Monocyte Activation Markers in Cerebrospinal Fluid Associated With Impaired Neurocognitive Testing in Advanced HIV Infection

Published in final edited form as:
J Acquir Immune Defic Syndr. 2012 July 1; 60(3): 234–243. doi:10.1097/QAI.0b013e318256f3bc.

Monocyte activation markers in cerebrospinal fluid associated with impaired neurocognitive testing in advanced HIV infection

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Abstract

Background—Activated monocytes/macrophages play a role in severe forms of HIV-associated neurocognitive disorders (HAND), but little is known about mechanisms driving milder forms that are prevalent despite combination antiretroviral therapy (cART). To examine relationships of monocyte activation markers to HAND of varying severity, we compared plasma and CSF biomarker levels to neurocognitive test scores in HIV+ subjects.

Methods—Plasma and CSF sCD14, CCL2, and IL-6 were measured by ELISA in 67 HIV+ subjects with nadir CD4 <300, and CSF inflammatory biomarkers were measured by multiplex assay in 14 subjects on suppressive cART.

Results—82% were on cART, with 31% having undetectable plasma VL. CSF sCD14 was increased in subjects with impaired neurocognitive testing (p=0.02), correlated inversely with global T scores in subjects with detectable but not undetectable plasma VL (p=0.02), and yielded higher AUROC values for predicting impaired T scores (0.659) than plasma or CSF VL and current or nadir CD4 counts in single-marker and multivariate models. CSF sCD14, IL-6, IL-8, CCL2, CCL3, CXCL10, and IFNγ were increased in subjects on suppressive cART regardless of cognitive status and predicted patient class in unsupervised analyses, with IL-8, CCL2, and IFNγ explaining most of the variance.

Conclusions—CSF sCD14 is associated with impaired neurocognitive testing in HIV patients on nonsuppressive cART, suggesting potential utility as a biomarker to monitor HAND progression. CSF sCD14, IL-6, IL-8, CCL2, CCL3, CXCL10, and IFNγ remain elevated in patients on suppressive cART regardless of cognitive status, implying ongoing intrathecal inflammation even in the absence of clinical manifestations.

Keywords

HIV; HIV-associated neurocognitive disorders; immune activation; cerebrospinal fluid; biomarkers

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Conflicts of Interest: The study authors report no disclosures or competing interests.

Introduction

HIV-associated neurocognitive disorders (HAND), consisting of asymptomatic neurocognitive impairment (ANI), minor cognitive-motor disorder (MCMD), and HIV-associated dementia (HAD), affect 20–50% of HIV-infected individuals despite widespread use of combination antiretroviral therapy (cART).[1–4] HIV enters the central nervous system (CNS) via trafficking of infected monocytes and lymphocytes, and activation of monocytes/macrophages is associated with severe forms of HAND in patients with evidence of ongoing viral replication.[5–10] However, little is known about mechanisms underlying milder forms of HAND in patients with cART-mediated virological suppression.

In the pre-cART era, CSF markers associated with more severe forms of HAND included CSF HIV RNA[11–14] and markers indicating intrathecal immune activation (CCL2,[15–19] neopterin,[20–22] β-2 microglobulin,[12, 23] TNF,[23–25] IL-6,[23, 24] and quinolinic acid[23, 24]). Since widespread use of cART, the prevalence of HAD has decreased but prevalence of milder forms of HAND has remained similar or increased.[1–3, 26] Furthermore, several studies show evidence of ongoing intrathecal immune activation despite cART. High CSF neopterin and β-2-microglobulin, both markers of immune activation, have been found in subjects with suppressed plasma viral loads (VL).[27–30] Elevated soluble CD14 (sCD14), a marker of monocyte activation, was detected in plasma[6, 31] and CSF[32] of HIV+ patients with neurocognitive impairment. High CSF levels of CCL2, a monocyte chemoattractant, have also been associated with cognitive impairment and altered metabolites in brain in cART-treated HIV+ patients.[33, 34] These studies provide evidence that immune activation continues to contribute to HAND pathophysiology in the cART era.

Recently, we found that plasma sCD14 was associated with impaired neurocognitive testing, particularly in attention and learning domains, in a cART era cohort of HIV+ subjects with advanced disease.[31] Here, we examined relationships between CSF monocyte activation markers (sCD14, CCL2, and IL-6) and neurocognitive test scores in HIV+ subjects from the same study cohort and used a multiplex assay to examine inflammatory cytokines/chemokines in plasma and CSF from subjects on suppressive cART.

Methods

Subjects

67 HIV+ subjects (98% with nadir CD4 counts < 300) with samples and data collected between 1999 and 2009 were from 4 sites (Manhattan HIV Brain Bank, National Neurological AIDS Bank, California NeuroAIDS Tissue Network, Texas NeuroAIDS Research Center) within the National NeuroAIDS Tissue Consortium (NNTC) (n=57) and from CNS Highly Activate Retroviral Therapy Effects Research (CHARTER) (n=10), a six-center observational cohort study. 82% were on cART, with 31% having undetectable plasma HIV RNA. 66 subjects were examined in our previous study,[31] with available CSF samples from the same time as plasma samples and neurocognitive testing. All subjects provided written informed consent under local institutional IRB approval. HAND clinical diagnoses were determined using established criteria[35] based on formal neurocognitive testing and neurological evaluation. Neuropsychological impairment due to other causes (NPI-O) was diagnosed when factors in addition to primary HIV could contribute to neurocognitive impairment (Table 1). Current substance abuse was determined by Psychiatric Research Interview for Substance and Mental Disorders (PRISM) or Composite International Diagnostic Interview (CIDI) and urine toxicology. Subjects with severe psychiatric disorders, a confounding neurological disorder, or active systemic infection were excluded. Plasma and CSF HIV RNA were $\log_{10}$ transformed for statistical analysis. Undetectable plasma and CSF VL values were assigned $\log_{10}$ values of 2.6 or 1.7, reflecting

sensitivity cutoffs of the assay; values below these cutoffs reflect lower assay sensitivity for some sites. Fifteen plasma samples from healthy donors testing HIV/HCV seronegative and 20 CSF samples from non-diseased controls were from Bioreclamation LLC (NY). Nineteen HIV/HCV seronegative plasma samples were from healthy donors at Research Blood Components (Brighton, MA) with written informed consent and IRB approval. Because there was no clinical information available for CSF samples from Bioreclamation, CSF samples were pre-screened for sCD14 and CCL2 levels by ELISA to identify samples with values outside normal ranges reported in the literature (<0.25 μg/ml and <1,000 pg/ml, respectively), which excluded 6 male and 10 female CSF samples with high CCL2 or sCD14 levels, respectively.

Neurocognitive testing

All subjects were administered an identical comprehensive test battery designed to assess seven categories of neurocognitive function.[36] Demographically corrected T global T scores were generated from individual T scores as described.[31, 36] T scores correlate negatively with severity of neurocognitive impairment, with values below 40 signifying impairment (40 corresponds to one standard deviation of 10 from a normalized mean of 50).

Biomarker quantification

sCD14, IL-6, and CCL2 were quantified by ELISA (R & D systems). A multiplex immunoassay (Bio-source 25-plex Human Cytokine Assay; Invitrogen) was used to measure IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, IFN-α, IFNγ, TNF, GM-CSF, CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β, CXCL10/IP-10, CXCL9/MIG, CCL11/eotaxin, and CCL5/RANTES using a Bio-Plex Workstation (Bio-Rad).

Data analysis

Data was analyzed using the Mann-Whitney test and Spearman correlation. Receiver operating characteristic (ROC) curves were generated to measure the ability of markers to predict neurocognitive impairment (global T score <40) in univariate and multivariate models. Hierarchical clustering was performed with dChip software[37, 38] using Euclidean distance and average linkage. Comparison criteria required the fold change (FC) between group means to exceed a specific threshold, with mean difference significant by unpaired t-test ($p<0.05$). Concentration values were $\log_2$-transformed and cytokines with >50% missing data were excluded from further analysis; 17 plasma and 7 CSF biomarkers met these criteria and were included for further analysis. The limit of detection (LOD) was provided by the manufacturer. In lowest-value mode,[39] the lowest detected value (LDV) corresponded to the mode among detectable values below the LOD. Missing values were imputed using the LOD unless >15% of samples had detectable values below the LOD; in this case, missing values were imputed using the LDV. Principal component analysis (PCA) and partial-least squares discriminant analysis (PLS-DA) were performed on the Metaboanalyst web portal[40] using normalized and auto-scaled expression values. The web portal utilizes the prcomp function of the stats package, and plsr function of the pls package of R. Class labels were permuted 2000 times to test whether differences between groups were significant.[41]

Results

Higher plasma and CSF sCD14 levels are associated with impaired neurocognitive test performance

The study cohort consisted of 67 HIV+ subjects with advanced disease (median current and nadir CD4 counts of 85 and 52 cells/μl, respectively), and high frequency of drug abuse

(49%) and HCV co-infection (36%) (Table 1). The majority (82%) were treated on cART, with 31% and 46% having undetectable plasma or CSF HIV RNA, respectively (<400 and < 50 copies/ml, respectively). First, we compared monocyte activation markers between HIV+ and uninfected subjects in plasma and CSF. Plasma and CSF levels of sCD14, CCL2, and IL-6 were higher in HIV+ compared to uninfected subjects ($p<0.05$) (Supplemental Digital Content 1). In HIV+ subjects, HIV RNA and sCD14 were 2–3 fold higher in plasma compared to CSF, while CCL2 levels were 2-fold higher in CSF than plasma ($p<0.0001$, Supplemental Digital Content 2). CD4 count correlated inversely with plasma and CSF VL, sCD14, and plasma CCL2, while plasma sCD14 correlated positively with IL-6 ($r=0.248$, $p=0.04$) (Supplemental Digital Content 3). Plasma and CSF sCD14 levels were strongly correlated ($r=0.487$, $p<0.0001$), while plasma and CSF CCL2 levels trended toward correlation ($r=0.234$, $p=0.06$). These results show that sCD14 levels are higher in plasma than in CSF, while CCL2 levels are higher in CSF than in plasma, and sCD14 levels in plasma and CSF are strongly correlated in HIV+ subjects.

To examine relationships between monocyte activation markers and HAND, we compared plasma and CSF levels of HIV RNA, sCD14, CCL2, and IL-6 to global T scores. Plasma and CSF sCD14 levels were higher in subjects with global T scores <40 (impaired) versus ≥40 (unimpaired) ($p=0.02$ and $p=0.022$, respectively) and correlated inversely with global T scores ($r=–0.263$, $p=0.03$ and $r=–0.282$, $p=0.02$, respectively) (Figure 1). In contrast, plasma and CSF CCL2 or IL-6 levels were similar between groups. ROC analysis for single markers demonstrated that sCD14 levels in plasma and CSF yielded higher Area Under the ROC Curve (AUROC) values for classification of global T scores <40 (0.657 and 0.659, respectively) compared to four conventional markers (plasma and CSF VL and current and nadir CD4 count; AUROC=0.541, 0.566, 0.607, and 0.598, respectively). In multivariate models for predicting T score <40, plasma and CSF sCD14 added incremental value to logistic regression models using age and cART, and improved AUROC values (by 0.12 and 0.14, respectively) when added to age and cART, whereas the four conventional markers did not improve the predictive ability of these models. Thus, elevated CSF sCD14 levels are associated with global T scores indicating neurocognitive impairment, and yield higher AUROC values for predicting impaired global T scores than four conventional markers in univariate and multivariate models.

Plasma and CSF sCD14, CCL2, and IL-6 levels do not distinguish between groups defined by HAND clinical diagnoses

We compared global T scores between subjects stratified into two groups: no neurocognitive impairment (no NCI) and HAND or NPI-O clinical diagnosis grouped together. As expected, global T scores were higher in subjects with no NCI compared to those with a HAND or NPI-O clinical diagnosis ($p<0.0001$, Supplemental Digital Content 4). We compared levels of plasma and CSF sCD14, CCL2, and IL-6 between the two groups but found no differences (Supplemental Digital Content 5). Thus, plasma and CSF sCD14, CCL2, and IL-6 did not distinguish between subjects defined by HAND clinical diagnoses.

Plasma and CSF sCD14 levels are associated with global T scores indicating neurocognitive impairment in viremic but not aviremic HIV+ subjects

Next, we compared relationships between monocyte activation markers and global T scores in plasma and CSF for subjects grouped according to detectable (≥400 HIV RNA copies/ml) or undetectable (<400 HIV RNA copies/ml) plasma VL. These groups had similar median age, but median current and nadir CD4 counts were lower in viremic compared to aviremic subjects (54 and 41 versus 249 and 71 cells/μl, $p<0.0001$ and 0.05, respectively). sCD14, CCL2, and IL-6 remained elevated in plasma and CSF in both viremic and aviremic subjects compared to controls (Figure 2A). Conversely, CCL2 and IL-6 were higher in CSF than

plasma of aviremic subjects. Plasma sCD14 correlated with CSF sCD14 (r=0.574, p <0.0001), while plasma and CSF sCD14 correlated inversely with global T scores (r= –0.354, p=0.017 and r=–0.297, p=0.047, respectively) in viremic but not aviremic subjects (Figure 2B and C). In aviremic subjects, CD4 count correlated inversely with CSF CCL2 levels (r=–0.598, p=0.003), while plasma CCL2 correlated with CSF CCL2 (r=0.520, p=0.018). Unexpectedly, aviremic subjects with global T scores <40 had lower CSF CCL2 compared to those with global T scores ≥40 (p=0.005). Because illicit drug use and HCV co-infection are comorbidities of HIV infection with immunomodulatory effects that may influence risk of HAND,[42–44] we examined performed subgroup analysis. Neither CSF nor plasma biomarkers levels showed significant differences when subjects were classified according to patterns of substance abuse (within 6 months) (opiate or cocaine users compared to non-users, n=19, 21, and 27, respectively) or HCV co-infection (data not shown). Thus, plasma and CSF sCD14 levels correlated inversely with global T scores in subjects with detectable but not undetectable plasma VL.

CNS penetration effectiveness (CPE) score is not associated with differences in CSF biomarker levels

To examine relationships between CNS penetration of cART regimens and biomarker levels, we compared CSF sCD14, CCL2, and IL-6 level to CNS penetration effectiveness (CPE) score, which assigns each drug a value of 0 (low), 0.5 (intermediate), or 1.0 (good penetration); scores are summed to determine overall CPE rank of a regimen.[45] The median value for 2008 CPE rank (2.0) was the cutoff for comparing biomarker levels in subjects with good (≥2) versus poor (<2) CPE; scores were then evaluated as continuous variables compared to CSF sCD14, CCL2, and IL-6 levels. Additionally, data was analyzed using the revised CPE 2010 scoring system.[46] No associations were found between CPE rank and CSF sCD14, CCL2, and IL-6 levels, or global T scores, using either CPE scoring system. Subjects with suppressed plasma VL (<400 copies/ml) also showed no significant association between CPE rank and CSF biomarker levels or global T scores. Thus, higher CPE scores did not appear to influence CSF monocyte activation markers or global T scores in this cohort.

Increased CSF inflammatory biomarkers distinguish HIV+ subjects on suppressive cART from uninfected controls regardless of cognitive status

To examine inflammatory biomarker patterns in plasma and CSF of HIV+ subjects with cART-mediated virological suppression, we used a multiplex assay to measure 18 plasma and 7 CSF biomarkers in 14 aviremic HIV+ subjects and 14 healthy uninfected controls. Univariate analysis identified 8 cytokines/chemokines (sCD14, IL-6, IL-8, CCL2, CXCL9, CXCL10, IL-2R, CCL11, and CCL3) with higher levels in plasma from aviremic HIV+ subjects compared to healthy controls (FC 1.37–4.38, p<0.05) (Supplemental Digital Content 6), while CCL4 showed a trend towards significance (FC 2.6, p=0.054). Similar analysis of matched CSF samples detected 7 cytokines/chemokines (CXCL10, CCL3, CCL2, IL-8, IFNγ, IL-6, and sCD14) at higher levels in aviremic HIV+ subjects compared to healthy controls (FC 1.82 – 13.09, p<0.05) (Supplemental Digital Content 6). IL-8, IFN γ, and CCL2 were the top- ranked CSF biomarkers distinguishing aviremic HIV+ subjects from healthy controls (FC 2.89 – 13.09, p<0.0001).

Next we examined whether aviremic HIV+ patients clustered according to clinical subgroups and covariates defined by neurocognitive diagnosis, global T score (impaired or unimpaired, corresponding to <40 and ≥40, respectively), and HIV disease markers. Supervised hierarchical clustering for 18 plasma and 7 CSF biomarkers across aviremic HIV + subjects and healthy controls showed that none of these biomarkers in plasma and CSF clustered aviremic HIV+ subjects according to neurocognitive diagnosis, global T score, or

current or nadir CD4 counts. When analysis was applied to only 7 plasma biomarkers detected in CSF, we identified a major cluster of 11/14 controls with low rate of misclassification (14.2%), second cluster of 6 aviremic HIV+ subjects, and third cluster with 6 aviremic and 3 control subjects (Figure 3A). Unsupervised hierarchical clustering of 7 CSF biomarkers revealed that aviremic HIV+ subjects and healthy controls segregated with 100% accuracy into 2 major clusters (Figure 3B). The aviremic HIV+ subject cluster separated into 2 subclusters based on differential CSF CCL3 levels, each subcluster consisting of 7 aviremic subjects including various HAND diagnoses. IL-8 and IFNγ levels were higher (FC 3.58, p=0.001 and FC 1.45, p=0.023, respectively), while CCL2 trended towards significance (FC 1.99, p=0.07), in the subgroup with higher versus lower CSF CCL3 levels. These findings show sCD14, IL-6, CCL2, CCL3, CXCL10, IL-8, and IFNγ are increased in CSF from aviremic HIV+ subjects compared to controls in a small study regardless of neurocognitive status, and identify IL-8, CCL2, and IFNγ as a CSF biomarker cluster.

Next, we performed PCA and PLS-DA to examine variance in the CSF biomarker data. Unsupervised analysis of 7 CSF biomarkers by PCA revealed that the first 3 components explain 88.7% of the variance and discriminate aviremic HIV+ subjects from controls with 100% accuracy (Figure 3C). Supervised analysis by PLS-DA revealed that aviremic HIV+ subjects and controls could be separated along the axis defined by 3 components explaining 83.9% of the variance (Figure 3D); permutation tests confirmed significant separation ($p<0.0005$). Variables important in projection (VIP) scores plot ranked IL-8, IFNγ, and CCL2 as the top 3 biomarkers explaining variance between the two groups (Figure 3D). For plasma biomarkers, PCA did not reveal significant discrimination between aviremic HIV+ subjects and controls, while PLS-DA predicted 56% of the variance along the axis defined by 3 components with significant separation (p=0.019). We further examined IL-8, IFNγ, and CCL2 by Spearman correlation; in aviremic HIV+ subjects, IL-8 correlated positively with IFNα and CCL2 in plasma, and with IFNγ and CCL2 in CSF (Figure 4). Additionally, CCL3 levels correlated with IL-8 and IFNγ ($r=0.815$, $p=0.0003$ and $r=0.728$, $p=0.003$, respectively) in CSF. These findings suggest that increased CSF sCD14, IL-6, CCL2, CCL3, CXCL10, IL-8, and IFNγ distinguishes HIV+ patients on suppressive cART from uninfected controls.

Discussion

In this study of HIV+ subjects with advanced disease, CSF sCD14 levels were higher in subjects with impaired neurocognitive test performance, and correlated inversely with global T scores. CSF CCL2 and IL-6 did not show these associations. AUROC values for predicting impaired global T score <40 were higher for plasma and CSF sCD14 than for current or nadir CD4 count or CSF or plasma VL, and remained significant after adjusting for age and cART. The predictive ability of CSF sCD14 in single-marker and multivariate models was at least as good as plasma sCD14 for predicting impaired global T scores. These findings extend our previous analysis of plasma sCD14 in the study cohort,[31] and suggest that CSF sCD14 is a useful biomarker to monitor intrathecal inflammation during HAND progression and therapeutic responses.

Despite cART-mediated virological suppression, monocyte/macrophage activation markers (e.g., neopterin, sCD14, CCL2, CCL3, CCL4, CCL5, CXCL10) are frequently detected in CSF.[6, 27, 29, 30, 33, 34, 47–50] In the present study, plasma and CSF sCD14 levels were associated with global T scores in subjects with detectable but not undetectable VL. Furthermore, increased CSF sCD14, IL-6, CCL2, CCL3, CXCL10, IL-8, and IFNγ robustly distinguished aviremic HIV+ subjects from controls regardless of cognitive status in a small study of 28 subjects. In unsupervised analyses, these CSF biomarkers discriminated

aviremic HIV+ subjects from controls with 100% accuracy, with IL-8, CCL2, and IFNγ explaining most of the variance between groups. IL-8, produced mainly by activated monocytes and NK cells, is a chemoattractant for neutrophils, T cells, and NK cells expressing CXCR1 or CXCR2. In a recent study, increased CSF IL-8, CCL2, and CXCL10 were strongly associated with cerebral metabolites related to neuronal injury and neuroinflammation in subjects with HAND[34]. Subgroup analysis of untreated subjects or those failing cART indicated no correlation of IL-8, CCL2, and CXCL10 with inflammatory pattern scores related to neuronal injury, suggesting cART alters relationships between these chemokines and cerebral metabolites. Consistent with these findings, we found clustering of CSF IL-8, CCL2, and IFNγ in heatmaps and positive correlations of CSF IL-8 levels with IFNγ and CCL2 in subjects on suppressive cART. These findings are consistent with a model in which IFNγ-driven pathways contribute to ongoing intrathecal immune activation in HIV+ patients despite cART-mediated virological suppression.

Another interesting finding was the identification of 2 clusters of aviremic HIV+ subjects distinguished by differential CSF CCL3 levels: one with higher CCL3 levels consisting of 5/7 subjects with impaired global T scores along with higher CSF IL-8/IFN-γ levels, and a second cluster with lower CCL3 levels consisting of 5/7 subjects with unimpaired T scores. Although the small sample size limits conclusions that can be drawn, this preliminary finding, together with previous studies implicating increased CSF CCL3 in HAND pathogenesis,[51, 52] suggests that CCL3 as a potential CSF biomarker that may distinguish clinical subgroups warrants further investigation. Further studies of larger cohorts of aviremic subjects followed longitudinally are needed to determine relationships of CSF IL-8, CCL2, and IFN-γ (and CCL3) to risk of developing HAND in HIV+ patients on suppressive cART and their utility as biomarkers to monitor intrathecal inflammation and therapeutic responses.

Although we found an association between CSF sCD14 levels and impaired neurocognitive test performance, we found no differences in plasma or CSF sCD14 levels between HIV+ subjects classified by HAND diagnosis. A previous study of CD14+/CD16+ monocytes in HAD patients[32] found higher levels of sCD14 in CSF from HIV+ individuals with HAD compared to controls, but differences were significant only for those with a diagnosis of moderate to severe dementia and sample sizes were small. We cannot exclude the possibility that our inability to detect differences in plasma or CSF biomarkers between HAND subtypes reflected methodological problems related to patient selection or criteria used for assigning a clinical diagnosis. Nonetheless, our findings suggest that T scores are more sensitive indicators of neurocognitive impairment than clinical diagnoses in the cART era, and using continuous descriptors rather than categorical diagnoses is important for demonstrating associations between biomarker levels and impaired neurocognitive function.

The relationship of CPE score to improvement in clinical outcome is not linear and might be explained by a delicate balance between viral suppression in the CNS and potential neurotoxicity of certain antiretroviral drugs.[46] We found no association between CPE score and CSF sCD14, CCL2, or IL-6 levels for the total cohort or aviremic subgroup. Similarly, a recent magnetic resonance spectroscopy study did not find an association between CPE score and commonly measured cerebral metabolites.[53] Although interpretation of our findings is limited by the small sample size, they are indicative of ongoing intrathecal inflammation in HIV+ patients regardless of CPE score.

Limitations of our study include its cross-sectional design and small sample size, which may have limited the power to detect significant associations. Also, narrow selection criteria used to define the study cohort (CD4 nadir <300) limit our findings to those with advanced HIV disease. We included NPI-O subjects, as many likely exhibit neurocognitive deficits

attributable to HIV, and there is site-to-site variation in assigning a diagnosis of NPI-O. Subgroup analysis by type of illicit drug use or HCV co-infection did not show significant differences in plasma or CSF sCD14, CCL2, or IL-6, consistent with other studies that failed to demonstrate associations between these comorbidities and elevated sCD14 or CCL2.[5, 54] Thus, these comorbidities are unlikely to account for associations between elevated sCD14 and impaired global T scores. The study cohort was from NNTC, which specifically recruits individuals with advanced disease, and CHARTER, which includes a large population of well-controlled HIV+ subjects, to represent a diverse population of HIV-infected individuals with broad range of viral loads. As such, the study cohort reflects a bias of urban cohorts with large populations of non-suppressed patients and IV drug users and results cannot be generalized to all populations.

In conclusion, plasma and CSF sCD14 levels are associated with impaired neurocognitive test scores in HIV+ patients on nonsuppressive cART, providing evidence that monocyte activation continues to contribute to HAND pathogenesis in the cART era. CSF IL-8, CCL2, CCL3, CXCL10, IFN-γ, and IL-6 levels remain elevated in patients on suppressive cART, even in subjects without cognitive impairment. Plasma and CSF sCD14 may be particularly useful as biomarkers to monitor systemic and intrathecal monocyte activation, HAND progression, and therapeutic responses in HIV+ patients on cART.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank NNTC and CHARTER sites for providing plasma samples and clinical data for AIDS patients. We also acknowledge support from the Harvard Center for AIDS Research Biostatistics Core and the Mount Sinai Institute for NeuroAIDS Disparities.

Financial support: Supported by National Institutes of Health Grant DA26322, DA28994, and MH083588 to D.G. and a Mount Sinai Institute for NeuroAIDS Disparities (MSINAD) Scholar Grant (funded through R25MH080663) to J.L.L. National NeuroAIDS Tissue Consortium (NNTC) sites were supported by National Institutes of Health Grants U01MH083501, R24MH59724, U01MH083506, R24MH59745, U01MH083507, R24 NS45491, 5U01MH083500, NS38841, U01MH083545, and N01MH32002. CNS HIV Antiretroviral Therapy Effects Research (CHARTER) was supported by N01MH22005. Core facilities were supported by the Harvard Center for AIDS Research and Dana-Farber Cancer Institute/Harvard Center for Cancer Research grants.

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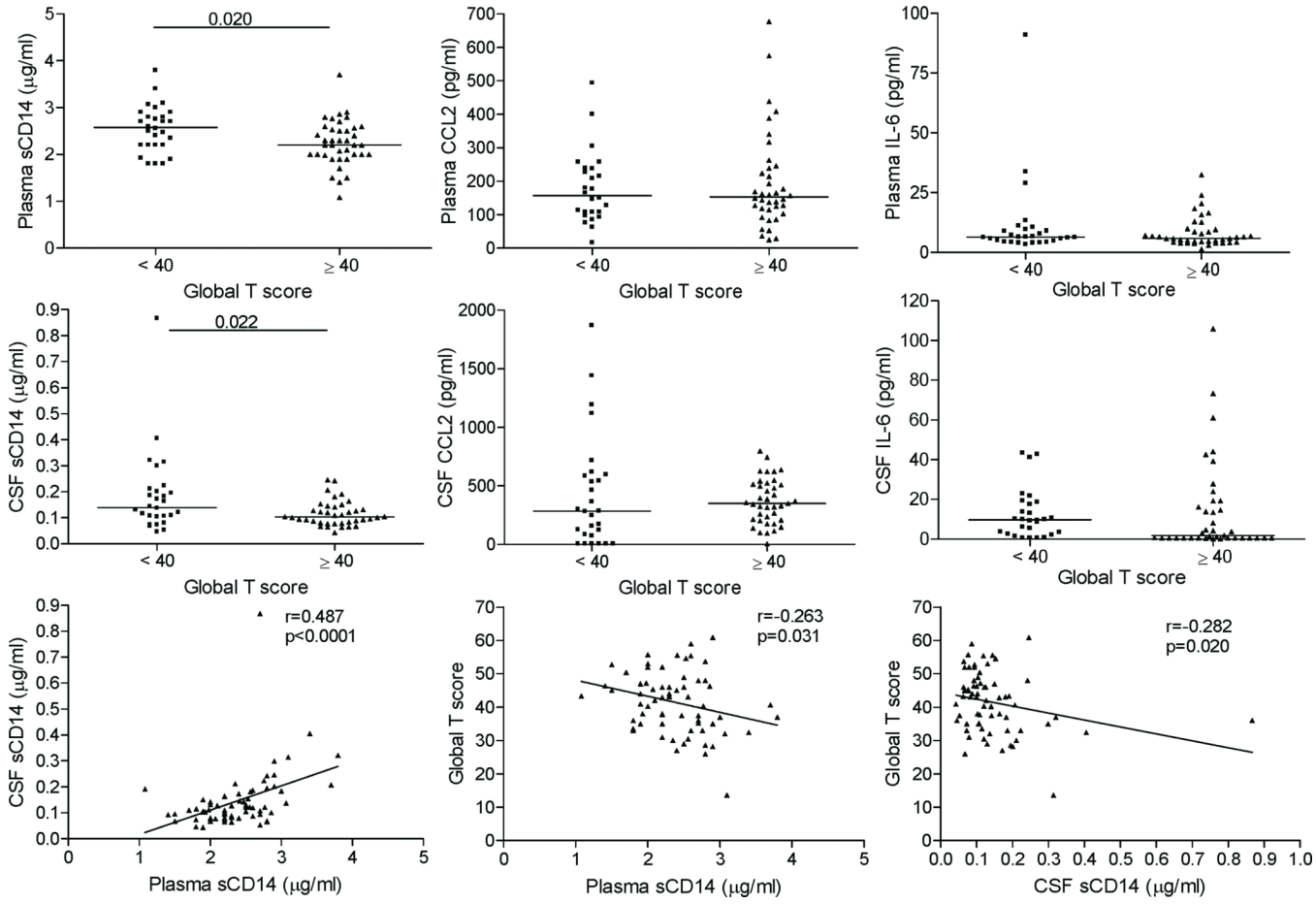

Figure 1. Higher plasma and CSF sCD14 levels are associated with global T scores indicating neurocognitive impairment

HIV+ subjects were grouped by global T score <40 or ≥40 and biomarker levels were compared. Plasma and CSF sCD14 levels were higher in subjects with global T scores <40 versus global T scores ≥40, and correlated inversely with global T scores, while no differences were seen for plasma or CSF CCL2 or IL-6 levels. Median values are indicated as horizontal lines. Statistical significance between groups was calculated using the Mann-Whitney test, and relationships between continuous variables were examined by Spearman correlation; significant differences ($p<0.05$) are indicated.

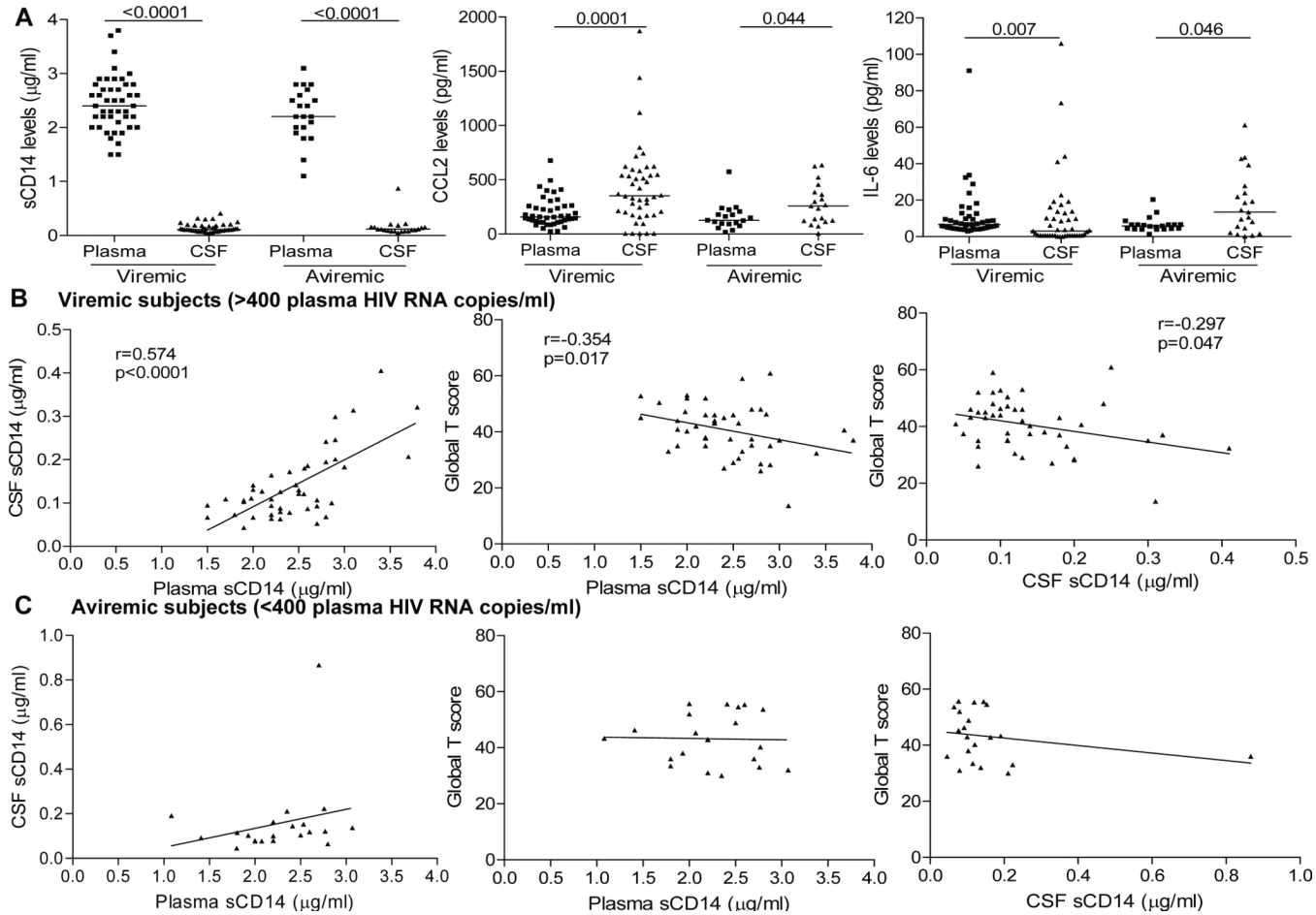

Figure 2. Plasma and CSF sCD14 levels correlate inversely with global T scores in viremic but not aviremic HIV+ subjects

A, sCD14, CCL2, and IL-6 levels in plasma and CSF of viremic and aviremic patients. B and C, Subgroup analysis of viremic (plasma VL >400 HIV RNA copies/ml) (B) and aviremic (plasma VL <400 HIV RNA copies/ml) (C) subjects showed positive correlation between CSF and plasma sCD14 levels and inverse correlation between plasma and CSF sCD14 levels and global T scores in viremic but not aviremic subjects. Statistical significance was determined by Mann-Whitney test (A) and Spearman correlation analysis (B and C); medians (A) and significant differences ($p<0.05$) are indicated.

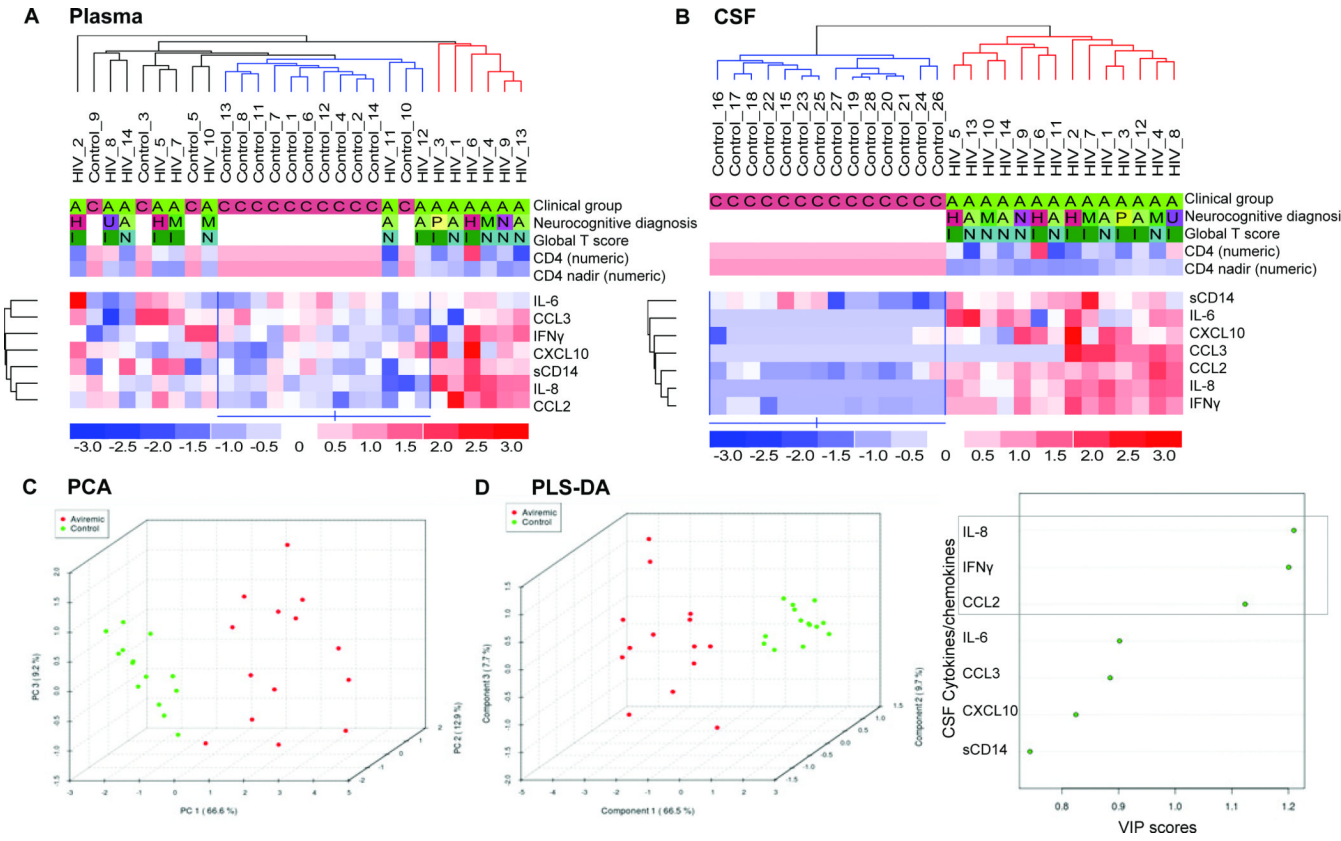

Figure 3. CSF inflammatory biomarkers distinguish HIV subjects on suppressive cART from healthy uninfected controls

A and B, Unsupervised hierarchical clustering by Euclidean distance and average linkage of 7 biomarkers (sCD14, IL-6, IL-8, CCL2, CCL3, CXCL10, and IFNγ) detected in both plasma (A) and CSF (B) of 14 aviremic HIV+ subjects and healthy controls using dChip software. Clustering analysis was also run across the following clinical groups and covariates as indicated above each heatmap: clinical groups (A, aviremic; C, healthy control), neurocognitive diagnosis (H, HAD; M, MCMD; A, Asymptomatic neurocognitive impairment; P, Neurocognitive impairment due to other causes; N, Normal; and U, Unknown), global T score (N, not impaired; I, impaired, corresponding to ≥40 and < 40, respectively), and current and nadir CD4 count. In heatmaps, red and blue represent increased and decreased levels relative to the mean level of a biomarker, respectively. Each column and row defines individual patients and cytokines/chemokines, respectively. Unsupervised clustering of plasma biomarkers (A) predicts a major cluster with 11/14 healthy controls (highlighted in blue) and second major cluster consisting of 6 aviremic subjects (highlighted in red), with increased expression of 7 biomarkers in aviremic HIV+ subjects compared to controls. Unsupervised hierarchical clustering of 7 CSF biomarkers segregates aviremic HIV+ subjects (highlighted in red) from controls (highlighted in blue) with 100% accuracy (B). Within the major cluster consisting of HIV+ subjects are two subclusters with differential CCL3 levels, each with 7 aviremic HIV+ subjects. C, PCA represented as a three dimensional scatter plot showing the top 3 principal components of cytokine/chemokine level data measured in aviremic HIV+ subjects (red dots, n=14) and controls (green dots, n=14). D, PLS-DA represented as a three dimensional scatter plot on the left panel shows the top 3 components of cytokine/chemokine level data measured in aviremic HIV+ subjects (red dots, n=14) and controls (green dots, n=14). The plot shows that 83.9% variance is explained by the first 3 components. Plot of variables important in

projection (VIP) (right panel) ranks IL-8, IFNγ, and CCL2 as the top 3 biomarkers accounting for separation between aviremic HIV+ subjects and uninfected controls.

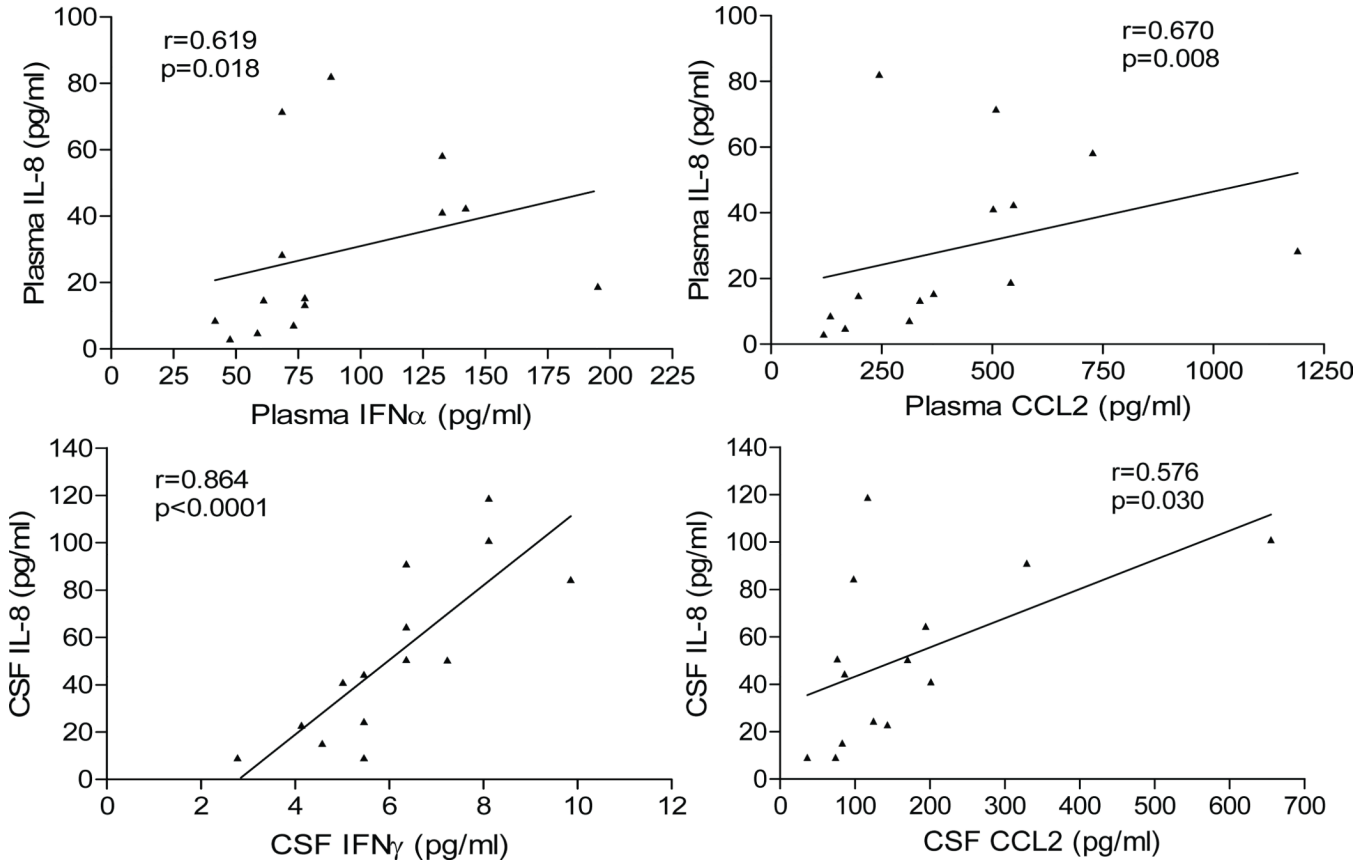

Figure 4. Relationships between IL-8 and other inflammatory biomarkers in aviremic HIV+ subjects on cART

Plots show positive correlations of inflammatory IL-8 with IFNα, and CCL2 in plasma (upper panel) and with IFNγ, and CCL2 in CSF (lower panel) of aviremic HIV+ subjects (Spearman correlation; $p<0.05$).

Table 1

Demographic and clinical characteristics of HIV+ subjects in the study cohort (n=67).

Age (years)	
Mean ± SD	46 ± 7
Median (range)	44 (32– 63)
Gender	
Male	55 (82%)
Female	12 (18%)
Race	
African American	31 (46%)
Caucasian	27 (40%)
Hispanic	5 (7%)
Other	4 (6%)
Risk factor	
Sexual transmission	27 (40%)
Intravenous drug abuse	40 (60%)
CD4 T cell count (cells/*μ*l)	
Mean ± SD	155 ± 162
Median (range)	85 (1– 688)
Nadir CD4 T cell count (cells/*μ*l)	
Mean ± SD	75 ± 93
Median (range)	52 (1 – 536)
Plasma HIV RNA (copies/ml)	
Mean ± SD	127,975 ± 236,077
Median (range)	13,500 (undetectable – 843,720)
> 400 copies/ml	45 (67%)
< 400 copies/ml	21 (31%)
Unknown	1 (1.5%)
CSF HIV RNA (copies/ml)	
Mean ± SD	3,554 ± 13,049
Median (range)	50 (undetectable – 75,000)
< 50 copies/ml	31 (46%)
cART	
Yes	55 (82%)
No	12 (18%)
ARV CPE Penetration rank 2008	
≥ 2	27 (49%)
< 2	28 (51%)
HCV co-infection	
Positive	25 (37%)
Negative	26 (39%)
Unknown	16 (23%)

HAND Diagnosis	
No impairment	19 (28%)
ANI	10 (15%)
MCMD	12 (18%)
HAD	11 (16%)
NPI-O*	12 (18%)
Unknown	3 (4%)

HAND, HIV-associated neurocognitive disorders; No NCI, no neurocognitive impairment; ANI, asymptomatic neurocognitive impairment; MCMD, minor cognitive-motor disorder; HAD, HIV-associated dementia; NPI-O, Neuropsychological impairment due to other causes.

*Neuropsychological impairment due to other causes (NPI-O) was diagnosed in patients with factors in addition to primary HIV that could contribute to neurocognitive impairment (past traumatic head injury (n=2), remote cerebral vascular accident (n=3), remote CNS toxoplasmosis (n=2), chronic hepatic cirrhosis (n=1), old frontal lesion (n=1), depression (n=1), and unknown NPI-O diagnosis (n=2)).