Invited Review

CROI 2022: Neurologic Complications of HIV-1, SARS-CoV-2, and Other Pathogens

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The 2022 Conference on Retroviruses and Opportunistic Infections featured new and important findings about the neurologic complications of HIV-1, COVID-19, and other infections. Long-term analyses identified that cognitive decline over time, phenotypic aging, and stroke are associated with various comorbidities in people with HIV. Neuroimaging studies showed greater neuroinflammation, white matter damage, demyelination, and overall brain aging in people with chronic HIV infection. Childhood trauma and exposure to environmental pollutants contribute to these neuroimaging findings. Studies of blood and cerebrospinal fluid biomarkers showed that systemic inflammation, neurodegeneration, endothelial activation, oxidative stress, and iron dysregulation are associated with worse cognition in people with HIV. Some animal studies focused on myeloid cells of the central nervous system, but other animal and human studies showed that lymphoid cells also contribute to HIV neuropathogenesis. The deleterious central nervous system effects of polypharmacy and anticholinergic drugs in people with HIV were demonstrated. In contrast, a large randomized controlled trial showed that integrase strand transfer inhibitor therapy was not associated with neurotoxicity. Studies of cryptococcal meningitis demonstrated the cost-effectiveness of single high-dose liposomal amphotericin and the prognostic value of the cryptococcal antigen lateral flow assay. People hospitalized with COVID-19 had more anxiety over time after discharge. The SARS-CoV-2 nucleocapsid antigen is present in cerebrospinal fluid in the absence of viral RNA. Systemic inflammation, astrocyte activation, and tryptophan metabolism pathways are associated with post-COVID-19 neurologic syndromes. Whether these processes are independent or intertwined during HIV-1 and COVID-19 infections requires further study.

Keywords: HIV, CROI 2022, COVID-19, SARS-CoV-2, neurologic complications, cognition, brain, CSF, neuroimaging

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Introduction

Central nervous system (CNS) effects of HIV-1 were an important theme at the 2022 Conference on Retroviruses and Opportunistic Infections. Presentations focused on HIV pathogenesis and CNS reservoirs, as well as on persistent neurologic dysfunction (as assessed by neuropsychologic testing, imaging, or cerebrospinal fluid [CSF] evaluations) in people with HIV who have virologic suppression. New data were presented on accelerated aging and the effects of aging-related comorbidities on brain function—themes that have become increasingly important as people with HIV live longer as a result of effective antiretroviral therapy (ART). New data also provided encouraging news for treating

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cognitive impairment and advancing the HIV cure agenda. This review focuses on major thematic areas that could inform new research initiatives and stimulate novel approaches for the clinical management of people with HIV.

Neuroimaging Findings

Persistent neuroinflammation may affect the CNS in early and chronic HIV infection and may have a variety of causes. Alagaratnam and colleagues evaluated if neuroinflammation, as measured by positron emission tomography imaging with the translocator protein radioligand [^{11}C]PBR28, would be lower in people with HIV who initiated ART during acute HIV infection than in those who initiated it later (Abstract 127). Within a small group of virologically suppressed people with HIV, those with chronic HIV infection had greater [^{11}C]PBR28 binding (ie, greater neuroinflammation) than people without HIV. In contrast, [^{11}C]PBR28 binding in people who initiated ART during acute HIV infection was similar to that in people without HIV, suggesting early ART initiation may mitigate neuroinflammatory responses. Bolzenius and colleagues showed an analysis of people with acute HIV infection in which brain volume changes were examined in a large Thai cohort (Abstract 419). Participants were categorized by stage of acute HIV infection (Fiebig stage). People in late Fiebig stages (III-IV) had significantly larger volumes in subcortical areas (including caudate, putamen, pallidum, and amygdala) than people without HIV, a finding attributed to possible infiltration of immune cells in response to HIV. Although this neuroinflammation may resolve with continued ART, it could also lead to brain structural changes. For this reason, longitudinal studies are needed that include soluble and cellular biomarkers and neuroimaging. Ham and colleagues studied the effects of childhood trauma (experiencing or witnessing physical or sexual abuse) on cognition, daily functioning, and brain morphology in adults with HIV who were receiving ART and were virologically well controlled (<200 HIV RNA copies/mL) (Abstract 418). They found that people with HIV who had no history of childhood trauma had larger brain volumes and better cognitive performance than those with such history. However, people with HIV who had childhood trauma and larger subcortical brain volumes had worse learning performance and greater functional impairments. Because the larger brain volumes observed in this cross-sectional analysis may reflect neuroinflammation, further study using longitudinal analyses that combine imaging with soluble and cellular biomarkers could aid understanding. Although continued suppression of HIV RNA with ART has salutary effects, the influence of events before HIV infection and ART initiation on brain volumes and cognitive performance are becoming increasingly clear.

A biomarker of oxidative stress was associated with a larger ratio of abnormal white matter to total white matter

One structural neuroimaging finding that has been consistently associated with cognitive performance is abnormal white matter. To further evaluate this association, Riggs and colleagues analyzed data from the CHARTER (CNS HIV Antiretroviral Therapy Effects Research) project (Abstract 408). The ratio of abnormal white matter to total white matter was measured via magnetic resonance imaging (MRI) in 55 people with HIV. Also, 15 soluble biomarkers were measured in blood and, in 1 subgroup, in CSF. Multivariable analyses identified that higher blood concentration of protein carbonyls, a biomarker of oxidative stress, was associated with a larger ratio of abnormal white matter to total white matter; also associated were older age and Black race. Changes in oxidative stress may result from several distinct processes, with protein carbonyl concentration reflecting an aggregate measure of physiologic stressors.

Neuroimaging studies also yielded evidence of neurodegeneration and axonal loss. Patel and colleagues used myelin water imaging, a novel neuro-

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imaging method, to investigate water within myelin bilayers (Abstract 417). They compared the myelin water fraction (MWF), a metric of myelin content, for virally suppressed adults with perinatal HIV with MWF for people without HIV. Global and frontal-lobe MWF was lower in people with HIV than in people without HIV. Lower global MWF was associated with worse executive function in people with HIV. These results indicate that decreased axonal myelination may occur early in perinatal HIV infection and may be associated with worse cognitive performance. However, longitudinal studies are needed that include larger numbers of people with HIV and matched controls to verify these results. Meade and colleagues examined the relationship between serum neurofilament light chain (NfL), a marker of axonal injury and neuronal loss, and white matter integrity, as measured by diffusion tensor imaging, within a cohort of chronically infected people with HIV who were virologically well controlled (Abstract 413). Higher serum NfL levels were associated with greater peripheral inflammation (ie, sCD14) and with reductions within structural connections in subcortical brain regions.

Additional risk factors may also affect brain structure and function on neuroimaging. Peterson and colleagues used machine-learning algorithms to evaluate changes in white matter within the brain with aging. In addition to HIV, older age and vascular disease are associated with changes to cerebral white matter that are detectable on diffusion-weighted MRI. These changes can be quantified using the brain age gap, the difference between true age and neuroimaging-predicted "brain age." In a large cohort of people with and without HIV, white matter aging (ie, progressive accumulation of microstructural damage) was evaluated with respect to age. Compared with people without HIV, people with HIV who had a detectable viral load had an additional 1.5 years of brain age gap per decade, suggesting premature aging. Greater white matter changes were also associated with worse cerebrovascular disease, as measured by Framingham risk score. Aging with detectable plasma HIV RNA and the presence of cardiovascular risk factors are associated with white matter pathology and may contribute to the development of cognitive impairment in people with HIV.

Environmental pollutants are increasingly recognized for their effects on lung, cardiovascular, and brain function. Wisch and colleagues evaluated a community-dwelling cohort of older people with HIV to assess the effects of common air pollutants, including fine particulate matter (PM2.5) and ozone, on cognitive performance and brain structure (Abstract 416). A 10-µg/mL increase in average exposure to PM2.5 in the week before assessment was associated with worse learning performance. Among people without HIV, greater ozone exposure was associated with smaller temporal lobe volume, but in people with HIV the opposite was true. These findings are consistent with previous literature that suggests a deleterious effect of PM2.5 on cognition.

Exposure to PM2.5 in the week before assessment was associated with worse learning performance

Aging, Comorbid Diseases, Biomarkers, and Neurologic Complications

Corbett and colleagues examined the prevalence of a new stroke event in people with HIV in South Africa (Abstract 424). A total of 884 strokes were identified, and among people with HIV (68% of whom were taking ART), hypertension and dyslipidemia were less prevalent than among people without HIV. In contrast, concurrent infections (including tuberculosis, syphilis, and cryptococcal infections) were more prevalent in people with HIV than in people without HIV.

People with HIV often have an excess of comorbidities, which may substantially affect mortality and quality of life. Ellis and colleagues attempted to predict long-term cognitive decline based on a simple assessment of comorbid conditions in people with HIV (Abstract 427). A simple comorbidity index (SCI), composed of presence or absence of hypertension,

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chronic obstructive pulmonary disease, and depression, was compared with other well-established measures including the Charlson Comorbidity Index, the VACS (Veterans Aging Cohort Study) index, and the Framingham cardiovascular index. Participants with a higher SCI at baseline had significantly worse neurocognitive decline over 12 years of follow-up. Individuals with 2 or more comorbidities at baseline had worse neurocognitive decline than individuals without comorbidities. Compared with other indices such as the VACS and Framingham indices, only the SCI was associated with cognitive decline. The importance of these comorbidities is rooted in their effects on daily functioning and quality of life. Prior studies have assessed overall quality of life, but fewer have assessed the influence of cognitive impairment in people with HIV on health-related quality of life. To address this issue, Alford and colleagues assessed health-related quality of life and its relationship to cognition in people with HIV, finding that cognitive impairment predicted worse health-related quality of life (Abstract 422).

Participants with a higher SCI at baseline had significantly worse neurocognitive decline over 12 years of follow-up

Aung and colleagues longitudinally studied 457 people with HIV yearly over 3 years. At baseline, 31% were cognitively impaired (Abstract 425). The cognitive performance of a minority of participants either (1) declined after 1 (6%) or 2 (7%) years of follow-up, or (2) improved after 1 (4%) or 2 (3%) years. Worse cognitive performance over time was associated with severe depression, worse ART acceptance, and lack of companionship. The investigators concluded overall that the rate of cognitive decline was low owing to good virologic control, which is supported by more integrated healthcare services. Han and colleagues compared phenotypic aging between people with and without HIV using a novel combination of biomarkers (Abstract 615). Phenotypic age was calculated using chronologic age and a combination of 9 blood-based biomarkers, including complete blood cell counts and inflammatory, metabolic, liver, and kidney-related parameters. To evaluate if phenotypic age was accelerated in people with HIV compared with people without HIV, the difference between chronologic and phenotypic age was calculated for both groups. Lower CD4+/CD8+ cell ratio and higher VACS index were associated with older phenotypic age in people with HIV. Within the entire cohort, male sex, current smoking, diabetes mellitus, frailty, and higher interleukin (IL)-6 level were associated with an elevated phenotypic aging value.

Lower CD4+/CD8+ cell ratio and higher VACS index were associated with older phenotypic age

Measurement of proteins and nucleic acids in extracellular vesicles isolated from body fluids may provide more precise evidence of cellular injury and abnormal intercellular communication. Guha and colleagues compared plasma and CSF concentrations of free and extracellular vesicle (EV)-associated biomarkers from 98 people with HIV with virologic suppression with concentrations of the same biomarkers from 86 people without HIV who were matched for age, sex, and race (Abstract 407). People with HIV were stratified by cognition (52% were impaired) and presence of vascular disease (23.5% had vascular disease). People with HIV had higher levels of plasma NfL and CSF amyloid beta (β)1-42 (Aβ42). CSF EV-associated Aβ42 level was lower (P=.0002) and CSF EV-associated tau/Aβ42 ratio was higher (P=.001) in people with HIV and cognitive impairment than in people with HIV without cognitive impairment. Lower CSF EV-associated Aβ42 (P<.0001), higher CSF EV-associated tau/Aβ42 ratio (P=.0003), and higher plasma NfL level (P=.098) were associated with worse cognitive performance in multivariable models that incorporated the whole group. Cognitive impairment in

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people with HIV was also associated with higher levels of plasma intercellular adhesion molecule (ICAM)-1 (P=.02), vascular cell adhesion molecule (VCAM)-1 (P=.004), and C-reactive protein (P=.02). People with HIV had higher levels of EV ICAM-1 and VCAM-1 than people without HIV (P<.0001), but these higher levels were not associated with cognitive impairment.

Using data and biospecimens from 376 participants of AIDS Clinical Trials Group (ACTG) clinical trials, Kalayjian and colleagues evaluated relationships between cognitive performance and biomarkers in plasma, including citrate, soluble (s) tumor necrosis factor receptor (TNFR) I and II, IL-6, sCD14, sCD163, intestinal fatty acid binding protein (IFAB), and vascular endothelial growth factor (Abstract 412). In multivariable models, higher concentrations of sTNFR I, sTNFR II, and sCD163 were associated with both prevalent and incident cognitive impairment. Meanwhile, higher IFAB concentration was associated with lack of prevalent cognitive impairment. In models for prevalent cognitive impairment, significant interactions were present between (1) vascular endothelial growth factor levels and sex and (2) citrate level and age. In mixed-effects models evaluating cognitive change over time, higher sTNFR I, sTNFR II, and citrate levels were associated with greater cognitive decline over time. Although prior research has identified links between inflammation biomarkers like sTNFR I and cognition, the findings with IFAB and citrate are relatively novel and may provide new insights into pathogenesis.

Lower FTL level was associated with worse cognitive performance over time

From the same ACTG cohort, Kaur and colleagues evaluated biomarkers of iron homeostasis (plasma ferritin heavy chain [FTH]1; urine T-cell immunoglobulin and mucin domain 1 [TIM-1], an FTH1 receptor; and plasma ferritin light chain [FTL]) (Abstract 406). Cross-sectional multivariable analysis of the entire cohort identified that lower FTL and higher TIM-1 levels were associated with cognitive impairment, and longitudinal analyses identified that a lower FTL level was associated with worse cognitive performance over time. Sex-specific cross-sectional analyses identified that a lower FTH level was associated with cognitive impairment in women, an association that held over time. In men, higher TIM-1 level was associated with worse cognitive performance. These data add to accumulating evidence that disordered iron metabolism contributes to cognitive performance in people with HIV and support a clinical trial of an iron-focused intervention.

In addition to its role in heme synthesis and oxygenation, iron is crucial to the function of many cell types and may further influence neurologic health by its association with inflammation and oxidative stress. Combined with the finding noted earlier linking protein oxidation and abnormal white matter volume, biomarker evidence is growing of the impact of oxidative stress on the CNS in treated people with HIV (Abstract 408). Mitochondria are central to cellular oxidant–antioxidant balance, and Volpe and colleagues reported on comparisons of mitochondrial haplogroups with cognitive performance in 691 women with HIV from the MACS/WIHS (Multicenter AIDS Cohort Study and the Women's Interagency HIV Study) Combined Cohort Study (Abstract 415). They found that mitochondrial haplogroup L2a was associated with better motor and speed-of-information-processing performance in Black women with HIV, and mitochondrial haplogroup B was associated with better motor performance in Hispanic women with HIV. Most mitochondrial research to date in people with HIV has been in men, so these findings provide important information on sex-related differences in genetic vulnerability to neurologic complications.

Biomarker investigations are affected by the sensitivity, accuracy, and reproducibility of biomarker assays, many of which are antibody based. The quality of these antibodies can vary over time, which can lead to inconsistent results. Newer methods, such as the single-molecule assay method,

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may better address these historical limitations. This method was used to develop an assay for NfL in blood, higher concentrations of which have been linked to cognitive impairment in people with HIV. To build on these findings, Trunfio and colleagues used the single-molecule assay method and other methods to measure NfL and Alzheimer's disease–related biomarkers in blood and CSF in 44 people with HIV; they found strong correlations between NfL concentrations in CSF and blood but not for other analytes (Abstract 411). When they compared biomarker levels with cognitive performance, they found that biomarker concentrations in blood correlated more strongly with cognitive performance than concentrations in CSF. Although the current research does not explain the underlying mechanisms of this surprising finding, it holds promise that diagnostic biomarkers for cognitive impairment may be measurable in blood, which would avoid the need to perform lumbar puncture.

Correlation between biomarker concentrations in CSF and blood was the focus of a poster from Kamkwalala and colleagues, who presented findings from the CHARTER cohort (Abstract 409). Comparing data from 2 assessments separated by 12 years, they found that biomarkers in CSF correlated more strongly with each oter over time than did biomarkers in blood, particularly for sTNFR II (P=7x10-29), Aβ42 (P=8x10-33), and total (P=4x10-26) and phosphorylated (P=2x10-54) tau. This pattern suggests that the influence of the mechanisms reflected by these biomarkers may be relatively fixed over time in people with HIV, which is a novel finding.

With the expansion of multiplex biomarker assay methods, accounting for type I error is increasingly important. The likelihood of type I error can increase with each additional biomarker. Additionally, many inflammatory biomarkers correlate with each other, which can also add to the likelihood of type I error or produce misleading results. One approach to managing this common error is through use of dimension-reduction methods. Tavasoli and colleagues used one such method, factor analysis, to evaluate the same biomarkers from the CHARTER cohort, again at 2 timepoints 12 years apart (Abstract 410). They found that higher levels of a factor combining sTNFR II and neopterin in CSF correlated with higher NfL levels in CSF, but only among people with HIV who were virally suppressed with ART at both timepoints (correlation coefficient [r], 0.36; P=8x10-6). This finding links neuroinflammation with neuronal injury over a long period of observation.

Brain Tissue Investigations in Animals and Humans

Although CSF and blood analyses can yield valuable insights into HIV pathogenesis in the brain, directly examining brain tissue should provide even more relevant data. To address this goal, White and colleagues simultaneously evaluated brain and cardiac pathology in CD8+ T-cell-depleted simian immunodeficiency virus (SIV)-infected macaques with AIDS (Abstract 403). Animals were grouped as having (1) cardiovascular disease (CVD) and encephalitis (SIVE) (CVD+SIVE), (2) CVD only or SIVE only, (3) SIV without encephalitis, or (4) none of the conditions. Animals with CVD+SIVE had more CD163+, CD206+, CD68+, and MAC387+ cardiac macrophages than the other groups. Animals with CVD+SIVE also had greater cardiac collagen deposition and higher sCD163 level than those with either CVD or SIVE only. Animals with CVD+SIVE had repeatedly higher CD14+CD16+ monocyte levels in blood at 3 timepoints after infection. A higher CD14+CD16+ monocyte level in the blood correlated with greater cardiac collagen deposition in the entire cohort. These findings link disease-related changes in the heart and the brain, confirming and extending clinical data that have found that cardiovascular disease increases the risk for cognitive impairment in people with HIV.

BLZ945 also decreased SIV DNA in 9 of the 11 analyzed brain regions

Given evidence that perivascular macrophages (PVMs) of the brain serve as HIV reservoirs and

that colony-stimulating factor 1 receptor (CSF1R) in PVMs is upregulated in SIV-infected rhesus macaques with encephalitis, Zablocki-Thomas and colleagues evaluated blockade of this receptor in the macaque model (Abstract 347). After CD8+ depletion, they compared untreated animals with animals treated with BLZ945, a selective brain-penetrating CSF1R kinase inhibitor, at high or low dose. They found that PVM count significantly correlated with SIV DNA in the CNS and that high-dose BLZ945 reduced PVMs. BLZ945 also decreased SIV DNA in 9 of the 11 analyzed brain regions. The findings support that inhibiting CSF1R kinase may hold promise in reducing HIV DNA level in the brain.

Mathews and colleagues evaluated glial HIV reservoirs in a triple-humanized mouse model (Abstract 390). In this model, microglia distribute throughout the brain, but astrocytes are limited to the corpus callosum, fornix, and near lateral ventricle. Human oligodendrocytes and neurons are also present in the brain. According to their canonical markers, 15 different clusters of cells were demonstrated. Of the 7 clusters showing myeloid markers, 6 showed the presence of HIV. Individual HIV genes were also found in most groups of myeloid cells. Transcriptomics revealed that 2 clusters had differential gene expression, with upregulation of interferon signaling and increased cytokines or chemokines but downregulation of most other signaling pathways. This humanized mouse model showed evidence of productive HIV infection in microglia but not astrocytes.

In a postmortem study using frontal cortex samples from the Manhattan HIV Brain Bank, Plaza-Jennings and colleagues characterized microglia using 10x chromium single-nucleus RNA-seq (snRNA-seq), integration site analysis, and high-throughput chromatin conformation capture (Abstract 391). Brain tissue from people with HIV who died with HIV encephalitis (HIVE) was compared with brain tissue from people without HIV. snRNA-seq revealed 188 upregulated genes (many related to the immune response) and 276 downregulated genes (many related to the antiviral immune response) in HIVE. HIV integration sites were found in highly expressed microglia genes and were over-represented among differentially expressed genes in HIVE microglia. Considering these and other findings, the investigators concluded that HIVE leads to widespread 3D-genome restructuring in microglia, including at immune loci, and that these same regions are targeted for viral integration.

In another postmortem study from the same group, Min and colleagues reported on HIV transcript findings using high-throughput snRNA-seq on frontal cortex from 3 groups: (1) people without HIV (n=2), (2) people with HIV and without HIVE (n=3), and (3) people with HIV and HIVE (n=3) (Abstract 402). Among the major brain cell types, HIV transcripts were elevated in a subset of microglia in people with HIV and HIVE but not in people with HIV and without HIVE. Dual RNA-FISH assays with probes complementary to the Iba1 microglial marker and HIV RNA confirmed that microglia express high levels of HIV. In people with HIV and HIVE, HIV transcripts were the top 0.3% most highly expressed transcripts in the microglial transcriptome. In addition, transcription of proinflammatory signaling genes was more elevated in people with HIV than in people without HIV, including purinergic receptors (P2X1, P2X7), NLRP3 inflammasome signaling components (NLRP3, Casp1, ASC), and proinflammatory cytokines (IL-1β, IL-18).

The Role of Lymphoid Cells in the CNS During HIV Infection

The preceding findings reinforce well-known associations between myeloid cells and neurologic complications in HIV disease, but increasing evidence supports a role for lymphoid cells as well. For example, Fox and colleagues studied 3 macaques 12 days postinfection with SIVmac251 (Abstract 393). SIV RNA was expressed by 3.67% of blood CD4+ T cells, less than 0.01% of blood monocytes, and 0.15% of brain myeloid and lymphoid cells. The infected blood CD4+ T cells were present in 2 populations distinguishable by the reciprocal expression of RNA for (1) the cytotoxic molecule granzyme B (GZMB) and (2) the transcription factor TCF1 (TCF7). Only the GZMB+TCF7- population of SIV-infected CD4+ T cells (which resemble cytotoxic T cells) were found in the brain, in addition to infected cells

characteristic of microglia (AIF+P2RY12+CD3D-). Flow cytometry confirmed the presence of GZMB+ CD4+ T cells in the brain, and immunohistochemistry/in situ hybridization identified CD4+ T cells expressing SIV RNA in the brain. This study suggests that HIV in the brain may derive from subsets of T cells, which runs contrary to existing belief that myeloid cells such as brain macrophages and microglia are primarily responsible for HIV replication in the brain. However, 12 days postinfection may be too early to identify all infected cells that may occur in the brain during chronic HIV.

DNA and cell-associated HIV RNA were detectable in up to 87.5% of participants, with the number of HIV-1 transcripts much higher in CSF-derived cells than in blood-derived cells

Another report supported the importance of lymphoid cells in the CNS. Zaunders and colleagues characterized cells isolated from CSF and blood of people with HIV taking suppressive ART by using 18-color flow cytometry and the Double-R πCode MicroDiscs assay (Abstract 126). Comparing these findings with information from brain magnetic resonance spectroscopy, they found that CSF cells were 91% memory T cells, and only a minority were monocytes or macrophages. DNA and cell-associated HIV RNA were detectable in 81% and 87.5% of participants, respectively. The number of HIV-1 transcripts was much higher in CSF-derived cells than in blood-derived cells (9226 vs 185 copies/10_6 CD4+ T cells, respectively). Participants who had more HIV-1 transcripts detected in CSF also had evidence of greater brain injury on magnetic resonance spectroscopy, particularly in frontal white matter and posterior cingulate cortex. Kincer and colleagues further supported the importance of lymphoid cells in production of HIV RNA in the CNS by focusing on symptomatic CSF viral escape, a condition typically characterized by having detectable HIV RNA in CSF and undetectable HIV RNA in blood (Abstract 130). The researchers used single-genome amplification and Illumina MiSeq deep sequencing with Primer ID to assess genetic diversity in partial *env* sequences (V1-V3) and drug resistance mutations. They found that most CSF-escape viral populations had either 1 (47%) or 2 major lineages (33%). A minority of populations were a highly diverse, recombinant population (16%). Relevant to the focus of this section on the contribution of lymphoid cells to HIV RNA in CSF, they also identified that all escape viruses were T-cell-tropic. This finding is similar to previous work that CSF HIV is mostlyT-cell-tropic.

The contribution of lymphoid cells to neurologic complications in HIV disease was further supported by 2 reports from SEARCH (Southeast Asia Research Collaboration in HIV) cohorts in Thailand. Mitchell and colleagues polyclonally expanded CD8+ T cells from blood and CSF of 15 people with HIV with acute infection and 6 people with HIV with chronic infection before and after ART initiation (Abstract 392). They then sequenced the T-cell receptor beta chain and measured functional HIV-specific responses. Within-sample clonality was measured with the Simpson clonality index and between-sample clonality with the Morisita index. Simpson clonality index was significantly higher in CSF-derived cells than in blood-derived cells before ART and after 24 and 96 weeks of ART, particularly among participants with acute HIV infection. Participants with acute HIV infection had higher Simpson clonality from CSF cells than participants with chronic HIV infection before ART but not at 24 or 96 weeks. The Morisita index was significantly higher (indicating less turnover) for cells derived from blood than for cells from CSF at weeks 24 and 96. Lastly, higher CSF Simpson clonality before ART correlated with increased frequencies of Env-, Nef-, and Rev/Tat-specific CD8+ T cells in the CSF at 24 weeks. The second SEARCH-cohort report, from Corley and colleagues, performed single-cell analyses of cells from CSF, lymph node, gut, and T-follicular cells from an ART-naive person with chronic HIV infection (Abstract 394). The team mapped out the cellular makeup of each tissue type, showing

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for example that all detectable HIV transcripts in the CSF compartment were from CD4+ memory T cells, similar to findings reported in Abstract 126. Clonotype tracking of sorted C-X-C chemokine receptor type 5 (CXCR5)+ T-follicular helper cells revealed exclusive shared clonotypes with lymph node only. On the basis of the capture across compartments of 71 single transcriptionally active HIV+ cells with a paired T-cell receptor, all T-cell clones were determined to be unique in cells containing HIV transcripts from CSF, gut, lymph node, and CXCR5+ T-follicular helper cells. Although this work focused on the logistical feasibility of international single-cell multiomics, the findings provide additional support for the influence of lymphoid cells in the CNS.

After ART interruption, only 1 of 6 animals rebounded in CSF compared with all 6 animals that responded in blood

Although debate has tended to focus on whether lymphoid or myeloid cells are more important in the CNS of people with HIV, both types likely influence pathogenesis, along with others such as astrocytes and oligodendrocytes. The report from Veenhuis and colleagues may shed light on the interplay between lymphoid and myeloid cells in the CNS (Abstract 129). In ART-treated SIVmac251-infected macaques, CD4+ T cells were depleted with CD4R1, an anti-CD4 antibody, and the macrophage-latent reservoir was assessed by an intact proviral DNA assay and a macrophage quantitative viral outgrowth assay. After ART interruption, only 1 of 6 animals rebounded in CSF compared with all 6 animals that responded in blood, further supporting the importance of lymphoid cells in HIV replication in the CNS. The investigators did, however, observe an increase in the reactivatable reservoir in the brain in the CD4+ T-cell-depleted animals, which reinforces that lymphoid and myeloid cells likely each have roles in maintenance of the brain reservoir.

Effects of Antiretroviral and Other Drugs on the CNS

ART Drugs and the CNS

Several questions remain about ART pharmacology in the CNS. These include questions about the extent of ART drug distribution into the brain and CSF, the influence of ART on control of HIV in the CNS, and the toxic effects of ART drugs in the CNS. In recent years, data have emerged to support that ART drugs may reach higher concentrations in the brain than in CSF. Higher concentrations should result in better control of HIV replication but could also increase the risk of toxicity. In a postmortem study from Uganda, Nicol and colleagues measured ART drug concentrations in blood and CSF from 65 deceased individuals with HIV, as well as in brain tissue in a subgroup (Abstract 450). They found that brain tissue concentrations were lower than CSF concentrations for tenofovir, lamivudine, and dolutegravir and higher than CSF concentrations for efavirenz. Concentrations across the 12 evaluated brain regions were heterogenous, but interindividual variability was greater than intraindividual variability. CSF concentrations did not correlate with mean brain concentrations of tenofovir or dolutegravir but strongly correlated for lamivudine ($r=0.90$; $P<.001$).

Brain tissue concentrations were lower than CSF concentrations for tenofovir, lamivudine, and dolutegravir and higher than CSF concentrations for efavirenz

The observation that efavirenz concentrations may be higher in the brain than in CSF may explain its well-documented neurotoxicity. The 8-hydroxy(OH) metabolite of efavirenz has previously been implicated in its neurotoxicity. Ranzani and colleagues investigated this concept further by measuring efavirenz and 8-OH-efavirenz in blood along with

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cognitive performance, mood, and sleep (Abstract 398). They confirmed that 8-OH-efavirenz concentrations in blood were associated with CNS adverse events including increased depressive symptoms and worse sleep as well as overall CNS symptom score. They posit that measuring 8-OH-efavirenz concentrations in blood in the clinic may identify people with HIV who are at risk for CNS complications. Interindividual variation in 8-OH-efavirenz concentrations is due in part to variation in genes involved in drug metabolism and elimination, like the gene that encodes cytochrome P450 2B6. Variation in this gene may be responsible for a severe but uncommon CNS complication of efavirenz: late-onset efavirenz neurotoxicity syndrome, which is associated with isoniazid coadministration. Investigators from South Africa characterized genetic variation in cytochrome P450 2B6 and in the N-acetyltransferase 2 gene that encodes a protein involved in the metabolism and elimination of isoniazid (Abstract 395). They identified that all participants who had late-onset efavirenz neurotoxicity syndrome were slow metabolizers of cytochrome P450 2B6 and slow acetylators of N-acetyltransferase 2; these participants also had efavirenz concentrations in blood that were more than 12 times higher than the upper limit of therapeutic range. Caution is advised when coadministering efavirenz and isoniazid, and ideally drug concentrations are measured or genotyping is performed when the drugs are used together.

Three other groups also reported on the effects of ART on the CNS. In addition to the CNS effects of efavirenz, a CNS safety signal has been reported for integrase strand transfer inhibitors (InSTIs), like dolutegravir. Analyzing data from ACTG clinical trials, O'Halloran and colleagues reported on people with HIV who (1) switched their ART to an InSTI-containing regimen; (2) underwent at least 2 cognitive assessments before the switch and 1 afterward; and (3) maintained viral suppression throughout observation (Abstract 400). The dataset was substantial, with 5824 assessments in 395 people with HIV over a mean duration of observation of 9 years pre- and 3 years post-switch. Overall cognitive performance (based on a 4-test composite) improved over time and the slopes were similar before and after the InSTI switch, supporting the conclusion that InSTI-containing regimens did not adversely affect overall cognition. Within the composite, the Hopkins Verbal Learning Test–Revised had a small but statistically significant decrease after the switch (P=.03). This may or may not have been significant after adjustment for numerous comparisons.

Given the declining use of efavirenz, a known neurotoxic drug, and growing data supporting that InSTIs are not neurotoxic, has the prevalence of cognitive impairment declined in people with HIV? Data from Mastrorosa and colleagues support a decline in prevalence (Abstract 132). Analyzing data from 1365 people with HIV who had 2383 assessments performed over 4 periods (2009-2011, 2012-2014, 2015-2017, 2018-2020) at a single center, they found that the prevalence of cognitive impairment substantially declined from 37.6% between 2009 and 2011 to 15.6% between 2018 and 2020. It was not clear if this may have been due to earlier treatment in the later time periods, but InSTI therapy was associated with lower risk of impairment in multivariable models. Although such analyses are prone to survivor bias (and other biases), they provide further support that current therapies are less neurotoxic than in the past. Another report supports the conclusion, however, that the findings may be at least partially due to the reversibility of ART neurotoxicity after a switch in therapy. Taramasso and colleagues identified 60 ART treatment–limiting CNS adverse events in 4751 people with HIV who were monitored over time (Abstract 399). Follow-up data were available for 52 of the 60 participants with CNS adverse events and identified that the effects resolved after discontinuation in nearly all (92.3%) and did not differ by use of an InSTI or another drug class.

The prevalence of cognitive impairment substantially declined from 37.6% between 2009 and 2011 to 15.6% between 2018 and 2020

Because CNS complications in people with HIV could result from persistent low levels of HIV RNA in the CNS and the resulting immune response, ART intensification could be beneficial for people with HIV who have CNS complications like cognitive impairment. To investigate this possibility, Letendre and colleagues performed a clinical trial in the ACTG, in which 191 people with HIV who had cognitive impairment and were taking suppressive ART were randomly assigned to intensify their existing ART regimen with a combination of (1) dolutegravir and maraviroc, (2) dolutegravir and placebo, or (3) dual placebo (Abstract 133). The study arms were well balanced, and consistent with practice effect, cognitive performance improved in all 3 arms. The improvement in cognitive performance in the active treatment arms did not differ from that in the dual-placebo arm and thus did not support the conclusion that ART intensification benefits people with HIV who have cognitive impairment. Of note, the overall cognitive performance of the active treatment arms also did not decline, adding further evidence against the neurotoxicity of InSTIs.

Improvement in cognitive performance in the active treatment arms did not differ from that in the dual-placebo arm and thus did not support the conclusion that ART intensification benefits people with HIV who have cognitive impairment

Concurrently Prescribed Drugs and the CNS

Along with findings on the diminished neurotoxicity of newer ART drugs, data were reported at the 2022 Conference on Retroviruses and Opportunistic Infections about the neuropsychiatric effects of the non-ART drugs prescribed to people with HIV. Doctor and colleagues presented cross-sectional data on the effects of anticholinergic drugs on falls and frailty in the multicenter POPPY (Pharmacokinetic and Clinical Observations in People Over Fifty) study (Abstract 35). Participants were categorized based on self-report of recurrent falls (at least 2 in 28 days) and Fried frailty criteria. Approximately one-quarter of participants (27%) used at least 1 anticholinergic drug, the most common being codeine, citalopram, loperamide, and amitriptyline. Multivariate modeling showed a trend between anticholinergic use and frequent falls as well as frailty. People with HIV who used 2 or more anticholinergic drugs were much more likely to have had recurrent falls (hazard ratio [HR], 3.6; confidence interval [CI], 1.4-9.4). This study supports avoiding the use of multiple an-ticholinergics in older people with HIV if possible.

People with HIV who used 2 or more anticholinergic drugs were much more likely to have had recurrent falls

Dastgheyb and colleagues investigated the influence of non-ART prescribed drugs by analyzing data from 920 virally suppressed people with HIV in various neurologic HIV cohorts assessed between 2015 and 2019 (Abstract 423). The participants reported use of a median of 6 prescribed drugs from more than 200 drug classes, which were analyzed using principal-components analysis to reduce the component number to 16. Another dimension-reduction method, self-organizing maps, was applied to cognitive performance and identified 10 cognitive profiles, which were compared with prescribed drug use and other data. Several prescribed drug components were associated with cognitive profiles, including one that combined several types of drugs that have CNS effects or are used to treat CNS conditions. These findings provide further evidence of the potential adverse effects of non-antiretroviral prescribed drugs on cognitive health.

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Neurologic Complications of COVID-19

The neurologic complications of COVID-19 are of continued interest. The syndrome of post-acute sequelae of COVID-19 (PASC) frequently includes neurologic symptoms. Eden and colleagues presented a cross-sectional study evaluating SARS-CoV-2 nucleocapsid antigen (N-Ag) levels in CSF and plasma (Abstract 131). They enrolled 44 hospitalized individuals with moderate to severe COVID-19 and 10 healthy COVID-19–negative controls; they also included specimens from 41 prepandemic control individuals. A total of 23 of the 44 had CNS symptoms (almost all with encephalopathy). None of the participants had SARS-CoV-2 RNA detectable in CSF. However, SARS-CoV-2 N-Ag was detected in 31 of 35 CSF specimens, and CSF concentrations highly correlated with N-Ag concentrations in plasma (r= 0.84; *P*<.001). CSF N-Ag concentrations correlated with CSF neopterin (r=0.38; *P*=.01) and CSF interferon gamma (IFN-γ) (r=0.42; *P*=.01). Regardless of CNS symptoms, participants with COVID-19 had higher CSF concentrations of β2-microglobulin, neopterin, IL-6, IL-10, and tumor necrosis factor (TNF)α; however, these concentrations were not significantly different when comparing COVID-19 participants with or without CNS symptoms. Those with CNS symptoms had higher concentrations of CSF IFN-γ, but not CSF IL-1β, IL-2, or NfL. Although the high detectability of N-Ag from CSF is notable, it may not necessarily indicate viral neuroinvasion given the high correlation with blood level and lack of viral RNA in CSF, although this finding could also indicate a nonproductive infection of glial cells, such as occurs when astrocytes are infected by HIV-1. A non-mutually exclusive explanation is that N-Ag could have properties that allow efficient crossing of the blood–CSF barrier. However, CSF N-Ag concentrations were only about one-tenth of blood concentrations.

SARS-CoV-2 N-Ag was detected in 31 of 35 CSF specimens

Peluso and colleagues analyzed 121 participants at 2 time points (median 52 days and median 123 days) after COVID-19 diagnosis (Abstract 631). Blood was collected for biomarker analysis at both time points, and participants were interviewed at the second visit for somatic symptoms, including CNS-related symptoms. At the first visit, participants with CNS symptoms had higher concentrations of glial fibrillary acidic protein (GFAP) (*P*=.02) and chemokine ligand 2 (CCL2) (*P*=.03). At both visits, those with CNS symptoms had higher concentrations of IL-6 (*P*=.006 and *P*=.01, respectively) and TNFα (*P*=.003 and *P*=.02, respectively). CNS symptoms were not associated with NfL, IL-10, IFN-inducible protein-10 (IP-10), or SARS-CoV-2 antibody concentration. In contrast, participants with CNS symptoms had significantly lower IFN-γ levels at the second visit. These findings suggest that CNS symptoms after COVID-19 may be associated with certain aspects of systemic inflammation that persist after the infection.

Antibodies against at least 1 SARS-CoV-2 antigen were detected in 7 of 10 CSF specimens

McAlpine and colleagues presented data from 34 individuals who had neurologic symptoms after acute COVID-19 diagnosis and 21 controls (Abstract 635). They measured levels of neopterin, NfL, total tau, GFAP, YKL-40, and soluble TREM2, and the presence of anti-SARS-CoV-2 spike, receptor-binding domain, and nucleocapsid antibodies in blood and CSF. The most common post-COVID-19 symptoms were cognitive impairment, depression/anxiety, fatigue, and headache. From blood, post-COVID-19 participants had significantly higher D-dimer (median, 0.44 vs 0.26 mg/L; *P*=.039) and GFAP (median, 84.4 vs 42.4 pg/mL; *P*=.006). Antibodies against at least 1 SARS-CoV-2 antigen were detected in 7 of 10 CSF specimens and in all 8 blood specimens in the post-COVID-19 group. This study adds to

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evidence that astrocyte activation, as evidenced by higher GFAP, may play a role in PASC.

Vergori and colleagues presented neuropsychologic testing data on 302 individuals after COVID-19 diagnosis (Abstract 632). Assessments were performed at 3, 6, and 12 months after COVID-19 diagnosis and included 10 neuropsychologic tests. Also included were questionnaires on depression, anxiety, and sleep. Nearly 60% of participants had been hospitalized, and hospitalized participants were more likely to be older, male, and have a high body mass index and at least 1 comorbidity. Lactate dehydrogenase and IL-6 levels were significantly higher in hospitalized participants than in those who were not hospitalized but there was no difference between the 2 groups in C-reactive protein level, lymphocyte count, or ferritin level. Hospitalized participants were more likely than nonhospitalized participants to be cognitively impaired at the 3-month visit (41.4% vs 11.1%, respectively; P=.004) but not at 6- or 12-month visits. Hospitalized participants had more anxiety symptoms at the 3- (P=.034) and 12-month visits (P<.001) (55.6% and 31.4, respectively) but less sleep disturbance at 3 months (P=.028). Depressive symptoms did not differ at any of the 3 timepoints. Anxiety appears to be common after hospitalization for COVID-19.

Higher quinolinic acid level was associated with worse cognitive performance in people who had COVID-19

The kynurenine pathway of tryptophan metabolism appears to be dysregulated during COVID-19 and may have a role in neuroCOVID outcomes. Cysique and colleagues evaluated 128 individuals at 2, 4, and 12 months after COVID-19 diagnosis (Abstract 634). Assessments evaluated cognition, olfaction, and mood. Blood testing included tryptophan, kynurenine, and several metabolites including quinolinic acid. Cognitive and olfaction impairment did not improve over the 3 visits, including when practice effects were accounted for. At the 2-month mark, disease severity was associated with anosmia (P=.05) but not cognition, and cognitive deficits were more common in those with anosmia (P=.05). Also, at 2 months, anxiety and depressive symptoms were associated with higher quinolinic acid concentration (P<.005). After an initial increase, quinolinic acid declined steeply by 12 months. In multivariate mixed-effects models, higher quinolinic acid level was associated with worse cognitive performance in people who had COVID-19. This study supports further investigation of the relationship between the kynurenine pathway and neuroCOVID syndromes.

To evaluate the effects of the COVID-19 pandemic on mental health in people with HIV, Bares and colleagues evaluated depression, anxiety, sleep, and alcohol intake in 95 individuals (50 people with HIV and 45 people without HIV) before the COVID-19 pandemic and again in early 2021 (Abstract 633). Mean depression severity and alcohol use increased significantly in both groups (P<.001 in people with HIV and P=.003 in people without HIV), and alcohol use was higher in men than in women (P=.002). The percentage of people (both with and without HIV) who moved into a more severe category of depression after the onset of the pandemic was identical (18%). Adverse mental health outcomes therefore appear to have increased as a result of the COVID-19 pandemic, but people with HIV may not fare worse than people without HIV.

Hypoxia-inducible factor 1-alpha (HIF1-α), a marker of tissue hypoxia, was upregulated in several brain regions of SARS-CoV-2–infected animals

In a nonhuman primate model of COVID-19, Fischer and colleagues infected 4 rhesus macaques and 4 African green monkeys with 2019-nCoV/USA-WA1/2020 and compared them with 2 controls of each primate species (Abstract 404). Microgliosis and neuronal cell death were present only in infected animals and were associated with the presence of

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virus in the brain. Similar to COVID-19 human studies, there was evidence of microhemorrhages in several brain regions. These findings were present regardless of acute respiratory distress syndrome status. Hypoxia-inducible factor 1-alpha (HIF1-α), a marker of tissue hypoxia, was upregulated in several brain regions of SARS-CoV-2–infected animals, except for cerebellum. However, SARS-CoV-2 was found only rarely in the brain and, when present, appeared to colocalize with the endothelial cell marker von Willebrand factor. This study shows that similar to humans, neuropathology in association with SARS-CoV-2 in the nonhuman primate model does not require direct viral neuroinvasion.

Other CNS Infections in People With HIV

In a cost-effectiveness study, Muthoga and colleagues examined data from the AMBITION-CM (Ambisome Therapy Induction Optimisation – Cryptococcal Meningitis) study, which showed the noninferiority of single high-dose liposomal amphotericin B in combination with 14 days of oral flucytosine plus fluconazole compared with World Health Organization (WHO) standard-of-care treatment (which includes 1 week of amphotericin deoxycholate plus flucytosine) for cryptococcal meningitis (Abstract 664). Costing tools were developed for each of the 5 countries involved (Botswana, Malawi, South Africa, Uganda, and Zimbabwe). The Malawi context was chosen for the primary analysis. Mean costs, cost-differences, and an incremental cost-effectiveness ratios were calculated. Additional sensitivity analyses were performed based on the potential for the liposomal amphotericin B regimen to reduce the length of hospital admission under real-world implementation conditions. For Malawi, the mean incremental cost-effectiveness ratio was US $128 (95% CI, $53-$257) per life-year saved with liposomal amphotericin B. Using a real-world laboratory monitoring schedule, the mean incremental cost-effectiveness ratio cost per life-year saved fell to US $80 (95% CI, $15-$275) with liposomal amphotericin B. For the other countries, incremental cost-effectiveness ratio cost per life-year saved ranged from US $92 to $152 for in-trial and US $71 to $121 for real world schedules. In the countries with higher costs for inpatient hospitalization (Botswana and South Africa), the liposomal amphotericin B regimen was cost-saving compared with the control treatment if patients could be discharged 1 or 2 days earlier. Extrapolating to high-income countries, cost savings with liposomal amphotericin B for cryptococcal meningitis may be even higher. Notably, the WHO issued a press release in April 2022 that endorsed the single-dose liposomal amphotericin protocol.

People with HIV with cryptococcal or tuberculous meningitis and detectable CMV DNA detected in blood had 59% increased hazard of death at 18 weeks

In an AMBITION-CM substudy, Leeme and colleagues examined the prognostic value of using a semiquantitative lateral flow assay to detect cryptococcal antigen from blood and CSF (Abstract 665). Using the Botswana and Malawi sites, the team evaluated mortality in participants with high or low blood cryptococcal antigen titer, which is dichotomized by the assay. Among 187 individuals whose titers were analyzed, 56% had high titers and 44% had low titers. People with high titers had significantly higher CSF fungal burden by colony forming units (CFU) per milliliter. Over 10 weeks of follow-up, those with high titers had an 88% increase in mortality hazard than those with low titer (P=.04). This difference remained significant after adjustment for age, sex, and treatment group. Use of this lateral flow assay could potentially risk-stratify people with cryptococcal meningitis.

In a substudy of the ASTRO-CM (Adjunctive Sertraline for the Treatment of HIV-Associated Cryptococcal Meningitis) and RifT (High Dose Oral and Intravenous Rifampicin for Improved Survival From Adult Tuberculous Meningitis) studies, Skipper and colleagues evaluated the association between cytomegalovirus viremia and mortality (Abstract 667).

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A total of 340 individuals with HIV and meningitis were evaluated, 90% of whom were from ASTRO-CM, and 36% of whom had detectable cytomegalovirus (CMV) from blood. Those with CMV viremia had lower hemoglobin concentration and were less likely to have CSF pleocytosis. Mortality at 18 weeks was 50% for participants with CMV viremia and 34% for participants without CMV viremia. In separate Cox proportional hazards modeling that accounted for low CD4+ T-cell count and Glasgow coma scale, people with cryptococcal or tuberculous meningitis and detectable CMV DNA in blood had 59% increased hazard of death at 18 weeks than people without CMV present. The findings may reflect more immune dysfunction in people with CMV viremia; the possible benefit of anti-CMV therapy is still untested but should be considered for future studies.

In a 3-country study in people with HIV living in sub-Saharan Africa, Kanyama and colleagues assessed the effects of implementation of a standardized management algorithm of CNS infections (Abstract 663). This algorithm included bedside rapid diagnostic tests and WHO-recommended treatments. In the preimplementation phase, only 10.1% of participants had microbiologically confirmed CNS infection; in the implementation phase, this level markedly improved with 75% (269/356) having a probable or confirmed CNS infection, and 77.3% (174/225) being microbiologically confirmed. In the implementation phase, the most common infection was cryptococcal meningitis (55%), driven by participants from Malawi and Tanzania. CNS toxoplasmosis was the most common infection in Cameroon. Median CD4+ T-cell count was lowest for cryptococcal meningitis (37/μL), followed by CNS toxoplasmosis (87/μL), tuberculous meningitis (104/μL), and bacterial meningitis (148/μL). Ten-week mortality was up to 45% for all infections except CNS toxoplasmosis, for which 10-week mortality was 21%. This study showed that standardized algorithms for CNS infections can drastically improve diagnostic certainty and guide management, which should improve survival.

All abstracts cited in the text appear in the CROI 2022 Abstract eBook, available online at www.CROIconference.org

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