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Biological And Clinical Markers Of Neuronal Injury In Primary And Chronic Hiv-1 Infection

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BIOLOGICAL AND CLINICAL MARKERS OF NEURONAL INJURY IN PRIMARY AND CHRONIC HIV-1 INFECTION

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The use of antiretroviral therapy (ART) has shifted the neurological manifestations of HIV-1 infection toward mild but debilitating HIV-associated neurocognitive disorder (HAND). Through two studies, we sought to characterize neuronal injury during primary and chronic HIV infection and to describe its relationship with HAND.

The aim of the first study was to quantify cerebrospinal fluid (CSF) and neuroimaging biomarkers of neuronal injury in primary HIV infection (PHI). We compared CSF levels of neurofilament light chain (NFL), tau, and amyloid proteins in 92 subjects with PHI and 25 controls and examined relationships with disease progression and neuroinflammation, neuropsychological testing, and proton-magnetic resonance spectroscopy (MRS). We hypothesized that PHI is characterized by increased CSF NFL that correlates with neuronal inflammation, and that tau and amyloid levels are normal in PHI. NFL was elevated in PHI ($p=0.0004$) and correlated with CSF neopterin ($r=0.38$, $p=0.0005$), IP-10 ($r=0.39$, $p=0.002$), WBCs ($r=0.32$, $p=0.004$), and CSF:plasma albumin ratio ($r=0.60$, $p<0.0001$). NFL correlated with decreased N-acteylaspartate and glutamate in the anterior cingulate, frontal white matter, and parietal gray matter ($r>0.30$, $p<0.05$). Beta-amyloid was elevated in PHI ($p=0.0005$) and correlated with time infected ($r=0.34$, $p=0.003$). Neither marker correlated with neuropsychological abnormalities. T-tau and amyloid precursor proteins did not differ between groups.

The aim of the second study was to characterize HIV-infected patients with neuro-symptomatic CSF ‘escape,’ defined as detectable CSF HIV RNA in the setting of

treatment-suppressed plasma levels or CSF RNA >1 log higher than plasma RNA. We conducted a retrospective case series of virologically controlled HIV-infected patients on ART with progressive neurological abnormalities who were determined to have CSF 'escape' at 4 urban medical centers in the United States and Europe. We recorded levels of CSF HIV RNA and inflammatory markers, clinical signs and symptoms, and magnetic resonance imaging (MRI) findings. We hypothesized that individuals with this condition would have inflammation in CSF and MRI studies, that CSF virus would be resistant to the ART regimen, and that symptoms would improve when ART was changed based upon central nervous system (CNS) drug penetration and resistance genotyping. 10 patients presented with sensory, motor, and cognitive abnormalities. Median CSF HIV RNA was 3900 copies/mL; median plasma HIV RNA was 62 copies/mL. Median $CD4^+$ T cell count was 482 cells/mm^3. All patients had been controlled <500 copies/mL for median 27.5 months and 5/10 had been suppressed <50 copies/mL for median 19.5 months. Patients were on a stable ART regimen for median 21 months. All had CSF pleocytosis or elevated CSF protein; 7/8 had MRI abnormalities; and 6/7 harbored CSF resistance mutations. Following optimization of ART, 8/9 patients improved clinically.

Although these processes occur at distinct time points in the disease, both neuronal injury during PHI and the development of symptomatic CSF 'escape' in chronic, well-treated infection are associated with, and possibly caused by, mechanisms involving immune activation and inflammation within the CNS. The inflammatory milieu induced by the activity of HIV in invading cells and triggering an immune response has important implications throughout the time course of infection, and may be particularly important for understanding the pathophysiology of HAND.

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INTRODUCTION

Thirty Years Later: HIV Beyond the Immune System

Thirty years after the report of five unexplained cases in Los Angeles of *Pneumocystis carinii* pneumonia in men suffering from what would become recognized as the acquired immunodeficiency syndrome (AIDS) [1], the human immunodeficiency virus (HIV) remains the subject of intense biochemical, molecular, clinical, and epidemiologic investigation. HIV is a blood-borne and sexually transmitted infection that has significant implications for both individual and public health and has disproportionately affected vulnerable and marginalized individuals and populations, including the poor and underserved, injection drug users, commercial sex workers, and men who have sex with men [2]. But the epidemic has also changed: HIV has no regard for gender, age, or sexuality and what once was largely a disease of young homosexual men has expanded to affect populations that are older, heterosexual, and female.

It is estimated that there are currently 34 million people living with HIV worldwide, that there are 2.5 million new infections annually, and that 1.7 million individuals die from the disease and its sequelae each year [2,3]. And while the greatest number of new infections and the worst outcomes occur in the lowest-resource settings in sub-Saharan Africa and southeast Asia, there are still nearly 50,000 individuals who are newly infected within the United States each year [2]. Despite the progress that has been made over the last three decades, the epidemic is far from over (Figure 1a-d).

While a better understanding of the virus' characteristics, including its pathogenesis and transmission patterns, has led to both prophylactic and therapeutic interventions, many questions about the disease's pathogenesis remain unanswered. In

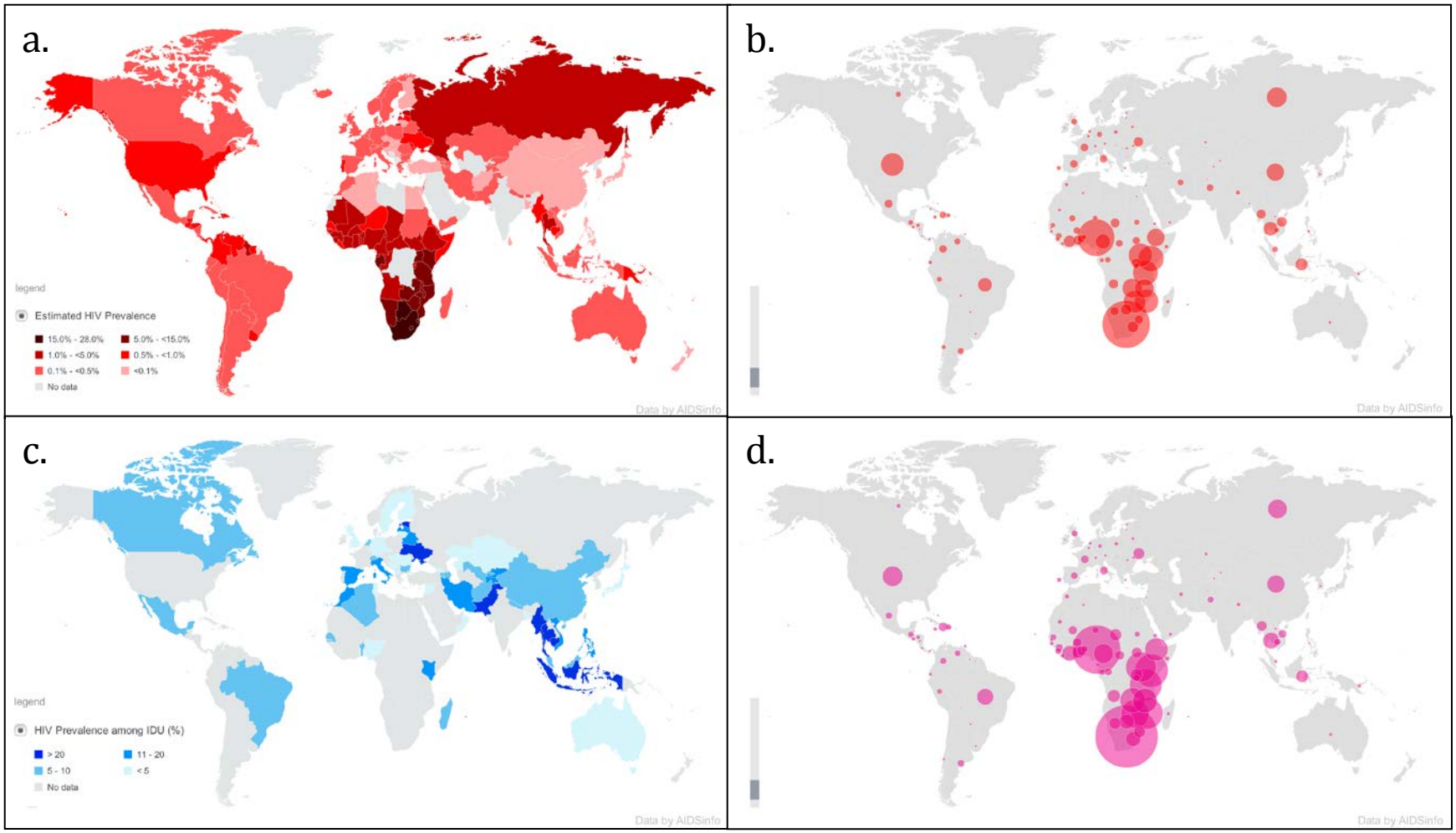

Figure 1. The epidemiology of HIV infection in 2011. a. Estimated HIV prevalence by country, demonstrating significant prevalence worldwide and high prevalence in sub-Sarahan Africa and Asia. b. Total number of adults living with HIV; size of circles corresponds with absolute number of individuals. c. HIV prevalence among injection drug users, note the especially high prevalence in Asian countries. d. Total number of women living with HIV infection worldwide; this has increased significantly over the last 20 years. Adapted from AIDSinfo at unaids.org [3].

particular, the last two decades have seen an increased focus on the effects of HIV beyond the immune system, including its end-organ effects in the cardiovascular, renal, and nervous systems. By extension, the study of the virus in these new contexts outside of the plasma has led to the recognition of a biological compartmentalization that allows for infection and injury of target tissue, independent evolution of the virus from its counterparts in the plasma, and the protection of the virus from systemic therapy [4,5]. The development of these distinct biological compartments, such as those in the breast [6,7] and genital tract [8,9], facilitates viral replication, complicates viral eradication, and leads to compartment-specific effects. One compartment that has received increased attention over the last decade is the central nervous system (CNS) [4,10-12].

HIV infection is associated with the establishment of a CNS reservoir of infection, as evidenced by the detection of HIV DNA in perivascular brain macrophages, microglial cells, and astrocytes [13-15], compartmentalization of HIV quasi-species in CNS tissues [16,17], and clinical cases of isolated CNS 'escape' from antiretroviral therapy (ART) [18,19]. This local infection leads to neurological injury and creates a sanctuary for ongoing HIV replication within CNS tissues. Understanding the initial establishment and clinical importance of this CNS compartment-specific infection has critical implications for strategies to optimize the lives of persons infected with HIV.

"Classic" Neurological Manifestations of HIV Infection

The neurological manifestations of HIV were first described in the early stages of the epidemic and were quickly recognized as some of the most dramatic sequelae of HIV infection. The AIDS Dementia Complex – an early-onset, progressive dementia

characterized by motor, psychological, and behavioral dysfunction – was the most dreaded of these complications and became the grim reality for many HIV-infected individuals once they developed AIDS [20,21]. Patients with AIDS were also at risk for developing CNS opportunistic infections with organisms like *Toxoplasma gondii*, *Cryptococcus neoformans*, and *Mycobacterium tuberculosis*, CNS lymphoma linked to Epstein-Barr virus, JC virus-associated progressive multifocal leukoencephalopathy, and meningitis related to bacterial, viral, and fungal pathogens. These neurological manifestations of the disease were dramatic and the prognosis in patients with such manifestations was grim.

Clinical manifestations of the AIDS Dementia Complex in the absence of antiretroviral therapy were mirrored in clear CNS abnormalities on pathological evaluation, including profound infiltration of immune cells into the CNS, with subsequent inflammation and HIV virions detected most abundantly in macrophages and microglial cells of the deep gray matter of the brain [21-23]. In chronic, established infection, even in patients without dementia, HIV DNA has been recovered from homogenized brain tissue and specific cell types in the CNS including macrophages, microglia, and astrocytes [17,22,24,25]. Neurons themselves, while not productively infected by HIV, appear to be damaged and undergo apoptosis through “indirect” pathways mediated by immune activation and inflammation within the CNS [26,27].

The introduction of combination antiretroviral therapy (cART) in the 1990s fundamentally altered the landscape of both systemic and neurological HIV disease. The profound immunodeficiency associated with HIV/AIDS that acted as the substrate for the “classic” neurological manifestations of the disease itself and the opportunistic infections

with which it was associated could now be significantly delayed or prevented, transforming the diagnosis of HIV infection from a rapid death sentence to a chronic illness. With these changes came a marked shift in the epidemiological and clinical characteristics of its neurological complications.

Neurological Manifestations of HIV in the Era of Antiretroviral Therapy

In general, antiretroviral therapy suppresses both plasma and cerebrospinal fluid (CSF) viral levels and improves neurological outcomes in patients infected with HIV [28]. Typically, plasma HIV RNA suppression is paralleled by suppression in the CSF, and the initiation of cART also limits the extent of immune activation in the CSF, as measured by white blood cell counts and immunological markers [29,30]. With systemic control of the virus and improved immune status in these patients has come a striking decline in the occurrence of neurological opportunistic infections, while the attenuation of viral replication and immune activation in the CNS has resulted in a decline in the incidence of the most dramatic forms of HIV-associated neurologic disease over the last two decades [31]. Nevertheless, even individuals with well-controlled HIV infection continue to experience neurological dysfunction which, although often less pronounced than the dementing illnesses experienced by many patients with AIDS thirty years ago, has the potential to seriously impact productivity and quality of life [32-35].

HIV-Associated Neurocognitive Disorder (HAND)

With improved outcomes and the recognition of a broad spectrum of neurological signs and symptoms associated with HIV have come a variety of classification systems for

clinicians and researchers to describe the manifestations of CNS disease experienced by patients with HIV and AIDS. What was previously defined in its most severe form as the AIDS-Dementia Complex [20,21] is now represented by a spectrum of disorders reflecting the variability in presentation, outcome, and impact of neurological disease.

HIV-associated neurocognitive disorder (HAND) comprises a diverse set of neurocognitive diseases, ranging from clinically asymptomatic impairment to severe dementia. HAND is a clinical diagnosis defined by abnormalities identified through neuropsychological testing and is subdivided into three categories of increasing severity: asymptomatic neurocognitive impairment (ANI), mild neurocognitive disorder (MND), and HIV-associated dementia (HAD) [36]. These diagnoses require a specific neuropsychological testing battery comprised of the following abilities: verbal/language, attention/working memory, abstraction/executive functioning, memory (learning and recall), speed of information processing, sensory-perceptual, and motor skills. While the incidence of the most severe manifestations of HAND (i.e., HAD) has decreased in the setting of widespread access to cART, mild-to-moderate HAND has persisted and has, in fact, become the most prevalent primary CNS complication of HIV infection [33]. The spectrum of HAND is delineated in the Table 1 and key differences between conditions are explained in the subsequent text.

Asymptomatic Neurocognitive Impairment (ANI)

The most benign and most common manifestation of HAND is asymptomatic neurocognitive impairment, which has been identified in approximately one-third of HIV-infected patients [33]. Decreased performance on neuropsychological testing in

Asymptomatic Neurocognitive Impairment (ANI)
1. Acquired impairment in cognitive functioning, involving at least two ability domains, documented by performance of at least 1.0 SD below the mean for age- and education-appropriate norms on standardized neuropsychological tests. 2. The cognitive impairment does not interfere with everyday functioning. 3. The cognitive impairment does not meet criteria for delirium or dementia. 4. There is no evidence of another preexisting cause for the ANI.
Mild Neurocognitive Disorder (MND)
1. Acquired impairment in cognitive functioning, involving at least two ability domains, documented by performance of at least 1.0 SD below the mean for age- and education-appropriate norms on standardized neuropsychological tests. 2. The cognitive impairment produces at least mild interference in daily functioning, as evidenced by at least one of the following: a) Self-report of reduced mental acuity, inefficiency in work, homemaking, or social functioning. b) Observation by knowledgeable others that the individual has undergone at least mild decline in mental acuity with resultant inefficiency in work, homemaking, or social functioning. 3. The cognitive impairment does not meet criteria for delirium or dementia. 4. There is no evidence of another preexisting cause for the MND.
HIV-Associated Dementia (HAD)
1. Marked acquired impairment in cognitive functioning, involving at least two ability domains; typically the impairment is in multiple domains, especially in learning of new information, slowed information processing, and defective attention/concentration. The cognitive impairment must be ascertained by neuropsychological testing with performance of at least 2.0 SD less than than demographically corrected means. 2. The cognitive impairment produces marked interference with day-to-day functioning such as work, home life, or social activities. 3. The pattern of cognitive impairment does not meet criteria for delirium (i.e., clouding of consciousness is not a prominent feature); or, if delirium is present, criteria for dementia need to have been met on a prior examination when delirium was not present. 4. There is no evidence of another, preexisting cause for the dementia (i.e., other CNS infection, CNS neoplasm, cerebrovascular disease, preexisting neurologic disease, or severe substance abuse compatible with CNS disorder).

Table 1. Classification and description of HIV-associated neurocognitive disorder (HAND) in the era of combination antiretroviral therapy. Key distinctions between the three disorders are described in the text. Adapted from Antinori et al., 2007 [36].

patients without obvious clinical signs and symptoms of impairment has led to the classification of ANI, which is characterized as a subclinical cognitive decline. The strict definition of ANI requires the presence of mild neuropsychological impairment, not attributable to comorbid conditions, involving 2 or more ability domains on neuropsychological testing. ANI specifically requires that criteria for a negative impact on everyday functioning *not* be met; this is how the condition is contrasted from mild neurocognitive disorder.

It is unclear whether ANI is a process predictive of neurological impairment later in the course of the disease, whether it contributes to neuropathological vulnerability, and whether early intervention with cART during ANI might prevent ongoing deterioration [37]. Additionally, the ANI designation is not specific for active brain injury, and may be complicated by confounding factors related to HIV disease, such as mood disorders or substance abuse. The contribution of such comorbidities might be difficult to ascertain in patients with ANI.

Mild Neurocognitive Disorder (MND)

Mild neurocognitive impairment is being increasingly recognized in individuals treated with cART, who typically have a relatively reconstituted immune system characterized by higher $CD4^+$ T cell counts and suppressed or undetectable viral loads. As the population of patients with chronic, well-controlled HIV infection continues to grow, so does the overall prevalence of MND, making this manifestation of HAND an important focus of scientific and clinical investigation. In the CHARTER study, 12% of individuals met the criteria for MND [33].

The diagnosis of MND is contingent upon the detection of abnormalities in neuropsychological testing, specifically with relation to attention, information processing, learning and memory, psychomotor speed, and executive function [38]. It is distinguished from ANI because these patients typically experience a subtle, but noticeable, decline in cognitive ability and increased difficulty in carrying out their activities of daily living. MND can also manifest through both pyramidal and extrapyramidal motor systems, producing symptoms such as ataxia, tremor, and incoordination that may worsen over time [39]. It is also believed that MND can result in behavioral effects, which are independent from those associated with mood disorders concomitant with HIV infection [40,41].

HIV-Associated Dementia (HAD)

What was initially described as the AIDS Dementia Complex twenty-five years ago [20,21] is now known as HIV-Associated Dementia (HAD), the most dramatic manifestation of HAND [36]. The diagnosis of the condition remains a challenge in clinical practice, as there are no diagnostic studies or laboratory tests that are specific for HAD [27,33,36,42]. Therefore, the identification of this disorder is reliant upon the recognition of a clinical syndrome and the exclusion of alternative diagnoses.

The diagnosis of HAD is based on progressive neurocognitive impairment and the exclusion of other conditions that can cause or exacerbate such impairment, including CNS opportunistic infections and tumors. It is further supported by high levels of HIV RNA in the CSF (typically >3 logs). HAD most typically occurs in patients with slowed cognitive processing in the context of long-standing HIV infection. Additionally, HAD is

still most commonly identified in patients off of antiretroviral therapy, with the prevalence in treated patients estimated to be as low as 2% [34,43-45]. The syndrome is often characterized by motor abnormalities such as slowed movement and spastic gait, as well as hyperactive deep tendon reflexes [20]. However, these signs and symptoms are not diagnostically specific for HAD, and further evaluation with computed tomography (CT) or magnetic resonance imaging (MRI) is often required. Neuroimaging is most appropriately used to first rule out more common AIDS-related neurological conditions including opportunistic infections and CNS lymphoma. With these diagnoses excluded, diffuse cerebral atrophy and subcortical or periventricular white matter changes are consistent with, although not specific for, HAD [46-48].

Diagnostic and Management Issues in HAND

Because HAND has no specific markers, it is necessary to rule out CNS opportunistic infections, neurosyphilis, delirium, toxic-metabolic disorders, psychiatric disease, delirium, and age-related dementia before making the diagnosis. While traditional neuroimaging is useful in ruling out other HIV-associated disease processes, including CNS lymphoma, CNS infections (opportunistic infections and abscesses), and inflammatory processes, there are no findings on standard neuroimaging with CT or MRI that are specific for HAND (although more severe disease does present with late-stage abnormalities on neuroimaging). Recently, an effort has been made to use more advanced neuroimaging techniques to identify mild HAND. These include brain mapping, structural imaging, functional MRI assessing brain perfusion and functional connectivity, and proton-magnetic resonance spectroscopy (proton-MRS), which has shown promise in

identifying metabolite abnormalities in patients with mild disease [49-62]. Nevertheless, the utility of these new modalities remains to be determined and the diagnosis of HAND is therefore reliant upon clinical findings.

Neuropsychological testing results have been shown to improve in the setting of drug therapy. Early studies of zidovudine (AZT) monotherapy indicated improvement in testing scores [63,64], and more recent studies have shown that cART decreases HAD while increasing the prevalence of milder HAND [65,66]. While cART might improve cognitive performance, this improvement is frequently incomplete and residual deficits remain [67,68]. There has therefore been a focus on using adjunctive therapy to attenuate the inflammatory events that are postulated to occur in the CNS of HIV-infected patients in the hope that this will result in improvements for those with mild HAND.

Efforts have been made to manage HAND with non-antiretroviral adjunctive therapies including memantine, selegiline, and nimodipine, but none of these have demonstrated efficacy [69]. Psychiatric drugs like valproic acid and lithium have been hypothesized to decrease HIV replication and neuroinflammation through their glycogen synthase-kinase 3-beta activity [70], as have serotonin reuptake inhibitors like citalopram and paroxitine through an unknown mechanism [70]. However, it remains to be seen whether these treatment adjuncts will result in improved recovery from HAND. Similarly, due to anti-inflammatory and antiviral effects, the antibiotic minocycline has been suggested as a potential therapy for HAND, but randomized studies have not indicated efficacy [71,72]. Methylphenidate has been successful for symptomatic management of fatigue [73] and slowing in patients with HAND [74], but this symptomatic relief does not alter the course of the disease.

The Etiology of HAND in the Era of Antiretroviral Therapy

Although the biological substrate of HAND in the setting of antiretroviral therapy is unknown, a number of mechanisms are possible. One potential mechanism is that injury occurring in the earliest stages of HIV infection is compounded over time and progresses to clinically detectable abnormalities (i.e., ANI, MND) later in the disease course. Such injury would begin during the period before treatment is initiated, and would continue along a trajectory that may or may not be mitigated by initiation of antiretroviral therapy. After several years, a combination of host susceptibilities and disease factors may result in the development of symptomatic neurological disease. While this is a plausible pathogenic mechanism for HAND, data regarding objective neurological injury during the earliest stages of HIV infection is currently lacking. It is unclear when in the disease course such injury begins and what the clinical implications of this injury may be.

Another possibility is that, due to the compartment-specific nature of CNS HIV infection, neurological injury occurs despite the initiation and continuation of systemically suppressive treatment. Even in individuals with no overt signs or symptoms of neurocognitive impairment, the presence of HIV in the CNS may result in constant low-level inflammation and immune activation that has been hypothesized to result in ongoing brain injury. CSF immune activation, brain inflammation detected by proton-MRS, and microglial activation in brain tissue persist in patients on long-term suppressive antiretroviral therapy started during the chronic stage of infection [25,75-77]. However, much remains unknown regarding the manifestations, etiology, and implications of compartmentalized HIV infection in patients on suppressive therapy. The correlates of neurological disease developed in this setting are unclear.

Below, primary HIV infection and central nervous system compartmentalization are discussed as potential contributors to neurological disease in patients with HIV. This background will provide an introduction to the two studies included in this thesis.

The Central Nervous System during Primary HIV Infection

While the study of neurological disease in HIV infection has traditionally focused on chronic and late-stage neurocognitive manifestations of the virus, recent work has drawn attention to the effects of HIV on the CNS much earlier in the disease course. Primary HIV infection, defined as the first year following the transmission of the virus, has been a focus of this work.

Primary HIV infection is characterized by a rapid and dramatic rise of HIV RNA levels in the plasma [78-80], accompanied by an increase in HIV antibody levels that are detectable within 2 weeks of transmission by fourth-generation enzyme immunosorbent assay (EIA) tests. In at least two-thirds of individuals, the period of seroconversion is accompanied by the acute retroviral syndrome, characterized by vague symptoms of fatigue, malaise, fever, and anorexia [78,81]. Within a few months of seroconversion, a partially effective immune response causes HIV RNA to stabilize at a reduced, chronic, individual-specific level that serves as the plasma viral “set point” [82]. This is a result of the increased activity of $CD8^+$ T cells in conjunction with a decreased reservoir of $CD4^+$ T cells available for infection by the virus [83,84]. The standard clinical time course of HIV infection is reviewed in Figure 2. Note the timing and clinical events of primary HIV infection, which include the acute retroviral syndrome, dissemination of the virus and invasion of lymphoid tissues, and the $CD4^+$ T cell nadir.

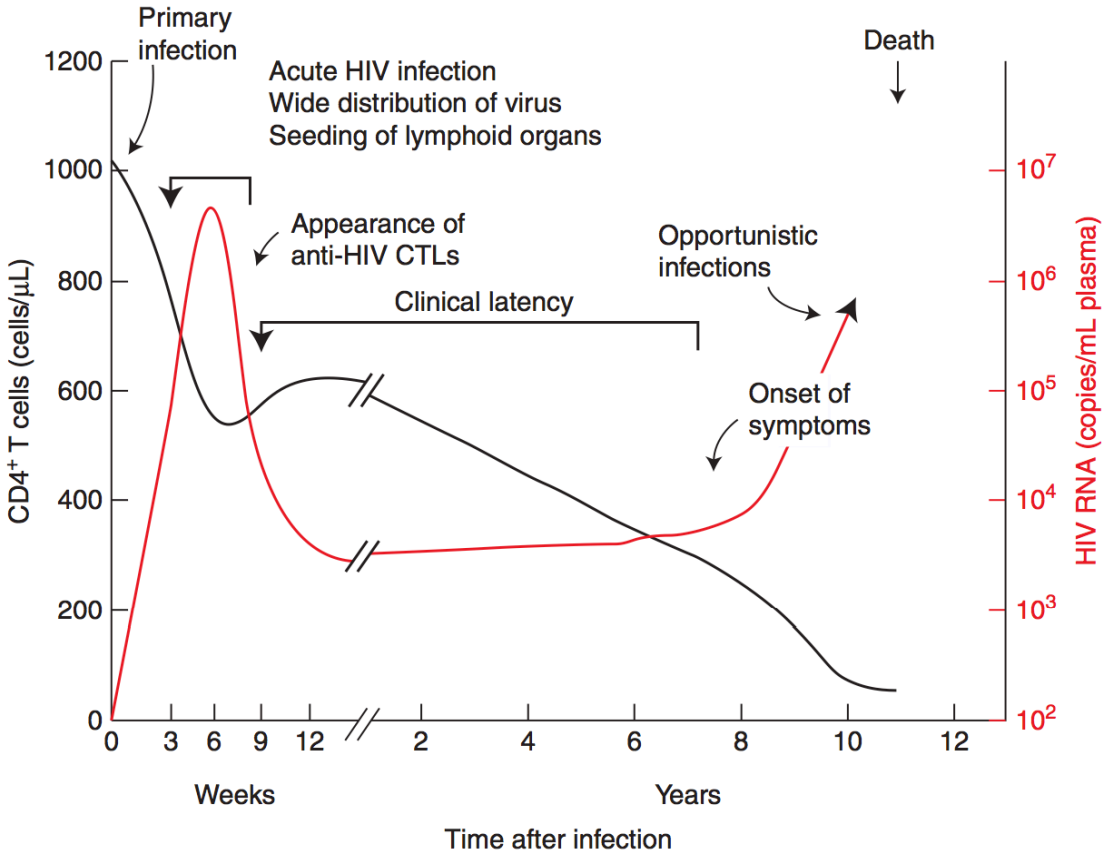

Figure 2. Typical time course of HIV-1 infection. Primary HIV infection refers to the first year following viral transmission, which includes the acute retroviral syndrome, widespread distribution of the virus, the seeding of lymphoid organs, and the $CD4^+$ T cell nadir. Patients develop anti-HIV cytotoxic T lymphocytes (CTLs or $CD8^+$ T cells) as the $CD4^+$ T cell count drops, leading to an increase in the $CD8^+/CD4^+$ T cell ratio. After a small recovery in the $CD4^+$ T cell count, infected individuals enter a long period of clinical latency in which they experience a slow decline in the $CD4^+$ T cell population. If treatment is not initiated, the $CD4^+$ T cell count falls to critically low levels and the patient develops AIDS. This period characterized by viral proliferation, increased risk of acquiring opportunistic infections, and the onset of symptoms consistent with end-organ damage in a variety of body systems. Ultimately, it leads to death from one or a combination of these causes. Adapted from Fauci and Desrosiers 1997, Cold Spring Harbor Laboratory Press [80]. Reproduced with permission from Cold Spring Harbor Laboratory, *Retroviruses*; reproduced with permission from Pantaleo et al 1993, *NEJM*, copyright Massachusetts Medical Society.

In addition to the symptoms of the acute retroviral syndrome, it has long been recognized that a subgroup of individuals newly infected with HIV develop neurological symptoms and signs around the time of seroconversion [85,86]. Evidence suggests that this occurs in up to 10% of individuals [87]. In addition, HIV can be found in the CSF and brain tissue of patients during the earliest stages of infection, in the weeks to months following viral transmission [81,87-89]. Recently, HIV has been identified in the CSF as early as eight days post-transmission [90], suggesting that this compartment is occupied by the virus very early in the disease course.

Studies have also shown that immune activation accompanies the presence of HIV virions in the CSF during primary infection [91]. The elevation in CSF white blood cell counts, neopterin, and inflammatory cytokines during the first months of infection suggest the possibility that CNS injury can also take place during this period, contrasting with the view that the damaging effects of HIV infection occur only after a long period of clinical latency.

The neurological manifestations associated with early HIV infection vary between individuals and a consistent underlying pathophysiology remains to be identified. One of the first syndromes to be linked with HIV infection was “aseptic” meningitis, characterized by a CSF lymphocytosis or clinical signs of meningitis [92]. Other individuals experience varying degrees of encephalopathy in the setting of meningoencephalitis, encephalitis, or encephalomyelitis [93,94]. Acute neuropathies, including facial nerve paralysis and optic neuritis, also occur frequently with seroconversion and are common in acute HIV infection [93,94].

These heterogeneous neurological syndromes associated with primary HIV infection share several common features. First, the onset is typically within three weeks following the symptomatic manifestations of the acute retroviral syndrome [85]. Second, the clinical signs and symptoms are typically self-limited and resolve without any specific intervention. Third, patients almost always have a seroconversion associated with the timing of symptoms. Taken together, this suggests that pathogenesis of these syndromes is likely due to a host-mediated autoimmune response in the setting of massive systemic immune activation that results from the rapid expansion of the virus in its new host once transmission has occurred [95].

Seroconversion characterized by a variety of non-neurological symptoms has been associated with more rapid disease progression [82,83]. Recently, there has been increased interest in determining whether early HIV infection has neurological consequences beyond the self-limited symptoms that are associated with the acute seroconversion syndrome. It remains to be seen whether these early signs of neuroinflammation related to objective neuronal injury, whether inflammation or injury predicts neurological outcomes in later stages of the disease, and whether the early initiation of antiretroviral therapy is able to ameliorate these processes even before systemic immunosuppression occurs. It is possible, therefore, that the period of primary HIV infection has important implications for the timing and clinical course of HAND.

HIV Compartmentalization in the Central Nervous System

By separating the CNS from the systemic circulation, the blood-brain and blood-CSF barriers affect the ability of antiretroviral agents to access the CNS compartment. In

addition, local spontaneous replication of HIV within this viral sanctuary may allow for the independent mutation of HIV virions such that the CNS virus “evolves away” from that in the periphery [4,5,10,12]. While the response of CSF HIV RNA levels to cART parallels that in the plasma, the rate of decay in the CSF may be more gradual in some patients [29,96-98], suggesting a compartmentalization characterized by slower cell turnover, extended macrophage release, or attenuated drug entry into the CNS.

Because of the blood-brain barrier, HIV in the CNS can be protected from the full effect of antiretroviral agents, especially those that are large or hydrophilic. The CNS penetration-effectiveness (CPE) index represents an effort to quantitatively estimate the relative ability of each antiretroviral agent to penetrate the CNS and interfere with CSF HIV replication. Each agent is assigned a “CPE score,” and a total regimen score can be calculated by summing the scores for individual agents. The CPE scores for various agents are reviewed in Table 2 [99].

Some studies have shown that antiretroviral regimens with higher CPE scores tend to be more successful at achieving HIV RNA suppression in the CNS [100,101]. But, while more potent HIV RNA suppression in this compartment might be expected to lead to better neurocognitive outcomes and more effective treatment of HAND, this has not necessarily been the case. Observational studies have suggested that the initiation of regimens with higher CPE scores may produce a cognitive benefit in patients with HIV-related neurological disease [102,103], but other studies have shown that HIV-infected individuals treated with regimens with higher CPE scores actually exhibit poorer neurocognitive performance despite suppression [101] or only benefit if they are on more than three drugs, which is the standard for most cART regimens [104].

	CNS Penetration-Effectiveness Score			
Drug Class	**4**	**3**	**2**	**1**
NRT Inhibitor	Zidovudine	Abacavir Emtricitabine	Lamivudine Stavudine	Didanosine Tenofovir Zalcitabine
NNRT Inhibitor	Nevirapine	Delavirdine Efavirenz	Etravirine	-
Protease Inhibitor	Indinavir/r	Darunavir/r Fosamprenavir/r Indinavir Lopinavir/r	Atazanavir Atazanavir/r Fosamprenavir	Nelfinavir Ritonavir Saquinavir Saquinavir/r Tipranavir/r
Entry Inhibitor	Vicriviroc	Maraviroc	-	Enfurvirtide
Integrase Inhibitor	-	Raltegravir	-	-

Table 2. Proposed CNS Penetration-Effectiveness (CPE) ranks for commonly used antiretroviral agents, 2010. NRT = nucleoside reverse transcriptase; NNRT = non-nucleoside reverse transcriptase; /r = ritonavir boosted. Adapted from Clifford DB at New York, NY: March 22, 2010, IAS-USA [99].

Recently, the inability of antiretroviral therapy to control the potential reservoir of HIV that exists in monocytes has been proposed as a possible explanation for continued neurocognitive impairment in the setting of cART [105]. Along these lines, a monocyte efficacy (ME) score has been proposed as another means of quantifying the ability of antiretroviral agents to affect neurological outcomes. Preliminary work using this score has suggested that that the ME score is a predictor of neurocognitive performance even when CPE score is not [106]. More work, including prospective studies, is needed to determine whether ME score is an adequate predictor of neurocognitive outcomes in patients with HIV.

The effort to classify antiretroviral regimens according to CPE or ME indices underscores the recognition of CNS HIV compartmentalization as an issue with important clinical consequences. It is especially important to understand the heterogeneous manifestations of this compartmentalization as a first step toward determining whether it might be relevant to or independent of the pathogenesis of HAND in these patients.

Structure of the Thesis

This thesis is divided into two primary "chapters" that comprise two projects conducted during 18 months of research on neurological manifestations of HIV infection. Each chapter focuses on a different process that might be relevant to our understanding of HIV-associated neurocognitive disorder, as described in the introductory sessions on primary HIV infection and CNS HIV compartmentalization.

The first chapter describes a translational research project focused on evidence of neurological injury in individuals with primary HIV infection. This project involved the study of CSF and neuroimaging biomarkers of neuronal injury and inflammation in a cohort of 92 individuals with newly acquired HIV infection and showed for the first time with these markers that objective neuronal injury occurs at this early time point. This work has been presented at the International Society for NeuroVirology's 11th Annual Symposium on NeuroVirology in May 2012 (appendix 1) and was recently accepted for publication in the *Journal of Infectious Diseases* [107].

The second chapter describes a clinical case series investigating a rare but important manifestation of neurological disease in patients with chronic, well-controlled HIV infection. This project added a substantial amount of information on the cerebrospinal fluid and imaging abnormalities in these patients to the limited data that was already present in the literature. It was presented at the 19th Annual Conference on Retroviruses and Opportunistic Infections (CROI) in March 2012 (see appendix 2) and was published in the journal *AIDS* in September 2012 [19].

Both of these studies are included in the thesis because they represent areas of HIV disease that have important implications for patients and our understanding of HAND, but about which relatively little is known. However, because they deal with fundamentally different time points and pathological processes in the course of HIV infection, they are better introduced, described, and discussed separately. Afterwards I will endeavor to synthesize the material from both projects to reach some general conclusions and discuss future directions of this work.

CHAPTER 1: CEREBROSPINAL FLUID AND IMAGING BIOMARKERS OF NEURONAL INJURY IN ANTIRETROVIRAL NAÏVE PATIENTS DURING PRIMARY HIV INFECTION

Chapter Background

As previously discussed, the extent of neurological injury during pre-symptomatic HIV infection, especially early infection, is not completely understood. Limited data suggest that neurological injury occurs in some individuals during primary HIV infection, defined as the first year following viral transmission, during which up to 10% of individuals develop neurological signs and symptoms and the virus can be detected in CSF and brain tissue [87]. HIV infiltrates the CSF within days of transmission [90] and immune activation occurs throughout primary infection [91], suggesting the potential for CNS injury in the earliest stages of infection.

This chapter describes a research study focused on identifying neuronal injury in individuals with primary HIV infection, in an effort to further characterize the neurological implications of the disease beginning at the earliest time point.

Cerebrospinal Fluid Biomarkers

Cerebrospinal fluid biomarkers have gained popularity as objective markers of neuronal injury in a variety of neurological and neurodegenerative disorders including Alzheimer's dementia, Parkinson's disease, multiple sclerosis, and amyotrophic lateral sclerosis [108-113]. Perturbations in the levels of these biomarkers have allowed researchers to distinguish static neurological abnormalities associated with previous neurological injury in individuals with HIV infection from active processes associated with ongoing neuronal injury. Over the last 10 years, there has been an effort to describe the changes in these

biomarkers that occur with different manifestations of HIV infection in the central nervous system, including HIV-associated dementia, other manifestations of HAND, and CNS opportunistic infections.

The biomarkers of interest in the present study are most commonly involved with neuronal stability and axonal assembly and are described below. Table 3 summarizes the abnormalities in each biomarker in individuals with HIV and Alzheimer's disease.

Neurofilament Light Chain

The light subunit of the neurofilament protein is a major structural component of myelinated axons and has been identified as a sensitive marker of axonal disruption. The neurofilament protein itself is associated with large myelinated neurons in the cerebral white matter, and is known for maintaining the caliber of the axon as well as its structural and functional integrity. In this way, the neurofilament protein is thought to play a crucial role in the ability of axons to conduct nerve impulses [114].

The light chain of the neurofilament protein has been demonstrated to be a sensitive marker of neuronal injury in a number of conditions, both chronic and acute. This includes disorders involving the degeneration of white matter tracts in the brain or spinal cord, such as amyotrophic lateral sclerosis or multiple sclerosis, as well as cerebral disorders such as Alzheimer's disease [110]. In an acute setting, there is a dramatic, dose-specific leakage of neurofilament light chain into the CSF, in proportion to the extent of ischemic injury [109,115,116].

Neurofilament light chain has been shown to be elevated in HIV-associated dementia and CNS opportunistic infections, with higher levels associated with worse

neurological disease [117] (Figure 3). Previously, neurologically asymptomatic HIV-1-infected individuals with chronic infection and $CD4^+$ T cell counts above 200 cells/uL were thought not to have elevated CSF neurofilament light chain, and this threshold was thought to be sufficient to prevent CNS disease [118]. However, further work in chronically infected subjects indicated that HIV-infected individuals with $CD4^+$ T cell counts above 200 cells/uL can have elevations in neurofilament light chain upon the cessation of cART [119], that lower $CD4^+$ T cell counts tend to be associated with higher neurofilament light chain [117], and that very low $CD4^+$ T cell counts are associated with increased neurofilament light chain concentrations [120]. However, neurofilament light chain was not elevated in the majority of patients with primary infection [117] and its utility during this time period remained unknown.

Tau Proteins: t-tau and p-tau

Tau is a ubiquitously expressed microtubule-associated protein that promotes axonal stability and participates in the maintenance of synapses within the central nervous system [121]. It is largely found in non-myelinated cortical axons [122]. Its hyperphosphorylated component, known as p-tau, is widely known for its association with neuronal injury in Alzheimer's disease, ischemic stroke, and Cruetzfeldt-Jakob disease [111,123]. It is notably not elevated in Parkinson's disease [112,124]. Elevated levels of tau are thought to be related to neuronal loss, which is a characteristic of all of these conditions; it has been hypothesized that tau levels might reflect the magnitude and rate of neurodegeneration.

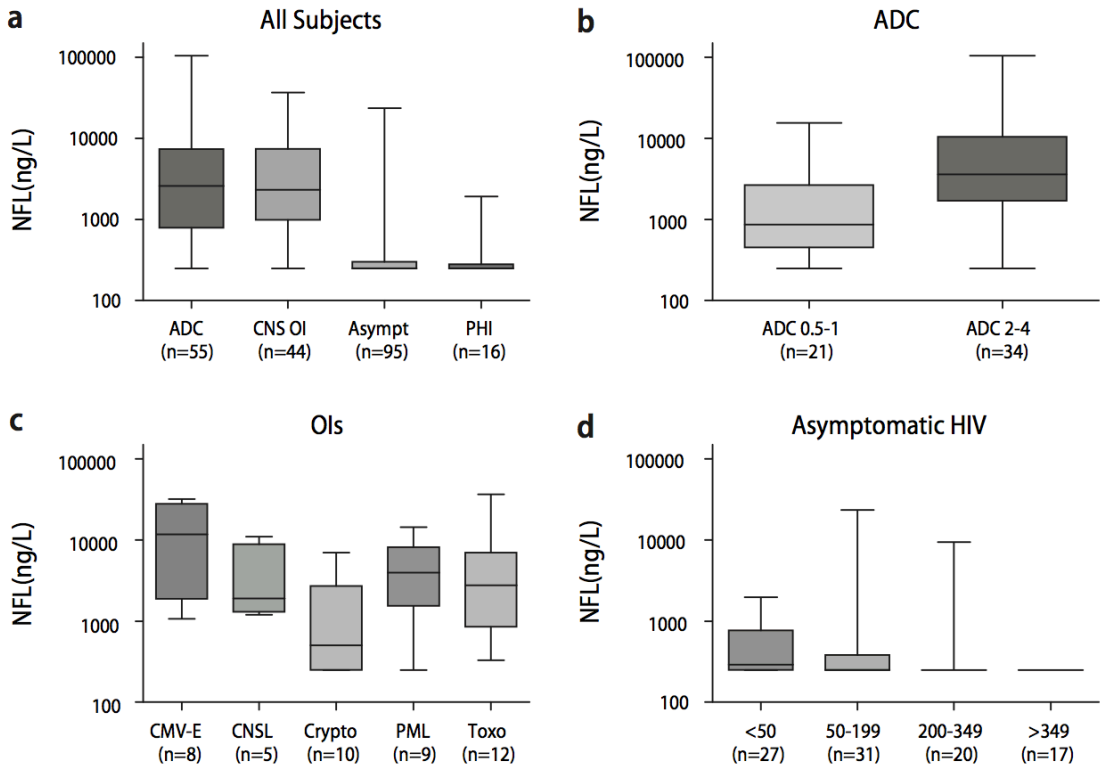

Figure 3. CSF neurofilament light chain concentrations in 210 HIV-1 infected individuals at different stages of disease, based on "less-sensitive" assay with lower limit of detection 250 ng/L. Box plots indicate median values, 25th and 75th percentiles, and ranges. a. Subjects divided into different groups, showing significant elevations in AIDS Dementia Complex (ADC) and CNS opportunistic infections (OI), and no elevations in neuroasymptomatic subjects (asympt) or subjects with primary HIV infection (PHI). b. Subjects with ADC staged according to the Memorial Sloan-Kettering Scale, indicating elevated neurofilament light chain in more severe ADC cases. c. Subjects with CNS OIs; CMV encephalitis (CMV-E), CNS lymphomas (CNSL), cryptococcal meningitis (Crypto), progressive multifocal leukoencephalopathy (PML) and toxoplasmosis (Toxo). d. Neurologically asymptomatic subjects grouped according to their CD4$^+$ cell counts, showing elevated neurofilament light chain with worsening CD4$^+$ T cell counts. From Abdulle, et al. [117]. Used with kind permission of Springer Science and Business Media.

Marker	PHI	NA HIV	HAND/HAD	CNS OI	AD
NFL	=[117]	=[117]	↑[117]	↑[117]	↑[110]
t-tau	No data	=[113] ↓[139]	↑[113,125] =[126,127]	=[113,127]	↑[113,123,133,139]
p-tau	No data	=[113] ↓[139]	↑[125] =[113]	=[113]	↑[113,123,133,139]
sAPP-α	No data	=[113]	↓[113]	↓[113]	=[113,123,128]
sAPP-β	No data	=[113]	↓[113]	↓[113]	=[113,123,128]
β-amyloid	No data	=[113]	=[113] ↓[125,139]	↓[113]	↓[113,123,128,133,138]
NAA/Cr	=[129,137] ↓[130,131]	↓[50,59]	↓[59]	No data	↓[132,133,134,135]
Glu/Cr	=[123,137]	↓[130,136]	↓[130,137]	No data	↓[134,135]

Table 3. Summary of previously described biomarker perturbations in HIV infection and Alzheimer's disease. PHI = primary HIV infection; NA HIV = neuroasymptomatic, chronic HIV infection; HAD = HIV-associated dementia; CNS OI = central nervous system opportunistic infections; AD = Alzheimer's Disease; NFL = neurofilament light chain, sAPP = soluble amyloid precursor protein; NAA = n-acetylaspartate; Glu = glutamate; Cr = creatine. Reference numbers provided.

Previous studies of tau proteins in HIV infection have been inconclusive in demonstrating the directionality of perturbations in these metabolites. One recent study demonstrated t-tau elevations in individuals with HAD, who displayed levels above those seen in HIV-uninfected controls and neuroasymptomatic HIV-infected individuals [113]. P-tau was not elevated in any CNS manifestations of HIV. The authors suggested that this was likely due to pathology in neurological HIV infection that more commonly affects subcortical pathways and does not result in the neurofibrillary tangles common in Alzheimer's disease [113]. Other studies have had conflicting results, with some showing elevated t-tau and p-tau in HAND or HAD [125,126] and others failing to show a consistent relationship [127,139,140]. Patterns in t-tau and p-tau proteins have not previously been explored in primary HIV infection.

Proteins of the Amyloid Processing Pathway

The amyloid processing pathway is a complex enzymatic pathway involving the proteolytic processing of transmembrane proteins that results in the generation of metabolites with varying pathological potential [128]. Beginning with full-length transmembrane amyloid precursor protein, the pathway may follow amyloidgenic or non-amyloidgenic routes. In the non-amyloidgenic route, transmembrane amyloid precursor protein is cleaved by an α-secretase into soluble amyloid precursor protein–α, which is present in the CSF but does not lead to the formation of pathological β-amyloid. In contrast, transmembrane amyloid precursor protein might be cleaved by a β-secretase, which results in the generation of soluble amyloid precursor protein–β. The formation of this protein results in the production of the β-C-terminal fragment (CTF) protein, which

in turn is cleaved by a γ-secretase to form β-amyloid peptides. The 1-42 version of β-amyloid aggregates to form the amyloid plaques seen in Alzheimer's disease. The CSF amyloid profile in Alzheimer's disease is therefore composed of normal or mildly elevated CSF sAPP–α and –β proteins with *decreased* CSF amyloid-beta 42, which results from increased deposition of the protein in the brain tissue and therefore lower levels in the CSF [123,128,138].

In HIV-infected individuals, soluble amyloid precursor proteins have been shown to decrease with late-stage neurological disease, including HIV-associated dementia and CNS opportunistic infections [113]. However, inconsistencies have been reported in terms of amyloid-beta 42, with some studies showing no change [113] and others revealing a decrease consistent with what is found in Alzheimer's disease [125,139]. Effects on these proteins have not been described during primary HIV infection.

Proton-Magnetic Resonance Spectroscopy

Proton-magnetic resonance spectroscopy (proton-MRS) is a non-invasive imaging modality that has been used to monitor neuronal injury through the analysis of cerebral metabolite abnormalities. N-acetylaspartate and glutamate are markers of neuronal health that deplete with injury [141,142] and are often expressed in terms of their ratio to creatine. Perturbations in metabolites measured through proton-MRS have been identified in a number of brain regions of subjects with Alzheimer's disease [132-137].

Work in macaques suggests that the neuronal manifestations of HIV as identified through proton-MRS abnormalities occur rapidly but are reversible with the initiation of cART [143]. In human studies, the N-acetylaspartate/creatine ratio declines in chronic

untreated HIV infection (i.e., [50,59]), and improves, but does not normalize, with therapy [144,145].

Some neuroimaging studies have suggested that neuronal injury occurs during primary HIV infection as evidenced by decreased N-acetylaspartate in the frontal cortex of newly infected individuals [130,131]. Other studies have failed to find group-level differences in metabolite levels between controls and patients with primary HIV infection at baseline [137,138]. Recent work using our cohort of participants with primary infection has demonstrated that, although there are no group differences at baseline compared with controls, abnormalities in subjects with primary infection worsen over time and improve with early initiation of cART [129]. Despite this progress, at the time of thus study no information linking CSF biomarker data with non-invasive proton-MRS metabolite data was available.

Statement of Purpose, Specific Aims, and Hypotheses

In this study, we sought to quantify CSF and neuroimaging biomarkers as a proxy for neuronal injury in individuals with primary HIV infection and to compare them with those in HIV-uninfected controls. The specific aims of the study were as follows:

1. To determine whether neurofilament light chain, a sensitive marker of neuronal injury, is elevated in subjects with primary HIV infection compared with HIV-uninfected control subjects.

2. To investigate potential mechanisms of neuronal injury by correlating abnormalities in neurofilament light chain in primary HIV infection with markers of CNS inflammation and viral load.

3. To determine whether tau and amyloid proteins are perturbed in primary HIV infection and to identify relevant correlates of these perturbations.

4. To identify cerebral metabolite abnormalities as measured using proton-magnetic resonance spectroscopy and to determine whether there is a relationship between non-invasive imaging and CSF biomarkers of neuronal injury during primary HIV infection.

5. To examine the relationship between CSF biomarkers of neuronal injury and neuropsychological testing performance during primary HIV infection.

We hypothesized that primary HIV infection is characterized by increased concentrations of CSF neurofilament light chain, and that this increase correlates with elevated concentrations of CSF markers of neuroinflammation and decreased concentrations of N-acetylaspartate and glutamate measured by proton-MRS. We expected tau and amyloid levels in primary HIV infection to not differ from controls, as has been previously reported [113] in chronically HIV-infected, neuroasymptomatic individuals.

Methods

Study Design and Participants

This was a cross-sectional study utilizing clinical signs and symptoms, biological samples, and laboratory test results from 92 antiretroviral naïve individuals with primary HIV infection enrolled between 1987 and 2011 at study sites in San Francisco, USA, Gothenburg, Sweden, and Sydney, Australia.

Participants were referred from physicians or counseling and testing centers based upon known or suspected recent HIV infection. Participants were eligible if they met criteria for primary infection based upon nucleic acid testing and less-sensitive ELISA studies according to the standard serologic testing algorithm for recent HIV seroconversion, commonly known as the STARHS algorithm [95]. Estimated number of days post-transmission was calculated assuming exposure occurred 14 days prior to the acute retroviral syndrome [81], or in the absence of symptoms, as halfway between the last negative and the first positive test result.

A neurologist screened the participants with a history and physical exam to detect exclusion criteria including active unrelated neurological disorders such as known prior stroke, seizure disorder, brain tumor, or CNS opportunistic infections. Subjects were screened with standardized inventories for comorbid mental illness and substance abuse, and the presence of these was recorded. Data was excluded from the analysis if participants demonstrated signs of intoxication and/or reported substance use on the day of the study visit.

Data regarding CSF HIV RNA and neurological symptoms have been previously reported in a subset of participants from this study [42,146], as have neurofilament light

chain results in 16 subjects using a different, less-sensitive assay [117] than the one that was used in this study.

Twenty-five HIV-uninfected volunteers provided comparison samples. All participants provided written informed consent in studies approved by the institutional review board or equivalent entity at each institution.

Specimen Sampling, Processing, and Laboratory Analysis

Participants underwent detailed neurological history and physical examination, as well as collection of blood and CSF specimens between 7:30am and 12:00pm at study visits. The timing of specimen collection controlled for potential diurnal variations in amyloid-beta 42 [147]. HIV RNA levels in cell-free CSF and plasma were measured by the ultrasensitive Roche Amplicor HIV-1 Monitor PCR (version 1.5; Roche Molecular Diagnostic Systems, Branchburg, NJ), Cobas TaqMan RealTime HIV-1 (version 1 or 2; Hoffmann-La Roche, Basel, Switzerland), or the Abbott RealTime HIV-1 (Abbot Laboratories, Abbot Park, IL, USA) assays at local sites. CSF total white blood cells (WBCs) and protein, and $CD4^+$ and $CD8^+$ T lymphocyte counts were measured on fresh samples by flow cytometry.

Cell-free CSF and blood plasma were aliquoted and stored within 6 hours of collection in -70°C or -80°C freezers monitored by National Institutes of Standards and Technology-certified thermometers. Neurofilament light chain concentration was measured using a new, highly sensitive, two-site enzymatic quantitative immunoassay with a lower limit of detection 50 ng/L (UMAN Diagnostics, Umea, Sweden). The upper-normal CSF neurofilament light chain levels at the laboratory were <380 (in subjects <30

years), <560 (30-39 years), <890 (40-59 years) and <1850 ng/L (>59 years) [148]. Reference values were obtained in the Zetterberg laboratory based on the analysis of 108 neurologically healthy HIV-uninfected individuals. Detection of t-tau, amyloid-beta 42, and soluble amyloid precursor proteins–α and –β used standard ELISAs. Blood and CSF neopterin measurements were performed in the laboratory of Dr. Fuchs employing commercially available immunoassays (BRAHMS Aktiengesellschaft, Hennigsdorf, Germany); interferon gamma-induced protein 10 (IP-10) and monocyte chemotactic protein 1 (MCP-1) measurements were performed locally using commercially available assays (R&D Systems, Minneapolis, USA).

Proton-Magnetic Resonance Spectroscopy

After standard clinical magnetic resonance imaging, proton-MRS was performed on a 4-Tesla Siemens/Bruker scanner (Siemens, Erlangen, Germany) in 53 primary HIV infection participants at baseline in the neuroimaging laboratory of Dr. Meyerhoff. Water-suppressed short echo-time (TE) single-volume STEAM spectra (TR/TE/TM=2000/12/10 ms, spectral width=2000 Hz, spectral data size=2048 points, 128 scans, total acquisition time=4:16 min) were acquired from four volumes selected on sagittal T1-weighted and axial T2-weighted images. Four different tissue types were targeted: anterior cingulate ($20\times20\times20mm^3$), frontal white matter ($15\times25\times20mm^3$), basal ganglia ($17\times35\times15mm^3$), and parietal grey matter ($25\times20\times20mm^3$). Figure 4 shows the proton-MRS voxel locations of these tissues. We chose these areas based on acute SIV studies in macaques that had shown changes in these regions [143,149,150].

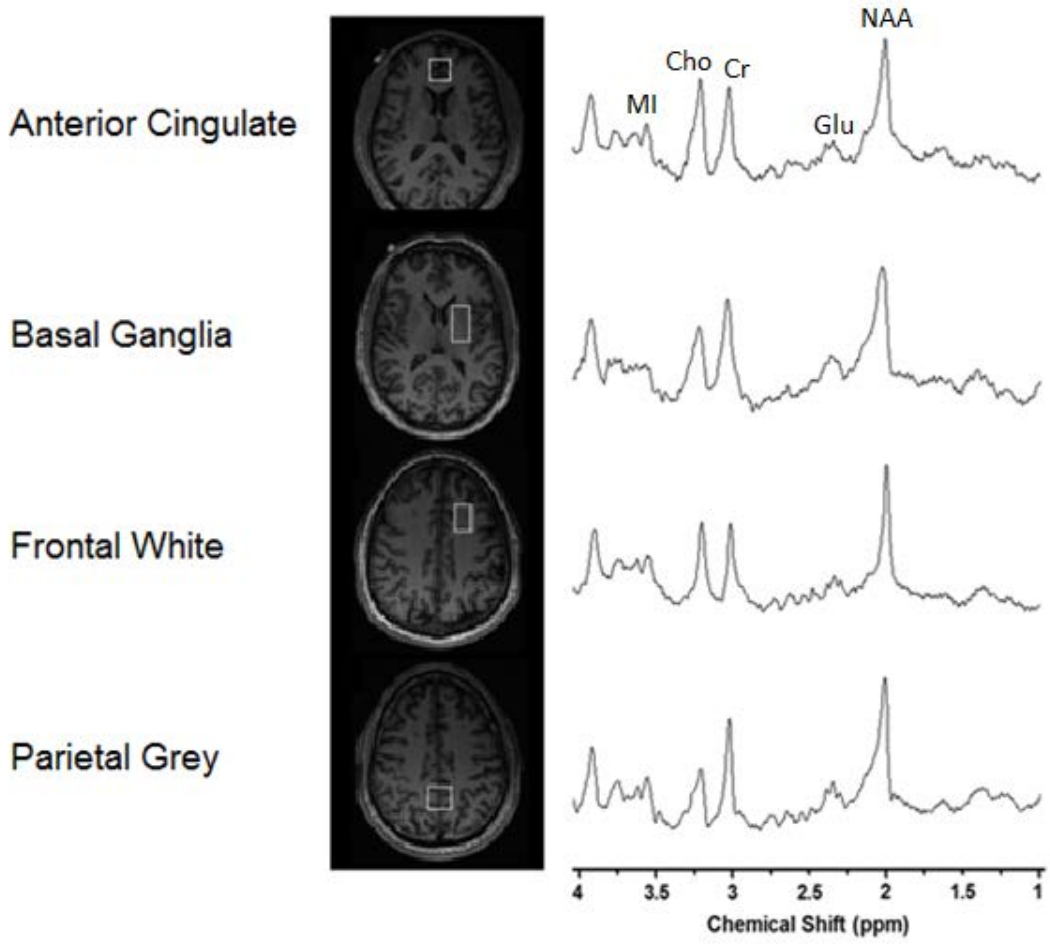

Figure 4. Overview of proton-magnetic resonance spectroscopy acquisition locations and characteristic spectral peaks for metabolites included in this study. Acquired using 4-Tesla Siemens/Bruker MR system. MI = myo-inositol, Cho = choline-containing metabolites, Cr = creatine, Glu = glutamate, NAA = N-acetylaspartate. From Young AC at Seattle, WA: March 3, 2012, CROI [129]. Used with permission.

Meyerhoff and others have previously described most of the proton-MRS processing methods [151]. Briefly, all MRI and single-volume proton-MRS data were stored in an SQL database, and were processed and analyzed with updated software tools developed in-house [152] or in routine use for many years. The spectral fitting software, SITOOLS, used a parametric model of known metabolite resonances and modeled spectral components, including those of macromolecules, to fit all spectral resonances and nonparametric parameters to the baseline. *A priori* spectral information used the frequencies, phases, and approximate relative amplitudes of all major metabolites at 4-Tesla and also included resonances for macromolecules. The obtained raw metabolite signal integrals were corrected for MRI-derived tissue and CSF contributions to the proton-MRS volumes, corrected for different receiver and transmitter settings when necessary, and then normalized to the cerebral water signal obtained from the corresponding volumes. For each metabolite, these adjusted signal integrals ("peak areas") were converted to metabolite ratios: glutamate/creatine, myo-inositol/creatine, N-acetylaspartate/creatine, and choline-containing metabolites/creatine. We excluded spectra if they exhibited poor signal-to-noise ratios, excessive water signal, or other significant artifacts. High-field proton-MRS at 4-Tesla has highly sensitive signal-to-noise detection and allowed us to determine individual peaks of glutamate and glutamine instead of Glx; this level of resolution is not possible with less powerful magnets.

Neuropsychological Testing

Neuropsychological testing was performed only at the San Francisco site and all participants were fluent in English. At baseline, a trained psychometrist administered a

neuropsychological testing battery composed of motor (timed gait, finger tap non-dominant hand, grooved pegboard), processing speed (trail making A, digit symbol), executive function (trail making B, verbal fluency), learning (RAVLT, figural memory), and memory (RAVLT delay, figural memory delay) domains. To control for the social and demographic variability in the group, z-scores for neuropsychological testing were used for all analyses, and were derived from comparing raw scores to age-, gender-, ethnicity-, and level-of-education-matched norms. We calculated a z-domain score by averaging all z-scores within that domain, and calculated an NPZ-4 score by summing grooved pegboard, digit symbol, finger tapping, and timed gait. A total z-score was calculated as a composite of all tests and a global deficit score was calculated in the standard manner [153].

Statistical Analyses

Non-parametric descriptive statistics used the Mann Whitney U-test and the Kruskal-Wallis test with post-hoc testing corrected with Dunn's multiple comparison, all performed with SPSS (version 19.0, SPSS Inc, Chicago, IL) and GraphPad Prism (version 5.0d, GraphPad Software, San Diego, USA). Correlations between measured parameters employed Spearman's rank correlation coefficient; parametric correlations and linear regression were also conducted for illustrative purposes. A multivariate regression model to investigate independent predictors of CSF neurofilament light chain included age, CSF neopterin, WBC, protein, IP-10, and CSF:plasma albumin ratio; these parameters had been identified as significant predictors in both the parametric and non-parametric univariate models.

Results

Study Participant and HIV Disease Characteristics

Table 4 shows background clinical and demographic information for primary HIV infection participants (n=92) and HIV-uninfected controls (n=25) included in the analysis. HIV-infected participants were an estimated median of 3.1 months post-transmission and were younger and more likely to be male than the controls. They displayed the decline in $CD4^+$ T cell count ($p<0.0001$) and the increased $CD8^+/CD4^+$ T cell ratio characteristic of early HIV infection ($p<0.0001$) and also demonstrated a CSF pleocytosis compared with HIV-uninfected controls ($p<0.0001$). 8/92 (8.7%) participants in the primary HIV infection group had previously experienced one or more neurological symptoms during seroconversion, and the majority of participants harbored infection with HIV-1 subtype B, as described in previous work [42].

CSF Markers of Neuronal Injury during Primary HIV Infection

In a subset of 32 participants, the new CSF neurofilament light chain assay demonstrated a high degree of correlation with the older, less sensitive assay that was used in previous studies ($r=0.8$, $p<0.0001$). Figure 5 shows comparisons of the six CSF biomarkers in this study between the primary HIV infection and HIV-uninfected control groups. Median neurofilament light chain in 82 primary HIV infection participants was elevated compared with 20 controls ($p=0.0004$; Figure 5a).

P-tau was elevated in 66 primary HIV infection participants compared with 23 controls ($p=0.016$, Figure 5b). There were no significant differences between groups in t-tau or soluble amyloid precursor proteins–α and –β (Figure 5c-e). Amyloid-beta 42 was

	PHI n=92	**HIV-uninfected** n=25	p-value
% Male	95.2%	80%	0.03
Age, *years*	36 (28-46)	43 (40-49)	0.001
Estimated Days of Infection	92 (52-152)	-	-
$CD4^+$ Count, *cells/uL*	536 (392-682)	836 (703-1056)	< 0.0001
$CD8^+$ Count, *cells/uL*	985 (161-9063)	550 (157-1031)	< 0.0001
Log_{10} Plasma VL	4.6 (4.0-5.2)	-	-
Log_{10} CSF VL	2.9 (2.0-3.6)	-	-
CSF Protein, *mg/dL*	41 (32-53)	47 (33-56)	0.71
CSF WBC, *cells/uL*	6 (2-11)	1 (0-2.5)	< 0.0001
% ARS Neuro Symptoms	8.7%	-	-

Table 4. Demographic and descriptive characteristics of primary HIV infection participants and HIV-uninfected controls. Values are shown as median (IQR) except where noted. PHI = primary HIV infection; VL = viral load; CSF = cerebrospinal fluid, WBC = white blood cells, ARS = antiretroviral syndrome. Used with permission [107].

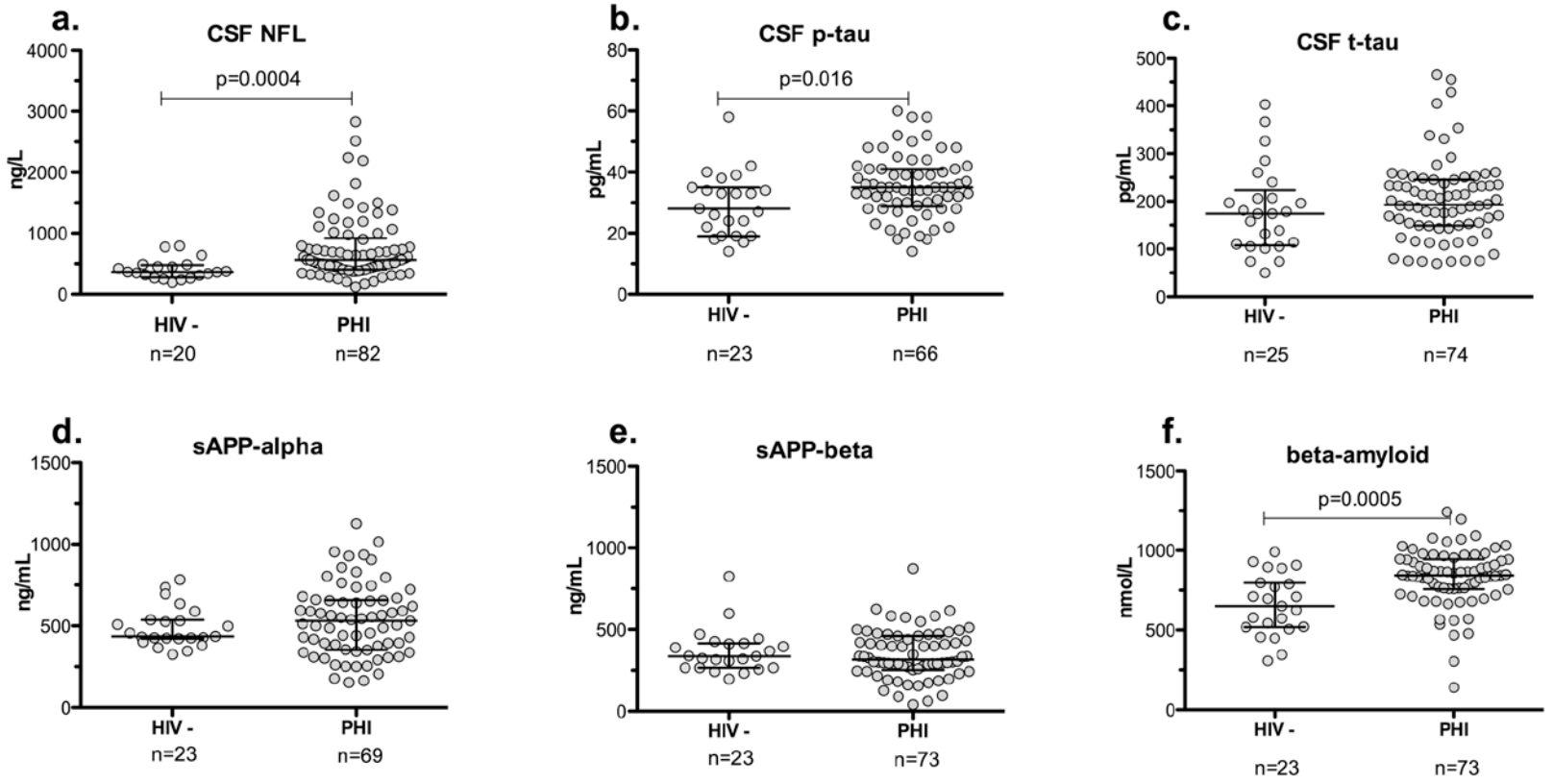

Figure 5. CSF biomarkers of neuronal injury in the primary HIV infection group and HIV-uninfected control group. Note the statistically significant elevations in NFL, p-tau, and amyloid-beta 42. PHI = primary HIV infection group, HIV- = HIV-uninfected control group, NFL = neurofilament light chain, p-tau = hyperphosphorylated tau, t-tau = total tau, sAPP = soluble amyloid precursor protein. Used with permission [107].

elevated in 73 primary HIV infection participants compared with 23 controls (p=0.0005; Figure 5f).

Figure 6a indicates that, when stratified by age, 36/82 (44%) participants had neurofilament light chain elevations above the upper limit of normal for their age group: 13/24 (54%) <30 years, 12/24 (50%) 30-39 years, 10/31 (32%) 40-59 years, and 1/3 (33%) >59 years. Figure 6b-c shows that participants who had experienced neurologically symptomatic seroconversion (n=8) did not have higher neurofilament light chain, amyloid-beta 42, or p-tau (not shown) than those who had been neurologically asymptomatic during seroconversion. Even when previously symptomatic participants were excluded, these biomarkers remained elevated in the primary infection group compared with controls.

Determinants of Elevated Neurofilament Light Chain during Primary HIV Infection

Figure 7 displays the correlations between neurofilament light chain and markers of viral and immune system activity in the CSF. CSF neurofilament light chain correlated with concentrations of inflammatory markers including CSF neopterin (r=0.38; p=0.0005) and IP-10 (r=0.39; p =0.002), WBC count (r=0.32; p=0.004), protein (p=0.59; p<0.0001) and CSF:plasma albumin ratio (r=0.60; p<0.0001). Significant correlations were not found between neurofilament light chain and $CD4^+$ T cell count, estimated days post-infection at sampling, plasma and CSF HIV RNA levels, or CSF MCP-1 (p=0.33; graph not shown).

Figure 8 shows the relationship between neurofilament light chain concentrations and metabolite analyses conducted with proton-MRS. Elevated levels of neurofilament

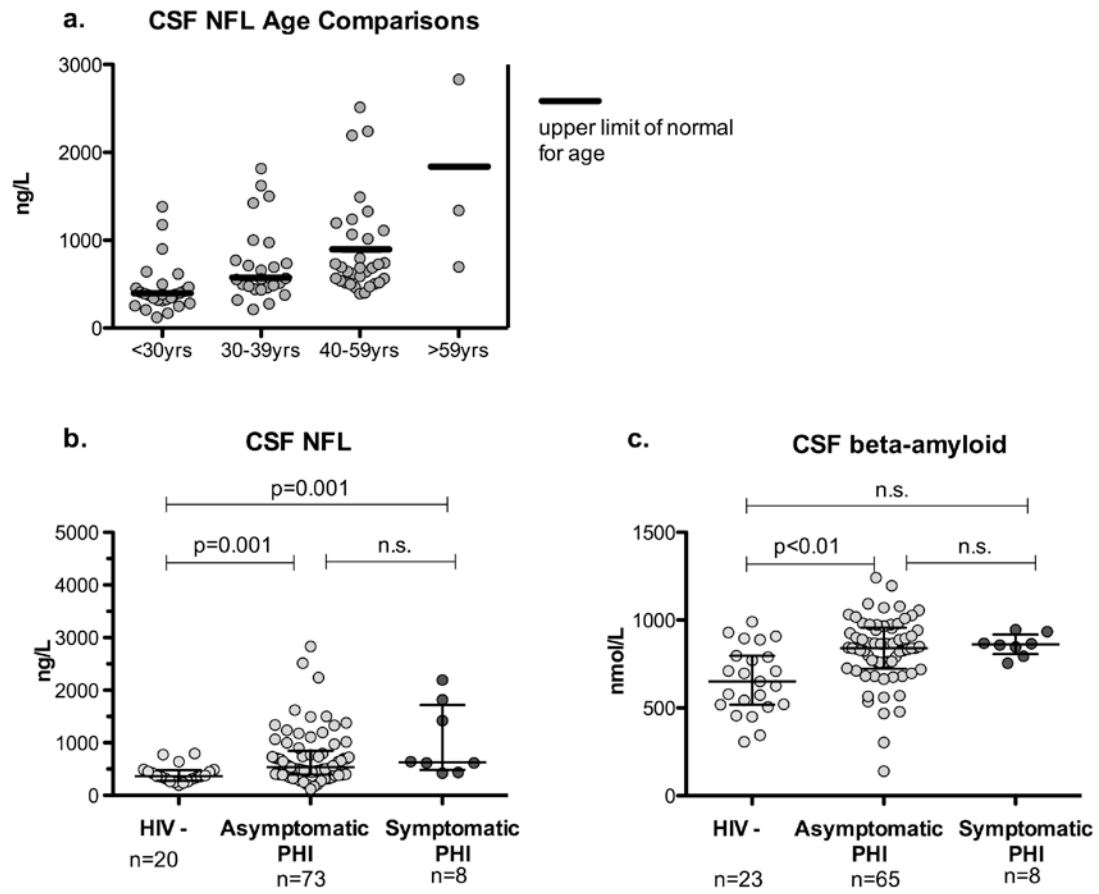

Figure 6. Biomarker measurements stratified by age and history of symptomatic seroconversion. a. CSF neurofilament light chain measurements stratified by age, with relation to the age-specific upper limit of normal. 44% of subjects have elevations above the upper limit of normal for their age group. b. CSF neurofilament light chain values stratified by history of symptomatic seroconversion, showing significant elevations regardless of presence or absence of previous neurological symptoms. c. CSF beta-amyloid values stratified by history of symptomatic seroconversion, showing significant elevation even when previously symptomatic patients are removed. NFL= neurofilament light chain, PHI = primary HIV infection.

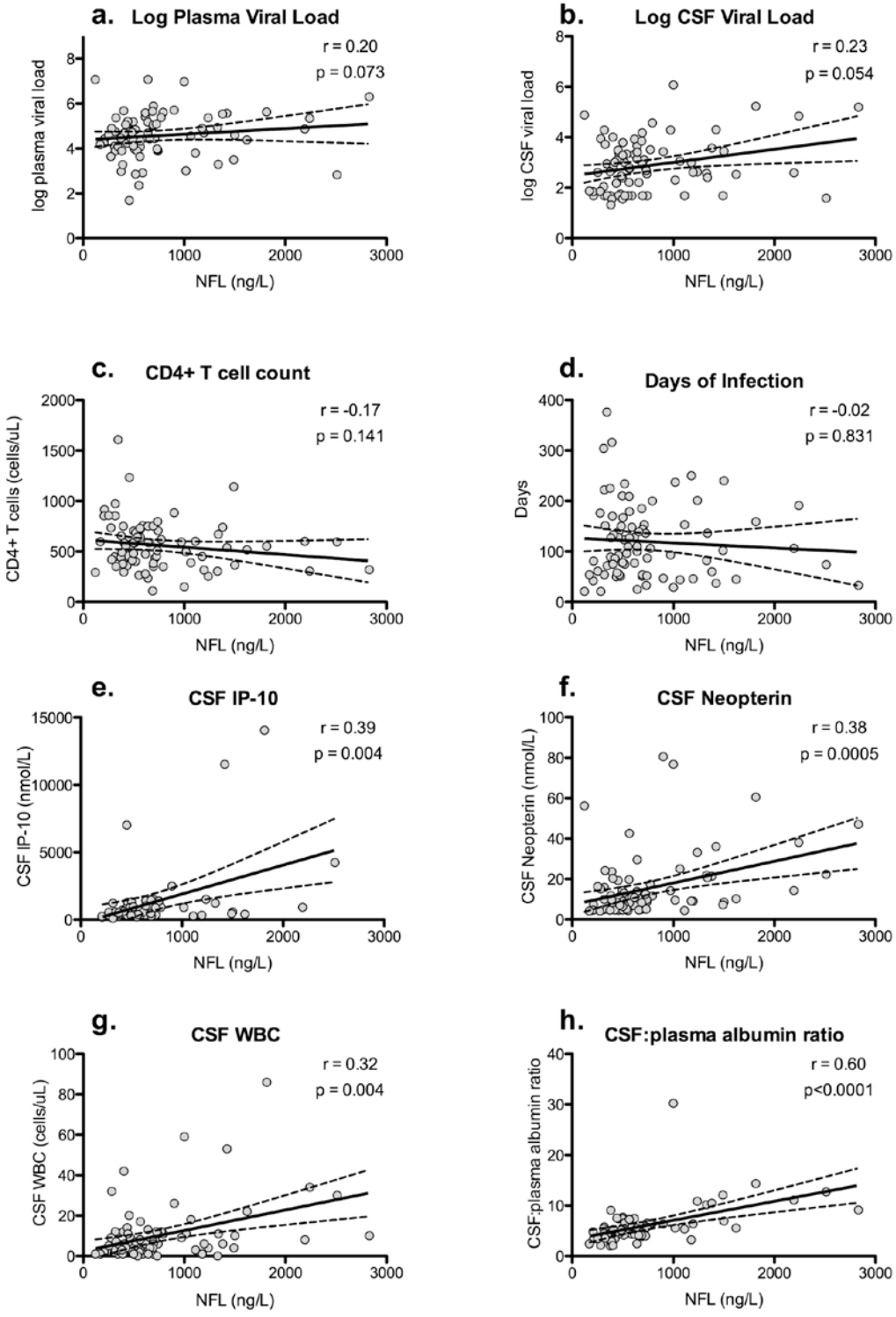

Figure 7. Selected correlates of neurofilament light chain levels in primary HIV infection. r represents the Spearman correlation coefficient and the corresponding p-value is displayed. Solid lines represent best-fit regression line and dashed lines represent 95% confidence intervals. Used with permission [107].

light chain correlated with low N-acetylaspartate/creatine and glutamate/creatine ratios in the anterior cingulate (r=-0.35, p=0.02; r=-0.40, p=0.009, respectively), frontal white matter (r=-0.43, p=0.003; r=-0.30, p=0.048, respectively), and parietal gray matter (r=-0.43, p=0.003; r=-0.47, p=0.001, respectively). Figure 9 demonstrates that N-acetylaspartate/creatine and glutamate/creatine ratios were correlated across these three brain regions ($r>0.50$, $p<0.001$). No significant correlations were present between neurofilament light chain and glutamate/creatine or N-acetylaspartate/creatine in the basal ganglia (Figure 8g-h) or with myo-inositol/creatine or choline/creatine from any region (data not shown).

Multivariate linear regression modeling was used to identify independent predictors of CSF neurofilament light chain in primary infection participants and revealed independent correlations with age, CSF WBCs, and CSF:plasma albumin ratio (adjusted r-square=0.624).

Determinants of Elevated Amyloid-beta 42 during Primary HIV Infection

Figure 10 shows correlations between amyloid-beta 42 and CSF markers of HIV disease and neuroinflammation. Amyloid-beta 42 did not correlate with age in the primary HIV infection group, but did correlate with levels of soluble amyloid precursor proteins–α and –β (r=0.27, p=0.03; r=0.30, p=0.015, respectively) and with estimated days post-infection (r=0.34, p=0.003). There were no significant correlations between amyloid-beta 42 and plasma or CSF viral load, CSF:plasma albumin ratio or CSF protein, neopterin, MCP-1, or IP-10. Notably, neurofilament light chain and amyloid-beta 42 did show a modest correlation (r=0.29, p=0.018; Figure 10g).

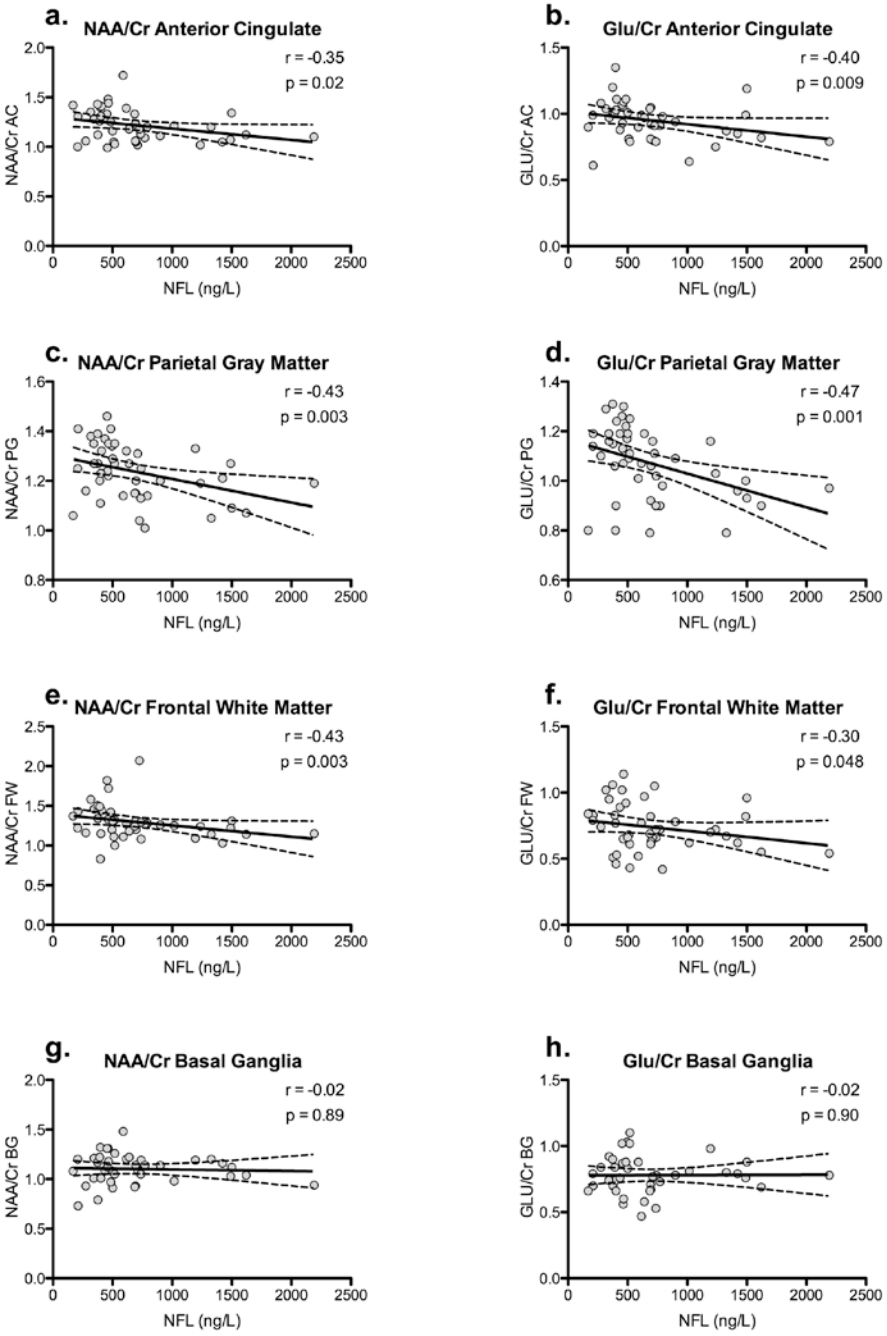

Figure 8. Correlations of neurofilament light chain levels with regional proton-magnetic resonance spectroscopy-derived metabolite ratios. r represents the Spearman correlation coefficient and corresponding p-value. Solid lines represent best-fit regression line and dashed lines represent 95% confidence intervals. Used with permission [107].

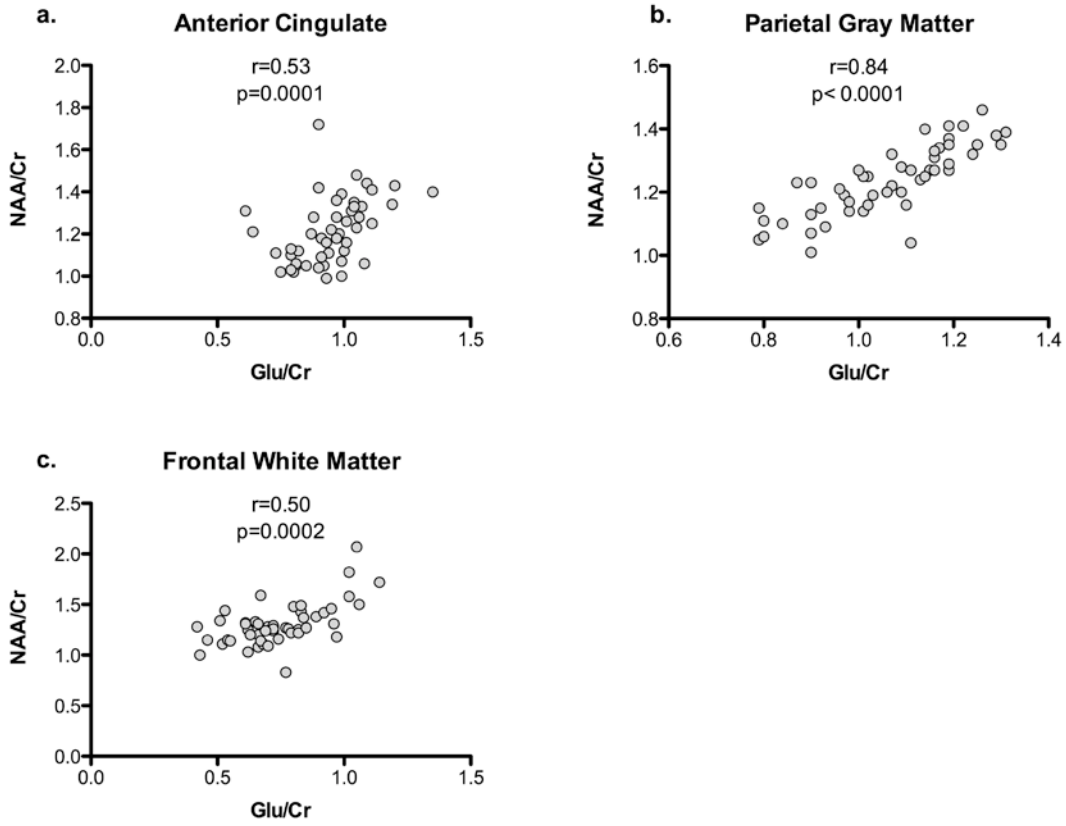

Figure 9. Correlations between N-acetylaspartate/creatine and glutamate/creatine measured by proton-magnetic resonance spectroscopy in selected brain regions. Note the high level of correlation across the three regions. r represents the Spearman correlation coefficient and the corresponding p-value is displayed.

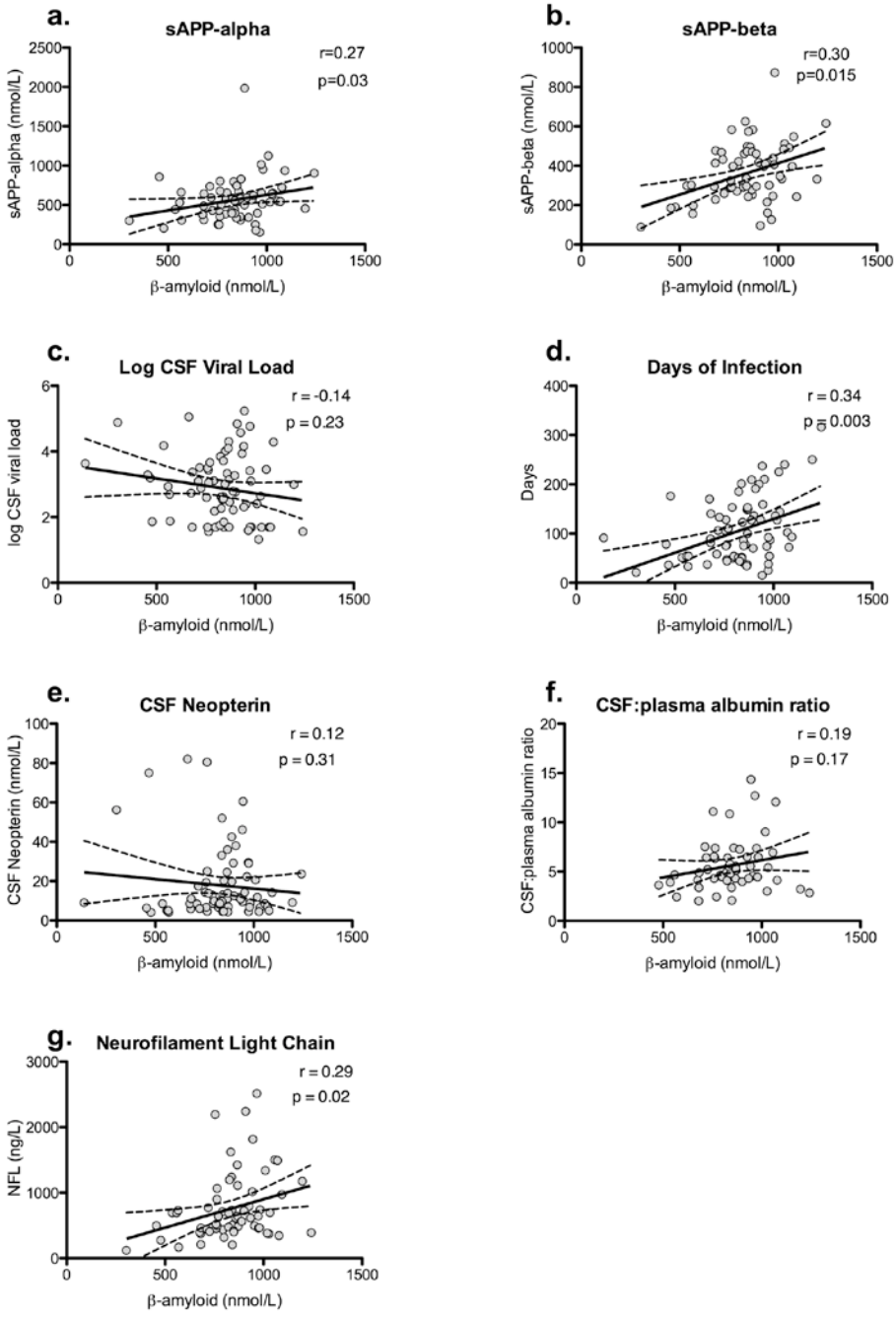

Figure 10. Selected correlates of amyloid-beta 42 in primary HIV infection. r represents the Spearman correlation coefficient and the corresponding p-value is displayed. Solid lines represent best-fit regression line and dashed lines represent 95% confidence intervals. Used with permission [107].

Neuropsychological Testing in Primary HIV Infection Participants

Figure 11 shows the results of neuropsychological testing in the primary HIV infection cohort at baseline and compares the mean for the HIV-infected group to a theoretical mean of 0 (normal neuropsychological performance). Overall, the primary HIV infection subjects did not differ from the theoretical mean in motor performance (mean -0.16; $p=0.10$) but performed worse than would be expected on tests of executive function (mean -0.44, $p<0.0001$), processing (mean -0.53, $p<0.0001$), memory (mean -0.39, $p=0.0001$), and learning (mean -0.61, $p<0.0001$).

Figure 12 shows the relationship between neuropsychological testing performance and neurofilament light chain measurements. There were no significant correlations between CSF neurofilament light chain and composite z-scores for motor function, processing speed, memory, or learning. Neurofilament light chain and the composite z-score for executive function tended to be correlated ($r=0.27$; $p=0.049$). There was no evidence of correlation between neurofilament light chain and the global deficit score. There were no significant correlations between amyloid-beta 42 or p-tau and any composite z-scores on neuropsychological testing (data not shown).

Discussion

The findings in this study demonstrate that several biomarkers of neuronal injury, including neurofilament light chain and amyloid-beta 42, are abnormal in the CSF of a subset of individuals with primary HIV infection and that neurofilament light chain concentration correlates with established proton-MRS markers of neuronal injury. This suggests that neuronal injury, in addition to viral replication [87], immune activation

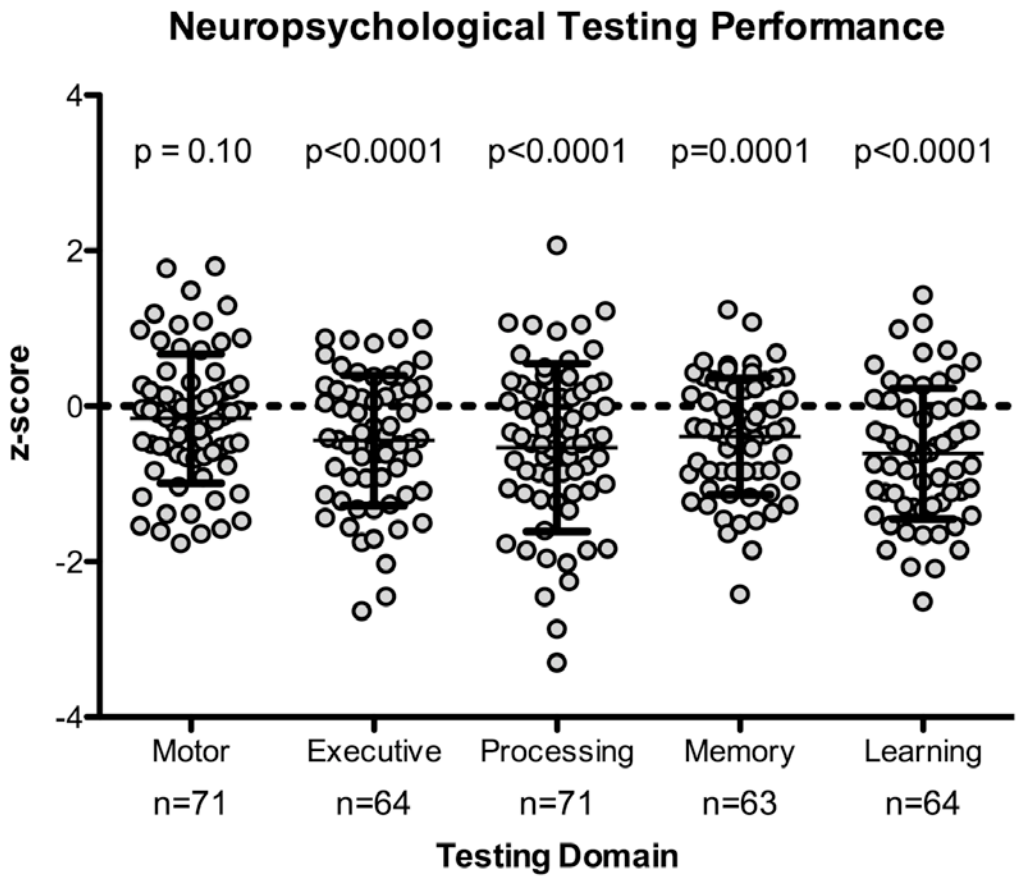

Figure 11. Neuropsychological testing performance at baseline (median of 3.1 months) in participants with primary HIV infection. "0" line indicates control z-domain score. Z-domain scores calculated as average of z-scores for each component of the domain (see text for domain components), with each z-score increment representing 1 standard deviation above or below 0. Bars represent mean and standard deviation for each component. P-values calculated using a one-sample t-test with theoretical mean of 0.

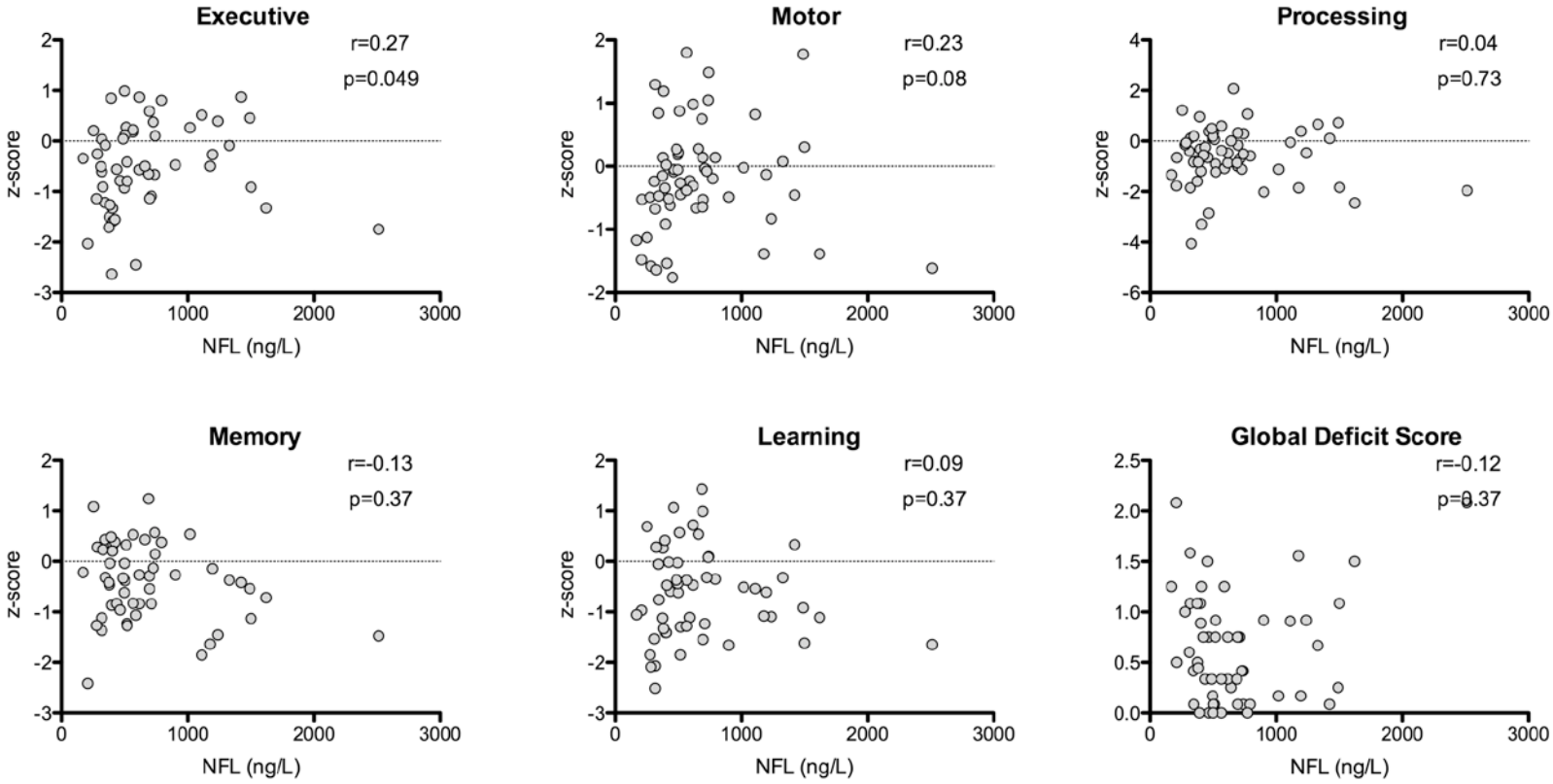

Figure 12. Correlations between composite domain-based neuropsychological testing performance and neurofilament light chain concentration in subjects with primary HIV infection. r represents the Spearman correlation coefficient and the corresponding p-value is displayed.

[29,154], and blood-brain barrier breakdown [87] occurs early in the course of HIV infection in some individuals.

The association of neurofilament light chain with markers of inflammation suggests a relationship between injury and immune activation in this setting. The finding that proton-MRS measures of cortical and white matter N-acetylaspartate/creatine and glutamate/creatine demonstrate associations with CSF neurofilament light chain concentrations is the first data to relate non-invasive neuroimaging markers of neuronal injury (i.e., proton-MRS) with CSF neural marker abnormalities in HIV infection.

Neuronal health and stability appears compromised in some brain regions, including the frontal white matter and parietal gray matter, in the earliest stages of HIV infection. The lack of a convincing correlation between neurofilament light chain levels and performance on common neuropsychological tests may suggest that neurofilament light chain elevation reflects subclinical injury during this stage of infection, but may also reflect shortcomings with regard to the sensitivity and/or specificity of neuropsychological testing during this time period.

Neurofilament Light Chain

Neurofilament light chain concentration serves as a sensitive indicator of CNS axonal injury in a number of neurodegenerative disorders including Alzheimer's disease, atypical Parkinsonian syndromes, and amyotrophic lateral sclerosis [110,123], as well as in multiple sclerosis [108] and traumatic brain injury [155]. CSF neurofilament light chain concentrations increase in untreated individuals with HIV-associated dementia and neurological opportunistic infections [117]. Moreover, neurofilament light chain may

predict who goes on to develop neurological disease [156], has been shown to decrease with the initiation of antiretroviral therapy [118], and increases with cART discontinuation [119]. This previous data suggests that neuronal damage occurs in the setting of the pathogenic activity of HIV in the central nervous system and the immune response of the host during other stages of HIV infection.

Previous work including a subset of patients from this cohort identified elevated neurofilament light chain in 4/16 (25%) subjects with primary HIV infection, but showed no significant difference compared with controls [117]. However, that work utilized a less-sensitive assay, with a lower limit of detection of 250 ng/L compared with the assay used in this study, which had a lower limit of detection of 50 ng/L. The two assays had a strong correlation when results were compared in a subset of subjects, but the newer, more-sensitive assay allowed for greater resolution of the lower end of the concentration scale and resolved points that were previously clustered together at the lower limit of detection using the earlier assay.

Our findings using this more-sensitive assay demonstrate increased neurofilament light chain in the group comparison, which has not previously been demonstrated in primary HIV infection [117]. The results also demonstrate that 44% of participants in this study had elevations in neurofilament light chain above the age-appropriate upper limit of normal for this marker. This finding suggests that, in at least a subset of participants, neurological injury occurs during primary HIV infection even in subjects who had not previously experienced neurological symptoms during seroconversion. Along with CSF HIV RNA and inflammatory markers, elevated neurofilament light chain levels may help to identify individuals with an active disease process and distinguish them from those

with static neurological abnormalities. Since it has been suggested that neurofilament light chain can predict neurologic disease progression [156], it is possible that this marker may identify individuals that could benefit from early pharmacologic intervention aimed at protecting the brain from neuronal injury.

It is also worth noting that a significant proportion of these participants (56%) did not demonstrate elevated neurofilament light chain, suggesting the possibility that an unknown viral or host factor increases the susceptibility of certain individuals to neurological injury during this period.

Correlates of Elevated Neurofilament Light Chain during Primary HIV Infection

Overall, the results of this analysis suggest an association between neurofilament light chain and inflammatory processes in the central nervous system, reflected in significant correlations with CSF neopterin and IP-10, as well as markers of CSF pleocytosis and blood-brain barrier breakdown. It is notable that such associations did not exist with amyloid-beta 42, implying that neurofilament light chain might be a more specific marker for inflammatory injury in the central nervous system.

During primary infection, neurofilament light chain was not associated with $CD4^+$ T lymphocyte count, which may reflect the fact that $CD4^+$ count during this period is a correlate of the acute systemic immune response to HIV acquisition rather than the duration and progression of infection. Neurofilament light chain also did not strongly associate with markers of infection, including plasma and CSF HIV RNA.

Because the N-acetylaspartate/creatine ratio is a putative marker of neuronal health, our results suggest that declining neuronal health is associated with increased

neuronal injury as identified through elevated CSF neurofilament light chain. This is particularly true in the parietal gray matter and frontal white matter, consistent with studies in animal models [149,150]. We also found a negative association between the glutamate/creatine ratio and neurofilament light chain. Elevated glutamate/creatine is a putative marker for excitotoxicity, but it is also considered a marker of neuronal integrity (i.e., [142]). Previous studies have shown that HIV-infected individuals with cognitive deficits have lower glutamate/creatine levels, particularly in the parietal gray matter but not the frontal white matter [142]. Here, we found a similar regional specificity in that high neurofilament light chain levels correlated strongly with low glutamate/creatine in the parietal gray matter, but not in the frontal white matter. The strong correlations between glutamate/creatine and N-acetylaspartate/creatine ratios across all brain regions emphasize the value of these metabolites in the assessment of neuronal health and suggest that the consistent regional metabolite correlations with neurofilament light chain are meaningful.

It is difficult to draw clear conclusions from the generally below-average performance on the tests for many neuropsychological domains, because performance on these tests may be confounded by a variety of factors related to HIV infection but unrelated to the pathogenesis of the disease. In addition, we found no convincing correlation between CSF neurofilament light chain and performance on most cognitive domains assessed by this circumscribed battery of tests. However, a modest association between higher NFL and poorer performance in tests of executive function may reflect a relationship between neural injury and impairment of this cognitive domain during early infection.

Tau Proteins

Tau and amyloid proteins are valuable in the identification of neurodegenerative disorders [138,157,158], but their utility in HIV infection is less clear. T-tau and p-tau patterns in HAND and HAD are inconsistent [113,125,140].

In this study, elevated p-tau occurred in the context of unchanged t-tau, a pattern different from that which is seen in chronic AIDS [125], Alzheimer's disease [158], and Creutzfeldt-Jakob disease [159]. The difference is weaker than that identified for neurofilament light chain or amyloid-beta 42, and we are therefore unable to conclude from this study whether t-tau and p-tau could be useful measures of neuronal injury in primary HIV infection.

Amyloid Proteins

Amyloid precursor proteins are cleaved by secretases into soluble amyloid precursor proteins–α and –β; cleavage to –β form generates a molecule leading to amyloid-beta 42 [113,128]. Amyloid-beta 42 decreases in Alzheimer's disease [138] and in HAND [139], HAD [125], and CNS Opportunistic Infections [113]. Pathological studies show amyloid deposition in brain tissue of HIV-infected individuals [160], but this marker has not been explored in primary HIV infection.

The elevation in amyloid-beta 42 discovered here could be accounted for by a number of mechanisms. It is unlikely to be due to the age of the participants in each group, as median levels for controls is concordant with those for young controls in other studies [113], and the median value in primary HIV infection participants is higher than in neuroasymptomatic HIV-infected individuals [113].

Elevated CSF amyloid-beta 42 has recently been associated with cerebral inflammation [161] and this elevation is associated with increases in markers of inflammation in the CSF, including TNF-α, IL-6, and IL-8. This suggests that acute cerebral inflammation results in the production of amyloid-beta 42. While our primary infection subjects presented with a neuroinflammatory picture (with elevated CSF WBCs, IP-10, and protein), amyloid-beta 42 did not correlate with markers of neuroinflammation in this group. However, the markers we measured differed from those for which correlations have been identified (TNF-α, IL-6, or IL-8) in other studies [161].

There are a number of other mechanisms that may cause or be associated with this unexpected elevation of amyloid-beta 42. In the plasma, a large fraction of this protein is bound to and transported by albumin [162]. The disruption of the blood-brain barrier in primary HIV infection may cause a translocation of amyloid-β-albumin complexes into the CSF [91], but we found no correlation between amyloid-beta 42 and CSF:plasma albumin ratio, which is a marker of blood-brain barrier breakdown. Low-density lipoprotein receptor-related protein (LRP) clears soluble amyloid-beta 42 by mediating endocytosis in macrophages [163], which go on to catabolize this protein. HIV tat protein inhibits LRP uptake and degradation of amyloid-beta 42 [164] and increases its intracellular, soluble component [165]. It is possible that viral replication in primary HIV infection results in tat-mediated inhibition of amyloid-beta 42 uptake and degradation within macrophages, which are known to be infected early by the virus [166]. While there was no correlation between amyloid-beta 42 and viral load, tat protein may be produced in such quantities during primary infection that the viral load itself is not the determinant of inhibited amyloid degradation.

Limitations

This cross-sectional study captured participants at a single time point; future work with longitudinal data from this group will help to determine how these biomarkers change with time. Our study participants were almost exclusively men, which poses a problem for generalizing these results to the increasing number of HIV-infected women. Because the median duration of infection was 3.1 months, it is unclear when during primary HIV infection these biomarker abnormalities begin to occur.

We defined our hypotheses before conducting our analyses, but still made many statistical comparisons; because of this, we attempted to exercise restraint in statistical interpretation and to correct for multiple comparisons. Nevertheless, we find the data, particularly in the contrasts in correlated variables between neurofilament light chain and amyloid-beta 42, to be a convincing starting point for further exploration of the mechanisms of neurologic injury during primary HIV infection.

CHAPTER 2: CEREBROSPINAL FLUID HIV "ESCAPE" ASSOCIATED WITH PROGRESSIVE NEUROLOGICAL INJURY IN PATIENTS ON ANTIRETROVIRAL THERAPY WITH WELL-CONTROLLED PLASMA VIRAL LOAD

Chapter Background

As discussed in the Introduction, the mechanisms and clinical implications of compartmentalization of HIV within the central nervous system is an area of growing scientific interest. This chapter focuses on a newly identified manifestation of this compartmentalization, which has recently been recognized as having important implications for patients with well-controlled, chronic HIV infection and may be help us understand the mechanisms behind HAND in the antiretroviral era.

CSF 'Escape'

Recently, Canestri *et al.* demonstrated the phenomenon of CSF/plasma HIV RNA discordance involving the development of new neurological symptoms in eleven patients with well-controlled plasma HIV [18]. These patients, in general, had chronic HIV infection managed long-term with antiretroviral therapy and went on to develop symptoms despite having a relatively well-controlled plasma viral load. In all cases, it was noted that although the virus was controlled or suppressed in the plasma, these patients had virus present at a concentration of 1 log greater in the CSF. Further investigation revealed that, in some cases, significant resistance mutations in the CSF viral subpopulation were present on genotyping. This suggested that the current treatment regimen had failed in the CNS despite its success in the plasma compartment. Some patients improved when their antiretroviral therapy regimen was optimized based upon

the results of genotyping and the analysis of presumed CNS drug exposure as measured through the CPE score.

This work has suggested that even in the setting of long-term plasma viral control, a subset of patients may go on to develop neurological symptoms because of a failure of control in the CSF compartment. Laboratory studies in these patients have revealed low-level viremia in the CSF in the setting of suppressed plasma HIV RNA and relatively preserved immune function. This phenomenon of CSF 'escape' was initially reported in individual cases [167-170] and culminated with the Canestri report. However, despite these publications, CSF 'escape' remains a poorly understood phenomenon. This is largely due to some of the criticisms of previous reports, in which some patients have been on monotherapy [18] or salvage therapy [167,168], or have had low $CD4^+$ T cell counts [18,169]. Imaging data has been generally sparse and information on follow-up has been limited.

Statement of Purpose, Specific Aims, and Hypotheses

In this project, we sought to add to the contributions of Canestri *et al.* [18] and previous reports [167-170] by further investigating the condition of CSF 'escape' in patients with well-controlled plasma HIV and preserved immune function. We also sought to provide more detailed background information regarding HIV disease course in these patients and to emphasize key portions of the clinical picture, including neuroimaging, that have not yet been described in detail. The specific aims of this project were as follows:

1. To identify cases of CSF 'escape' according to a strict case definition that excludes patients who have been generally non-compliant with mediations, have been on unusual or incomplete regimens, or have an unknown disease history.

2. To characterize these cases in terms of patient demographics and disease history, including nadir $CD4^+$ T cell count, HIV plasma viral load, neurological signs and symptoms, and antiretroviral regimen.

3. To characterize the cerebrospinal fluid and imaging findings in these patients.

4. To investigate antiretroviral resistance patterns and CNS penetration efficacy of regimens before and after CSF escape was identified.

5. To hypothesize host and disease mechanisms contributing to the development of CSF HIV 'escape.'

In this chapter, we report a group of patients from four institutions in the United States and Europe. Each patient presented with new-onset neurological symptoms in the context of low or undetectable plasma HIV levels, underwent neurological studies including lumbar puncture and CSF analysis, and was noted to have CSF 'escape.' Antiretroviral therapy regimens were optimized based upon drug susceptibility and penetration as measured through resistance genotyping and CPE. This study adds to a growing body of evidence regarding the rare condition of CSF 'escape' associated with progressive neurological disease in otherwise well-controlled HIV infection.

Methods

Study Initiation

In July 2011, a patient (patient 1000) was referred by his primary care HIV provider (N.A.) and was seen in HIV neurology clinic by two of the authors (M.J.P. and S.S.). The patient had been diagnosed with HIV in 1990 and had been compliant with medications, as evidenced by low (<50 copies/mL) or undetectable viral loads throughout most of the time since diagnosis. He presented with a complaint of worsening headaches and intractable vertigo. A complete workup, including lumbar puncture, revealed that the only laboratory abnormality was slightly elevated CSF HIV RNA level (460 copies/mL) in the setting of <50 copies/mL in the plasma. An exhaustive investigation for other contributing comorbidities, including opportunistic infections, did not reveal any additional contributing factors. The patient was diagnosed with CSF/plasma discordance.

A literature review of the case presentation revealed few publications on this topic. Upon further discussion with the patient's primary clinician, she recalled one other case from the Nathan Smith Clinic. We conducted a review of the previous case and identified it as another example of CSF/plasma discordance or CSF 'escape.' It was decided to assemble a case report and to identify similar cases among our research collaborators. Consent was obtained from patients to have their data included in a report.

We drafted a screening protocol for distribution to collaborators in the United States and Europe. This protocol defined a case as "generally well-controlled HIV-infected patients on antiretroviral therapy presenting with neurological symptoms in the setting of previous viral suppression who are determined to have detectable CSF viral load on lumbar puncture." We requested information on disease factors, medication

regimen, CSF studies, brain imaging, EEG, and initial consult and follow-up notes (see appendix 3). These materials were requested electronically and in person at trips to collaborating institutions, where additional visits were scheduled with patients if they were still being followed. All material was compiled in a central repository at Yale.

Study Design and Patient Characteristics

In this study, we retrospectively compiled cases of HIV-infected patients on antiretroviral therapy who presented with neurological signs and/or symptoms in the context of plasma HIV RNA suppression and underwent evaluation, including CSF studies. Subjects were identified by clinicians in our research collaboration at four urban academic centers in San Francisco, USA, Milan, Italy, New Haven, USA, and Gothenburg, Sweden.

All patients were on stable combination antiretroviral therapy regimens with either suppressed (<500 copies/mL) or undetectable (<50 copies/mL) plasma HIV RNA. Patients with symptoms attributable to other neurologic or psychiatric causes were excluded. CSF and concurrent plasma samples were obtained either by the primary clinical team for diagnostic purposes or in the context of research studies in separate local protocols that were approved by the institutional review board or local equivalent at each institution. Clinical brain MRIs were obtained prior to lumbar puncture on varied local 1.5-Tesla scanners in the majority of subjects. We included patients found to have CSF ‘escape,’ defined as detectable CSF HIV RNA in the setting of plasma levels <50 copies/mL or CSF RNA >1 log higher than plasma RNA level as previously defined by Canestri and colleagues [18].

Laboratory and Statistical Methods

HIV RNA levels were measured in cell-free CSF and plasma using the ultrasensitive Amplicor HIV Monitor (version 1.5; Roche Molecular Diagnostic Systems, Branchburg, NJ), Cobas TaqMan RealTime HIV-1 (version 1 or 2; Hoffmann-La Roche, Basel, Switzerland), or the Abbott RealTime HIV-1 (Abbot Laboratories, Abbot Park, IL, USA) assays at local sites. For uniformity, 50 copies/mL was used as the lower limit of quantitative detection in this analysis. Paired blood and CSF measurements used the same assay. CSF total WBCs and protein, and $CD4^+$ and $CD8^+$ T lymphocyte counts by flow cytometry were measured at each local laboratory on fresh samples. Blood and CSF neopterin measurements employed commercially available immunoassays (BRAHMS Aktiengesellschaft, Hennigsdorf, Germany) and were performed in one laboratory. HIV resistance genotyping was performed where available in CSF samples harboring adequate HIV RNA levels for amplification. Genotyping was interpreted according to the International Antiviral Society-USA guidelines [171].

As a means to approximate expected effectiveness of ART in the CNS, we used proposed CNS penetration-effectiveness (CPE) scores using the 2010 version developed by Letendre and colleagues [33] to calculate a “raw” CPE score for each regimen at the time when discordance was identified. These are reviewed in Table 2. In an effort to take into account effective resistance in a consistent, quantitative way, we calculated an “adjusted” CPE score based upon the genotyping results of CSF viral isolates. When a mutation to a particular drug in the regimen was identified, the individual CPE score for that drug was arbitrarily designated “0” so that its contribution to the regimen would effectively be removed from the calculation.

Descriptive analyses were undertaken to characterize these patients and are reported as percentages or median value (range) for continuous variables.

Results

Between February 2000 and August 2011, 10 patients with chronic but well-controlled HIV infection and preserved immune status presented with new neurological symptoms and were recognized as meeting the criteria for CSF 'escape.' The clinical and demographic characteristics of these patients are described in Table 5.

The patients consisted of 8 men and 2 women with a median age of 47.5 years (range, 26-55 years). The median time since HIV diagnosis was 16.2 years (range, 9.4-21.7 years). At the time of the neurologic episode, the patients had been on a stable regimen for a median of 21 months (range, 9-60 months). These regimens consisted of at least 2 NRTIs plus a PI in 9/10 cases; the PI was boosted with ritonavir in 8/9 cases. Individual patients had additional components to their regimen, including integrase or fusion inhibitors. None were on mono- or dual-therapy.

The median duration of HIV RNA suppression below 500 copies was 27.5 months (range, 2-96 months). The median duration of HIV RNA suppression below 50 copies was 19.5 months (range, 2-96 months). The median $CD4^+$ T cell count at presentation was 482 cells/mm^3 (range, 290-660 cells/mm^3). The median nadir $CD4^+$ T cell count was 35 cells/mm^3 (range, 4-222 cells/mm^3).

Three patients had a previous neurological abnormality. These included a presumed cerebellar meningioma that had been stable for many years (patient 1000), labyrinthitis and right sensorineural deafness (patient 8000), and CNS lymphoma that had

		Historical Data			Time of Presentation			
Site* Patient	Date of Presentation (month/year)	Age/Sex (years)	Nadir $CD4^+$ T cell count (cells/mm^3)	Documented time stable plasma HIV† (copies/mL: months)	$CD4^+$ T cell count (cells/mm^3)	Plasma HIV RNA (copies/mL)	Drug regimen	Neurologic Signs/Symptoms
SF 7066	02/2000	45/M	55	<50: n/a <500: 23	318	380	DDI SGC RTV	Cognitive impairment Gait ataxia
MI 9000	05/2003	46/F	15	<50: 28 <500: 28	305	372	3TC d4T LPV/r	Coma Tremor Vertigo
SF 1034	03/2004	51/M	80	<50: 2 <500: 2	588	<50	3TC ZDV LPV/r	Cognitive impairment Gait ataxia Tremor Weakness
SF 7071	07/2004	49/M	8	<50: 30 <500: 30	444	<50	3TC ZDV EFV LPV/r T-20	Cognitive impairment Gait ataxia Sensory impairment
SF 4065	02/2007	49/M	4	<50: 2 <500: 7	520	184	DDI TDF ATV/r	Cognitive Impairment Diplopia Dysphagia Gait ataxia
NH 2000	03/2007	55/M	60	<50: 96 <500: 96	308	<50	3TC ABC LPV/r	Aphasia Gait ataxia Sensory impairment Tremor
GS 5168	05/2008	45/F	55	<50: 47 <500: 60	660	118	3TC TDF ATV/r	Cognitive impairment
MI 8000	08/2010	45/M	222	<50: 27 <500: 27	545	<50	3TC ABC FPV/r	Cognitive impairment Dysarthria Sensory impairment Vertigo
MI 7000	01/2011	26/M	9	<50: 4 <500: 12	290	98	FTC TDF ATV	Diplopia Dysarthria Gait ataxia Headache Tremor
NH 1000	08/2011	49/M	180	<50: 12 <500: 43	627	<50	FTC TDF ATV/r RAL	Aphasia Cognitive impairment Gait ataxia Headache Vertigo

Table 5. Demographic information and HIV history of patients with CSF/plasma discordance. SF = San Francisco; MI = Milan, Italy; NH = New Haven; GS = Gothenburg, Sweden; n/a = not applicable; * = site-specific research protocol identifiers are provided where available; † = where <500 copies/mL is considered "good control" and <50 copies/mL is considered "undetectable," length of time <500 copies/mL includes times when patient was <50 copies/mL. See table 7 for drug abbreviations. Used with permission [107].

resolved completely with initiation of ART, without radiotherapy, several years before (patient 5168).

Clinical and MRI Manifestations

The neurological abnormalities present in this patient group (Table 5) occurred sub-acutely (>2 weeks) in 9/10 patients and were acute (<2 weeks) in 1 patient (patient 9000). They comprise a variety of sensory (in 3 patients), motor (in 9 patients), and cognitive (in 8 patients) manifestations. Imaging in 7/8 patients at the time of presentation showed MRI abnormalities consisting of white matter hyperintensities on T2-weighted and FLAIR sequences (Table 6). Figure 13a-d shows representative imaging examples from patients 2000 and 7000 during the initial studies for neurologic symptoms.

CSF and Brain Pathology

CSF pleocytosis and biochemical abnormalities were found in all 10 patients (Table 6). 8/9 patients had elevated CSF protein levels ≥ 60 mg/dL. The median protein level was 105 mg/dL (range, 46-170 mg/dL). CSF pleocytosis was observed in 9/10 patients, with median 14.5 cells/mm^3 (range 0-200 cells/mm^3). All samples were negative for bacteria, fungi, and other viruses by standard microbiological tests at each institution, including JC virus DNA studies for progressive multifocal leukoencephalopathy (PML). Two samples (patients 1000 and 7000) had low-level (<5000 copies/mL) EBV DNA [172]. CSF from patient 5168 had previously been positive for EBV in the past when she suffered from CNS lymphoma, but CSF EBV titers were negative during and throughout the time of CSF HIV escape in this patient.

Site Patient	Plasma HIV RNA (copies/mL)	Cerebrospinal Fluid Analysis				
		HIV RNA (copies/mL)	Protein (mg/dL)	WBC (cells/mm^3)	CSF Neopterin (nmol/L)	MRI Findings
SF 7066	380	9056	162	50	76.3	Not done
MI 9000	372	8000	170	0	-	Diffuse white matter abnormalities
SF 1034	<50	378	89	6	-	Patchy periventricular white matter abnormalities
SF 7071	<50	8320	60	33	-	Not done
SF 4065	184	4570	74	14	-	Patchy subcortical/ periventricular white matter abnormalities with involvement of corpus collosum and cerebellum
NH 2000	<50	613	77	28	-	Symmetric subcortical/ periventricular white matter abnormalities extending into cerebellum
GS 5168	118	3230	n/a	9	37.6	Slight deformity of frontal ventricular horns, stable compared to previous examinations. Otherwise normal.
MI 8000	<50	134	121	15	-	Diffuse white matter abnormalities
MI 7000	98	5200	137	200	-	Lenticular/posterior internal capsule/cingular cortex white matter abnormalities extending into cerebellum; diffuse pial contrast enhancement
NH 1000	<50	460	46	11	-	Cortical/subcortical/periventricular white matter abnormalities

Table 6. Neurological studies in patients with CSF/plasma discordance. WBC = white blood cells; SF = San Francisco; MI = Milan, Italy; NH = New Haven; GS = Gothenburg, Sweden; n/a = not available. Used with permission [107].

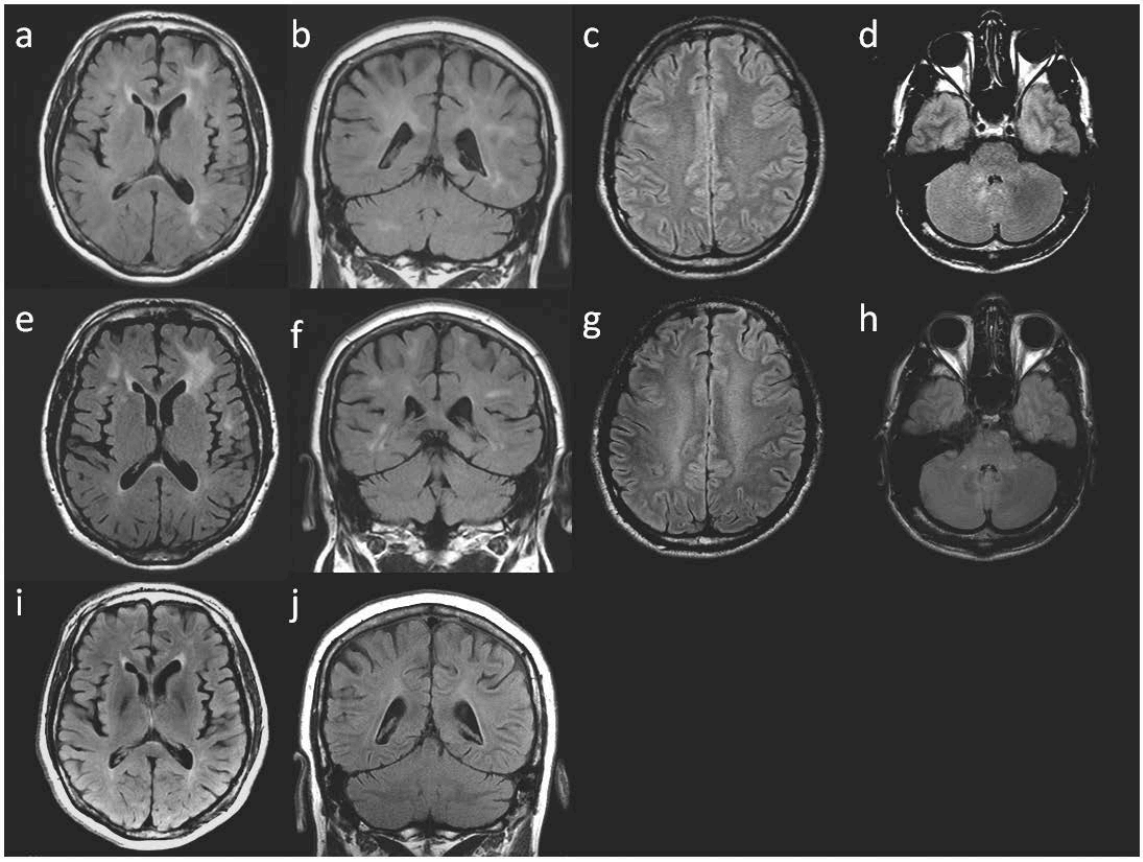

Figure 13a-j. Selected MRI images for Patients 2000 and 7000. Panels a-d show imaging at the time of neurologic workup when CSF 'escape' was initially detected for patients 2000 (a,b) and 7000 (c,d), demonstrating diffuse T2-prolongation (a,b) and suggesting focal lesions (d) at the time of CSF 'escape.' Panels e-h show follow-up imaging for patient 2000 at 111 days and patient 7000 at 60 days. Even though neurological symptoms had resolved in both cases, imaging still shows diffuse leukoencephalopathy (e,f) and hyperintense, diffuse signal alteration of bilateral white matter (h), despite improvement of previous focal lesions (h). Panels i and j show imaging for patient 2000 at 567 days follow-up, demonstrating significant interval decrease in T2-prolongation. Used with permission [107].

CSF neopterin was measured in two patients at the time of CSF 'escape.' Patient 7066 had a CSF neopterin level of 76.3 nmol/L with a plasma level of 12 nmol/L; patient 5168 had a CSF neopterin of 37.6 nmol/L with a plasma level of 8 nmol/L. Reference ranges for HIV-uninfected subjects are <5.8 nmol/L in CSF and <8.8 nmol/L in plasma [173]; for successfully ART-treated HIV-infected subjects, mean 10.8 nmol/L in CSF [174].

Two patients (1034 and 4065) underwent brain biopsy at the time of CSF 'escape,' revealing dense, perivascular lymphocytic infiltrates in the white matter with extension into the surrounding parenchyma. Immunoperoxidase staining showed a mixture of mature and immature B- and T-lymphocytes, with $CD8^+$ predominance.

HIV RNA in CSF and Plasma

By definition, all patients had CSF HIV replication at initial evaluation, with a median of 3900 copies/mL (range, 134-9056 copies/mL). All had a plasma HIV RNA <500 copies/mL and 5/10 had a plasma HIV RNA <50 copies/mL at the time CSF 'escape' was discovered. The median plasma viral load was 62 copies/mL (range, <50-380 copies/mL). For the 5 patients with controlled but detectable plasma HIV RNA (>50 but <500 copies/mL), the CSF HIV RNA was at least 1 log higher than the plasma HIV RNA.

Figure 14 shows longitudinal plasma data for these patients, indicating plasma control <500 copies/mL in 7/10 patients over the previous 1000 days. Of these, 5/7 had HIV RNA below the limit of detection (<50 copies/mL) for the previous 1000 days. One patient had a transient increase in plasma viral load during this period (patient 4065), but had been well-controlled previously and following this increase. Two had viral loads that

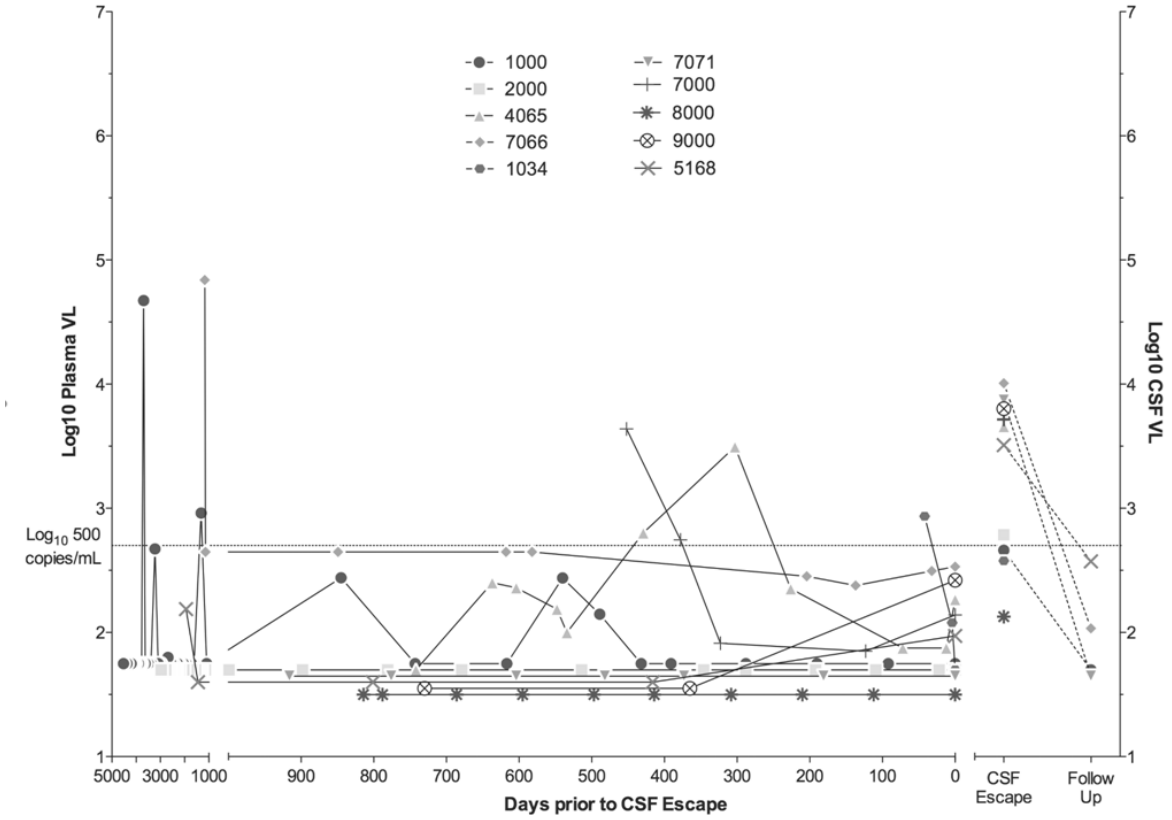

Figure 14. Longitudinal plasma HIV RNA levels for patients with CSF 'escape'. HIV RNA is calculated in days prior to time CSF/plasma discordance was detected (time "0"). Reference dotted horizontal line indicates $\log_{10}$ 500 copies/mL. Corresponding CSF HIV RNA levels are indicated on the right axis of the graph at the time when CSF escape was identified ("CSF Escape") and at a standardized follow-up time point, where repeat CSF was available ("Follow-up"). Used with permission [107].

had more recently declined to <500 copies/mL (patients 1034 and 7000). These patients were included because they presented with new neurologic symptoms in the absence of alternate pathogens or focal lesions as determined through imaging or brain biopsy, with CSF 'escape' in the setting of preserved immune status and declining plasma HIV RNA.

Viral Resistance and CNS Penetration

Table 7 indicates the results of CNS genotyping and CNS penetration calculations. 6/7 patients on whom resistance gentoyping was conducted in the CSF had NRTI mutations, 5/7 patients had PI mutations, and 2/7 patients had NNRTI mutations. One patient had no mutations detected on CSF genotyping.

The original antiretroviral regimens for these patients had a median CPE score of 6.5 (range, 3-13). When adjusted for resistance, the median adjusted CPE score was 1 (range, 0-9). Regimens were revised in 9/10 subjects based on CSF findings. The revised regimens (see Table 7) had a median raw CPE score of 11 (range, 7-16) and median adjusted CPE score of 4 (range, 4-10).

Changes after Treatment Intervention

Eight out of nine patients demonstrated clinical improvement following neurologic evaluation and ART regimen optimization. One patient did not improve (patient 5168) and one patient died from septic shock secondary to presumed bowel ischemia before treatment was modified (patient 1034).

Follow-up CSF was available in 4/9 patients and demonstrated reduced CSF HIV RNA levels (from median 5775 copies/mL to median 66 copies/mL) at a median of 70

		Initial Regimen			New Regimen		
Site Patient	**Resistance Mutations Detected in CSF**	**Drugs in Regimen**	**Raw CPE**	**Adjusted CPE**	**Drugs in Regimen**	**Raw CPE**	**Adjusted CPE**
SF 7066	Not done	DDI SGC RTV	3	n/a	ABC NVP IDV/r	11	n/a
MI 9000	NRTI: K65R, K70R, V75I, F77L, F116Y, Q151M, R211K NNRTI: none PI: I54V, A71V, V77I, V82F, L90M	3TC* d4T** LPV/r****	7	0	TDF* NVP APV/r T-20	9	8
SF 1034	Not done	3TC ZDV LPV/r	9	n/a	Not done	n/a	n/a
SF 7071	NRTI: D67N, T69D, K70R, L74V, T215F, K219Q NNRTI: V108I, Y181C, G190A, F227L PI: L10I, K20I, M36I, M46I, I50V, Q58E, L63P, A71V, L90M	3TC ZDV**** EFV*** LPV/r***** T-20	13	2	3TC TDF ZDV**** LPV/r***** T-20	11	4
SF 4065	NRTI: L74V, M184V, Y115F NNRTI: Y181C, F227L PI: L63P, A71T, V77I, I85V	DDI* TDF ATV/r**	3	1	3TC* ABC*** ZDV LPV/r*	12	4
NH 2000	NRTI: M41L, E44D, D67N, V118I, M184V, L210W, T215Y NNRTI: none PI: I13V, K20R, M36I, I54V, L63P, V82A	3TC* ABC* LPV/r****	5	0	3TC* ABC* ZDV* NVP DRV/r*	16	4
GS 5168	NRTI: M41L, V75A, M184I NNRTI: none PI: M36I, L63P	3TC* TDF ATV/r*	6	1	FTC* TDF DRV/r	7	4
MI 8000	Not done	3TC ABC FPV/r	8	n/a	3TC ABC ZDV FPV/r	12	n/a
MI 7000	NRTI: none NNRTI: none PI: none	FTC TDF ATV	9	9	3TC ZDV DRV/r	9	9
NH 1000	NRTI: M184I NNRTI: none PI: none	FTC* TDF ATV/r RAL	9	6	FTC* TDF ZDV ATV/r RAL	13	10

Table 7. HIV drug regimens and resistance profiles in patients with CSF/plasma discordance
CPE = central nervous system penetration effectiveness; SF = San Francisco; MI = Milan, Italy; NH = New Haven; GS = Gothenburg, Sweden; * denotes number of resistance mutations to each drug in regimen; n/a= not applicable; drug abbreviations: DDI = didanosine, SGC = saquinavir, RTV = ritonavir, 3TC = lamivudine , d4T = stavudine, LPV = lopinavir, ZDV = zidovudine, EFV = efavirenz, T-20 = enfuvirtide, TDF = tenofovir, ATV = atazanavir, ABC = abacavir, FPV = fosamprenavir, FTC = emtricitabine, RAL = raltegravir, NVP = nevirapine, IDV = indinavir, APV = amprenavir, DRV = darunavir, /r = boosted with ritonavir. Used with permission [107].

days following change in drug regimen (range, 11-189 days). In 3/4 cases, discordance between CSF and plasma resolved at this follow-up point; in 1 case, discordance persisted at a lower level (patient 5168 with 340 copies/mL in the CSF); this patient's abnormalities did not improve after 189 days on the new regimen.

Figure 13e-h shows short-term follow-up imaging for patients 2000 and 7000. At 60 days, MRI for patient 7000 showed resolution of most focal lesions, but the development of a diffuse leukoencephalopathy despite resolution of symptoms. Similarly, patient 2000 had persistent diffuse white matter hyperintensities on MRI at 111 days, with subsequent significant decrease at 346 and 567 days follow-up.

Discussion

We report 10 cases of elevated CSF HIV RNA in the setting of plasma suppression in patients with well-controlled HIV infection, with long-term plasma control and $CD4^+$ T cell counts indicating preserved immune status at the time when neurologic symptoms developed. These cases demonstrate an unusual but clinically important phenomenon of CSF 'escape' associated with incident neurologic signs and symptoms in patients with chronic treated HIV infection.

Patient Characteristics and Clinical Presentation

The patients we report comprise a representative sample of those living with ART-treated HIV. This includes individuals with persistently suppressed plasma HIV RNA over many years (patient 2000), those who have been under control for a number of years (patients 1000, 7066, 7071, 9000, and 5168), those with a recent 'blip' (patient 4065), and those with an unclear history who are coming under control (patients 1034 and 7000). The

common clinical picture of neurologic abnormalities across this spectrum of patients suggests that CSF 'escape' is a relevant consideration in a variety of clinical contexts.

Patients experienced a variety of neurologic symptoms including cognitive, sensory, and motor impairment. Onset was most often subacute, impairment varied in severity, and abnormalities progressed over time. Overall, the neurologic symptoms reflect a level of debilitation that was significant and involved a range of functional domains. They reported a loss of function consistent with the significant impact that neurological symptoms are known to have on quality of life [32].

Immune Status

Despite relatively reconstituted immune status at the time of evaluation, all patients had $CD4^+$ T cell nadirs <250 cells/mm^3, with many below 100 cells/mm^3, consistent with a previous report of a median nadir $CD4^+$ count of 55 cells/mm^3 in similar patients [18]. A history of advanced immunosuppression may confer increased risk for prior local CNS infection and compartmentalization [175], which, despite peripheral $CD4^+$ improvement, fails to be entirely suppressed by ART. Clinically, the $CD4^+$ nadir might be an important consideration in the assessment of patients with new neurological abnormalities.

Imaging Results

MRI findings were consistent among patients and with those reported in previous cases [18,167,169]. Furthermore, they are similar but not identical to those classically identified in typical HIV-associated dementia in patients off of ART. White matter hyperintensities on T2-weighted and FLAIR imaging suggest a generalized inflammatory process consistent with diffuse encephalitis, and similar to findings reported in patients

failing antiretroviral therapy [176]. Comparison of MRI results at the time of presentation (Figure 13a-d) and short and long-term follow-up (Figure 13e-h, 13i-j, respectively) suggests that this process is associated with findings on imaging that may persist after the resolution of symptoms, and may take months to years to resolve completely. Still, the nature of these imaging findings remains incompletely understood.

Antiretroviral Regimens and Patient Adherence

One concern with previously reported cases of CSF 'escape' has been that some patients have been on atypical, incomplete, outdated, or "last-resort" salvage regimens [18]. All patients in our study were on appropriate multi-drug combination ART regimens before they developed symptoms, although some older regimens may be outdated by current standards. No patients in our study were on mono- or dual-therapy.

Preserved immune status and suppression of plasma viremia suggest adherence with ART, though the contribution of suboptimal adherence cannot be ruled out. Theoretically, partially reduced adherence may lead to insufficient drug concentrations in the CSF while maintaining satisfactory concentrations in plasma. CSF drug levels may therefore be an important consideration in this subset of patients, as has been suggested elsewhere [18]. CSF 'escape' may arise secondary to differences in susceptibility between HIV subpopulations in blood and CSF [177-180] due to the selection of resistant virus in the context of sub-therapeutic drug levels in the CNS compartment [181].

CNS Drug Penetration and Viral Resistance

While it has been argued that CNS drug penetration may be an important factor in the pathogenesis of CSF 'escape' [104], these cases indicate that viral resistance should also

be considered. Resistance to at least one drug in the regimen was common. The "adjusted" CPE score represents a first attempt to incorporate resistance into a numerical calculation of drug effectiveness, and conservatively assumes that a single mutation will confer complete resistance to a drug, though, in fact, the drugs may remain partially effective despite the mutations. While it is unclear to what extent clinical improvement resulted from treatment interventions in the cases included here, most patients improved when their regimens were adjusted with regard to both penetration and resistance. This suggests that regimen modifications should be based on more than penetration alone.

CNS Inflammation

Taken together, the range and quality of neurological dysfunction and the MRI findings in these patients have substantial overlap with typical findings in HIV-associated dementia. However, despite this overlap, these are not identical to those in HAD and we believe that the etiology of these findings is different than that of HAD in the absence of treatment. In accordance with previous reports [18,167,169], markedly elevated CSF total protein levels and WBC counts in our subjects compared to healthy HIV-uninfected controls and neuro-asymptomatic HIV-infected subjects on "successful" ART [30] indicate that a CNS inflammatory response is occurring in these patients. The pronounced inflammation and $CD8^+$ T cell infiltration noted on brain biopsy suggests that CSF 'escape' in the setting of an immune system reconstituted by systemically successful ART is associated with a degree of local inflammation distinct from typical HAD or HIV encephalitis. CSF neopterin, which is elevated in HAD and reduced by ART [174,182,183], was markedly increased in comparison to plasma neopterin and typical values of CSF neopterin in HIV-infected, ART-suppressed subjects. This provides

evidence that in CSF ‘escape,’ inflammation may be relatively compartmentalized in the CNS. The role of inflammation in this disorder may determine the distinct neurotropism for these lesions, as reflected in MRI and clinical symptoms.

Given the observation that symptomatic CSF ‘escape’ is accompanied by CNS inflammation, a moderately reconstituted immune system may play an important role in both eliciting a symptomatic inflammatory response and in providing a substrate for ongoing discordant HIV replication within the CNS. Since all of the subjects had preserved immune function and none had recently initiated ART, typical immune reconstitution inflammatory syndrome (IRIS) was not considered the primary cause of these abnormalities. Nevertheless, the combination of persistent CNS infection and relatively preserved immune response, including an HIV-specific response, may generate immunopathology in cases of CSF ‘escape.’ This is analogous to IRIS [184], but may differ in that it represents not the effects of immune reconstitution, but rather a “stable state” of antigen and immune response within the CNS.

Limitations

This analysis is limited by its retrospective approach, which utilized chart reviews and was constrained to studies previously performed during clinical evaluation and research protocols. It is unclear what the prevalence of CSF ‘escape’ may be in the general HIV-infected population, as patients with minor neurologic complaints are relatively unlikely to undergo detailed CNS evaluations. Our follow-up data are limited in many cases because further studies were not pursued once symptoms resolved.

IMPLICATIONS OF THE THESIS RESEARCH

Each of these projects adds to what is known regarding neurological manifestations of HIV in the era of combined antriretroviral therapy and contribute to the understanding of HAND. The first study answers important questions about the implications of the very first stages of HIV infection in the central nervous system and reveals that neuronal injury occurs earlier than previously thought. This provides a foundation for the development of further evidence that injury occurring before antiretroviral therapy is initiated contributes to the etiology of HAND. The second study contributes to the understanding of the activity of HIV within the central nervous system compartment and shows how its very presence can have a profound clinical significance for individuals who are otherwise living with HIV as a chronic disease. A better understanding of the phenomenon of CSF 'escape' will help clinicians to more specifically tailor therapy toward the CNS and will help scientists to understand how the independent evolution of virus within the CNS could contribute to the development of HAND.

Implications for Primary HIV Infection

Our finding of neuronal injury during primary HIV infection has important implications for the understanding of HIV pathogenesis and management. Previously, neuronal injury was thought to be a product of prolonged infection. The presence of elevated CSF NFL compared to the upper limit of normal for age in 44% of our subjects adds to a growing body of evidence suggesting that neurological injury is present beginning in primary HIV infection.

It is particularly notable that the elevations in neurofilament light chain and amyloid-beta 42 appear to occur through different pathways, with the former correlating

with multiple CSF and metabolic markers of neuroinflammation and the latter related to some other process, such as disruption of the blood-brain barrier or decreased breakdown secondary to viral inhibition. This suggests that we may need to invoke more than just infection or inflammation alone in characterizing the CNS perturbations present during primary HIV infection.

It has long been known that primary HIV infection may be complicated by central and peripheral nervous system involvement. In this study, evidence of neuronal injury was found in patients even without clinical evidence of neurological symptoms. Furthermore, evidence of neuronal injury did not correlate with clinical signs of abnormalities in neuropsychological testing. Further study is required to determine whether this injury is sub-clinical, if the lack of correlation is due to the relative insensitivity of this type of testing, whether the injury resolves with or without treatment, and if it has long-term neurological implications.

Although treatment with cART is able to suppress viral levels in both the plasma and CSF, a proportion of patients have ongoing brain atrophy and neurological impairment for unclear reasons [185]. One possible explanation for this phenomenon is that neurological injury begins accruing early in the disease course, including during primary HIV infection. Thus, while initiation of cART typically occurs once an immunological threshold is crossed after several years of infection, CNS injury might begin soon after seroconversion, in the setting of early neuroinvasion and immune activation. If this were the case, it would provide additional evidence for early pharmacological intervention in HIV infection aimed at mitigating CNS injury.

Implications for Central Nervous System Compartmentalization

Our description of CSF 'escape' in the patients we describe here contributes to the literature on this rare but clinically significant process. It is crucial that all physicians who care for patients with HIV, not just neurologists, be aware of this unusual manifestation of HIV disease. These cases reflect that new neurological symptoms in the context of standard cART regimens and well-controlled plasma HIV infection should not be dismissed and instead warrant an evaluation of the CSF to determine whether viral replication is occurring and, if so, whether the virus in the CSF compartment possesses resistance to the regimen being used to control the virus in the plasma compartment.

While the investigation of CSF HIV is standard practice in the guidelines for HIV management in many European countries, this is not the case in the United States. CSF HIV viral load and genotyping are difficult tests to order at many U.S. medical centers, and are generally not a standard offering by most hospital laboratories. This descriptive analysis of 10 cases of CSF HIV 'escape' demonstrates that CSF HIV analysis can be an important diagnostic tool and should be available to clinicians for the purpose of measuring HIV RNA concentration and identifying resistance, especially in patients who develop new neurological symptoms that cannot otherwise be explained. These cases underscore the need for further investigation into the mechanism and consequences of HIV replication and persistence in the CNS.

General Implications for HIV-Associated Neurocognitive Disorder

Both of these studies reflect what is becoming an increasingly accepted fact about the pathogenic mechanisms of HIV infection – that the body's reaction to HIV can be as damaging as the activity of the virus itself. Although the two processes described here,

primary HIV infection and CSF HIV 'escape,' take place at distinct time points in the course of the disease, both appear to be associated with, and possibly caused by, mechanisms of immune activation and inflammation within the central nervous system. The inflammatory milieu that is induced by the activity of HIV in invading cells and triggering an immune response has important implications throughout the time course of infection, and may be particularly important for our understanding of HAND.

In the earliest stages of primary infection, the virus crosses the blood-brain barrier for the first time and initiates a cascade of cytokines that contribute to breakdown of that barrier, as well as CSF pleocytosis, macrophage and lymphocyte activation, interference with neuronal synthesis and maintenance pathways, and ultimately neuronal injury that can be detected by biomarker and neuroimaging perturbations. Whether these patients become symptomatic at the time of these perturbations or later in the disease course remains to be seen. And while control of the virus through the initiation of antiretroviral therapy can decrease the viral load and quiet the immune response, the CNS compartment remains particularly vulnerable to further insult. In some patients, this means the development of some degree of mild HAND, or worse, HIV-associated dementia late in the course of unsuccessfully or sub-optimally treated infection. In others, the initiation of therapy might lead to a profound immune reconstitution that precipitates the perivascular inflammation and leukoencephaloathy consistent with IRIS [186-188]. And in others still, minimal non-adherence, reduced drug activity, or some yet-unknown factor may result in the development of a low-level CSF viral proliferation in the setting of suppressed or well-controlled plasma virus. This can initiate an immune cascade that, although meant to respond to the replicating virus, may ultimately end up feeding its proliferation by providing a substrate to infect.

FUTURE DIRECTIONS

While we have identified cross-sectional changes in biomarkers indicating neuronal damage during primary HIV infection, it would be useful to characterize the onset of abnormal CSF neurofilament light chain and its longitudinal changes over the time course of primary infection and early HIV disease. We have access to a cohort of HIV-infected individuals from Thailand from whom we have collected CSF at the time of seroconversion. Analysis of these samples will provide insight into the timing of neuronal injury and help to determine whether this is present before the median 3.1 month time point in the current study. It will also be instructive to study subjects with neuro-asymptomatic advanced (CD4 <200) and non-advanced (CD4 >200) chronic HIV infection to determine whether the latter group continues to show evidence of neuronal damage later in the course of infection. These studies will fill an important gap in the understanding of the pathophysiological consequences of HIV infection between primary HIV infection and late-stage AIDS. Whether biomarker abnormalities during primary infection predict later HAND and HAD is unknown. The subjects in the primary infection study were all antiretroviral naïve at baseline, but many began treatment at subsequent visits. The analysis of longitudinal trends in NFL in untreated subjects and the comparison of NFL trends during periods with and without therapy in subjects who initiate cART will indicate whether neurological damage continues throughout the course of infection and whether initiation of therapy can attenuate this damage. Data using proton-MRS metabolites suggest that worsening neuronal injury may be mitigated by the initiation of therapy [129].

With regard to CSF ‘escape,’ a greater understanding of this condition will come from the publication of more detailed cases of the disease process and its clinical

manifestations. In addition, more in-depth neuroimaging and tissue analysis will help to distinguish the pathophysiological mechanisms of this condition from those seen in IRIS. Finally, a better assessment of medication adherence in these patients will help to determine whether this is a determining factor in the pathogenesis of the disease, as will further research into issues related to HIV compartmentalization in the CNS.

Progress in both of these areas of research will help to better describe and understand the pathophysiological mechanisms responsible for HAND.

CONCLUSIONS

Thirty years after the identification of the very first cases of HIV and AIDS, the scientific community has made great strides toward understanding the pathogenesis, clinical manifestations, and public health implications of the disease. But three decades of scientific research have generated as many questions as they have answered, and millions of individuals are still acquiring the virus each year. The central nervous system is becoming increasingly recognized as a crucial frontier in the battle against HIV disease, both because of the dramatic neurological sequelae of HIV infection and the difficulty in accessing and controlling the virus within this compartment. The content of this thesis makes a modest contribution to the growing understanding of the impact of both acute and chronic HIV infection on the central nervous system, and underscores that a great deal of work is yet to be done in this area of investigation. As HIV/AIDS continues its shift toward becoming a chronically managed disease, it is the responsibility of physicians and scientists to more fully understand how infection with this virus affects a patient from the moment of transmission, through years of chronic infection, until the moment of death or, hopefully within this generation, cure.

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AUTHOR CONTRIBUTIONS

BIOLOGICAL AND CLINICAL MARKERS OF NEURONAL INJURY IN PRIMARY AND CHRONIC HIV-1 INFECTION. Michael J. Peluso, Dieter Meyerhoff, Julia Peterson, Evelyn Lee, Andrew Young, Francesca Ferretti, Antonio Boschini, Rudy Walter, Nancy Angoff, Kevin Robertson, Dietmar Fuchs, Bruce Brew, Paola Cinque, Lars Hagberg, Henrik Zetterberg, Magnus Gisslén, Richard Price, and Serena Spudich, Department of Neurology, Yale University School of Medicine, New Haven, CT.

The work included in this thesis is the product of an international collaboration of clinician-scientists and study organizers. The purpose of this section is to ensure the proper attribution of each component of the work by section.

Introduction

The Introduction was written in its entirety by MJP and reviewed by SS Portions of the Introduction will be edited for inclusion as a chapter on "AIDS and Other Retroviral Infections of the Nervous System" in the Fifth Edition of *Aminoff's Neurology and General Medicine*, to be published in 2013.

Chapter 1: Cerebrospinal fluid and imaging biomarkers of neuronal injury in antiretroviral naïve patients during primary HIV infection

MJP was responsible for the coordination of the transfer and acquisition of study CSF biomarker, neuropsychological, and proton-MRS neuroimaging data, compilation and organization of the original raw datasets and maintenance of the study database, designing and developing the research questions and research hypotheses, performing all statistical analyses and interpreting the results, articulating the implications of the research, leading the other authors in discussion of the interpretation and impact of the work, and presenting the work at international meetings. The CSF biomarker assays (i.e.

ELISAs) were conducted off-site in the laboratories of our collaborators using assays that are not commercially available in the United States. SS designed and implemented the original research study and provided weekly mentorship in all of the above areas of the project. All authors contributed in the interpretation of data led by MJP.

Additional individual author contributions were as follows: DJM contributed in design, implementation, acquisition, and processing of proton-MRS studies; RWP contributed in study design, implementation, and data collection; JP contributed in study coordination, data collection, and analysis of neuropsychological data; EL contributed in study coordination and data collection; AY organized the proton-MRS data; RW acquired and processed MRS data; DF contributed in laboratory analysis of CSF inflammatory markers; BB and PC contributed in subject recruitment; KR contributed in designing and scoring the neuropsychological studies; LH and MG contributed in subject recruitment and laboratory analysis of CSF biomarkers; HZ contributed in laboratory analysis of CSF biomarkers.

Chapter 2: Cerebrospinal fluid HIV “escape” associated with progressive neurological injury in patients on antiretroviral therapy with well-controlled plasma viral load

MJP was responsible for the study design, the development of the study protocol, the coordination of transfer of data from external sites, the abstraction of all relevant patient data from original medical records and raw data provided by the Yale University, University of California-San Francisco, San Raffaele Scientific Institute, and University of Gothenburg sites, the compilation and organization of study data and maintenance of the database, performing all statistical analyses and interpreting the results, articulating

the implications of the research, leading the other authors in discussion of the interpretation and impact of the work, and presenting the work at international meetings. Patients were initially identified by their respective clinicians (NA, SS, RWP, AB, PC, and MG) for inclusion in the study; afterwards, their records were obtained by MJP. SS proposed the idea for the study after receiving a patient referral from NA and provided weekly mentorship in all of the above areas of the project. All authors contributed in the interpretation of data led by MJP.

Additional individual author contributions were as follows: FF assisted with the acquisition of medical record data on patients 7000, 8000, and 9000; JP and EL assisted with the acquisition of medical record data on patients 7066, 1034, 7071, and 4065; DF provided laboratory support for the conduction of neopterin analyses; MG was involved in study conception and provided data on patient 5165; NA was involved with study conception and the review of interim data; RWP was involved with study conception and data collection on the San Francisco patients in the context of other research studies; PC participated in study conception.

APPENDICES

Plasma/CSF Viral Discordance Case Series
Patient Information Checklist

Cases: Generally well-controlled HIV+ patients on HAART presenting with neurological symptoms in the setting of previous viral suppression who are determined to have detectable CSF viral load on lumbar puncture.

General Information
❑ Year of HIV diagnosis
❑ Nadir CD4 (if known)
❑ Known co-infections or opportunistic infections
❑ Note containing H&P for neurological symptoms
❑ HIV regimen at time of visit for neurological symptoms
❑ # of months on this regimen
❑ list of previous HIV regimens, with dates (if available)
❑ # of months of VL suppression (either < detection threshold or undetectable)
❑ Complete longitudinal information on plasma VL and CD4 count from diagnosis until present (usually available as a flowsheet in medical records)

Lumbar Puncture Information
❑ Date of LP
❑ Plasma CD4 at time of LP
❑ Plasma VL at time of LP
❑ CSF chemistries and cytology (including protein, glucose, RBC, WBC, nucleated cells, %lymphs, %monos)
❑ CSF VL
❑ CSF HIV genotype (if done)

Additional Information
❑ Brain imaging results
❑ EEG results
❑ New HIV regimen
❑ Follow-up note indicating improvement

Primary HIV-1 infection is characterized by elevation of cerebrospinal fluid biomarkers indicating early neuronal damage

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Background

- Neurologic impairment is detected in up to 50% of patients with HIV, even in the setting of antiretroviral therapy[1]
- Cerebrospinal fluid (CSF) biomarkers of neuronal injury, such as neurofilament light chain (NFL), are elevated in subjects with advanced HIV-infection and HIV-associated dementia[2]
- We hypothesized that evidence of neuronal injury might be detected in subjects recruited during primary HIV infection (PHI), within the first year after HIV transmission

Methods

- In antiretroviral naïve subjects with PHI, CSF NFL was analyzed using a new, highly-sensitive, two-site enzymatic quantitative immunoassay with a lower limit of detection of 50 ng/L
- Detection of t-tau, β-amyloid, and soluble amyloid precursor protein-alpha and -beta (sAPP-α; sAPP-β) used standard ELISAs
- To investigate mechanisms, we examined levels of biomarkers with respect to other laboratory parameters of CNS HIV infection
- Analyses employed Mann-Whitney test, Spearman correlations

NFL	Structural component of myelinated axons
T-tau	Associated with microtubules in cortical axons
β-Amyloid	Abnormal amyloid protein in Alzheimer's disease
sAPP-α,β	Amyloid synthesis and processing
Neopterin	Marker of macrophage activation
IP-10	Cytokine recruiting lymphocytes
MCP-1	Cytokine recruiting monocytes

Results

	PHI n=84	HIV- n = 25	p-value
% Male	95.2%	80%	0.03
Age	36 (18-61)	43 (26-66)	0.002
Days Infected	96.5 (15-376)	-	-
CD4 Count	546 (111-1608)	836 (488-1627)	< 0.001
CD8 Count	985 (161-9063)	550 (157-1031)	< 0.001
Log Plasma VL	4.6 (1.7-7.1)	-	-
Log CSF VL	2.9 (1.3-6.1)	-	-
CSF Protein	41 (21-343)	47 (21-65)	0.416
CSF WBC	6 (0-86)	1 (0-6)	< 0.001
% Neuro Sx	10.7%	-	-

Table 1. Descriptive and demographic characteristics of PHI subjects and HIV-uninfected controls. Data reported as percentages or median (range). VL= viral load.

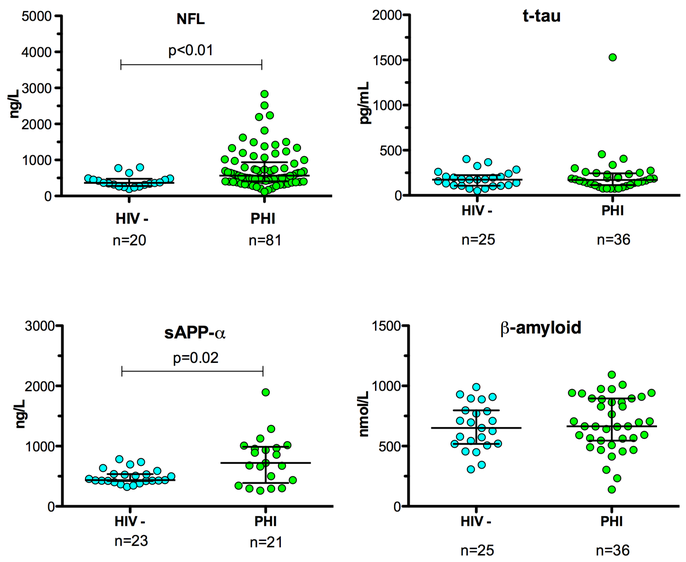

Figure 1. CSF biomarker comparisons between PHI subjects and HIV-uninfected controls. Lines represent median; range. HIV- = HIV uninfected; PHI = primary HIV infection; NFL = neurofilament light chain; sAPP = soluble amyloid precursor protein.

Variable	Spearman r	p-value
Log Plasma VL	0.23	0.040
Log CSF VL	0.23	0.040
Days of Infection	-0.01	0.903
NPZ-4	0.18	0.154
CSF WBC	0.33	0.003
CSF Protein	0.61	<0.0001
CSF:plasma albumin	0.59	<0.0001
T-tau	0.51	0.004
P-tau	0.42	0.058
Amyloid-β 42	0.51	0.018
sAPP-a	0.28	0.232
sAPP-β	0.07	0.758
Blood Neopterin	0.23	0.053
CSF Neopterin	0.40	0.002
IP-10	0.42	0.001
MCP-1	0.16	0.235

Table 2. Correlates of NFL in PHI subjects. VL = viral load; NPZ-4 = composite neuropsychologic Z-score; WBC = white blood cells; sAPP = soluble amyloid precursor protein.

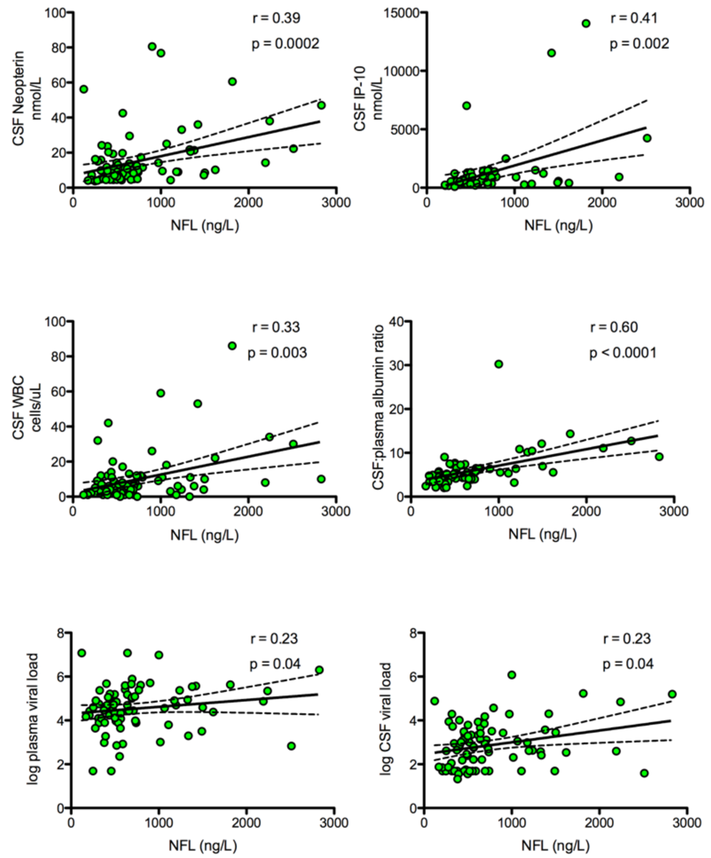

Figure 2. Correlates of NFL in PHI subjects. Bold line indicates linear approximation of relationship; dotted lines represent 95% confidence intervals.

Conclusions

- Biomarkers of neuronal damage are elevated in subjects with PHI compared to HIV-uninfected controls
- NFL, a sensitive marker of neuronal injury, correlates with markers of CSF inflammation during PHI
- These findings suggest that HIV-related neuronal damage starts during early HIV-infection and is mediated by neuroimmune activation during this period

Acknowledgments & References

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Poster
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Discordance between Plasma and Cerebrospinal Fluid HIV in Virologically Controlled Patients Presenting with Neurologic Symptoms

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Introduction

- Elevated HIV RNA levels in the cerebrospinal fluid (CSF) can be associated with HIV encephalitis (HIVE) and HIV-associated dementia (HAD)
- Antiretroviral therapy (ART) suppresses plasma and CSF HIV RNA and improves neurologic outcomes in patients with HIV
- A subset of patients may develop neurologic symptoms in the setting of long-term plasma viral control[1]

Methods

Study Design Retrospective case series of virologically controlled HIV-infected patients on ART with incident neurologic abnormalities, determined to have CSF 'escape,' defined as detectable CSF HIV RNA in the setting of suppressed plasma levels or CSF HIV RNA >1 log higher than plasma RNA[1]

Measurements Clinical signs and symptoms, historical virologic parameters, magnetic resonance imaging (MRI), CSF parameters, viral resistance and drug penetration in the CNS, and treatment intervention

Results

Table 1. Demographics and HIV history of patients with CSF 'escape'

	Historical Data			Time of Presentation			
Site, ID	Age/ Sex (years)	Nadir CD4+ T cell count (cells/mm^3)	Stable plasma HIV† (copies/mL: months)	CD4+ T cell count (cells/mm^3)	Plasma HIV RNA (copies/mL)	Drug regimen	Neurologic signs/symptoms
SF 7066	45/M	55	<50: n/a <500: 23	318	380	DDI SGC RTV	Cognitive impairment Gait ataxia
MI 9000	46/F	15	<50: 28 <500: 28	305	372	3TC d4T LPV/r	Coma Tremor Vertigo
SF 1034	51/M	80	<50: 2 <500: 2	588	<50	3TC ZDV LPV/r	Cognitive impairment Gait ataxia Tremor
SF 7071	49/M	8	<50: 30 <500: 30	444	<50	3TC ZDV EFV LPV/r T-20	Cognitive impairment Gait ataxia Sensory impairment
SF 4065	49/M	4	<50: 2 <500: 7	520	184	DDI TDF ATV/r	Diplopia Dysarthria Dysphagia Gait ataxia
NH 2000	55/M	60	<50: 96 <500: 96	308	<50	3TC ABC LPV/r	Aphasia Gait ataxia Sensory impairment Tremor
GS 5168	45/F	55	<50: 47 <500: 60	660	118	3TC TDF ATV/r	Cognitive impairment
MI 8000	45/M	222	<50: 27 <500: 27	545	<50	3TC ABC FPV/r	Cognitive impairment Dysarthria Sensory impairment Vertigo
MI 7000	26/M	9	<50: 4 <500: 12	290	98	FTC TDF ATV	Diplopia Dysarthria Gait ataxia Tremor
NH 1000	49/M	180	<50: 12 <500: 43	627	<50	FTC TDF ATV/r RAL	Aphasia Cognitive impairment Gait ataxia Tremor Vertigo

SF = San Francisco; MI = Milan, Italy; NH = New Haven; GS = Gothenburg, Sweden; n/a = not applicable; † = length of time <500 copies/mL includes times when patient was <50 copies/mL

Table 2. Neurologic studies in patients with CSF 'escape'

	Plasma HIV RNA (copies/mL)	Cerebrospinal Fluid Analysis			
Site, ID		HIV RNA (copies/mL)	Protein (mg/dL)	WBC (cells/mm^3)	MRI Findings
SF 7066	380	9056	162	50	Not done
MI 9000	372	8000	170	0	Diffuse white matter abnormalities
SF 1034	<50	378	89	6	Patchy periventricular white matter abnormalities
SF 7071	<50	8320	60	33	Not done
SF 4065	184	4570	74	14	Patchy subcortical/ periventricular white matter abnormalities with involvement of corpus collosum and cerebellum
NH 2000	<50	613	77	28	Symmetric subcortical/ periventricular white matter abnormalities extending into cerebellum
GS 5168	118	3230	n/a	9	Slight deformity of frontal ventricular horns, stable compared to previous examinations. Otherwise normal.
MI 8000	<50	134	121	15	Diffuse white matter abnormalities
MI 7000	98	5200	137	200	Periventricular/lenticular/posterior internal capsule/ cingular cortex white matter abnormalities extending into cerebellum; diffuse pial contrast enhancement
NH 1000	<50	460	46	11	Cortical/subcortical/periventricular white matter abnormalities

WBC = white blood cells; n/a = not available

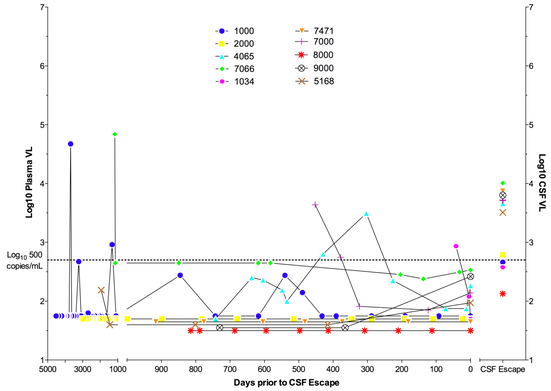

Figure 1. Longitudinal plasma HIV RNA levels prior to CSF 'escape.' HIV RNA calculated in days prior to CSF 'escape' (time "0"). Corresponding CSF HIV RNA levels are indicated on the right axis of the graph ("CSF Escape").

Summary of Key Findings:
Virologic History and Time of Presentation
Data reported as medians

• Duration HIV Infection	16.2 years	• CSF HIV	3900 copies/mL
• Time < 500 copies/mL	27.5 months	• Plasma HIV	62 copies/mL
• Time < 50 copies/mL	19.5 months	• CD4+ T cells	482 cells/uL
• Duration on Regimen	21 months	• CD4+ Nadir	35 cells/uL

Table 3. Drug regimens and resistance profiles in patients with CSF 'escape'

Site, ID	Initial Regimen	Raw CPE	Resistance Mutations Detected in CSF	Initial Regimen Adjusted CPE	New Regimen	New Regimen Adjusted CPE
SF 7066	DDI SGC RTV	3	Not done	n/a	ABC NVP IDV/r	11†
MI 9000	3TC* d4T** LPV/r****	7	NRTI: K65R, K70R, V75I, F77L, F116Y, Q151M, R211K NNRTI: none PI: I54V, A71V, V77I, V82F, L90M	0	TDF* NVP APV/r T-20	8
SF 1034	3TC ZDV LPV/r	9	Not done	n/a	Not done	n/a
SF 7071	3TC ZDV**** EFV*** LPV/r****** T-20	13	NRTI: D67N, T69D, K70R, L74V, T215F, K219Q NNRTI: V108I, Y181C, G190A, F227L PI: L10I, K20I, M36I, M46I, I50V, Q58E, L63P, A71V, L90M	2	3TC TDF ZDV**** LPV/r****** T-20	4
SF 4065	DDI* TDF ATV/r**	3	NRTI: L74V, M184V, Y115F NNRTI: Y181C, F227L PI: L63P, A71T, V77I, I85V	1	3TC* ABC*** ZDV LPV/r*	4
NH 2000	3TC* ABC* LPV/r****	5	NRTI: M41L, E44D, D67N, V118I, M184V, L210W, T215Y NNRTI: none PI: I13V, K20R, M36I, I54V, L63P, V82A	0	3TC* ABC* ZDV* NVP DRV/r*	4
GS 5168	3TC* TDF ATV/r*	6	NRTI: M41L, V75A, M184I NNRTI: none PI: M36I, L63P	1	FTC* TDF DRV/r	4
MI 8000	3TC ABC FPV/r	8	Not done	n/a	3TC ABC ZDV FPV/r	11†
MI 7000	FTC TDF ATV	9	NRTI: none NNRTI: none PI: none	9	3TC ZDV DRV/r	9
NH 1000	FTC* TDF ATV/r RAL	9	NRTI: M184I NNRTI: none PI: none	6	FTC* TDF ZDV ATV/r RAL	10

Raw CPE = central nervous system penetration effectiveness score[2]; "adjusted" CPE calculated by assigning drugs with known resistance mutation as "0" and re-calculating CPE; * denotes number of resistance mutations to each drug in regimen according to International Antiviral Society-USA Guidelines; n/a= not available; † = raw score, adjusted score not available because genotyping not done

Additional Findings

- Plasma neopterin normal or slightly elevated in 2 patients
  - 12 nmol/L, 8 nmol/L (reference 8.8 nmol/L in HIV-uninfected subjects)
- CSF neopterin elevated in 2 patients
  - 76.3 nmol/L, 37.6 nmol/L (reference 10.8 nmol/L in ART-treated subjects)
- Brain biopsies in 2 patients
  - dense, perivascular lymphocytic infiltrates in white matter with extension into surrounding parenchyma.
  - mixture of mature and immature B- and T-lymphocytes, CD8+ predominance

Changes after Treatment Intervention

- 8/9 patients demonstrated clinical improvement
- Follow-up CSF available in 4 patients; 3/4 had discordance resolve
  - Median pre-intervention CSF HIV 5775 copies/mL at time of CSF "escape"
  - Median post- intervention CSF HIV 66 copies/mL at median 70 days on new regimen

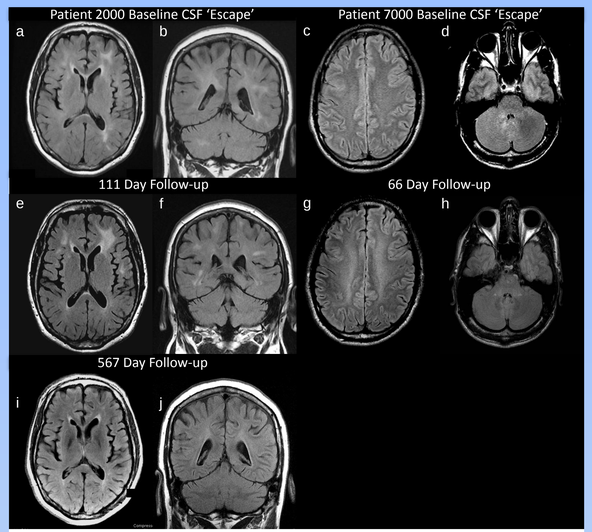

Figure 2a-j. MRI images. Initial MRI shows diffuse T2-prolongation (a,b) and focal lesions (c,d). Follow-up shows diffuse leukoencephalopathy despite symptom resolution (e-h) in the short-term and significant improvement at 567 days (i,j).

Summary & Conclusions

- New neurologic symptoms in the context of well-controlled plasma HIV warrant an evaluation of the CSF to determine whether viral replication is occurring
- CSF HIV analysis can be an important diagnostic tool and should be available to clinicians for the purpose of measuring HIV RNA and identifying resistance
- A moderately reconstituted immune system may play an important role in both eliciting a symptomatic inflammatory response and in providing a substrate for ongoing discordant HIV replication within the CNS
- Further investigation is needed into the mechanism and consequences of HIV replication and persistence in the CNS

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