The concept that better versus poorer CNS penetration and effectiveness of antiretroviral medications might differentially impact the efficacy of treatment in the brain versus the periphery has been the subject of intensive scrutiny over the past 10 years.[13,14] One new concern related to this fundamental issue is that although subjects on reduced-drug regimens designed to limit systemic toxicity, cost, and nonadherence associated with combination antiretroviral therapy may do well from a systemic virologic standpoint, it is possible that these regimens may not adequately suppress HIV in the CNS compartment. Stephan and colleagues (Abstract 473) studied 24 subjects on boosted, dual PI therapy and 131 subjects on classic triple-therapy regimens followed in the Frankfurt HIV Treatment Cohort. The median CSF HIV RNA level in the dual PI group was 600 copies/mL versus 50 copies/mL in the triple-therapy group. Although

a lower proportion of subjects in the dual PI group had plasma HIV RNA levels less than 50 copies/mL, the CSF-to-plasma HIV RNA ratios were higher in the dual PI group. Furthermore, HIV RNA increased in CSF over time in the dual PI group, raising the question of whether dual PI therapy may allow for progressive viral escape in the CNS during long-term antiretroviral treatment.

To examine whether a regimen with presumed better penetration and effectiveness in the CNS may have beneficial effects in the CNS, Tiraboschi and colleagues (Abstract 492) assessed 12 subjects with plasma HIV RNA levels less than 40 copies/mL (median suppression duration, 6.5 years) and neurocognitive impairment for baseline CSF measures while on tenofovir, emtricitabine, and efavirenz, and 9 of these subjects after a change to abacavir, lamivudine, and maraviroc. CSF parameters included HIV RNA measured using an assay with a lower limit of detection of 2.5 copies/mL and inflammatory and neural biomarkers including neopterin, TNF-α, monocyte chemoattractant protein (MCP)-1, and total tau. CSF HIV RNA on this highly sensitive assay was noted to decline after a switch in antiretroviral regimens in subjects with detectable levels of HIV RNA, though the difference was not statistically significant. Of the inflammatory and neural markers, the change in regimens was associated with a modest, nonstatistically significant decline in CSF neopterin and reduced levels of CSF TNF-α. Whether these findings are associated with reduced neurotoxicity or improved efficacy of these antiretroviral regimens within the CNS is unclear, but these preliminary findings suggest the need for further close examination of CNS end points in switch studies of patients on distinct antiretroviral regimens.

The possibility that certain antiretroviral medications may be toxic to the brain has been a subject of concern since the advent of antiretroviral treatments. Although the consensus of experts in the field is that given current data, the benefits of suppressive antiretroviral therapy in the CNS far outweigh possible risks, evidence has emerged in recent years that certain antiretroviral therapies may cause neurologic morbidity in some individuals. As noted above, Abstract 35 suggests that PI therapy may be associated with increased vascular pathology that in turn correlates with presence of neurologic impairment in HIV-infected persons.

The HIV nonnucleoside reverse transcriptase inhibitor (NNRTI) efavirenz has also been recognized as being associated with neurologic impairment that may lead to intolerable adverse effects in some individuals. The mechanisms behind efavirenz-related neurotoxicity are poorly understood. Funes and colleagues (Abstract 454) performed a study investigating whether interference with mitochondrial function, thought to mediate efavirenz-associated cellular toxicity in liver cells, may also underlie neuronal dysfunction in the setting of efavirenz. Their in vitro experiments assessing the effects of efavirenz on glial cells and neurons derived from human cell lines indicate that cellular oxygen consumption, cell viability, and intracellular adenosine triphosphate (ATP) production were reduced in the presence of efavirenz and that these effects were more profound in neurons than in astrocytes. Primary cultures of rat neurons also showed decreased ATP production and overall cell number if treated with efavirenz. These investigators conclude that because of the efavirenz-mediated effects noted on cell bioenergetic pathways, mitochondrial inhibition may be a key aspect of CNS toxicity due to efavirenz and that selectively more profound effects may be noted in neurons than in glial cells in the brain.

Potential Significance of Early HIV Infection and Treatment to CNS Injury

Abnormalities detected in persons on suppressive antiretroviral therapy who were started on treatment during chronic HIV infection may reflect processes such as viral persistence, ongoing low-level inflammation, progressive vascular disease, and other active processes. However, an alternate or additional possibility is that some damage was accrued in the nervous system prior to the start of HIV treatment that was incompletely reversed by antiretroviral therapy. Studies have indicated that HIV invades the nervous system in the first weeks of infection, accompanied by immune activation that likely establishes a substrate for local CNS infection and pathogenesis. However, only limited studies have examined whether local replication in the CNS may exist during the first years after infection.

Sturdevant and colleagues (Abstract 32) examined paired CSF and plasma samples from 72 subjects with primary HIV infection in the PISCES (Primary Infection CNS Events Study) cohort recruited at a median of 3.5 months after infection and longitudinally followed up for as long as 2 years. Using single-genome amplification of the envelope (*env*) gene, they identified distinct patterns of phylogenetic relationships between the CNS compartment and the blood and found evidence of viral compartmentalization based on sequences in 12% of subjects. In serial sampling, they found that viral compartmentalization or marked pleocytosis suggestive of ongoing replication was maintained over time in 20% of subjects, and diversification of CSF variants was also observed over time, indicating viral evolution locally within the CNS in the first 2 years of HIV infection. These locally evolving CNS HIV variants, as well as all plasma and CSF *env*, when analyzed with an affinofile cell entry assay, appeared adapted to replicate in cells with high CD4 receptor density, suggestive of T lymphocytes. These data suggest that even within the first 2 years of infection, HIV can establish an autonomous infection within the CNS compartment in some HIV-infected individuals.

Another study of subjects from the PISCES cohort examined the effect of antiretroviral therapy initiated in the first year after HIV infection on CSF and blood biomarkers associated with CNS disease in HIV infection. Peterson and colleagues (Abstract 30) studied

26 men enrolled in an observational study at a median of 4.5 months after initial HIV exposure who subsequently started combination antiretroviral therapy for reasons outside of the protocol at a median of approximately 8 months after infection. Laboratory biomarkers were assessed pretreatment and 6 months to 12 months (median 9 months) posttreatment. Improvements were noted in all biomarkers of intrathecal immune activation, including CSF neopterin, and infection. Importantly, all CSF measures, including CSF neopterin, normalized in comparison with an age-matched control group of 20 HIV-uninfected people, and in contrast to findings of persistently elevated CSF immune activation markers in subjects starting treatment during chronic infection. These findings suggest that early identification of HIV infection and prompt initiation of antiretroviral therapy may prevent or reduce signs of intrathecal immune activation, the pathologic substrate of HAND, to normal levels.

In a related study, Peluso and colleagues (Abstract 31) examined the effect of immediate antiretroviral treatment during acute infection on NFL, the protein product released during degeneration of myelinated axons that has been examined as a marker of active neuronal injury in neurodegenerative dementias and HIV infection. In Bangkok, Thailand, 32 subjects with acute HIV infection (median 18 days postinfection) and 33 subjects with chronic HIV infection had initial blood and CSF sampling at enrollment, prior to antiretroviral treatment. Although at this visit, HIV was detected in most subjects in blood and CSF, CSF NFL was elevated above expected values for age in 10 of 33 chronic but only 1 of 32 acute subjects, suggesting that acute HIV infection is not typically characterized by neuronal injury. All subjects in both groups initiated immediate combination antiretroviral therapy. After 48 weeks of treatment in the chronic group, 5 of 10 subjects with samples available had elevated CSF NFL for age; only 1 of 26 in the acute group manifested this abnormality after 24 weeks. These findings thus imply that very early initiation of antiretroviral therapy may halt processes that facilitate development or persistence of neuronal injury in chronic infection on antiretroviral therapy.

Examining data from the same cohort in Bangkok, Thailand, Valcour and colleagues (Abstract 447) examined whether in those subjects with acute HIV infection, treatment with standard combination antiretroviral regimens of efavirenz, emtricitabine, and tenofovir versus standard regimens plus maraviroc and raltegravir had a differential effect on neurologic outcomes. Sixty-two subjects with a median duration of 17 days of HIV infection at enrollment were randomized to the 2 study arms. From baseline (pre–antiretroviral therapy) to 24-month follow-up, the subset of subjects with samples available had improvement in all CSF (total n = 16), neuropsychologic (total n = 62), and magnetic resonance spectroscopy (total n = 43) measures, with no difference in improvement by study arm. Although for some of these end points, sample size may not have been adequately powered at this stage of the study to detect differences between arms, these data so far suggest that no CNS benefit is conferred by intensification with a C-C chemokine receptor type 5 (CCR5) inhibitor and an integrase inhibitor during acute HIV infection.

Ndhlovu and colleagues (Abstract 444) described patterns in blood monocyte phenotype evident in 17 subjects with acute HIV infection in this same cohort in Bangkok, Thailand prior to antiretroviral therapy initiation. Detection of nonclassic monocytes ($CD14^{low}$ $CD16^{++}$) was statistically significantly higher in Fiebig I versus Fiebig III infection. As these investigators have previously found that HIV DNA burden in monocytes correlates with the presence of HAND in chronic HIV infection, it is likely that this early activation and dynamic alteration in monocyte phenotypes in subjects in the earliest stages of infection sets the stage for important aspects of the neuropathogenesis of HIV.

Novel Alternative Mechanisms of HIV Neuropathogenesis

Although HIV-related neurologic injury is associated with pathologic immune activation in the CNS that can be improved if not always ameliorated by antiretroviral therapy, many key aspects of the specific pathways and mechanisms of this damage are yet to be elucidated. Several presentations at CROI 2014 suggested alternate or novel mechanisms of HAND neuropathogenesis.

Kallianpur and colleagues (Abstract 458) examined whether brain iron deficiency, defined by increased RNA expression of the transferrin receptor, associated with neuropsychologic performance. In 274 donors from the National NeuroAIDS Tissue Consortium (NNTC) who were neuropsychologically evaluated within 6 months prior to death, RNA expression of the transferrin receptor was measured in brain autopsy tissue (frontal cortex). In this subject group with a very high rate of HAND diagnoses (85%), presence of milder and more severe forms of HAND and performance on specific neuropsychologic tests independently correlated with transferrin receptor RNA expression. Thus, iron transport or iron deficiency in the brain may relate to neurologic injury in HIV-infected persons.

In a related study, Kallianpur and colleagues (Abstract 489) examined the relationship between red blood cell (RBC) indices and presence of cognitive impairment in 1235 subjects from the CHARTER study. RBC count, mean cell volume, mean cell hemoglobin, and hemoglobin were each associated with measures of neuropsychologic performance or detection of impairment. As these cell indices may reflect iron status and are reduced in the presence of chronic inflammation, these results may directly implicate iron deficiency in the pathogenesis of HAND or, alternately, may demonstrate changes in iron status in concert with neurologic dysfunction through a common pathway of chronic inflammation.

Cassol and colleagues (Abstract 34) employed metabolite profiling, or

metabolomics, of CSF samples derived from 46 HIV-infected subjects enrolled in the NNTC or CHARTER studies and 54 -uninfected controls to identify metabolites associated with HIV infection. Of the 107 named metabolites identified, 15 metabolites distinguished HIV-infected subjects on antiretroviral therapy from -uninfected controls. Using pathway analysis, these 15 metabolites associated with alterations in pathways associated with neurotransmitter production, mitochondrial function, oxidative stress, and accumulation of metabolic waste. Interestingly, some of these altered metabolite pathways overlapped with pathways altered in aging HIV-uninfected subjects. Further, a more circumscribed set of metabolites associated with the presence of HAND, including markers of glial cell activation and systemic and intrathecal markers of immune activation. Overall, this study approach yielded possible new mechanisms of pathogenesis in HAND, providing potential new biomarkers for investigation and targets for therapeutic strategies to ameliorate CNS injury in HIV infection.

In an investigation of a potential novel host determinant of HIV-related neurologic dysfunction, Hulgan and colleagues (Abstract 465) examined mtDNA haplotypes, patterns of single nucleotide polymorphisms in mtDNA associated with mitochondrial function in 1046 predominantly antiretroviral therapy–treated subjects enrolled in the CHARTER study. These mtDNA haplotypes were identified and distinct haplotypes were then correlated with the presence of neurocognitive impairment, assuming that differential rates of HAND may associate with distinct bioenergetics and oxidative stress related to the different haplotypes. One of the haplogroups identified, considered to be a Native American mtDNA haplogroup, was found in Hispanic participants to be associated with statistically significantly lower risk of neurocognitive impairment. This finding suggests that a potential genetic determinant for risk of HAND may be altered mitochondrial function, leading to altered neuroimmune activation or bioenergetics in the face of HIV infection.

Although host genetics likely plays a role in response to HIV infection, viral genetics, including within-compartment viral diversity and viral compartmentalization within the CNS, has been recognized as being associated with HAND. Wagner and colleagues (Abstract 471) used next generation sequencing (454 platform) of HIV-1 DNA derived from peripheral blood mononuclear cells to assess for dual HIV infection in 16 neurocognitively normal and 18 impaired HIV-infected subjects on antiretroviral therapy enrolled in the CHARTER study. Based on their criteria requiring divergent populations with bootstrap values of 90% or higher, 7 of 18 subjects with impairment versus only 1 of 16 neurocognitively normal subjects had dual HIV infection, suggesting a role for dual infection itself or very high within-compartment diversity in the pathogenesis of HAND. Evering and colleagues (Abstract 472) focused more specifically on differences between paired blood and CSF samples in subjects with chronic HIV infection in the CHARTER study. They used single genome amplification and sequencing of *env* to compare HIV variants in the blood and CSF of 15 subjects, identifying distinct patterns of compartmentalization across disease states. They then identified specific positions at which there were statistically distinct amino acids in the CSF versus blood to generate hot spots that might be more intensively examined in larger numbers of subjects as potential CSF compartmentalization–specific patterns or amino acids determining specific neurotropism or local viral evolution.

Conclusion

Neurologic presentations garnered a great deal of interest at CROI 2014, galvanized by the recognition that the CNS is likely still a site of perturbation in some HIV-infected persons despite successful antiretroviral therapy. An additional frontier of neuroHIV research is a focus on whether the CNS may serve as a reservoir of persistent and either latent or smoldering HIV infection that may be an impediment or consideration in some people in efforts to eradicate or cure HIV infection. A single small study by Rasmussen and colleagues (Abstract 482) assessing the effect of panobinostat, a potent histone deacetylase (HDAC)-inhibitor, on CSF markers is an initial step to examine the potential beneficial versus injurious effects of targeted cure strategies on the CNS. In 11 subjects treated with panobinostat who consented to lumbar puncture for CSF collection, no rise in CSF HIV RNA levels (measured using an assay with a limit of detection of 3.8 copies/mL) nor change in neural marker or immune activation levels was noted in the final dosing week after a round of 3 cycles of every other week panobinostat. These preliminary findings in a small number of subjects, though reassuring, provide evidence for the feasibility of CNS monitoring during such eradication approaches that should likely be considered in planning and implementing future cure studies.

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A list of all cited abstracts appears on pages 632-638 of this issue. Abstracts are published in *Top Antivir Med.* 2014;22(e-1):1-570, a special online issue available at www.iasusa.org/pub.

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