Invited Review

CROI 2021: Neurologic Complications of HIV-1 Infection or Covid-19

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The 2021 Conference on Retroviruses and Opportunistic Infections (CROI) featured a timely review of the neurologic complications of COVID-19 as well as new research findings on mechanisms by which SARS-CoV-2 may affect the brain. CROI included new and important findings about the neurologic complications of HIV-1, human polyomavirus 2 (also known as JC Virus), and cryptococcus. New long-term analyses of cognition in people with HIV-1 identified that cognitive decline over time is associated with multimorbidity, particularly diabetes, chronic lung disease, and vascular disease risk conditions. These conditions are associated with aging, and the question of whether people with HIV are at risk for premature aging was addressed by several reports. New findings from large analyses of resting state networks also provided valuable information on the structural and functional networks that are affected by HIV-1 infection and cognitive impairment. Several reports addressed changes after initiating or switching antiretroviral therapy (ART). Findings that will improve understanding of the biologic mechanisms of brain injury in people with HIV were also presented and included evidence that host (eg, myeloid activation, inflammation, and endothelial activation) and viral (eg, transcriptional activity and compartmentalization) factors adversely affect brain health. Other research focused on adjunctive therapies to treat HIV-1 and its complications in the central nervous system. This summary will review these and other findings in greater detail and identify key gaps and opportunities for researchers and clinicians.

Keywords: HIV, CROI 2021, COVID-19, SARS-CoV-2, neurologic complications, cognition, brain, CSF, neuroimaging, aging, neurotoxicity

Introduction

The effects of HIV-1 and SARS-CoV-2 in the central nervous system (CNS) was an important theme of several presentations at the 2021 *virtual* Conference on Retroviruses and Opportunistic Infections (CROI). Presentations focused on HIV pathogenesis and CNS reservoirs' persistent neurologic dysfunctions (as assessed by neuropsychiatric testing, imaging, or cerebrospinal fluid [CSF] evaluations) in virologically controlled people with HIV. New data were also presented on premature aging and the effects of aging-related comorbidities on brain function, which have become increasingly important as people with HIV age into their seventh decade and beyond. New data also provide encouraging news for reducing the neurotoxicity of antiretroviral therapy (ART), for treating cognitive impairment, and for advancing the HIV cure agenda. This review will focus on major thematic areas that may inform new research and stimulate further discussion of clinical management of HIV infection.

Observational Findings on the Effects of HIV-1 on the Brain

New Data on Cognition and Mood

People with HIV are highly diverse and differ in many characteristics including age, sex, race, ethnicity, drug use, and living location and conditions. A substantial proportion of people with HIV contracted their infection more than 20 years ago and are now aging into their seventh decade and beyond because of the benefits of ART. In the United States, this group has been the particular focus of research cohorts such as the MWCCS (MACS/WIHS Combined Cohort Study) and the CHARTER (CNS Antiretroviral Therapy Effects Research) study. CHARTER investigators reported on cognition and depression in people with HIV after more than a decade of follow up (Abstract 101). The 397 participants had been followed up for a mean of 12.4 years and had a mean age of 56 years. Nearly all took ART (mean duration, 15.3 years) and 91.9% had a plasma HIV RNA level below 200 copies/mL. Nearly a quarter (23.4%) had evidence of cognitive decline, compared with 5% of a normative population of people without HIV. Decline was associated with the presence of several comorbid conditions, including chronic lung disease (P=.021), diabetes (P=.004), major depressive disorder (P=.016), and hypertension (P=.021), as well as longer duration of ART (P=.048), nonuse of antihypertensive

In the CHARTER study, cognitive decline over 12 years was associated with chronic lung disease, diabetes mellitus, major depressive disorder, and hypertension

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drugs ($P = .001$), and lifetime cannabis use disorder ($P = .043$) (model $P < .0001$). Speed of information processing, working memory, motor functioning, and verbal fluency were the cognitive abilities that contributed the most to global decline.

The HNRP (HIV Neurobehavioral Program) at the University of California San Diego reported on cognitive decline in a larger group of participants (n = 1195), but over a shorter period of observation (mean, 7.1 years), finding that a commonly used index of comorbid conditions, the Charlson Comorbidity Index, was associated with more rapid global cognitive decline, particularly in working memory ($P = .007$) and executive functioning ($P = .001$) (Abstract 328). The medical conditions that were most strongly associated with cognitive decline were diabetes, mild liver disease, and congestive heart failure.

A continuing debate in the field concerns the extent to which HIV and its effects on the immune system drive brain injury compared with comorbid conditions like diabetes, as this influences treatment approaches to managing conditions like cognitive impairment and depression. The CHARTER analysis did not find that either HIV RNA level or CD4+ cell count was associated with cognitive decline but the HNRP analysis found that lower CD4+ cell count was statistically significantly associated with cognitive decline in with in-person analyses. Of note, neither analysis included measures relevant to activity of the HIV reservoir among people with HIV who are taking suppressive ART, such as low-level HIV RNA production (eg, using a single copy assay) or cell-associated HIV DNA. Since HIV-1 infection increases the risk for many of these conditions (eg, diabetes, heart disease) compared with the general population, it may indirectly be responsible for cognitive impairment even in the absence of direct effects on the brain.

Another approach was used by Lam and colleagues to determine if people with HIV were at greater risk for incident dementia (Abstract 330). They analyzed data from more than 165,000 electronic health records from the Kaiser Permanente system and compared the incidence of dementia between people with HIV on ART (n = 11,302) and people without HIV (n = 154,620) who were followed up in primary care clinics between 2000 and 2016. Incident all-cause dementia diagnoses were identified using the International Classification of Diseases (ICD) codes that were confirmed via chart review for more than 300 randomly selected patients. A total of 264 people with HIV and 2006 people without HIV developed dementia during follow up. Incidence of dementia was 4.4 (people with HIV) and 2.1 (people without HIV) per 1000 person-years, for an incident rate ratio of 2.0 (95% confidence interval [CI], 1.8-20). The incidence rate ratio was highest in younger people with HIV but declined in older people with HIV, a pattern that has been identified elsewhere.

Chan and colleagues reported results from people who were first assessed in Thailand shortly after contracting HIV in the SEARCH (South East Asia Research Collaboration on HIV) program. This group focused on longer term changes (6 years) with regard to depression instead of cognition (Abstract 340). Prior studies have found that depressive symptoms, like cognitive performance, often improve after initiation of ART, but most have reported on shorter term change in people with chronic HIV infection. The focus of the Thai cohort on people with acute HIV infection is a strength that better allows indexing changes from the time of infection and ART initiation. Participants completed the 9-item Patient Health Questionnaire (PHQ-9) for depression symptoms and the Distress Thermometer (DT) for anxiety/stress at baseline (before ART initiation) and then again 12, 24, and 96 weeks after ART initiation and every 48 weeks thereafter. At baseline, the prevalence of moderate (21%) and moderate to severe (27%) depression symptoms were high. Those individuals who had a higher baseline PHQ-9 score also had a higher plasma HIV RNA level and a greater frequency of acute retroviral syndrome. As expected, both the PHQ-9 and DT scores improved after starting ART and were at subclinical levels after 24 weeks. In those who maintained viral suppression on ART for 6 years, depressive symptoms remained stable or improved.

As people with HIV age, they appear to develop aging-related diseases such as diabetes and hypertension more frequently than people without HIV. This has raised concerns about premature aging of people with HIV, which may be linked to the inflammation that can persist even during suppressive ART. An important question is whether people with HIV will also develop Alzheimer's disease more frequently or at a younger age than people without HIV. Several research groups have identified that cognitive impairment in people with HIV has different phenotypes, and this has been categorized by the National Institute of Mental Health in a Research Domain Criteria (RDoC) framework.[1] Using criteria from the Alzheimer's disease field, Moore and colleagues previously identified that a subgroup of people who were categorized as having HIV-associated neurocognitive disorder (HAND) may actually have amnestic mild cognitive impairment (aMCI), a distinction that has implications for therapeutic management. In another analysis based at the University of California San Diego, Moore and colleagues classified 264 people with HIV as having either aMCI (n = 25), HAND (n = 65), neither (n = 81), or both (n = 95) in order to determine if this influenced their progression over time (Abstract 331). The 4 groups did not differ in duration of HIV disease, ART use, or plasma HIV RNA level, but did in age, CD4+ cell count, and baseline cognitive performance, with the impaired groups performing worse than the group with neither aMCI nor HAND. Over a mean of 4.2 years of follow up, none of the groups had significant decline based on fully adjusted models, which was contrary to hypotheses. The absence of cognitive decline would seem to be reassuring, but since 3 of the 4 groups had cognitive impairment at baseline, this also means that the impairment did not improve, which could mean that the injury is irreversible or that new therapies are needed for people with

HIV who remain cognitively impaired even if they are already taking suppressive ART. This group also analyzed Alzheimer's disease-related biomarkers (amyloid-β 1-42 [Aβ42] and phosphorylated tau *p*-tau] 181) in CSF from

NAA, a marker of neuronal health, was lower at baseline but normalized after 24 months of ART. In contrast, choline, an inflammation marker, continued to be elevated 24 months after ART initiation

31 people with HIV who were older than 50 years and enrolled in the NNTC (National NeuroAIDS Tissue Consortium), finding that a higher p-tau/Aβ42 ratio, which is typical of people with Alzheimer's disease, was associated with worse Learning and Delayed Recall. Together, these (and other) findings support that people with HIV may develop Alzheimer's disease-type pathology, but they do not support the conclusion that people with HIV are at greater risk than the general population.

New Data on Imaging

In addition to the depression findings (Abstract 340), the SEARCH program also reported on the effects on white matter brain metabolism of 2 years of ART that was initiated following acute HIV infection (Abstract 163). A total of 37 people with HIV were assessed by magnetic resonance spectroscopy (MRS) within the left frontal white matter (FWM) prior to ART (baseline) and 24 months after ART initiation. Compared with people without HIV, people with HIV had lower total N-acetylaspartate (NAA) level, a marker of neuronal health, at baseline but values normalized after 24 months of ART. In contrast, choline, an inflammation marker, continued to be elevated 24 months after ART, supporting the conclusion that people with HIV do not only have persistent systemic inflammation during suppressive ART but persistent neuroinflammation as well. In Abstract 318, the potential difference in neuroimaging measures as a function of sex was examined. Kelly and colleagues compared brain volume proportion in virologically-controlled people with HIV (n=286) and people without HIV (n=105) of both sexes to assess how biologic sex may modify the effects of HIV on the brain. Total brain, white matter, and gray matter proportions were plotted as a function of age. In general, brain volume proportions were higher in women than in men irrespective of HIV status. Greater loss of white matter volume over time was seen in men with HIV than in women with HIV. Consistent with this observation, men with HIV also had higher concentrations of the axonal biomarker, neurofilament light, than women with HIV (P=.02). These findings reinforce the importance of designing mechanistic and therapeutic research that focuses on potential sex-based differences.

With the growth of imaging datasets in recent years, high dimension analytical methods, such as machine learning, are increasingly used in neuroHIV

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research. For example, Luckett and colleagues identified potential relationships between HIV infection, structural and functional organization of the brain, cognition, and aging (Abstract 146). These authors used a machine learning-based approach within a cohort of virologically controlled people with HIV (n=297) on ART and people without HIV (n=1509) to identify resting-state networks that relate to cognitive impairment. The salience (SAL) and parietal memory networks most strongly distinguished people with HIV (either cognitively impaired or unimpaired) from people without HIV. Comparing cognitively impaired people with HIV with those without HIV, additional involvement of the frontal parietal network (FPN) was observed. Limiting the dataset to just people with HIV, the SAL, FPN, basal ganglia, and ventral attention network most strongly distinguished cognitively impaired from unimpaired participants. A deep learning model was also used to generate voxelwise maps of resting-state networks that identified changes in resting-state network topology among the 3 groups. Anatomically, the greatest differences in resting-state network topology identified by deep learning occurred in the dorsal and rostral lateral prefrontal cortex, anterior cingulate, parietal regions, and caudate. These results support that different resting-state networks are affected by HIV and cognitive impairment.

Petersen and colleagues also used machine learning to study brain aging among people with HIV. They examined brain structure (gray and white matter volumes) and function (cerebral cortical blood flow) to characterize deviations from typical aging across the lifespan, finding evidence of accelerated brain aging in people with HIV, that is, machine learning-based overestimation of the actual age of older people with HIV, compared with people without HIV or younger people with HIV. They also showed that cerebral blood flow reductions were specific to older people with HIV who had detectable HIV RNA, and people with HIV with suppressed viral load were similar to people without HIV. Finally, imaging-based estimates of brain aging correlated with cognition, especially motor functioning. These findings suggest that imaging-based brain aging may be a useful noninvasive biomarker of neuropathology and cognitive impairment in people with HIV.

Viral Mechanisms in the Pathogenesis of HIV Disease in the Brain

The observational findings in the preceding section are very valuable, but they do not address the biologic mechanisms of brain injury, which are essential for designing interventions to treat the CNS complications of HIV disease. These mechanisms can be broadly categorized into viral mechanisms and host mechanisms. Before the widespread use of potent ART, published data supported that compartmentalization of HIV in the CNS occurred in a substantial proportion of people with HIV and conferred greater risk of brain injury. With expanding use of ART, clues that HIV itself may continue to affect brain health trajectory are emerging. These clues include data on cell-associated HIV DNA, CSF viral escape, and HIV proteins such as Tat. Joseph and colleagues reported on a new concept, the compartmentalized CSF viral load (CCVL, specifically the product of the percent of CSF-derived sequences in variable regions of the HIV envelope [V1-V3] that are CSF-specific and the quantity of HIV RNA in CSF) (Abstract 341). Among 50 people with HIV assessed in the Rakai Cohort in Uganda, individuals with higher CCVL had worse global cognitive performance (P=.049, particularly speed of information processing), more depressive symptoms (P=.003), and worse self-reported functioning (P=.044) compared with individuals with lower CCVL. This composite measure had larger effect sizes in relation to these outcomes than did each of the individual components used to calculate it. Whether the pre-ART CCVL influences brain health trajectory following ART initiation remains to be proven, but prior research from this group suggests that it may.[2] Using brain tissue from the NNTC, the same group also examined the phylogenetic relationship between envelope sequences from brain, CSF, and blood from 5 people with HIV who had subtype B HIV-1 infection, had HIV-associated dementia, and were not taking ART at the time of death. Three of the participants had brain- and CSF-derived sequences that were compartmentalized from blood-derived sequences. Although the divergence of brain- and blood-derived sequences has been demonstrated many times, the investigators extended their work to show that HIV DNA concentrations were highest in tissue from the basal ganglia, the frontal lobe (white matter), and the occipital lobe. Using the Affinofile assay that allows the density of CD4+ expression to vary, they also found that Env proteins derived from the compartmentalized sequences from CSF and brain had greater affinity for CD4 and thus enter cells with low CD4 density more efficiently, which is typical of the myeloid cells that HIV-1 productively infects in the brain.

Higher compartmentalized CSF viral load was associated with worse global cognitive performance, more depressive symptoms, and worse self-reported functioning

Compartmentalization of HIV-1 in the brain has crucial implications for the HIV cure agenda. To better understand the nature of HIV-1 persistence in the brain, the Manhattan Brain Bank site of the NNTC isolated neuronal and glial nuclei from postmortem frontal cortex collected from 27 decedents (6 people with HIV encephalitis, 15 people with HIV without encephalitis, and 6 people without HIV) and created integration site sequencing libraries 10X chromium single nucleus RNA-sequencing (snRNA-seq). They identified 1279 integration sites, predominantly from the glial cell fraction from those with HIV encephalitis. Glial integration sites were found preferentially in introns, gene dense regions, and active regions of the genome. Glial integration sites showed a stronger preference for integration into short interspersed nuclear element repeats than T-cell integration sites and contained a significantly lower proportion of clonal (5% vs 18%; P < .0001) and recurrent (13% vs 30%;P<.0001) integration sites. Based on snRNA-seq, the investigators were able to identify multiple clusters of cells (eg, excitatory neurons, astrocytes) but HIV expression was only present in those who had HIV encephalitis, primarily in microglia. Differential expression analysis revealed that microglia with active viral transcription had greater expression of core markers of activation (secreted phosphoprotein 1[SPP1], lipoprotein lipase apolipoprotein E [LPL APOE], FMS-related receptor tyrosine kinase 1[FLT1]) and decreased expression of markers of proliferation. These findings are somewhat diminished by many of the findings being present only in those who had HIV encephalitis, a condition that is typically characterized by high levels of HIV expression and that has a greatly reduced incidence in the modern ART era. Even when no HIV transcripts were detected, however, the investigators still found evidence of enhanced microglial activation, suggesting that low-level HIV may still be present even if they did not detect it.

Two groups reported on HIV RNA or DNA quantification in peripheral blood mononuclear cells (PBMCs), CD4+ T cells, or monocytes. The neuroHIV group at St. Vincent's Hospital in Sydney, Australia, extracted cellular DNA and RNA from CSF and blood collected from 20 people with HIV who were taking suppressive ART and used a more sensitive method than has typically been used to quantify HIV-1 transcripts and HIV-1 DNA, specifically the Double-R assay that is based on πCode MicroDiscs platform (Abstract 162). An 18-color flow cytometry showed that cells in the CSF were 91% memory T cells, with roughly equal memory CD4+ and CD8+ T cells. Other CSF cells were 3.1% CD14+CD16+ monocytes, 2.0% natural killer (NK) cells, and 0.4% B cells. HIV-1 RNA transcripts were quantified in CSF in 90% of participants (compared with about 10% in prior reports) and HIV-1 DNA was quantified in CSF in 80% of participants (compared with about 50% of historical controls). Concentrations of both were higher in CD4+ T cells from CSF than in PBMCs, although the significance of this finding is reduced since the comparison of the

CD4+ T-cell subset in CSF with the total mononuclear cell fraction from blood will amplify the differences between the compartments. Participants were also assessed with 1H-magenetic resonance spectroscopy and greater transcriptional activity in CSF cells correlated with lower NAA (ie, worse neuronal integrity) in FWM (P=.04) and posterior cingulate cortex (P=.055).

Greater HIV transcriptional activity in CSF cells correlated with worse neuronal integrity in frontal white matter and posterior cingulate cortex

Using the same overall design as prior SEARCH program analyses (ie, identifying people with acute HIV infection and then following them up over time after ART initiation), investigators isolated monocytes from cryopreserved PBMCs from 30 people with HIV and quantified total monocyte HIV-1 RNA by real-time polymerase chain reaction prior to ART and 96 weeks after ART initiation. Monocyte HIV-1 RNA was detected in 17 (57%) participants at baseline, but only in 3 of 30 (10%) at 96 weeks. The investigators compared these findings with a panel of soluble myeloid activation and proinflammatory biomarkers in blood and performance on a screening battery of 3 neuropsychologic tests and found that participants who had detectable monocyte HIV RNA at baseline had higher neopterin concentrations and worse performance on 2 of the neuropsychologic tests (Color Trails 1, P=.014, and Trailmaking A, P=.05). Taken together, these findings support that HIV itself may continue to affect brain health trajectory, even during suppressive ART, but sufficiently sensitive methods are needed.

Many groups have found evidence of CSF viral escape, which is generally defined as detectable HIV-1 RNA in CSF when it is undetectable in blood or, when HIV-1 RNA in blood is detectable, having HIV-1 RNA at least 0.5 $\log_{10}$ copies/mL higher in CSF than in blood. Its presence can occur with severe neurologic symptoms or with no symptoms at all and some, but not all, studies have found associations with ART (eg, use of HIV protease inhibitors) and HIV-1 (eg, presence of drug resistance mutations) characteristics. Investigators from the Swiss HIV Cohort and the NAMACO (Neurocognitive Assessment in the Metabolic and Aging Cohort) study sought to validate these findings by reporting on CSF collected from 287 people with HIV. CSF viral escape was present in 29 (10.1%), of whom 18 (62%) had suppressed plasma HIV-1 RNA and 11 (38%) had detectable plasma HIV-1 RNA. Characteristics of patients were comparable whether or not they had CSF viral escape, including demographics, cardiovascular and metabolic comorbidities, time since HIV diagnosis (12 vs 16 years, respectively; P = .40), CD4 + T-cell count (553 vs 611 cells/μL; P=.10), CNS Penetration-Effectiveness score (7 vs 8; P=.20), neurocognitive diagnosis, or presence of magnetic resonance imaging (MRI) abnormalities. These findings confirm that CSF viral escape occurs in a minority of people with HIV, but its pathologic significance continues to be unclear, although it may have implications for achieving functional cure in the people who have it.

Host Pathogenesis of HIV Disease in the Brain

The persistent inflammation that occurs in people with HIV increases their risk for numerous aging-related comorbidities, including vascular disease and diabetes. Since inflammation and its downstream consequences have been linked to worse cognition and depression, understanding which of these most strongly influences brain health trajectory is important for directing therapy at the most impactful target. Guha and colleagues studied the potential role of cerebrovascular disease and its contribution to cognitive impairment in people with HIV by focusing on biomarkers that distinguish vascular cognitive impairment from HAND (Abstract 320). They measured soluble biomarkers of vascular injury (ICAM-1, VCAM-1, C-reactive protein), inflammation (interferon [IFN]-α, interleukin [IL]-1β, IL-6, IL-8, IL-15, C-X-C motif chemokine ligand 10 [CXCL10], chemokine ligand 2 [CCL2], vascular endothelial growth factor [VEGF]), and brain injury (total tau, glial fibrillary acidic protein [GFAP], YKL-40) in CSF and blood from 143 people with HIV on ART and 64 people without HIV from the NNTC and the CHARTER Study. Overall, people with HIV had higher levels of intercellular adhesion molecule (ICAM)-1, C-reactive protein, IL-8, IL-15, CXCL10, and VEGF in plasma and higher levels of C-reactive protein, CXCL10, VEGF, and GFAP in CSF than people without HIV. Among people with HIV, those with HAND had higher plasma ICAM-1, vascular cell adhesion protein (VCAM)-1, C-reactive protein, and YKL-40 as well as brain injury biomarkers (CSF total Tau, GFAP, YKL-40) than those without HAND. Furthermore, cerebrovascular disease was more prevalent among people with HIV who had HAND than among those without HAND and was associated with higher VCAM-1 and YKL-40 levels in plasma and higher total Tau and YKL-40 levels in the CSF. Overall, these results support that vascular disease may be more closely related to brain injury in people with HIV on ART than inflammation. The importance of vascular disease was further supported by Cooley and colleagues, who examined the effect of cardiovascular disease risk on white matter integrity, as measured by diffusion tensor imaging in people with HIV (Abstract 319). The Framingham cardiovascular disease risk score was calculated for 166 virologically well-controlled people with HIV and 48 people without HIV. Cognitive performance and fractional anisotropy of major white matter tracts in the brain were compared between low, moderate, and high cardiovascular disease risk for the 2 groups. Results indicated that a moderate or high cardiovascular disease risk was associated with worse cognitive performance (psychomotor speed) and lower fractional anisotropy within several major white matter

tracts including the frontal aslant, frontal occipital, and inferior longitudinal fasciculus for both people with HIV and people without HIV. Since the Framingham cardiovascular disease risk score contains several modifiable components (eg, smoking, blood pressure, and cholesterol), the results suggest that treating these conditions may improve white matter injury in people with HIV.

Molsberry and colleagues also investigated cardiovascular disease risk factors and explored the relationship between statin use and cognitive performance over time in participants from the MACS (Multicenter AIDS Cohort Study) (n = 1407) (Abstract 338). In addition to their lipid lowering effect, statins have anti-inflammatory properties that can improve endothelial function and enhance dynamic cerebral blood flow. These effects could potentially improve cognitive performance. Using multivariable-adjusted linear regression to compare cognitive test performance prior to and after statin initiation, statin use was not associated with improved performance on any neuropsychologic test on the first test completion after statin initiation. Further analysis identified that people with HIV who initiated statins tended to have, on average, a faster rate of cognitive decline. HIV serostatus did not modify this association. These results caution against use of statins to protect or improve cognition.

El-Kamari and colleagues studied the association between cognitive performance and biomarkers of inflammation, insulin resistance, and body fat composition (by dual-energy X-ray absorptiometry [DEXA]) in ART-treated people with HIV (n = 65) and people without HIV (n = 33) (Abstract 329). Cognitive function was evaluated using Cognivue (6 cognitive domains and 2 performance parameters). People with HIV had worse overall cognitive performance and had worse performance in multiple domains (visuospatial, memory, executive function, naming/language, delayed recall, and abstraction). Among people with HIV, worse cognitive performance in multiple domains was associated with higher biomarkers of inflammation in blood (IL-6, soluble tumor necrosis factor receptor (TNFR)-I, soluble TNFR-II, C-reactive protein). Higher body fat composition (total percent fat and visceral adipose tissue) was also associated with worse cognition. These results contrast with those of Abstract 320 and support targeting inflammation but, of note, these investigators did not include vascular biomarkers in their analysis for comparison.

Vecchio and colleagues evaluated sex differences in 16 soluble biomarkers of inflammation and immune activation in CSF in relation to cognitive performance in a cohort of virally suppressed people with HIV (n = 83) in Uganda (Abstract 321). Overall, men performed worse on various cognitive measures including timed gait, motor skills, executive performance, and semantic fluency than women, and men had stronger associations between biomarkers and cognition than did women. Thus, inflammation may more strongly influence cognition in Ugandan men than women with HIV. Further supporting a role for inflammation in men, Anderson and colleagues presented data from the MACS in the United States on GlycA, a composite blood biomarker of glycosylated proteins that reflects acute phase reactants (including alpha1-acid glycoprotein, haptoglobin, and others) in 843 men, 63% of whom had HIV-1 infection (Abstract 324). In multivariable analyses, higher GlycA was associated with impairment when incorporating 5 other biomarkers individually (C-reactive protein, IL-6, CCL2, sCD14, and soluble CD163). The association between GlycA and impairment was driven by people with HIV, particularly those with a higher C-reactive protein level (odds ratio, 1.84; 95% confidence interval, 1.17-2.89). This study provides more evidence that systemic inflammation plays a role in cognition among people with HIV.

Myeloid cells remain critically important in the pathogenesis of HIV-1 in the brain. Several reports extended work in this area, including Veenhuis and colleagues, who used a test-validation approach to assess myeloid cell proportions from 2 independent cohorts of virologically suppressed women with HIV-1 infection in Baltimore (n = 19) and New York City (n = 18) (Abstract 322). A higher proportion of intermediate (CD14+CD16+) monocytes, which express medium levels of CCR2, high levels of CX3CR1, are (C-C chemokine receptor type 5) CCR5 positive, and have proinflammatory effects, was associated with lower global cognitive performance at the time of cognitive testing ($P = .006$) and approximately 1 year before cognitive testing ($P = .02$). A higher proportion of classical monocytes was also associated with better cognition ($P < .05$). In contrast, no associations were found between monocyte subsets and mental health indicators, such as symptoms of depression or anxiety, although lower CD4+ T-cell proportion was associated with higher perceived stress ($P = .03$).

Collazo-Rodriguez and colleagues aimed to determine if exosomes from blood of women with HIV-1 infection induced a shift in uninfected monocytes toward this pathologic intermediate phenotype. They found that exosomes from women with HIV-1 were taken up by both uninfected classical and intermediate monocytes and that they caused a shift in phenotype toward intermediate monocytes and the extent of the shift did not depend on whether women had cognitive impairment or not, although exosomes from women who had cognitive impairment caused a more rapid shift toward the intermediate monocyte phenotype. Because of this well demonstrated importance of myeloid cells in the pathogenesis of HIV-1 in the brain, Hammonds and colleagues evaluated the performance of 4 myeloid cell models, specifically 2 microglial model cell lines (C20, HMC3) and 2 sources of primary cell-derived microglia (monocyte-derived microglia [MMG] and induced pluripotent stem cell-derived microglia [iPSC-MG]) (Abstract 351). Significant differences were observed upon gene expression profiling, with MMG and iPSC-MG clustering closely with primary human microglial cells, and C20 and HMC3 exhibited marked differences. Consistent with these differences,

iPSC-MG and MMG were readily infected with R5-tropic HIV-1, and C20 and HMC3 required pseudotyping for infection. HIV replication dynamics and HIV-1 particle capture, however, differed noticeably between MMG and iPSC-MG. Based on these and other findings, the investigators concluded that the iPSC microglia model provided a more authentic HIV-1 model system than the alternatives. These investigators are developing a 3-dimensional cerebral organoid model using these and other cells.

As noted earlier, people with HIV may experience earlier brain aging than people without HIV-1 and understanding the biologic mechanisms by which

Higher epigenetic age acceleration was associated with worse performance on attention and working memory in people with HIV but not in people without HIV

this occurs is a key gap in the field. In addition to imaging-based methods, brain aging can also be inferred by estimating cellular aging using methods such as DNA methylation or mitochondrial DNA (mtDNA). Shiau and colleagues measured DNA methylation in whole blood using Illumina EPIC Arrays in 69 people with HIV and 38 people without HIV who lived in New York City. The National Institutes of Health (NIH) Toolbox Cognition Battery was used to assess cognitive performance across 5 domains. Overall, chronologic age correlated with DNA methylation-estimated biologic age, but people with HIV had higher mean epigenetic age acceleration (EAA) and extrinsic epigenetic age acceleration (EEAA) than people without HIV. Higher EAA was associated with worse performance on attention and working memory in people with HIV but not in people without HIV.

Solanky and colleagues assessed associations between HIV-1 infection and either the quantity of mtDNA or of the mitochondrial common deletion from a buccal specimen, finding that people with HIV (n=124) had higher levels of both measures than people without HIV-1 (n=25) ($P<.0001$) (Abstract 326). When they compared these mitochondrial measures with a panel of soluble biomarkers in CSF and blood, they found that higher mtDNA level was associated with higher soluble TNFR-II ($P=.042$) and higher amyloid-β 1-42 ($P=.0005$) levels even after adjusting for HIV serostatus and demographic characteristics. The association with amyloid-β 1-42 was present in the subgroup of people with HIV, even after adjusting for duration of HIV and ART, and nadir and current CD4+ T cell count (model, $P<.0001$). A higher level of the mitochondrial common deletion was associated with higher soluble TNFR-II level in blood ($P=.004$) but not for CSF biomarkers.

Volpe and colleagues also worked with mtDNA but used MutPred pathogenicity scores to evaluate the influence of mtDNA variants on cognitive performance (Abstract 325). Among 744 people with HIV, the presence of any deleterious mtDNA variant was associated with motor impairment ($P=.03$), even in multivariable analyses. In ancestry-stratified multivariable analyses, people of European ancestry (n=317) also trended toward having an association with speed of information processing and people of African ancestry (n=357) trended toward having an association with working memory (P values = .06-.08). Overall, these results support that biomarkers that have been linked to premature biologic aging in people with HIV are also associated pathologic events in the CNS.

Interventional Findings on the Effects of HIV-1 on the Brain

Several clinical trials related to the CNS were presented. Yacoub and colleagues reported findings from a randomized, placebo-controlled clinical trial of intranasal insulin in 21 people with HIV who were taking suppressive ART and who had at least mild cognitive impairment. Six participants prematurely discontinued (3 due to nasopharyngeal irritation). Compared with placebo, intranasal insulin was associated with improvements in global cognition ($P=.029$) at 24 weeks, which was

Compared with placebo, intranasal insulin was associated with improvement in global cognition at 24 weeks, which was driven by improvements in verbal memory, visual memory, and attention

driven by improvements in verbal memory, visual memory, and attention. On a personal note, this study was led by Dr Ned Sacktor, whose untimely death in late 2020 cannot overshadow the many important contributions he made to the field.

Considering the importance of myeloid cells in the pathogenesis of HIV-1 disease in the brain, many have considered that CCR5 antagonists, such as maraviroc, might have particular benefit in the brain. Shikuma and colleagues reported findings from a randomized, placebo-controlled trial of adjunctive therapy with maraviroc in people with HIV who had sustained plasma HIV RNA suppression and at least mild cog nitive impairment (Abstract 333). Participants were randomized 2:1 and followed up for 48 weeks, resulting in 39 participants who completed all evaluations. At baseline, those randomly assigned to the maraviroc arm had worse global ($P=.002$) and motor ($P=.001$) performance. Participants in the maraviroc arm had significantly greater improvements in the combined learning/memory domain ($P=.009$) than participants in the placebo arm. No other domains significantly changed between the treatment arms. Of note, the study included people with HIV with very mild impairment who might not meet criteria for HAND (as minimal as neuropsychological testing performance (NPZ),-0.5), and the learning/memory domain change was not

statistically significant after adjusting for type I error.

With ongoing development of new ART drugs and treatment strategies, switch studies continue to provide important, clinically relevant data for people with HIV and their medical practitioners. Perez-Valero and colleagues performed an open-label switch study in which participants either continued a suppressive regimen of dolutegravir/lamivudine/abacavir or switched to darunavir/cobicistat/emtricitabine/tenofovir alafenamide (Abstract 339). Neuropsychiatric symptoms were evaluated with the Hospital Anxiety Scale, Hospital Depression Scale, Pittsburgh Sleep Quality Index, and neuropsychiatric adverse events by the NIH Division of AIDS criteria. At 4 weeks, participants who switched had a reduction in symptoms in 3 of the 4 categories (anxiety, neuropsychiatric adverse events, and sleep) compared with those who did not switch (n = 69 evaluable participants). This was particularly true for participants who had moderate to severe disturbances in sleep quality and sleep latency. Because the switch entailed a change of several drugs, it is unclear which medication(s) may have driven the results.

Calcagno and colleagues also reported a switch study in which people with HIV were randomly assigned to continue a suppressive ART regimen or to change to a regimen that was designed to minimize potential neurotoxicity (darunavir/cobicistat/emtricitabine/maraviroc) (Abstract 335). The study was interrupted for slow accrual after 38 people with HIV had been randomly assigned and had been followed up for 24 weeks. In the switch arm, parietal delta waves on electroencephalography, which can indicate brain injury, decreased ($P = .022$) as did phosphorylated Tau in CSF ($P = .002$) and liver fibrosis as measured by Fibroscan ($P = .038$).

In another switch strategy, Vergori and colleagues reported the results of a single-arm study in which 109 people with HIV on efavirenz/emtricitabine/tenofovir disoproxil fumarate switched to bictegravir/emtricitabine/tenofovir alafenamide (Abstract 334). Change in psychiatric symptoms and 5 domains of cognition were assessed at 48 weeks, at which point symptoms of depression and anxiety were less severe and sleep problems were less common. Global cognitive performance also improved, particularly executive function, attention/working memory, and learning/memory. Since this trial also switched several drugs, improvements cannot be attributed to a single drug. Importantly, the absence of both a comparison arm and the incorporation of practice effects in the analysis of the neuropsychologic test results are limitations of the study.

Another report that has implications for switching ART regimens in the clinic is the report on doravirine concentrations in CSF (Abstract 358). A total of 14 plasma and 15 CSF samples were collected with most participants replacing an integrase strand transfer inhibitor with doravirine. At week 4, 1 participant met a common definition of CSF viral escape with detectable HIV RNA CSF (32 copies/mL) but undetectable in blood plasma. Total doravirine concentrations in CSF were approximately 13% of those in blood. In CSF, doravirine was mostly unbound to drug-binding proteins (76.1%). The total CSF; unbound plasma ratio of doravirine was 0.99, supporting the conclusion that doravirine crosses the blood-brain barrier primarily via passive diffusion.

An additional switch study may have implications for brain health. Serrano-Villar and colleagues reported the effects of switching from a 3-drug to a 2-drug regimen on inflammation biomarkers over time in 148 people with HIV evaluated in the Spanish AIDS Research Network (Abstract 527). In this nonrandomized trial that included many 3- and 2-drug ART regimens, investigators found that participants who remained on a 3-drug regimen experienced a slow decline of numerous biomarkers over time (IL-6, C-reactive protein, soluble CD14, soluble CD163 and D-dimer). In contrast, switching to a 2-drug regimen was associated with increases in IL-6, C-reactive protein, and D-dimer (all P values $\leq .01$) over 3 years, after adjusting for covariates. Although 2-drug regimens have largely been safe for the brain in shorter term analyses, these findings raise concerns about their long-term effects on inflammation and coagulation.

Relevant to the HIV-1 cure agenda, McMahan and colleagues presented an interim analysis of a trial using the programmed cell death protein-1 (PD-1) blocker pembrolizumab (Abstract 345). Six people with HIV with virologic suppression for at least 12 months and CD4+ T-cell count above 350/mL were given a single dose of pembrolizumab. No grade 3 or 4 adverse events occurred but grade 1 or 2 adverse events involving blood, metabolic, and gastrointestinal systems were observed. PD-1+ CD4+ and PD1+ CD8+ T cells in CSF decreased at 3 weeks and appeared to rebound at 24 weeks. Cell-associated HIV DNA in CSF decreased by 46% after 3 weeks and remained persistently decreased by 7.7% after 24 weeks. The findings suggest that single-dose is generally safe in people with HIV and may decrease HIV in the CNS, but this was an interim analysis of a small number of participants within an ongoing study.

A single dose of pembrolizumab reduced cell-associated HIV DNA in CSF by 46% at week three with persistent decrease of 7.7% at week 24

There was also a CNS trial using the nonhuman primate model. Garcia-Mesa and colleagues presented a study in SIV-infected rhesus macaques of dimethyl fumarate, which regulates expression of antioxidant, anti-inflammatory, and cytoprotective genes via its effects on nuclear erythroid 2-related factor 2-antioxidant response element (Nrf2-ARE) pathway (Abstract 347). Five of 9 animals were treated with dimethyl fumarate, followed by examination of 11 brain regions. Several antioxidant enzymes (GPX-1, NQO1, HO-1, and PRDX1) were higher in the brains of the dimethyl fumarate-treated animals

in various brain regions. Dimethyl fumarate was also associated with lower oxidative stress end products (3-nitrotyrosine and 8-hydroxydeoxyguanosine), particularly in the brainstem, as well as lower mitochondrial redox state in both frontal cortex and brainstem. Although SIV RNA concentrations in CSF and blood did not change following dimethyl fumarate, the investigators did not quantify SIV RNA or DNA in brain tissue.

Findings on the Effects of SARS-CoV-2 or Other Infections on the Brain

SARS-CoV-2 Infection

Accumulating evidence shows that infection with SARS-CoV-2 can lead to neurologic complications. In an invited oral presentation, Benedict Michael discussed ongoing work on neuroCOVID in the United Kingdom (Abstract 51). He first contextualized SARS-CoV-2 in relation to other viruses that cause neurologic disease, including herpesviruses, West Nile virus, influenza virus, and Japanese encephalitis virus. He discussed the fact that viruses do not necessarily need to be neuroinvasive to cause neurologic disease, and that some viruses cause harm to the CNS by parainfectious and autoimmune pathways. This led to the initiation of numerous neuroCOVID studies, including a large surveillance system to monitor neuroCOVID cases, which had identified 511 cases to date in the United Kingdom. More than half (54%) involved a cerebrovascular event, which appears to be more common in older patients, and neuropsychiatric syndromes are more common in younger patients. However, cerebrovascular cases were generally younger than non-COVID-19 stroke cases collected from other surveillance systems. Patients with cerebrovascular events presented around the same time as the onset of respiratory symptoms, and patients with other neurologic symptoms more frequently presented about 2 weeks after onset of respiratory symptoms. The most significant predictors of poor outcome overall were older age and frailty. This surveillance system could serve as a model for other countries to monitor neuroCOVID cases.

In an oral presentation, Farhadian and colleagues from Yale and UCSF reported a cross-sectional study of immune responses in CSF collected from 6 hospitalized patients with SARS-CoV-2 infection and 11 healthy controls (Abstract 165). All 6 hospitalized patients had some degree of neurologic symptoms, including encephalopathy, headache, and seizures. None had elevated leukocytes or detectable SARS-CoV-2 RNA in CSF, although some had elevated total protein in CSF. Single-cell mRNA sequencing was performed, showing upregulation of pathways from both CD4+ and CD8+ T cells in CSF (including IL-1 and IL-12 responses) that were not upregulated in blood. Quantification of cytokines confirmed increase in IL-1β and IL-12 p70 in CSF, which again differed from blood (other biomarkers such as CCL2 and IL-8 were elevated in blood). Based on distinct CSF plasma cell clusters that were found in the SARS-CoV-2-infected patients, anti-SARS-CoV-2 antibodies were profiled and monoclonal antibodies were derived, resulting in the identification of 2 antispike antibodies from blood and one from CSF. The monoclonal antibodies were then incubated with mouse brain tissue, resulting in 4 CSF antibodies (including the antispike antibody) that had antineural immunoreactivity. This was followed by incubation of brain sections with whole CSF from SARS-CoV-2-infected patients, which again demonstrated increased antineural immunoreactivity compared with controls. These important findings support that the neurologic consequences of SARS-CoV-2 infection may result from CNS-specific immune responses, including from the humoral immune response. One limitation of the project is that it did not include biospecimens from people with SARS-CoV-2 infection who did not have neurologic complications.

Anti-SARS-CoV-2 antibodies were profiled and monoclonal antibodies were derived, resulting in the identification of 2 antispike antibodies from blood and 1 from CSF. The monoclonal antibodies were then incubated with mouse brain sections, resulting in 4 CSF antibodies that had antineural immunoreactivity

Using an in vitro model, Clough, Mahajan, and colleagues examined the effects of SARS-CoV-2 on the blood-brain barrier (Abstract 346). Human brain microvascular endothelial cells were treated with recombinant SARS-CoV-2 spike protein and heat-inactivated SARS-CoV-2. Compared with controls, cells treated with SARS-CoV-2 had increases in hypoxia inducible factor (HIF)1/2, nitric oxide synthase, the NLRP3 inflammasome, and several cytokines including TNF-α and IL-6. ACE2 (necessary for SARS-CoV-2 cell entry) was upregulated in treated brain microvascular endothelial cells, and blood–brain barrier integrity was decreased by 30% in the SARS-CoV-2 treated samples. Lastly, levels of 4 different tight junction proteins decreased in the SARS-CoV-2 treated samples compared with controls. Based on this study, SARS-CoV-2 appears to have a detrimental effect on the blood–brain barrier, which may be responsible for some of its neurologic sequelae.

Human Polyomavirus 2 Encephalitis

Progressive multifocal leukoencephalopathy (PML), caused by human polyomavirus 2 (also known as JC virus), still occurs in people with HIV, even in some individuals with CD4+ T-cell counts greater than 200/μL. Pinnetti and colleagues (Abstract 336) presented a small open-label study of 5 people with HIV with PML who were taking ART and who were treated with the PD-1 blocker, pembrolizumab. They observed a decrease in PD-1 expression on CD4+ and CD8+ T cells in blood and CSF in all participants, as well as

a decrease in human polyomavirus 2 DNA in CSF and an increase in human polyomavirus 2-specific T cells in blood. However, 2 participants died after a relatively short period and 1 participant experienced immune reconstitution inflammatory syndrome (IRIS). Although the changes in disease indicators is promising, more research is needed on pembrolizumab and other therapies for PML in people with HIV to evaluate safety and efficacy.

Cryptococcosis

Cryptococcal meningitis continues to be a devastating opportunistic infection in people with HIV with low CD4+ cell counts, particularly in sub-Saharan Africa. Drain and colleagues reported a prospective laboratory screening study for cryptococcal antigen in Umlazi Township, South Africa (Abstract 564). This study (n=908) occurred in 3 chronologic phases. Clinician-directed central laboratory cryptococcal antigen testing occurred in the first phase, followed by reflex central laboratory cryptococcal antigen testing based on CD4+ T cell count in the second phase, and then followed by point-of-care CD4+ T cell count and cryptococcal antigen testing by lateral flow in the third phase. Participants in the point-of-care phase were more likely to start ART and less likely to be lost to follow up than those in the clinician-directed phase. There was also a trend toward more frequent diagnosis of cryptococcal meningitis in the point-of-care phase. Thus, point-of-care CD4+ T cell count and cryptococcus testing may be beneficial in settings where low CD4+ T cell counts are common.

Peripheral Neuropathy

Complications of the peripheral nervous system remain common despite ART, although their severity is markedly reduced akin to the pattern observed with cognitive impairment in the modern treatment era. Ellis and colleagues (Abstract 348) evaluated the relationship between peripheral neuropathy pain symptoms and gut microbiota in 373 adults (72% people with HIV, 90% of whom were virologically suppressed). Peripheral neuropathy pain symptoms were more common and more severe in people with HIV ($P<.02$). More severe pain was associated with lower gut microbial diversity (determined by 16S rRNA sequencing) in people with HIV but not in people without HIV. Specifically, change to *Lachnospira* species from either *Ruminococcus* ($P=.007$) or *Streptococcus* ($P=.001$) species was significantly associated with peripheral neuropathy pain symptoms in people with HIV, raising the possibility that manipulation of the microbiome could be an approach to peripheral neuropathy in future studies of people with HIV.

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