Assessing the Effect of Time From Infection and Systemic Inflammation on HIV-Associated Neurocognitive Disorder

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Abstract

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Despite advances in HIV treatment, HIV-Associated Neurocognitive Disorder (HAND), a condition marked by neurocognitive slowing in HIV patients, has persisted, even in those patients with well-controlled disease. There is some thought that this process is due to chronic inflammation in the plasma and central nervous system (CNS) that accompanies HIV infection. Our study explored the relationship between baseline levels of inflammation during the first 100 days of HIV infection, time from initial infection, and performance on a series of neurocognitive performance tests (NPT) among men who have sex with men (MSM) and transgender women (TW) in Lima, Peru. We measured levels of 33 different inflammatory biomarkers in the plasma and CSF of participants in the SABES cohort of newly HIV+ MSM and TW, and used single-regression analysis to assess the relationship between individual biomarkers and neurocognitive performance NPT over 96 weeks. After adjustment for multiple comparisons, we found significant negative associations between levels of Plasma YKL40, Plasma IL-16, Plasma IL-6, CSF TNF-β, CSF IL-16, CSF TNF- α, and CSF IP-10, and NPT score at multiple timepoints.

Introduction:

In recent years, great strides have been made in the treatment of persons living with HIV (PLWH) through the introduction of combination antiretroviral therapy (cART). Despite dramatic reductions in morbidity and mortality, this population remains at elevated risk for a variety of chronic medical problems, including serious neurological conditions.[1] While introduction of cART has significantly reduced the incidence of HIV Encephalopathy and AIDS Dementia Complex, other forms of neurocognitive disability that limit medication adherence and increase mortality have persisted, despite viral suppression and ready availability of cART.[2,3]

The most common of these neurologic complications is HIV-Associated Neurocognitive Disorder (HAND), an entity with three subgroups: Asymptomatic Neurocognitive Impairment (ANI), Mild Neurocognitive Disorder (MND), and HIV-Associated Dementia (HAD), conditions diagnosable by neurocognitive performance testing (NPT).[1,4] While the pathogenesis of HAND remains incompletely understood, its presence during systemic viral suppression has cast doubt on the theory that HAND is purely due to viral replication in the CNS.[5] A new wave of research into the role of persistent inflammation, present even in well-controlled disease, suggests that HIV infection may lead to ongoing immune system activation, contributing to HAND through the actions of certain inflammatory cytokines and chemokines.[6,7]

The central role of monocytes in the pathogenesis of early HIV infection has made inflammatory biomarkers associated with monocyte activation a key focus of study.[8] CSF and serum immune markers associated with neurological dysfunction and linked to monocyte activation include Interleukin – 16 (IL-16)[9], Soluble CD-14 (sCD14);[10] Monocyte Chemoattractant Protein 1 (MCP-1);[11,12] Interferon-Gamma-Induced Protein 10 (IP-10/CXCL10),[10] Neopterin, and Soluble CD-163 (sCD163), among others.[7] While CNS inflammation appears central to the development of HAND, the dynamics of CNS immune activation and its neurocognitive consequences in early HIV infection require further exploration.

Rigorous study of the immune response in relation to estimated time from infection (ETI) is required to better characterize these inflammatory processes, and their potential effects on patients' cognition. In any analysis of cognitive response to inflammation, it is essential to consider the element of ETI due to the fluctuation of viral load and biomarkers in early HIV infection. Recent studies have suggested that early initiation of cART can reduce levels of inflammatory biomarkers in the CNS, further demonstrating that time between infection and cART initiation is an important consideration.[12]

No biomarker has emerged as clearly predictive of poor or improved neurocognitive outcomes, suggesting an urgent need for greater understanding of the clinical implications of CNS inflammation. For these reasons, this nested prospective cohort study uses the estimated date of detectable infection (EDDI) calculated using HIV testing history and test result,[13] laboratory analyses of key CNS and plasma biomarkers, and longitudinal NPT data to draw connections between ETI, inflammation as detected in plasma and CSF, and neurocognitive outcomes in PLWH. If key biomarkers could predict the development, progression, or reversal of HAND, this would greatly expand knowledge about the disease process itself, raising the possibility of anti-inflammatory treatments for HAND.

Specific Aims:

1. Describe the time-dependent inflammatory response to HIV infection in the plasma and CSF among ART-naïve patients with recently acquired HIV infection.
Hypothesis: During early HIV infection, viral replication is associated with an initial strong inflammatory response that recedes over time.

1a. Determine the relationship between time from HIV infection and viral load in the plasma and CNS.
Hypothesis: Plasma and CSF viral loads decrease linearly from the initial peak in the weeks following ETI, defined as the time from calculated EDDI to date of enrollment in participants with acute or very recent HIV infection.

1b. Determine whether ETI, as reflected by time from calculated EDDI to enrollment, has a linear relationship with peripheral or CNS inflammation, as reflected by concentrations of a variety of inflammatory biomarkers in CSF and Plasma.
Hypothesis: Following an initial peak, inflammation will decrease linearly in the months following HIV infection among ART-naïve patients.

2. Determine whether neuroinflammation during early HIV infection is correlated with lower current and future neurocognitive performance.

2a. Determine whether baseline levels of inflammatory biomarkers in the CNS relate to initial NPT scores among ART-naïve HIV patients.
Hypothesis: High levels of CNS inflammatory biomarkers are associated with poor baseline NPT score.

2b. Determine whether baseline levels of inflammatory biomarkers in the plasma and CNS at the time of cART initiation predict NPT score change over 96 weeks.
Hypothesis: Higher levels of key inflammatory biomarkers at the time of cART initiation are predictive of less improvement in NPT score over time.

Methods:
Study design: Nested Prospective Longitudinal Cohort Study
Project period: Participants were recruited and data collected between 2015 and 2017; results were analyzed in 2019.
Study population: Participants were recruited from the SABES Study in Lima, Peru. In SABES, HIV-negative men who have sex with men (MSM) and transgender women (TW) at high risk for HIV infection were monitored monthly by antibody and viral RNA testing for new HIV infection. Participants diagnosed during acute or recent HIV infection (see below) were eligible for a follow-on study of ART initiation. Consenting HIV-infected participants were randomized either to receive cART immediately, or to defer cART for 24 weeks, provided the participant did not meet the CD4 threshold for ART initiation at the time per local guidelines (initially CD4+ ≤350 then CD4+ ≤500). Two-hundred and sixteen participants met inclusion criteria and entered

the treatment phase of the trial. These patients were then followed for 48 weeks, and a variety of outcomes recorded before study participants were referred to the Peruvian Ministry of Health (MoH) for ongoing free HIV care. The SABES trial is described in greater depth elsewhere.[14] A subset of 87 SABES participants enrolled in a neurological sub-study. Blood samples were collected by venipuncture, CSF samples were collected by lumbar puncture at enrollment, and NPT evaluations were conducted at regular intervals. After 48 weeks, a companion study was conducted among participants being followed in the MOH ART program which collected NPT data and biologic specimens through 96 weeks.

Study Population:
Participants were eligible for SABES if they have been assigned male gender at birth, had at least one male sex partner in the past year, and were 18 years of age or older. In addition, they must have been unaware of their HIV status and have been at high risk for acquiring HIV, defined as: being a partner of a person with newly diagnosed HIV infection; seeking HIV testing because they had symptoms of acute retroviral syndrome; or reporting high-risk sexual behavior. HIV-infected SABES study participants, all of whom were diagnosed and enrolled during either acute or recent HIV infection, were eligible for the neurological sub-study. Acute infection was defined as positive plasma HIV RNA test in a person with a negative third-generation HIV antibody test. Recent infection was defined as a positive third-generation rapid HIV test that was confirmed by a separate enzyme immunoassay with a documented negative third-generation HIV-antibody or HIV-RNA test in the previous 90 days.

Exclusion criteria: Participants were excluded from the SABES study if they had received any version of an HIV vaccine; were receiving medications that were cytotoxic, nephrotoxic, or immunosuppressive; had any adverse reaction to the cART formulations used in the study, or had alcohol or substance dependency issues that would complicate their adherence to the medication regimen. For the neurological sub-study, prospective participants were also ruled ineligible if they had past head injury or neurological disease prior to HIV infection.

Biomarkers: Upon enrollment in the substudy, samples of participant serum and CSF were collected. These baseline samples were then analyzed for the presence and levels of a set of 33

biomarkers associated with immune activation and neuronal damage. Only baseline cytokine measurements were considered, providing a single measure of immune activation and neuronal damage at the time of study enrollment. Samples of plasma and CSF were analyzed for levels of IL-1a, IL-1β, IL-2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-16, IL-23p40, IP-10, TNF-α, MIP-1α, MIP-1β, IFN-γ, IFN- α2a, TNF-β, SDF-1α, and MCP-1 using Meso Scale Discovery Uplex (Meso Scale Diagnostics, Rockville, MD), a multiplex plate-based chemiluminescence assay. Plasma and CSF samples were analyzed for levels of Neopterin, Neurofilament Light Chain, soluble CD-163, S100-B, and soluble CD14 (in plasma only) by ELISA. Plasma and CSF samples were analyzed for levels of VCAM-1, Amyloid B, Osteopontin, MMP-2, MMP-9, TIMP-1, YKL-40, and Tau Proteins using a Luminex fluorescent bead-based immunoassay. CSF Samples were additionally analyzed for viral load, red blood cell (RBC), and leukocyte count, total protein, and CD4+ T cells (cells/mm^3) and CD8+T Cells (cells/mm^3). Plasma samples were additionally analyzed for levels of High-Sensitivity C Reactive Protein (hsCRP), soluble CD-14, and D-dimer.

NP Testing: HAND was diagnosed by a battery of neurocognitive tests accompanied by clinical evaluation, in accordance with the current clinically validated consensus definition.[15,16] Participants underwent NPT and neurological evaluation by local clinicians and psychologists, testing cognitive domains of psychomotor speed, gross and fine motor speed, executive skills, attention, memory, learning, and language fluency, using specific tests selected from ACTG 5199, the "International Neurological Study." The tests administered were previously validated for use in Spanish.[17] The tests include Timed Gait, Grooved Pegboard Dominant, Grooved Pegboard Non-Dominant, Finger Tap Dominant, Finger Tap Non-Dominant, Color Trails 1, Colors Trail 2, Semantic Verbal Fluency, Stroop Color, Stroop Word, Stroop Color Word, and Activities of Daily Living. NPT was performed at study enrollment (baseline), and at weeks 12, 24, 48, 72, and 96. NPT results were tabulated as Z-scores,[18] using a comparator group of HIV-negative MSM recruited at the clinics where the SABES study was conducted.

Statistical Analysis: Simple linear regression was used to evaluate associations between key biomarkers, NPT performance, and ETI, first using ETI as a predictor of baseline biomarker levels, and then using baseline biomarker levels as predictors of change in NPT score over time. Analyses used $\log_{10}$-transformed concentrations of biomarkers listed above, except for

untransformed concentrations of plasma D-dimer and CRP, in accordance with convention. For aim 1a, we analyzed the relationship between viral load and time from EDDI to enrollment date, an estimate of ETI,[19] using ETI as a predictor of viral load. For aim 1b, we analyzed the association between inflammatory biomarkers and ETI, using ETI as a predictor of inflammatory marker level at the time of enrollment. For aim 2a we used simple linear regression with biomarker levels in CNS and plasma as predictors of baseline NPT score. For aim 2b, individual linear regressions were run using baseline biomarker levels as predictors of change between Z score at baseline and Z score at weeks 12, 24, 48, 72, and 96. To prevent confounding by treatment start time, analyses of longitudinal neurocognitive outcomes in aim 2b included only data from participants enrolled in the immediate treatment group, those who started cART at enrollment. To adjust for multiple comparisons in aims 1b, 2a, and 2b, post-hoc power analysis was conducted using Bonferroni and Benjamini-Hochberg (BH) methods. Importantly, in health studies involving multiple comparisons, use of the BH False Discovery Rate is preferable to the often overly-conservative Bonferroni adjustment.[20]

Results:

Table 1: Cohort Characteristics per study arm: number, age, educational attainment, income, CSF provision, Estimated Time of Infection based on EDDI-enrollment interval, sexual orientation.

Table 1: Cohort Characteristics			
	Complete cohort	**Immediate Study Arm**	**Deferred Study arm**
N	87	42	45
Age Years, Mean (SD)	27.1 (7.5)	27.38 (7.6)	26.8 (7.5)
Education* Years, Mean (SD)	12.9 (3)	13.3 (3)	12.5 (3)
Monthly Income $US, Mean (SD)	247.2 (218)	289.1 (266.4)	208.1 (153.2)
Provided CSF sample	29	13	16
ETI (EDDI-Enrollment Interval days), Mean (SD)	43.3 (18.5)	44.7 (19.8)	41.6 (17.1)
Sexual orientation:			
Cis-MSM	74	35	39
Transgender Woman	13	7	6

**Vocational training and formal education including primary and secondary school*

Table 2: Viral Load vs ETI		
Analyte	**β-Coefficient (change in log 10 copies/mm^3 per day of infection)**	**p-value**
Plasma Viral Load	-0.037	<0.0001
CSF Viral Load	-0.024	0.0287

Table 2: ETI as predictor of viral load in plasma and CSF. Results of analyses with unadjusted p-values <0.05 presented.

Aim 1a. As shown in figure 1 and table 2, ETI was significantly negatively correlated with viral load in the plasma (-.0367 log10 copies/ml/day, p-value <.0001), and in the CNS (-0.02365 log10 copies/ml/day, unadjusted p-value 0.029).

Table 3: Biomarker Level vs ETI			
Analyte	**B-Coefficient (change in log 10 ug/ml analyte/day of infection)**	**unadjusted p-value**	**Benjamini-Hochberg**
Negative Linear Association			
CSF Total Protein	-0.238	0.0043	**0.0259**
CSF IFN-y	-0.015	0.0034	**0.0235**
CSF IL23p40	-0.014	0.0139	0.0529
Plasma IFN-a2a	-0.014	0.0373	0.1049
CSF IL-10	-0.012	0.0221	0.0729
CSF IL-6	-0.01	0.0005	**0.0051**
Plasma IFN-y	-0.01	0.0004	**0.0045**

CSF Neopterin	-0.009	0.0028	**0.0212**
CSF S100B	-0.009	0.0259	0.082
Plasma IL-10	-0.008	0.0001	**0.0015**
CSF MIP-1a	-0.007	<0.0001	**0.0003**
CSF Neurofilament Light Chain	-0.007	0.0098	**0.0472**
CSF VCAM-1	-0.006	0.002	**0.0169**
CSF YKL40	-0.006	0.0436	0.1124
Plasma VCAM-1	-0.006	<0.0001	**0.0003**
CSF CD-163	-0.005	0.0001	**0.0015**
Plasma IP-10	-0.005	0.0213	0.0729
CSF IL-2	-0.004	0.011	**0.0487**
CSF TNF-α	-0.004	0.0115	**0.0487**
CSF IL-16	-0.004	0.0421	0.1124
CSF IL-7	-0.004	0.0444	0.1124
Plasma S100B	-0.004	0.0125	0.0501
Plasma MCP-1	-0.003	0.0048	**0.0261**
Plasma Neopterin	-0.003	0.0157	0.0569
Positive Linear Association			
Plasma CD-163	0.004	0.0099	**0.0472**
Plasma MMP-9	0.005	0.0005	**0.005**
Plasma YKL40	0.005	0.0044	**0.0259**

Plasma IL-1β	0.015	0.0276	0.084

Table 3: ETI as predictor of Biomarker Levels in CSF and Plasma. Results of analyses with unadjusted p-values <0.05 presented, BH-adjusted p-values <0.05 in ***bold.***

Aim 1b. We observed significant relationships between ETI and multiple canonical inflammatory biomarkers. As shown in table 3, the unadjusted analysis showed a significant relationship between 16 CSF and 12 plasma biomarkers. In the BH-adjusted analysis, CSF biomarkers that were significantly *negatively* correlated with time from infection included: MIP-1a, CD-163, IL-6, VCAM-1, Neopterin, IFN-y, Total Protein, Neurofilament Light Chain, IL-2, TNF-α. The peripheral biomarkers negatively associated with ETI after BH adjustment included: VCAM-1, IL-10, IFN-y, and MCP-1, and CD-163. Plasma CD-163, YKL-40 and MMP9 were significantly *positively* correlated with ETI.

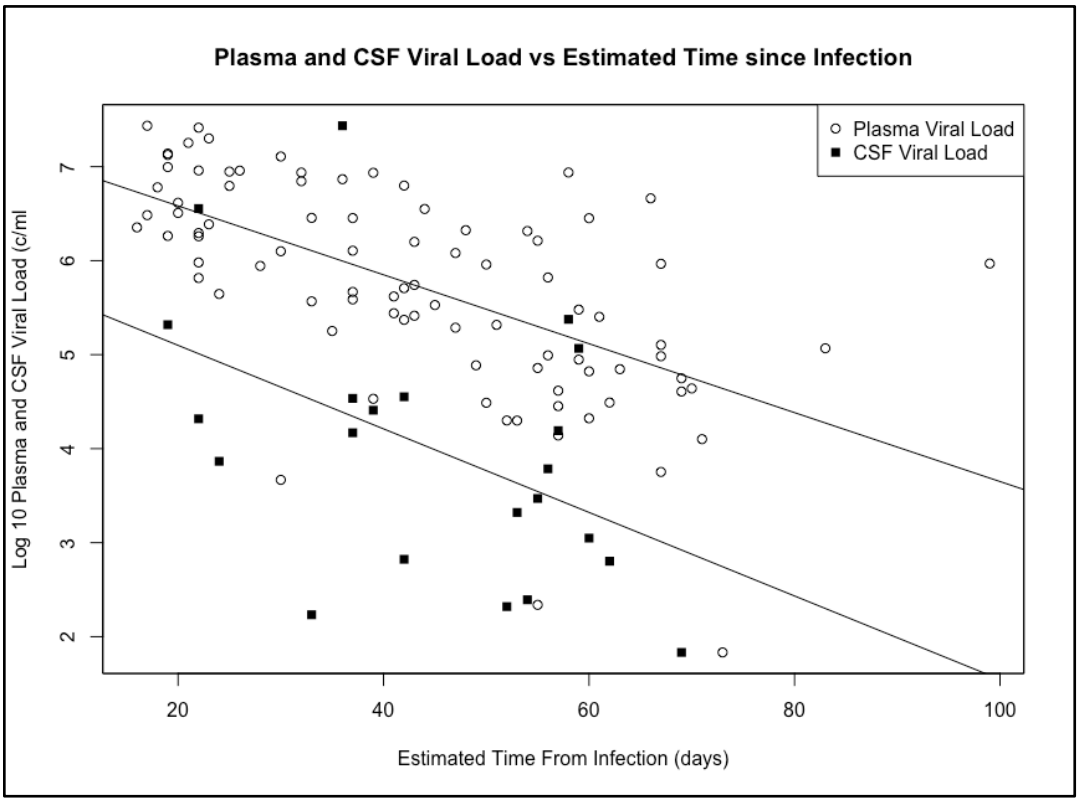

Figure 1: Simple linear regressions of Plasma and CSF viral loads as predicted by ETI. Viremia in the plasma and CSF declined from an elevated level during early HIV infection.

Aim 2a. When analyzing the association of biomarker levels at enrollment with simultaneously conducted NP testing outcomes, 3 markers had unadjusted p values of $p<0.05$ (see table 4). CSF MIP-1a was strongly negatively associated with baseline neurocognitive performance, while plasma IL-1a and plasma IL-12 both had a positive association. However, none of these associations was significant following adjustment for false discovery rate.

Table 4: Biomarkers vs Baseline NPT score (Total Z-score)			
Biomarker	**β-Coefficient (change in z-score per log 10 ug/ml change in analyte)**	**unadjusted p-value**	**Benjamini-Hochberg**
Plasma IL-1a	0.233	0.0042	0.3158
Plasma IL-12	0.315	0.0150	0.5715
CSF MIP-1a	-1.179	0.0340	0.6982

Table 4: Baseline Biomarker level as predictor of Baseline NPT Z-Score. Results of analyses with unadjusted p-values <0.05 presented, BH-adjusted p-values <0.05 in ***bold.***

Table 5: Neurocognitive Performance Testing Performance Over Time					
Time Point	**Week 0**	**Week 12**	**Week 48**	**Week 72**	**Week 96**
Mean	0.13	0.33	0.52	0.56	0.5
Median	0.05	0.34	0.53	0.6	0.46
Standard Deviation	0.42	0.46	0.5	0.51	0.53

Table 5: Mean and median NPT scores rose over time in the immediate arm of the study.

Figure 2: Key Biomarkers Correlated with Decline in NPT Score

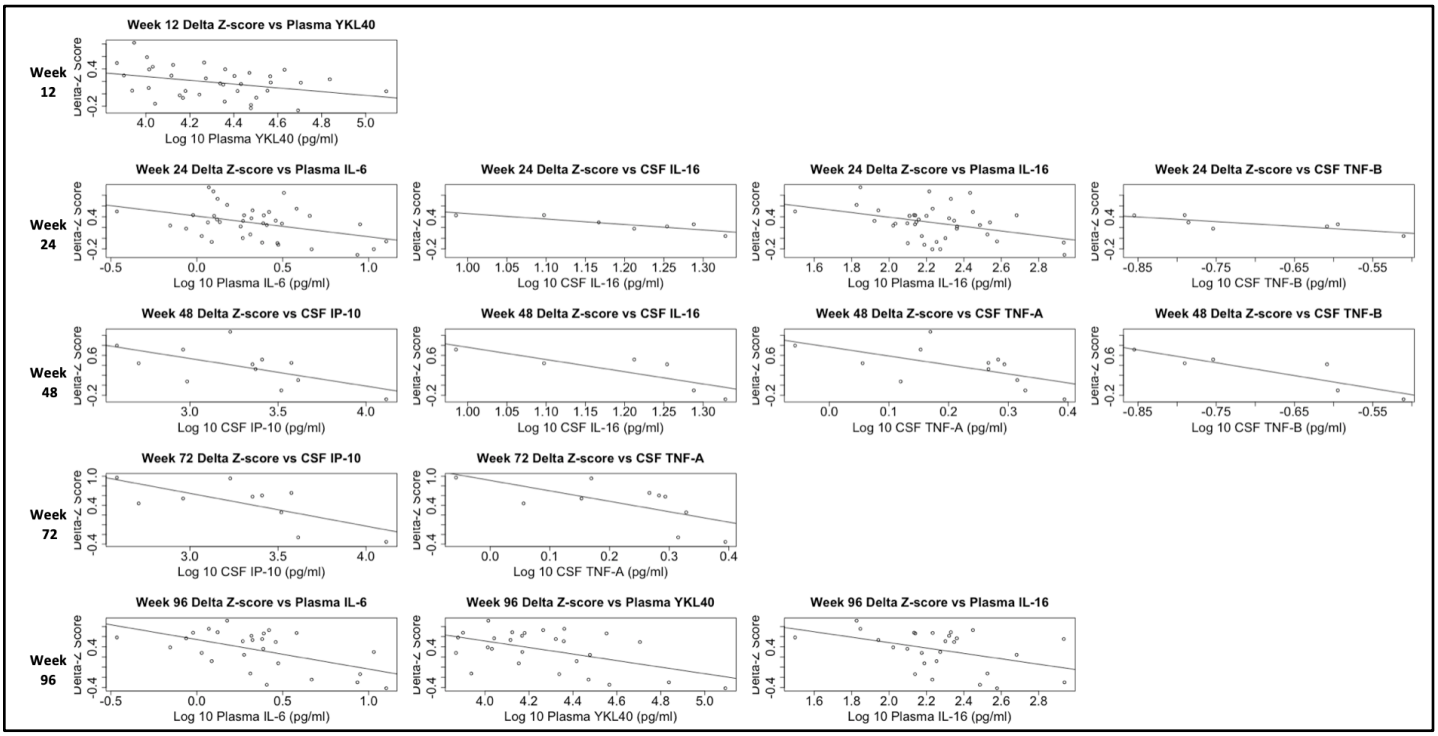

Figure 3: Biomarkers as Predictors of Delta-Z Score at weeks 12, 24, 48, 72, and 96. Only biomarkers with unadjusted p-values <.05 at two or more timepoints are graphed.

Table 6: Baseline Biomarkers vs Change in NPT score (Delta Z-score)			
Biomarker	**Coefficient (change in Z-score per log 10 ug/ml change in analyte)**	**unadjusted p-value**	**Benjamini-Hochberg**
Week 12			
Plasma YKL40	-0.304	0.0366	0.7215
Plasma CD-163	-0.291	0.0498	0.7215
Week 24			
Cerebrospinal Fluid Analytes			
CSF IL-16	-1.016	0.0105	0.3651
CSF SDF-1a	-1.451	0.0204	0.3868
CSF TNF-β	-0.874	0.0286	0.4347
Plasma Analytes			
Plasma IL-6	-0.429	0.0100	0.3651
Plasma IL-16	-0.429	0.0144	0.3651

Plasma MIP-1a	-0.422	0.0386	0.4889
Week 48			
Cerebrospinal Fluid Analytes			
CSF TNF-β	-2.572	0.0193	0.4639
CSF IP-10	-0.555	0.0351	0.4639
CSF TNF-α	-1.802	0.0366	0.4639
CSF IL-16	-2.450	0.0470	0.5101
Plasma Analytes			
Plasma IL-1a	-0.267	0.0104	0.4639
Plasma IL-2	-0.434	0.0329	0.4639
Plasma IL-12	-0.254	0.0343	0.4639
Week 72			
CSF IP-10	-0.677	0.0271	0.6815
CSF TNF-α	-2.157	0.0345	0.6815
Week 96			
Plasma IL-6	-0.582	0.0025	0.1865
Plasma YKL40	-0.645	0.0066	0.2163
Plasma D-dimer	-0.645	0.0085	0.2163
Plasma IL-16	-0.533	0.0225	0.4273

Table 6: Baseline biomarker level as predictor of change from baseline NPT Z-score. Results of analyses with unadjusted p-values <0.05 presented, BH-adjusted p-values <0.05 in bold.

Aim 2b. In the immediate arm as a whole, mean and median total Z score rose over the course of the study (see table 5). However, in our analysis of the relationship between baseline inflammatory biomarkers and change in NPT Z-score, measured over 96 weeks, multiple biomarkers significantly predicted a negative change in Z score at specific time points (see table 6). Of the 76 biomarkers tested, 21 had unadjusted p-values <0.05. Seven of these achieved significance at more than one time point between weeks 12 and 96. These included Plasma YKL40 at Weeks 12 and 96; Plasma IL-16 at weeks 24 and 96, Plasma IL-6 at weeks 24 and 96,CSF TNF-β at weeks 24 and 48, CSF IL-16 at weeks 24 and 48, CSF TNF- α at weeks 48 and 72, and CSF IP-10 at weeks 48 and 72; (see figure 2).

Discussion:

Our analysis of peripheral and CNS inflammatory biomarkers in relation to ETI and NP test results provided new insights into the nature of the inflammatory milieu during early HIV infection.

Aim 1a. We investigated the viral load in the CNS shortly after HIV acquisition, where the relationship between viral load and time from infection are less well understood than in the periphery. Our results showed high initial levels of HIV RNA in both the plasma and CSF to be associated with shorter ETIs, and lower levels of HIV RNA associated with longer ETIs, with a higher viral load in plasma than CNS at most points in time, consistent with the contemporary understanding of HIV compartmentalization. These results are consistent with other studies' conclusions that HIV is detected at higher levels in the CNS[21] and plasma during early, relative to chronic, infection.[22] However, the subsequent drop in viral load, particularly in the CNS, provides a clearer understanding of the rapidly changing levels of viral replication in the weeks following infection.

Aim 1b. The multiple significant negative associations between key CSF and plasma biomarkers and ETI suggest that cytokine-mediated inflammation does decline linearly from an elevated level during the first 100 days of HIV infection. These findings align with data from Sereti et al, who documented elevated inflammatory biomarkers including IL-6 and TNF - α in plasma during acute HIV infection in ART-naïve individuals participating in a prospective study of ART initiation in Thailand (RV254/SEARCH010),[23, 24] as well as Spudich and colleagues, who showed that immune activation and viral proliferation occur during early HIV infection in both the plasma and CNS.[25] Our results, based on precise measurements of cytokine levels in a large population of closely-surveilled participants enrolled shortly after infection but before ART initiation reinforce the robust body of literature on the pattern of inflammatory biomarkers in plasma during the first 100 days of HIV infection. Further, our results provide a more detailed view of the strength of association and rate of decline in inflammatory activity in the CNS during the same time period. They confirm the findings of Spudich et al, that even in the absence of cART, the inflammatory milieu of early HIV infection in both plasma and CSF is characterized by an initial peak in inflammation, followed by a decline to a reduced level in the majority of inflammatory biomarkers we measured. There were 3 plasma biomarkers, MMP-9, CD-163, and

YKL40, that had a positive linear association with ETI. Though it is generally well-understood that low-level systemic inflammation persists in plasma after initial peak viremia,[26] the observed data provide a more detailed picture of the initial peak and subsequent decline in CNS inflammation during this time.

Aim 2a. Our findings of a significant negative correlation between baseline CSF MIP-1a, and positive correlations between plasma IL-1a, plasma IL-12 and baseline NPT score highlight the link between HIV-induced inflammation and neurocognitive performance in early HIV infection[27]. MIP-1a is a product of astrocytes and microglia, and competes with gp120 for CCR5 binding sites, inhibiting a key mediating step in HIV infection. This functionality has been shown to prevent gp120-driven neuronal apoptosis in vitro, which is hypothesized to have a neuroprotective effect.[28] However, MIP-1a also acts as a powerful transendothelial chemoattractant of monocytes, dendritic cells, and NK cells, possibly mediating HIV entry into the CNS, as well as CNS inflammation and subsequent neuronal damage.[29, 30] Less is known about IL-1a as it relates to HAND. One study found a significant negative correlation between CSF IL-1a and NPT performance on a specific task (the Digit Symbol Test), which was not completed by our participants.[31] This suggests a detrimental effect of IL-1a on cognition, a finding not consistent with our results, which showed an improvement in Z-score correlating with higher plasma IL-1a levels. Finally, IL-12 is known to be an important component in suppressing HIV by causing differentiation of HIV- resistant macrophages rather than through an inflammatory cascade, which may account for its apparent neuroprotective effects.[32] An important element to consider when making comparisons between our study and other studies is the element of ETI. It is possible that an initial robust immune response to acute HIV infection might be protective during the first 96 weeks, only becoming detrimental after years of chronic illness, when inflammation itself, rather than viral replication, is causing neurocognitive decline. This phenomenon has been observed chiefly in older HIV/AIDS patients with chronic infection,[33] and it is possible that a different process is occurring in our much younger cohort, undergoing acute HIV infection.

Aim 2b. Our findings showed that baseline levels of certain inflammatory biomarkers in the plasma and CNS were significantly associated with poorer neurocognitive performance over

time, as measured by a drop in NPT score from baseline. These effects persist despite the observed positive "learning effect" expected as a result of repeated neurocognitive testing on the same participants. Specifically, elevated baseline values of plasma YKL-40, CSF TNF-β, CSF and Plasma IL-16, Plasma IL-6, CSF TNF-α, and CSF IP-10 at the time of diagnosis all appeared to have a significant negative correlation with NP function (Delta-Z score) at more than one subsequent time point. While other biomarkers did achieve significance in the unadjusted analysis at a single timepoint, the fact that these 7 recurred at least twice during the time-point analysis suggests that the observed effect is consistent, rather than an artifact of multiple comparisons.

These results contribute to a growing body of literature on key inflammatory biomarkers that appear to be central to HAND. Multiple studies have demonstrated a contributory role of plasma IL-6 and CSF IP-10 (among others),[10,34,35] while others have shown a neuroinflammatory cascade that is largely governed by the actions of TNF-α, IL-1b, and IL-16 through their actions on microglia, astrocytes, and perivascular macrophages in the CNS.[35-37] Interestingly, though plasma YKL 40 has not been clearly correlated with poor neurocognitive performance in the past, it is a known marker of microglial activation, one of the signaling pathways thought to be most responsible for neuroinflammation that contributes to HAND, and elevated CSF YKL 40 has been associated with both SIV encephalitis in macaques, and HIV encephalitis in humans.[35] IL-16, which was predictive of NPT trajectory in CSF at weeks 24 and 48, and in plasma at week 96, is also implicated in microglial immune activation, and has been observed at elevated levels in histopathologic studies of the brains of HIV encephalitis patients.[36,37] These findings suggest that IL-16 is a persistent marker of macrophage proliferation and activation in both CNS and plasma, possibly contributing to neurocognitive deficits. The fact that baseline plasma IL-16 rather than CSF IL-16 level was correlated with NPT outcomes at later time points could be due to the fact that the sample size for plasma analytes (n = 42) was considerably larger than for CSF (n = 13) . Finally, while TNF- β appears to play a role in HIV-associated neuroinflammation and viral replication,[38] it has not yet been clearly connected to neurocognitive performance.

Our study had a number of key strengths. First, the close surveillance of difficult-to-reach HIV-uninfected participants allowed for the rapid diagnosis and study of early HIV infection in a key

population (mean ETI at time of enrollment: 43.3 days). Secondly, the fact that our cohort is relatively youthful allowed for analysis of neurocognitive performance in the absence of the possible confounder of aging and unknown infection or treatment duration, a common problem in studies of HAND. Thirdly, longitudinal neurocognitive performance testing with validated tools allowed for reliable measurement of neurocognitive performance over time. Finally, our study made use of state of the art immunoassays to quantify 76 CSF and plasma biomarkers in baseline samples at the time of diagnosis and before cART initiation, providing a detailed view of the systemic immune response to acute HIV infection. The principle weaknesses of the study are small sample sizes (especially for CSF biomarkers), multiple comparisons, measurement of biomarkers at only one timepoint per participant, assumption of linearity for calculated beta-coefficients, and the relative imprecision of the Estimated Date of Detectable Infection calculation used to estimate ETI . The small number of participants included in the CSF analyses limited the power of the study, but the fact that so many associations initially appear significant despite the small sample size suggests that these relationships are quite strong. Though this study did contain a large number of individual comparisons, this was due in large part to the exploratory nature of the analysis and the wide variety of biomarkers measured. The fact that in the exploratory analysis of biomarker level and change in NPT at different timepoints, 7 biomarkers retained their significance after Benjamini-Hochberg adjustment suggests that there are true, strong relationships that should be more thoroughly investigated.

Most significantly, this exploratory analysis supports the position that while no single diagnostic marker is perfectly matched with neurocognitive function, a panel of several biomarkers, including Plasma YKL-40, CSF TNF-β, CSF and Plasma IL-16, Plasma IL-6, CSF TNF- α, and CSF IP-10, sampled at baseline, may have predictive value for the development of neurocognitive impairment 12-96 weeks after infection. This study also builds on work by Valcour, Sereti and others[23, 39] by using a broad panel of biomarkers to characterize the inflammatory milieu in the plasma and CNS during early HIV infection. Our findings further suggest that high levels of certain inflammatory biomarkers in early HIV infection are associated with a negative change in NPT score over time. The implications of these findings when taken together, that inflammation may be predictive of neurocognitive deficits, and that inflammation appears highest in early HIV infection, may reinforce rationale for earlier HIV diagnosis and

treatment, as well as a possible anti-inflammatory strategy in early infection to mitigate the risk of HAND. Moving forward, further investigation of these cytokines with more closely targeted, higher-powered studies is warranted.

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