Vascular injury markers associated with cognitive impairment in people with HIV on suppressive antiretroviral therapy

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Objective: Human immunodeficiency virus (HIV)-associated neurocognitive disorders (HAND) remain prevalent despite viral suppression on antiretroviral therapy (ART). Vascular disease contributes to HAND, but peripheral markers that distinguish vascular cognitive impairment (VCI) from HIV-related etiologies remain unclear.

Design: Cross-sectional study of vascular injury, inflammation, and central nervous system (CNS) injury markers in relation to HAND.

Methods: Vascular injury (VCAM-1, ICAM-1, CRP), inflammation (IFN-γ, IL-1β, IL-6, IL-8, IL-15, IP-10, MCP-1, VEGF-A), and CNS injury (NFL, total Tau, GFAP, YKL-40) markers were measured in plasma and CSF from 248 individuals (143 HIV+ on suppressive ART and 105 HIV− controls).

Results: Median age was 53 years, median CD4$^+$ cell count, and duration of HIV infection were 505 cells/μl and 16 years, respectively. Vascular injury, inflammation, and CNS injury markers were increased in HIV+ compared with HIV− individuals ($P < 0.05$). HAND was associated with increased plasma VCAM-1, ICAM-1, and YKL-40 ($P < 0.01$) and vascular disease ($P = 0.004$). In contrast, inflammation markers had no significant association with HAND. Vascular injury markers were associated with lower neurocognitive T scores in age-adjusted models ($P < 0.01$). Furthermore, plasma VCAM-1 correlated with NFL ($r = 0.29$, $P = 0.003$). Biomarker clustering separated HAND into three clusters: two clusters with high prevalence of vascular disease, elevated VCAM-1 and NFL, and distinctive inflammation profiles (CRP/ICAM-1/YKL-40 or IL-6/IL-8/IL-15/MCP-1), and one cluster with no distinctive biomarker elevations.

Conclusions: Vascular injury markers are more closely related to HAND and CNS injury in PWH on suppressive ART than inflammation markers and may help to distinguish relative contributions of VCI to HAND.

Introduction

Combination antiretroviral therapy (ART) has reduced HIV-related morbidity and mortality, but mild forms of HIV-associated neurocognitive disorders (HAND), con–sisting of asymptomatic neurocognitive impairment (ANI) and mild neurocognitive disorder (MND), remain prevalent in people with HIV (PWH) [1–3]. The mechanisms underlying HAND in PWH on suppressive ART are not well characterized [3,4]. Although viral reservoirs in brain can impact neuroinflammation and cognitive function [3,5,6], other factors including

systemic immune activation/inflammation, oxidative stress, metabolic abnormalities, blood-brain barrier (BBB) dysfunction, and ART drug neurotoxicity contribute to mechanisms involved in HAND in ART-treated PWH [4,7−9]. Comorbidities including vascular disease and substance use disorder also impact the development of HAND [3,4,10,11]. The identification of biomarkers associated with cognitive impairment and central nervous system (CNS) injury due to HIV-related vs. non-HIV-related etiologies is important for understanding biologically defined subtypes (biotypes) of HAND and development of tailored interventions [2,12,13].

Vascular disease is more prevalent in PWH compared with the general population [14−16]. The increased prevalence of atherosclerosis, coronary artery disease, and cerebrovascular disease reflects HIV-related factors including immune activation/inflammation, metabolic abnormalities, and effects of some ART drugs, as well as high rates of smoking and substance use [14−18]. Due to earlier onset and progression of vascular disease, PWH are also at increased risk for vascular cognitive impairment (VCI) at younger ages compared with the general population [10,19]. Given evidence that VCI contributes to cognitive impairment in PWH on ART [17,20,21], vascular disease has emerged as a significant contributing factor to HAND in the current ART era [10,19,22−25]. Accordingly, biomarkers that can distinguish VCI from other causes of cognitive impairment will be useful for diagnosis and clinical management.

The relationship between markers of vascular injury and HAND in virally suppressed PWH is unclear. Vascular injury markers [e.g. vascular cell adhesion molecule (VCAM-1), intercellular adhesion molecule (ICAM-1), selectins] are increased in PWH on ART [14,18,25−28]. Increased expression of endothelial activation markers such as VCAM-1 and ICAM-1 is associated with systemic and arterial inflammation in PWH on ART [18,27,29]. Consistent with these findings, inflammation markers including interleukin (IL)-6, C-reactive protein (CRP), interferon gamma-induced protein (IP)-10, monocyte chemoattractant protein (MCP)-1, and vascular endothelial growth factor (VEGF)-A are associated with vascular disease in ART-treated PWH [18,30−32]. Plasma and/or cerebrospinal fluid (CSF) neurofilament light chain (NFL), total and phosphorylated Tau, glial fibrillary acidic protein (GFAP), and YKL-40 (also known as Chitinase 3-like 1, CHI3L1) are biomarkers of CNS injury and glial activation in PWH [12,33−39]. Here, we evaluated the relationship between vascular injury markers and HAND by assessing plasma and CSF markers of vascular injury (ICAM-1, VCAM-1, CRP), inflammation (IL-1β, IFN-γ, IL-6, IL-8, IL-15, IP-10, MCP-1, VEGF-A), and CNS injury and glial activation (NFL, total Tau, GFAP, YKL-40) in relation to cognitive impairment in PWH on suppressive ART.

Methods

Study participants

Plasma and CSF samples from 248 eligible individuals ($n = 143$ PWH on ART and $n = 105$ HIV− controls) were collected during 2006−2016 (Supplementary Digital Content 1, http://links.lww.com/QAD/C941). HIV-positive samples were from the National NeuroAIDS Tissue Consortium (NNTC) [40] and CNS HIV Anti-Retroviral Therapy Effects Research (CHARTER) study [41]. All individuals were enrolled with written informed consent and institutional review board (IRB) approval at each study site. All participants were over age 30 years and PWH were taking three or more ART drugs for at least 1 year with current viral suppression in plasma (<200 HIV RNA copies/ml). Eight individuals had plasma viral load (VL) above 200 copies/ml (median VL 357 copies/ml [IQR 323−425]) but suppressed VL < 200 copies/ml at prior visits. PWH with HIV-associated dementia (HAD) or neuropsychological impairment due to other causes (NPI-O) were excluded because HAD diagnoses are rare in the current ART era and NPI-O reflects confounding diagnoses. Plasma and CSF samples from HIV− individuals without a diagnosed neurological disease (from Bioreclamation LLC, Westbury, New York, USA) were group-matched for age, gender, and race.

Assessment of cognitive impairment and medical comorbidities

PWH were administered a comprehensive neuropsychological test battery designed to assess seven neurocognitive domains. Demographically corrected global neurocognitive T scores were generated from individual test T scores as described [42]. T scores correlate negatively with severity of cognitive impairment, with values <40 (corresponding to 1 standard deviation of 10 below a normalized mean of 50) signifying cognitive impairment. HAND clinical diagnoses were determined using established criteria [43] based on neurocognitive testing and neurological evaluation. PWH were classified as cognitively impaired if they had a clinical HAND diagnosis of ANI or MND, corresponding to mild cognitive impairment with no interference or mild interference with everyday functioning, respectively. A history of medical comorbidities (e.g. hypertension, hyperlipidemia, diabetes, coronary artery disease, myocardial infarction, cerebrovascular disease, ischemic stroke, lacunes) was obtained from self-report, medical records, and review of medications and lab values as described [44]. Traditional cardiovascular risk factors were defined based on two or more visits with self-reported use of medications for these conditions or laboratory test values as follows: hypertension, systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg (stage 2 hypertension); hyperlipidemia, total cholesterol >240 mg/dl; diabetes, hemoglobin A1C ≥6.5%.

Meso Scale Discovery assays

Plasma and CSF samples were centrifuged at 1200 rpm for 5 min at 4°C to remove cells, and supernatants were aliquoted and stored at −80°C. Vascular injury (ICAM-1, VCAM-1, CRP), inflammation (IFN-γ, IL-1β, IL-6, IL-8, IL-15, IP-10, MCP-1, VEGF-A), CNS injury and glial activation (NFL, total Tau, GFAP, YKL-40) markers were measured in plasma and CSF using the Meso Scale Discovery (MSD) platform (Rockville, Maryland, USA). Vascular injury markers were measured using V-Plex vascular injury panel 2, inflammation markers using a U-Plex custom panel, and CNS injury and glial activation markers using R-Plex custom panels according to manufacturer's protocols. Plates were read on Meso Sector S 600 imager and data analyzed using MSD discovery workbench 4.0 software.

Measurement of cerebrospinal fluid/plasma albumin ratio

Albumin concentrations in paired plasma and CSF samples were measured using the BCG albumin assay kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Absorbance was measured at 570 nm, concentrations calculated using standard curves, and CSF/plasma albumin ratio (Qalb) calculated as an indirect indicator of BBB permeability.

Statistical analysis

Demographics, clinical covariates, and biomarker levels were compared between two groups of interest using the chi-square test for categorical variables and Mann−Whitney U test for continuous variables. Relationships between continuous variables were analyzed by Spearman's rank correlation. Associations between plasma vascular or CNS injury markers and global neurocognitive T scores were examined in linear and logistic regression models adjusted for age with T scores as a continuous or binary (<40 vs. ≥40) dependent variable, respectively, and $\log_{10}$-transformed plasma biomarkers as predictors. A two-sided P-value <0.05 was considered statistically significant (no Bonferroni correction was applied). These analyses were performed using GraphPad Prism, version 9.0 (GraphPad Software, San Diego, California, USA). Backward elimination starting with the full model (six biomarkers adjusted for age) followed by sequential elimination of biomarkers with the smallest contribution was used to develop an optimal regression model based on lowest Akaike information criterion (AIC) value. For biomarker-driven clustering of HAND ($n = 71$ after omitting three cases with missing values), the following R packages were used (R version 4.2.0; R Foundation for Statistical Computing, Vienna, Austria): principal component analysis (PCA) was performed with 15 biomarkers using the FactoMineR package, K-means clustering was performed using the stats package, and clusters were visualized using the factoextra package.

Results

Study cohort

Demographic and clinical characteristics of the study population are shown in Table 1 and overview of the study design is shown in Supplementary Digital Content 1, http://links.lww.com/QAD/C941. The cohort consisted of 143 PWH (median age 53 years [IQR 47−58 years]), 85% male, 71% white, with median duration of HIV infection 16 years, CD4$^+$ cell count 505 cells/μl, and nadir CD4$^+$ cell count 88 cells/μl. All PWH were on ART (59% on a protease inhibitor, 19% on an integrase inhibitor) with 94 and 96% having plasma and CSF VL <200 copies/ml and <50 copies/ml, respectively. Among PWH, 52% were diagnosed with mild forms of HAND (59.4% ANI, 40.6% MND). Cardiovascular and cerebrovascular disease (CVD) (e.g. coronary artery disease, myocardial infarction, ischemic stroke, silent brain infarcts, lacunes; hereafter termed vascular disease) was more prevalent among PWH with HAND (26%), particularly MND (40%), compared with no HAND (7%) ($P = 0.004$ and $P = 0.0006$, respectively) (Table 1 and Supplementary Digital Content 2, http://links.lww.com/QAD/C941). Prevalence of two to three cardiovascular risk factors and tobacco smoking was also higher in HAND (30 and 55%, respectively) vs. no HAND (17 and 39%) ($P = 0.12$ and $P = 0.05$, respectively). PWH with vascular disease were slightly older (median age 56 vs. 51 years; $P = 0.02$), with higher prevalence of tobacco smoking (75 vs. 42%; $P = 0.003$), hypertension (63 vs. 44%; $P = 0.09$), hyperlipidemia (38 vs. 22%; $P = 0.10$), and HAND (79 vs. 46%; $P = 0.003$) and lower neurocognitive T scores (median 42 vs. 49; $P = 0.002$) compared to those without vascular disease (Supplementary Digital Content 2, http://links.lww.com/QAD/C941).

Association of vascular injury markers with human immunodeficiency virus-associated neurocognitive disorders

To evaluate relationships of vascular injury markers to HAND, we compared levels of plasma ICAM-1, VCAM-1, and CRP between groups by HIV status, HAND, and vascular disease (Fig/ 1a). Plasma ICAM-1 and CRP, but not VCAM-1, were increased in PWH compared with HIV− controls ($P = 0.004$, $P = 0.0005$, and $P = 0.11$, respectively). Among PWH, ICAM-1 and VCAM-1 increased ($P = 0.01$), and CRP showed an increasing trend ($P = 0.08$) in HAND vs. no HAND. We further examined the association of vascular injury markers with ANI and MND (Supplemental Digital Content 3, http://links.lww.com/QAD/C941). VCAM-1 was higher in PWH with MND compared with ANI ($P = 0.04$) or no HAND ($P = 0.001$), while ICAM-1 was higher in ANI and showed an increasing trend in MND ($P = 0.007$ and $P = 0.18$, respectively). Plasma VCAM-1, but not ICAM-1 or CRP, was higher in PWH with vs. without vascular disease ($P = 0.049$) (Fig. 1a). Neither HIV

Table 1. Demographic and clinical characteristics of study participants.

	HIV− ($n = 105$)	HIV+ ($n = 143$)	No HAND ($n = 69$)	HAND ($n = 74$)	P-value
Age (years)	54 (47–61)	53 (47–58)	53 (47–58)	52 (47–58)	0.98
Male gender (n, %)	74 (70)	121 (85)	62 (90)	58 (78)	0.07
Race (n, %)[a]					0.98
Black	22 (27)	36 (25)	18 (26)	18 (24)	
White	58 (72)	101 (71)	48 (70)	53 (72)	
Other	1 (1)	6 (4)	3 (4)	3 (4)	
Current smoking (n, %)	48 (52)	68 (48)	27 (39)	41 (55)	0.07
Current alcohol use (n, %)		67 (47)	34 (49)	34 (46)	0.74
BMI (kg/m^2)	28 (23–34)	26 (22–30)	25 (22–28)	27 (22–33)	0.12
Diabetes (n, %)		21 (15)	8 (12)	13 (18)	0.35
Hypertension (n, %)		67 (47)	32 (46)	35 (47)	1.00
Hyperlipidemia (n, %)		35 (25)	14 (20)	21 (28)	0.33
2–3 Cardiovascular risk factors (n, %)[c]		35 (25)	12 (17)	22 (30)	0.12
Cerebrovascular disease/CVD (n, %)		24 (17)	5 (7)	19 (26)	0.004
Neurocognitive T scores		48 (42–53)	52 (49–57)	42 (38–47)	<0.0001
Global clinical rating		4 (3–5)	3 (2–4)	5 (4–6)	<0.0001
CSF protein		37 (31–47)	41 (32–50)	36 (30–45)	0.07
CSF WBC (cells/μl)		2 (1–3)	2 (1–3)	1 (1–4)	0.77
Duration of HIV infection (years)		16 (11–21)	16 (11–21)	16 (11–21)	0.90
Plasma viral load <200 copies/ml		134 (94)	65 (94)	69 (95)	1.00
CSF viral load <50 copies/ml[b]		122 (96)	60 (97)	61 (94)	0.68
CD4$^+$ cell count (cells/μl)		505 (328–734)	504 (339–827)	506 (291–681)	0.41
Nadir CD4$^+$ cell count (cells/μl)		88 (17–211)	88 (13–244)	91 (21–186)	0.84
ART use (n, %)					0.69
Protease inhibitors		84 (59)	41 (59)	43 (58)	
Integrase inhibitors		27 (19)	12 (17)	15 (20)	
Duration of ART (years)		10 (6–14)	10 (6–14)	9 (5–14)	0.67

Median (interquartile range) are shown unless otherwise indicated. P-values for two-group comparisons between HAND vs.no HAND were calculated using chi-square test for categorical variables and Mann–Whitney U test for continuous variables; bold font denotes $P < 0.05$. HAND diagnoses were asymptomatic neurocognitive impairment (ANI, $n = 44$) or mild neurocognitive disorder (MND, $n = 30$). ART, antiretroviral therapy; BMI, body mass index; CVD, cardiovascular disease; HAND, HIV-associated neurocognitive disorders; WBC, white blood cells.
[a]Race, current smoking, and BMI data not available for 24, 13, and 57 HIV- individuals.
[b]CSF viral load data not available for 16 HIV+ individuals.
[c]2–3 cardiovascular risk factors included diabetes, hyperlipidemia, and hypertension.

infection nor HAND were associated with increased levels of these markers in CSF (Fig. 1b).

Central nervous system injury markers in relation to human immunodeficiency virus-associated neurocognitive disorders and vascular disease

Next, we examined associations of CNS injury and glial activation markers with HIV, HAND, and vascular disease (Fig. 2). HIV infection was associated with increased plasma NFL and GFAP ($P = 0.004$ and $P = 0.005$, respectively), and increased CSF total Tau and GFAP ($P = 0.03$ and $P < 0.0001$, respectively). HAND was associated with increased plasma and CSF YKL-40 ($P = 0.004$ and $P = 0.003$, respectively), and trend toward increased CSF Tau ($P = 0.07$) compared with no HAND (Fig. 2a). Although we did not detect association of plasma NFL with HAND, we observed an increasing trend of plasma NFL ($P = 0.06$) in MND vs. ANI (Supplemental Digital Content 3, http://links.lww.com/ QAD/C941) and increased plasma NFL and GFAP in PWH with vs. without vascular disease ($P = 0.004$ and $P = 0.04$, respectively) (Fig. 2a). There was no significant association of HAND with CSF/plasma albumin ratio (Qalb) (median 4.9 vs. 3.7 in HAND vs. no HAND, respectively; $P = 0.68$).

Vascular injury markers correlate with lower neurocognitive test scores

Next, we evaluated relationships between vascular or CNS injury markers and global neurocognitive T scores, which reflect severity of HAND [9]. Plasma NFL, YKL-40, VCAM-1, and ICAM-1 levels correlated negatively with global T scores (Fig. 2b), while no significant correlations were detected between GFAP or CSF biomarkers and global T scores. In linear regression models adjusted for age (Table 2), higher plasma NFL ($\beta = -4.06$, $P = 0.019$), YKL-40 ($\beta = -3.57$, $P = 0.001$), VCAM-1 ($\beta = -3.57$, $P = 0.003$), ICAM-1 ($\beta = -9.05$, $P = 0.008$), and CRP ($\beta = -8.37$, $P = 0.007$), but not GFAP ($P = 0.2$) were associated with lower global T scores. The optimal model included two biomarkers, VCAM-1 and YKL-40, based on backward elimination to select the model with lowest AIC value (595, 595, 593, and 589 for the full model, VCAM-1, YKL-40, and both biomarkers, respectively). The models were not adjusted for gender or race because global T scores were not significantly different between males vs. females ($P = 0.97$) or white vs. nonwhite race ($P = 0.75$). We further assessed association of CNS and vascular injury markers in logistic regression models with cognitive impairment defined as global T scores <40 vs. ≥40, corresponding to the impaired vs. unimpaired range,
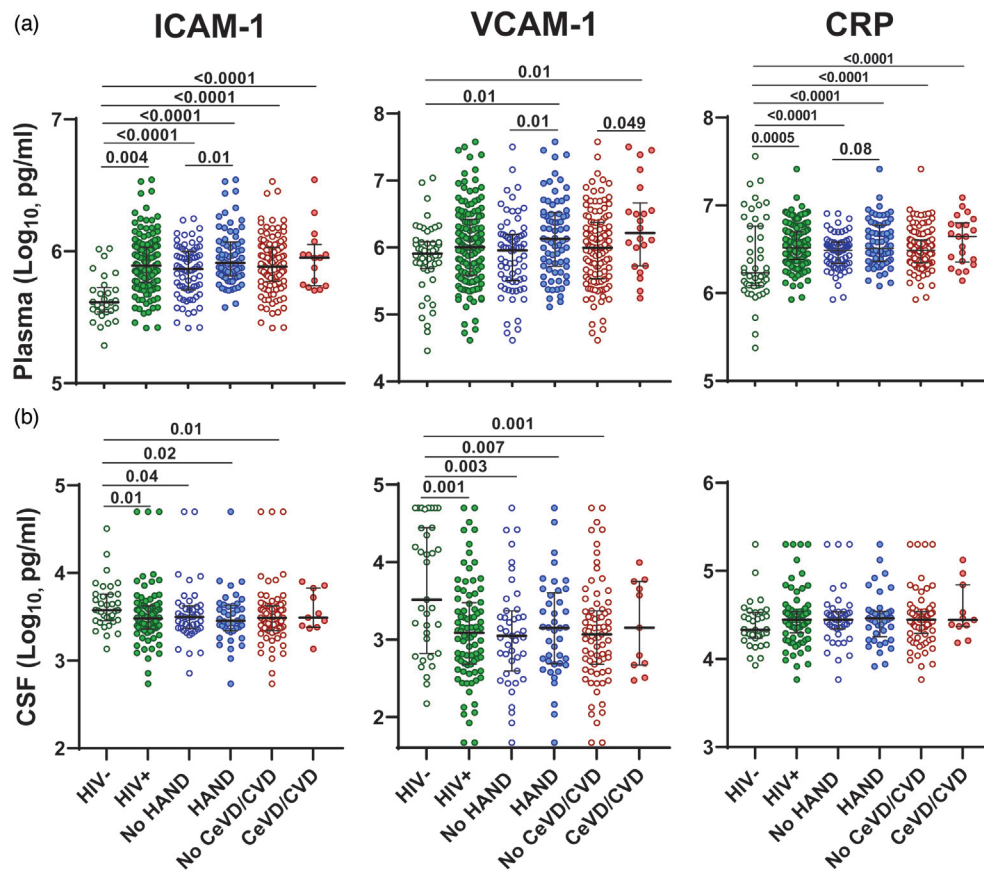
Fig. 1. HIV infection and HAND are associated with increased plasma vascular injury markers. (a) Association of plasma ICAM-1, VCAM-1, and CRP with HIV infection, HAND, and vascular disease. (b) Association of CSF ICAM-1, VCAM-1, and CRP with HIV infection, HAND, and vascular disease. Medians and IQRs are indicated as horizontal and vertical lines, respectively. Statistical significance was calculated using Mann–Whitney U test; significant differences ($P < 0.05$) are indicated. CRP, C-reactive protein; CSF, cerebrospinal fluid; HAND, HIV-associated neurocognitive disorders; ICAM-1, intercellular adhesion molecule-1.

respectively (Supplemental Digital Content 4, http://links.lww.com/QAD/C941). Plasma NFL ($\beta = 1.057$, $P = 0.03$), YKL-40 ($\beta = 1.23$, $P = 0.004$), VCAM-1 ($\beta = 1.22$, $P = 0.003$), and ICAM-1 ($\beta = 2.38$, $P = 0.03$) but not GFAP ($P = 0.09$) were associated with global T scores <40 in these models.

Vascular injury markers correlate with central nervous system injury and glial activation markers

To assess inter-relationships between vascular and CNS injury markers in PWH, we performed correlation analyses. Plasma VCAM-1 correlated with plasma NFL and YKL-40 ($r = 0.29$, $P = 0.003$; $r = 0.17$, $P = 0.04$, respectively), while plasma ICAM-1 and CRP correlated with plasma YKL-40 ($r = 0.45$, $P < 0.0001$ and $r = 0.443$, $P < 0.0001$, respectively) and CSF YKL-40 ($r = 0.279$, $P = 0.01$ and $r = 0.288$, $P = 0.008$) (Fig. 2b). CSF YKL-40 correlated with plasma NFL, CSF NFL, and CSF GFAP ($r = 0.233$, $P = 0.075$; $r = 0.377$, $P = 0.0005$ and $r = 0.230$, $P = 0.04$, respectively). Plasma ICAM-1, CRP,

and NFL correlated with CSF YKL-40, but showed no significant association with increased Qalb (Supplemental Digital Content 5, http://links.lww.com/QAD/C941).

Inflammation markers in relation to human immunodeficiency virus-associated neurocognitive disorders and vascular disease

Given that inflammation is a contributing factor to development of HAND [4,8,37,45], we examined inflammation marker levels by HIV status, HAND, and vascular disease. Plasma IL-1β, IL-8, IP-10, MCP-1, and VEGF-A were higher ($P = 0.02$, $P = 0.002$, $P < 0.0001$, $P = 0.003$, and $P < 0.0001$, respectively) in PWH compared with HIV− controls (Fig. 3a). Plasma IL-6 was increased in HAND, ANI, or MND, vs. no HAND ($P < 0.0001$, $P = 0.01$, and $P < 0.0001$, respectively), while IL-15, IFN-γ, and VEGF-A were increased in MND compared with no HAND ($P = 0.01$, $P = 0.02$, and $P = 0.04$, respectively) (Fig. 3a and Supplemental Digital Content 3, http://links.lww.com/QAD/C941). Plasma IL-6 and MCP-1 were increased ($P = 0.03$ and
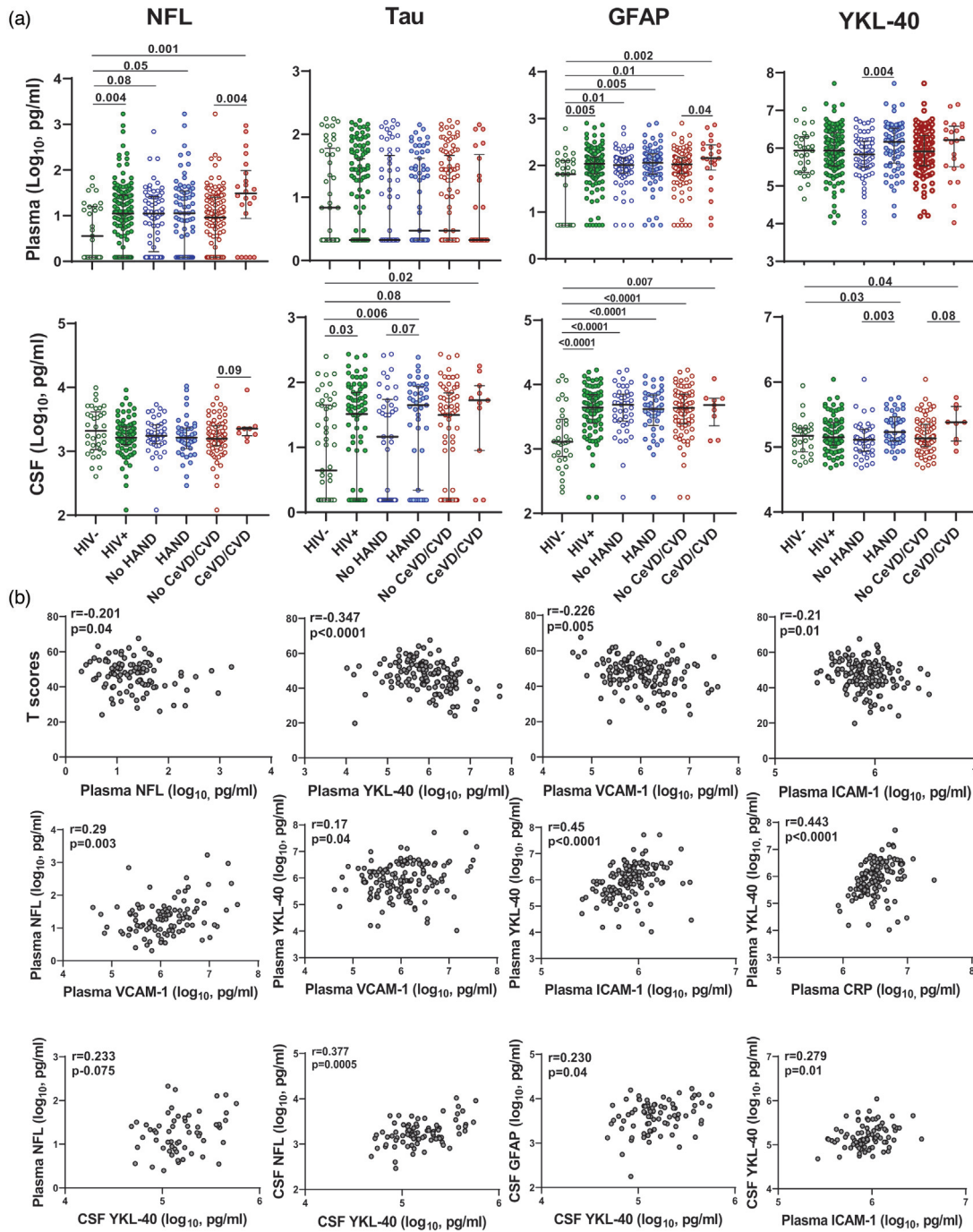
Fig. 2. Vascular injury markers are associated with lower neurocognitive *T* scores and correlate with CNS injury markers. (a) Association of plasma (top) and CSF (bottom) NFL, total Tau, GFAP, and YKL-40 with HIV infection, HAND, and vascular disease. Medians and IQRs are indicated as horizontal and vertical lines, respectively. Statistical significance was calculated using Mann–Whitney *U* test; significant differences (*P* < 0.05) are indicated. (b) Plasma NFL, YKL-40, ICAM-1, and VCAM-1 levels correlate negatively with global neurocognitive *T* scores, plasma VCAM-1 correlates positively with plasma NFL, and plasma VCAM-1, ICAM-1, and CRP correlate positively with plasma YKL-40 in PWH (middle panels). Inter-relationship of plasma and CSF CNS injury and glial activation markers with plasma vascular injury markers are shown in the bottom panels. Relationships between continuous variables were analyzed by Spearman's rank correlation (significant correlations *P* < 0.05). CNS, central nervous system; ICAM-1, intercellular adhesion molecule-1.

Table 2. Association of plasma NFL, GFAP, YKL-40, ICAM-1, VCAM-1, and CRP with global neurocognitive *T* scores in linear regression analysis adjusted for age.

Variable	β	SE	95% CI	P-value
Intercept	50.21	2.38	45.5 to 54.9	<0.0001
NFL	−4.06	1.70	−7.42 to −0.70	0.019
Intercept	53.12	4.52	44.2 to 62.1	<0.0001
GFAP	−2.88	2.26	−7.34 to 1.58	0.20
Intercept	69.16	6.61	56.1 to 82.2	<0.0001
YKL-40	−3.57	1.08	−5.72 to −1.43	0.001
Intercept	101.60	20.27	61.5 to 141.7	<0.0001
ICAM-1	−9.05	3.40	−15.77 to −2.33	0.008
Intercept	68.93	7.18	54.7 to 83.1	<0.0001
VCAM-1	−3.57	1.19	−5.92 to −1.21	0.003
Intercept	102.60	20.04	63.0 to 142.2	<0.0001
CRP	−8.37	3.05	−14.41 to −2.34	0.007
Intercept	83.31	8.68	66.1 to 100.5	<0.0001
YKL-40	−3.05	1.09	−5.19 to −0.90	0.006
VCAM-1	−2.91	1.18	−5.26 to −0.57	0.015

Multivariable linear regression models were fit for global *T* scores in PWH (n = 140) as the dependent variable adjusting for age. Independent variables (plasma NFL, GFAP, YKL-40, ICAM-1, VCAM-1, CRP) were log10 transformed. The optimal model based on backward elimination and lowest AIC values included both YKL-40 and VCAM-1. SE, standard error; CI, confidence interval.

P = 0.01, respectively) and IL–15 showed an increasing trend (*P* = 0.05) (Fig. 3a) in PWH with vs. without vascular disease. Among CSF markers, only IP-10 showed a significant difference by HIV status (*P* = 0.02), and none were significantly associated with HAND (Supplemental Digital Content 6, http://links.lww.com/QAD/C941).

Biomarker clustering separates human immunodeficiency virus-associated neurocognitive disorders into clusters distinguished by inflammation profiles, prevalence of vascular disease, and progression to HIV-associated dementia

K-means clustering based on 15 plasma biomarkers in 71 individuals with HAND combined with dimensionality reduction by PCA identified three distinct clusters (Fig. 3b). Among 71 individuals with HAND, 62 had one or more follow-up visits with neurocognitive evaluations at 6- or 12-month intervals including 10 (16%) who progressed to HAD within 2.5 years. The first and second principal components (Dim1 and Dim2) explained 22.8 and 17.2% of the variance, respectively, mainly driven by IL-6, IL-8, IP-10, VCAM-1, MCP-1, VEGF-1, and NFL (Dim1) and CRP, ICAM-1, YKL-40, VEGF-A, and IL-15 (Dim2) (Fig, 3b and Supplemental Digital Content 7, http://links.lww.com/QAD/C941). Biomarker and clinical characteristics according to each cluster are shown in Fig. 3c and Supplemental Digital Content 8, http://links.lww.com/QAD/C941. Cluster 1 (*n* = 30) represented a reference profile with no distinctive biomarker elevations, younger age (13% over age 60), and relatively lower rates of hypertension (37%), hyperlipidemia (23%), vascular disease (7%), and progression to HAD within 2.5 years (8%). Cluster 2 (*n* = 30) represented a mixed profile characterized by increased plasma VCAM-1, NFL, total Tau, CRP, ICAM-1, and YKL-40, more individuals over age 60 (20%), and relatively higher rates of hypertension (47%), vascular disease

(27%) and progression to HAD within 2.5 years (21%). Cluster 3 (*n* = 11) represented a vascular profile characterized by increased plasma VCAM-1, NFL, IL-6, IL-8, IL-15, and MCP-1, high proportion of individuals over age 60 (46%), and highest rates of hypertension (82%), hyperlipidemia (64%), vascular disease (64%) and progression to HAD within 2.5 years (25%). Regarding other distinctive features, cluster 3 had more current smokers compared with clusters 1 and 2 (73 vs. 50 and 57%, respectively), while cluster 2 had lower global *T* scores compared with clusters 1 and 3 (median 40.9 vs. 43.6 and 43.5, respectively; *P* = 0.043).

Discussion

In this study, vascular injury markers VCAM-1, ICAM-1, and CRP were associated with HAND and lower global neurocognitive *T* scores in PWH on suppressive ART. Furthermore, VCAM-1 correlated with the neuronal injury marker NFL. Consistent with these findings, cardiovascular risk factors and diagnoses were more prevalent in HAND vs. no HAND and biomarker clustering identified HAND subgroups distinguished by high prevalence of vascular disease and progression to HAD. In the current ART era, HAND frequently has non–HIV-etiologies, with vascular disease being one of the most common [10,19−21,46], and neurocognitive profiles can resemble VCI [10,17,19−21,24,25,46]. Although plasma inflammation markers (e.g., IL-1β, IL-8, IP-10, MCP-1) were increased in PWH, only IL-6 was associated with HAND (particularly MND). These findings suggest that vascular injury markers have a stronger association with cognitive impairment and CNS injury in virally suppressed PWH than inflammation markers and may help to distinguish relative contributions of VCI to HAND.
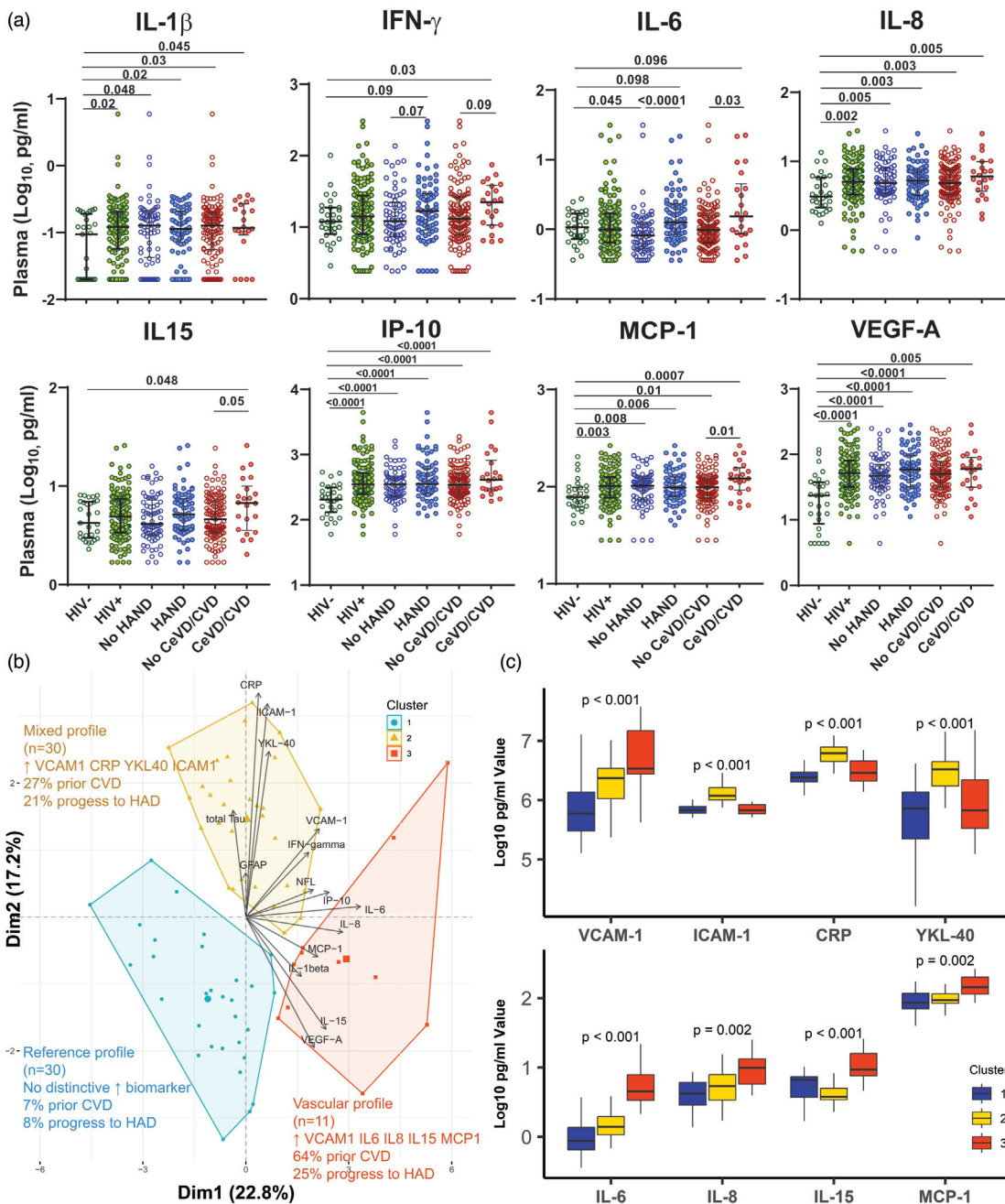
Fig. 3. Association of plasma inflammation markers with HIV infection, HAND, and vascular disease. (a) Association of plasma IL-1β, IFN-γ, IL-6, IL-8, IL-15, IP-10, MCP-1, and VEGF-A with HIV infection, HAND, and vascular disease. Medians and IQRs are indicated as horizontal and vertical lines, respectively. Statistical significance was calculated using Mann–Whitney U test; significant differences ($P < 0.05$) are indicated. (b and c) K-means clustering of 15 plasma biomarkers combined with dimensionality reduction by principal component analysis (PCA) among PWH with HAND ($n = 71$) identifies three clusters distinguished by inflammation marker profiles, prevalence of prior vascular disease, and progression to HAD within 2.5 years. Contributions of each biomarker to Dim1 and Dim2 are shown on the PCA plot (b) and Supplemental Digital Content 7, http://links.lww.com/QAD/C941. Statistical significance was calculated using the Kruskal–Wallis H test. HAND, HIV-associated neurocognitive disorders; IL, interleukin.

Markers of endothelial dysfunction, including VCAM-1 and ICAM-1, are predictors of severity and progression of vascular disease and have been associated with VCI in the general population [47]. Furthermore, increased VCAM-1 was associated with deficits in executive function, processing speed, and working memory in a recent study of 84 PWH [25]. VCAM-1 and ICAM-1 are adhesion molecules that accelerate vascular inflammation and

atherosclerosis by increasing attachment of circulating leukocytes to endothelial cells, while CRP is a marker of systemic inflammatory responses that promote vascular disease [18]. Our finding that ICAM-1 and CRP were increased in ART-treated PWH vs. HIV- controls is consistent with previous reports [27,28,32]. In contrast to some previous studies [25,26], we did not detect a significant increase in VCAM-1 in ART-treated PWH vs. HIV- controls. Nonetheless, VCAM-1 and ICAM-1 were associated with HAND, VCAM-1 was higher in MND compared with ANI, and VCAM-1, ICAM-1, and CRP were associated with lower global *T* scores in age-adjusted regression models. Moreover, VCAM-1 correlated positively with NFL, suggesting association with ongoing CNS injury. Accordingly, VCAM-1 is a potential biomarker for elevated risk of VCI in PWH and may be useful for monitoring responses to interventions targeting vascular disease.

IL-6, CRP, and MCP-1 are inflammation markers associated with vascular disease in PWH [30,32,48]. Despite marked reduction of inflammation in PWH on ART, low-level inflammation persists and contributes to vascular and neurological comorbidities [5,8,18,45]. Our finding that PWH with vascular disease had higher plasma IL-6 and MCP-1 compared to those without vascular disease is consistent with previous studies [30,32,49] and role of inflammation in promoting atherosclerosis [15,18,27,31]. Furthermore, biomarker clustering separated HAND into three clusters: two clusters with high prevalence of vascular disease, elevated VCAM-1 and NFL, and distinctive inflammation profiles (CRP/ICAM-1/YKL-40 or IL-6/IL-8/IL-15/MCP-1) and one cluster with no distinctive biomarker elevations, which may represent legacy effects of untreated HIV and its complications [3]. Together, these findings raise the possibility that targeting vascular inflammation may be beneficial to prevent or ameliorate VCI in PWH [48].

YKL-40, a biomarker of cardiovascular disease and neuroinflammation [50−52], correlated positively with vascular injury markers and was increased in plasma and CSF of PWH with HAND vs. no HAND. Moreover, the best model explaining the association between plasma biomarkers and neurocognitive performance included the combination of YKL-40 and VCAM-1. YKL-40 can be generated from multiple sources including macrophages/microglia, astrocytes, chondrocytes, neutrophils, and fibrotic liver tissue, and is involved in peripheral and CNS inflammation, tissue remodeling, and vascular disease [50,52]. Although the role of CSF YKL-40 as a prognostic marker for HAND [34,39] and Alzheimer's disease [51] has been documented, the role of plasma YKL-40 in relation to cognitive impairment in PWH is not well studied and warrants further evaluation to understand its relationship to biologically defined subtypes of HAND.

Plasma NFL is a sensitive biomarker of CNS injury in HAND and other neurological disorders [33,38,53−55]. In our study, plasma NFL was increased in PWH compared with uninfected controls, albeit not significantly different between HAND vs. no HAND. Plasma NFL correlated negatively with global *T* scores and the association remained significant in models adjusted for age, suggesting an association with subclinical CNS injury. Moreover, vascular disease was associated with increased plasma NFL. Previous studies detected increased plasma or CSF NFL in people with cerebral small vessel disease-related white matter injury [56,57], a common pathology in PWH [21,24,46] and correlate of VCI in the general population [47]. Total Tau, a biomarker of neurodegeneration in Alzheimer's disease and other neurodegenerative disorders, showed an increasing trend in CSF, but not plasma, in HAND compared with no HAND. Previous studies of CSF total Tau in PWH reported inconsistent findings, which may reflect differences in study design such as cohort age, viral suppression, and severity of cognitive impairment [12,35,36,58]. Further studies are warranted to understand the prognostic value of NFL, Tau, and other markers of CNS injury in older PWH in relation to current and future risk of cognitive impairment.

We acknowledge several limitations of the study. Our study was limited by the sample size and fewer participants with available CSF samples, which limited statistical power to detect some associations. Additionally, we did not validate the biomarker-defined clusters in a separate cohort. Although we detected a modest increase of CSF NFL in cognitively impaired PWH in a previous study that included some participants with HAD or viremia [59], similar to findings reported by others [33,38,45,55], we did not detect an association of HAND with CSF NFL in the present study. Other limitations are the low proportion of PWH on integrase inhibitors (19%) and newer ART drugs, lack of available neuroimaging data, and lack of data to address potential confounders such as medical comorbidities, mental health disorders, and socioeconomic status. Additionally, we did not evaluate the relationship of increased VCAM-1 to deficits in specific cognitive domains [25]. Lastly, as this was a cross-sectional study performed in the United States, we could not evaluate prospective associations of biomarkers with future development of HAND or findings that may differ across international settings. Longitudinal studies of larger cohorts including older participants, newer ART drugs, and diverse populations are needed to overcome these limitations and identify biomarkers associated with progression of vascular injury and HAND in ART-treated PWH.

In summary, we present evidence that peripheral markers of vascular injury are more closely associated with HAND and CNS injury in PWH on suppressive ART than markers of inflammation. Our findings are consistent

with the three-hit model proposed by Jakabek *et al.* [20] in which HIV infection, aging, and vascular disease contribute to brain aging in PWH, and suggest that vascular injury markers, particularly VCAM-1, may help to distinguish relative contributions of VCI to HAND. Further studies are warranted to better understand the utility of VCAM-1 and other peripheral markers of vascular injury for identification of HAND biotypes [11,13] and development of tailored interventions in virally suppressed PWH on ART.

Acknowledgements

Conflicts of interest
There are no conflicts of interest.

References

1. Wang Y, Liu M, Lu Q, Farrell M, Lappin JM, Shi J, *et al.* **Global prevalence and burden of HIV-associated neurocognitive disorder: a meta-analysis.** *Neurology* 2020; **95**:e2610–e2621.
2. Nightingale S, Dreyer AJ, Saylor D, Gisslen M, Winston A, Joska JA. **Moving on from HAND: why we need new criteria for cognitive impairment in people with HIV and a proposed way forward.** *Clin Infect Dis* 2021; **73**:1113–1118.
3. Winston A, Spudich S. **Cognitive disorders in people living with HIV.** *Lancet HIV* 2020; **7**:e504–e513.
4. Saylor D, Dickens AM, Sacktor N, Haughey N, Slusher B, Pletnikov M, *et al.* **HIV-associated neurocognitive disorder — pathogenesis and prospects for treatment.** *Nat Rev Neurol* 2016; **12**:234–248.
5. Chen MF, Gill AJ, Kolson DL. **Neuropathogenesis of HIV-associated neurocognitive disorders: roles for immune activation, HIV blipping and viral tropism.** *Curr Opin HIV AIDS* 2014; **9**:559–564.
6. Gabuzda D, Yin J, Misra V, Chettimada S, Gelman BB. **Intact proviral DNA analysis of the brain viral reservoir and relationship to neuroinflammation in people with HIV on suppressive antiretroviral therapy.** *Viruses* 2023; **15**:1009.
7. Anderson AM, Jang JH, Easley KA, Fuchs D, Gisslen M, Zetterberg H, *et al.* **Cognitive and neuronal link with inflammation: a longitudinal study in people with and without HIV infection.** *J Acquir Immune Defic Syndr* 2020; **85**:617–625.
8. McArthur JC, Johnson TP. **Chronic inflammation mediates brain injury in HIV infection: relevance for cure strategies.** *Curr Opin Neurol* 2020; **33**:397–404.
9. Lyons JL, Uno H, Ancuta P, Kamat A, Moore DJ, Singer EJ, *et al.* **Plasma sCD14 is a biomarker associated with impaired neurocognitive test performance in attention and learning domains in HIV infection.** *J Acquir Immune Defic Syndr* 2011; **57**:371–379.
10. Cysique LA, Brew BJ. **Vascular cognitive impairment and HIV-associated neurocognitive disorder: a new paradigm.** *J Neurovirol* 2019; **25**:710–721.
11. Aung HL, Alagaratnam J, Chan P, Chow FC, Joska J, Falutz J, *et al.* **Cognitive health in persons with human immunodeficiency virus: the impact of early treatment, comorbidities, and aging.** *J Infect Dis* 2023; **227**:S38–S47.
12. Bandera A, Taramasso L, Bozzi G, Muscatello A, Robinson JA, Burdo TH, *et al.* **HIV-associated neurocognitive impairment in the modern ART era: are we close to discovering reliable biomarkers in the setting of virological suppression?** *Front Aging Neurosci* 2019; **11**:187.
13. Mukerji SS, Petersen KJ, Pohl KM, Dastgheyb RM, Fox HS, Bilder RM, *et al.* **Machine learning approaches to understand cognitive phenotypes in people with HIV.** *J Infect Dis* 2023; **227**:S48–S57.
14. So-Armah K, Benjamin LA, Bloomfield GS, Feinstein MJ, Hsue P, Njuguna B, *et al.* **HIV and cardiovascular disease.** *Lancet HIV* 2020; **7**:e279–e293.
15. Demir OM, Candilio L, Fuster D, Muga R, Barbaro G, Colombo A, *et al.* **Cardiovascular disease burden among human immunodeficiency virus-infected individuals.** *Int J Cardiol* 2018; **265**:195–203.
16. Gutierrez J, Albuquerque ALA, Falzon L. **HIV infection as vascular risk: a systematic review of the literature and meta-analysis.** *PLoS One* 2017; **12**:e0176686.
17. McIntosh EC, Tureson K, Rotblatt LJ, Singer EJ, Thames AD. **HIV, vascular risk factors, and cognition in the combination antiretroviral therapy era: a systematic review and meta-analysis.** *J Int Neuropsychol Soc* 2021; **27**:365–381.
18. Stein JH, Hsue PY. **Inflammation, immune activation, and CVD risk in individuals with HIV infection.** *JAMA* 2012; **308**:405–406.
19. Brew BJ. **Has HIV-associated neurocognitive disorders now transformed into vascular cognitive impairment?** *AIDS* 2016; **30**:2379–2380.
20. Jakabek D, Rae CD, Brew BJ, Cysique LA. **Brain aging and cardiovascular factors in HIV: a longitudinal volume and shape MRI study.** *AIDS* 2022; **36**:785–794.
21. Gutierrez J, Porras TN, Yoo-Jeong M, Khasiyev F, Igwe KC, Laing KK, *et al.* **Cerebrovascular contributions to neurocognitive disorders in people living with HIV.** *J Acquir Immune Defic Syndr* 2021; **88**:79–85.
22. Wright EJ, Grund B, Robertson K, Brew BJ, Roediger M, Bain MP, *et al.* **Cardiovascular risk factors associated with lower baseline cognitive performance in HIV-positive persons.** *Neurology* 2010; **75**:864–873.
23. Samboju V, Cobigo Y, Paul R, Naasan G, Hillis M, Tsuei T, *et al.* **Cerebrovascular disease correlates with longitudinal brain atrophy in virally suppressed older people living with HIV.** *J Acquir Immune Defic Syndr* 2021; **87**:1079–1085.
24. Sanford R, Strain J, Dadar M, Maranzano J, Bonnet A, Mayo NE, *et al.* **HIV infection and cerebral small vessel disease are independently associated with brain atrophy and cognitive impairment.** *AIDS* 2019; **33**:1197–1205.
25. Saloner R, Sun-Suslow N, Morgan EE, Lobo J, Cherner M, Ellis RJ, *et al.* **Plasma biomarkers of vascular dysfunction uniquely relate to a vascular-risk profile of neurocognitive deficits in virally-suppressed adults with HIV.** *Brain Behav Immun Health* 2022; **26**:100560.
26. Graham SM, Rajwans N, Jaoko W, Estambale BB, McClelland RS, Overbaugh J, *et al.* **Endothelial activation biomarkers increase after HIV-1 acquisition: plasma vascular cell adhesion molecule-1 predicts disease progression.** *AIDS* 2013; **27**:1803–1813.

27. Kulkarni M, Bowman E, Gabriel J, Amburgy T, Mayne E, Zidar DA, *et al.* **Altered monocyte and endothelial cell adhesion molecule expression is linked to vascular inflammation in human immunodeficiency virus infection.** *Open Forum Infect Dis* 2016; **3**:ofw224.

28. Fourie CM, Schutte AE, Smith W, Kruger A, van Rooyen JM. **Endothelial activation and cardiometabolic profiles of treated and never-treated HIV infected Africans.** *Atherosclerosis* 2015; **240**:154–160.

29. Tawakol A, Lo J, Zanni MV, Marmarelis E, Ihenachor EJ, Mac-Nabb M, *et al.* **Increased arterial inflammation relates to high-risk coronary plaque morphology in HIV-infected patients.** *J Acquir Immune Defic Syndr* 2014; **66**:164–171.

30. Hsu DC, Ma YF, Hur S, Li D, Rupert A, Scherzer R, *et al.* **Plasma IL-6 levels are independently associated with atherosclerosis and mortality in HIV-infected individuals on suppressive antiretroviral therapy.** *AIDS* 2016; **30**:2065–2074.

31. Williams JW, Huang LH, Randolph GJ. **Cytokine circuits in cardiovascular disease.** *Immunity* 2019; **50**:941–954.

32. Duprez DA, Neuhaus J, Kuller LH, Tracy R, Belloso W, De Wit S, *et al.* **Inflammation, coagulation and cardiovascular disease in HIV-infected individuals.** *PLoS One* 2012; **7**:e44454.

33. Gisslen M, Price RW, Andreasson U, Norgren N, Nilsson S, Hagberg L, *et al.* **Plasma concentration of the neurofilament light protein (NFL) is a biomarker of CNS injury in HIV infection: a cross-sectional study.** *EBioMedicine* 2016; **3**:135–140.

34. Hermansson L, Yilmaz A, Axelsson M, Blennow K, Fuchs D, Hagberg L, *et al.* **Cerebrospinal fluid levels of glial marker YKL-40 strongly associated with axonal injury in HIV infection.** *J Neuroinflammation* 2019; **16**:16.

35. Ellis RJ, Chenna A, Petropoulos CJ, Lie Y, Curanovic D, Crescini M, *et al.* **Higher cerebrospinal fluid biomarkers of neuronal injury in HIV-associated neurocognitive impairment.** *J Neurovirol* 2022; **28**:438–445.

36. Rubin LH, Sundermann EE, Moore DJ. **The current understanding of overlap between characteristics of HIV-associated neurocognitive disorders and Alzheimer's disease.** *J Neurovirol* 2019; **25**:661–672.

37. Cassol E, Misra V, Morgello S, Gabuzda D. **Applications and limitations of inflammatory biomarkers for studies on neurocognitive impairment in HIV infection.** *J Neuroimmune Pharmacol* 2013; **8**:1087–1097.

38. Jessen Krut J, Mellberg T, Price RW, Hagberg L, Fuchs D, Rosengren L, *et al.* **Biomarker evidence of axonal injury in neuroasymptomatic HIV-1 patients.** *PLoS One* 2014; **9**:e88591.

39. Peluso MJ, Valcour V, Phanuphak N, Ananworanich J, Fletcher JL, Chalermchai T, *et al.* **Immediate initiation of cART is associated with lower levels of cerebrospinal fluid YKL-40, a marker of microglial activation, in HIV-1 infection.** *AIDS* 2017; **31**:247–252.

40. Morgello S, Gelman BB, Kozlowski PB, Vinters HV, Masliah E, Cornford M, *et al.* **The National NeuroAIDS Tissue Consortium: a new paradigm in brain banking with an emphasis on infectious disease.** *Neuropathol Appl Neurobiol* 2001; **27**:326–335.

41. Heaton RK, Clifford DB, Franklin DR Jr, Woods SP, Ake C, Vaida F, *et al.* **HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER Study.** *Neurology* 2010; **75**:2087–2096.

42. Woods SP, Rippeth JD, Frol AB, Levy JK, Ryan E, Soukup VM, *et al.* **Interrater reliability of clinical ratings and neurocognitive diagnoses in HIV.** *J Clin Exp Neuropsychol* 2004; **26**:759–778.

43. Antinori A, Arendt G, Becker JT, Brew BJ, Byrd DA, Cherner M, *et al.* **Updated research nosology for HIV-associated neurocognitive disorders.** *Neurology* 2007; **69**:1789–1799.

44. Lorenz DR, Mukerji SS, Misra V, Uno H, Gelman BB, Moore DJ, *et al.* **Multimorbidity networks associated with frailty among middle-aged and older people with HIV.** *AIDS* 2021; **35**:2451–2461.

45. Gisslen M, Keating SM, Spudich S, Arechiga V, Stephenson S, Zetterberg H, *et al.* **Compartmentalization of cerebrospinal fluid inflammation across the spectrum of untreated HIV-1 infection, central nervous system injury and viral suppression.** *PLoS One* 2021; **16**:e0250987.

46. Moulignier A, Savatovsky J, Assoumou L, Lescure F-X, Lamirel C, Godin O, *et al.* **Silent cerebral small-vessel disease is twice as prevalent in middle-aged individuals with well controlled, combination antiretroviral therapy–treated human immunodeficiency virus (HIV) than in HIV-uninfected individuals.** *Clin Infect Dis* 2018; **66**:1762–1769.

47. Cipollini V, Troili F, Giubilei F. **Emerging biomarkers in vascular cognitive impairment and dementia: from pathophysiological pathways to clinical application.** *Int J Mol Sci* 2019; **20**:2812.

48. Titanji B, Gavegnano C, Hsue P, Schinazi R, Marconi VC. **Targeting inflammation to reduce atherosclerotic cardiovascular risk in people with HIV infection.** *J Am Heart Assoc* 2020; **9**:e014873.

49. Borges AH, O'Connor JL, Phillips AN, Ronsholt FF, Pett S, Vjecha MJ, *et al.* **Factors associated with plasma IL-6 levels during HIV infection.** *J Infect Dis* 2015; **212**:585–595.

50. Deng Y, Li G, Chang D, Su X. **YKL-40 as a novel biomarker in cardio-metabolic disorders and inflammatory diseases.** *Clin Chim Acta* 2020; **511**:40–46.

51. Olsson B, Lautner R, Andreasson U, Ohrfelt A, Portelius E, Bjerke M, *et al.* **CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis.** *Lancet Neurol* 2016; **15**:673–684.

52. Kjaergaard AD, Johansen JS, Bojesen SE, Nordestgaard BG. **Role of inflammatory marker YKL-40 in the diagnosis, prognosis and cause of cardiovascular and liver diseases.** *Crit Rev Clin Lab Sci* 2016; **53**:396–408.

53. Khalil M, Teunissen CE, Otto M, Piehl F, Sormani MP, Gattringer T, *et al.* **Neurofilaments as biomarkers in neurological disorders.** *Nat Rev Neurol* 2018; **14**:577–589.

54. Barro C, Chitnis T, Weiner HL. **Blood neurofilament light: a critical review of its application to neurologic disease.** *Ann Clin Transl Neurol* 2020; **7**:2508–2523.

55. Peterson J, Gisslen M, Zetterberg H, Fuchs D, Shacklett BL, Hagberg L, *et al.* **Cerebrospinal fluid (CSF) neuronal biomarkers across the spectrum of HIV infection: hierarchy of injury and detection.** *PLoS One* 2014; **9**:e116081.

56. Peters N, van Leijsen E, Tuladhar AM, Barro C, Konieczny MJ, Ewers M, *et al.* **Serum neurofilament light chain is associated with incident lacunes in progressive cerebral small vessel disease.** *J Stroke* 2020; **22**:369–376.

57. Meeker KL, Butt OH, Gordon BA, Fagan AM, Schindler SE, Morris JC, *et al.* **Cerebrospinal fluid neurofilament light chain is a marker of aging and white matter damage.** *Neurobiol Dis* 2022; **166**:105662.

58. Milanini B, Valcour V. **Differentiating HIV-associated neurocognitive disorders from Alzheimer's disease: an emerging issue in geriatric NeuroHIV.** *Curr HIV/AIDS Rep* 2017; **14**:123–132.

59. Guha D, Mukerji SS, Chettimada S, Misra V, Lorenz DR, Morgello S, *et al.* **Cerebrospinal fluid extracellular vesicles and neurofilament light protein as biomarkers of central nervous system injury in HIV-infected patients on antiretroviral therapy.** *AIDS* 2019; **33**:615–625.