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Limited correlation between systemic biomarkers and neurocognitive performance before and during HIV treatment

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Abstract

The AIDS Clinical Trials Group (ACTG) study A5303 investigated the associations between neuropsychological performance (NP) and inflammatory biomarkers in HIV-infected participants. Fifteen NP tests were administered at baseline and week 48 to 233 ART naïve participants randomized to maraviroc- or tenofovir-containing ART. Neurocognition correlated modestly with markers of lymphocyte activation and inflammation pre-ART (percent CD38+/HLA-DR+(CD4+) ($r = -0.22$, $p = 0.02$) and percent CD38+/HLA-DR+(CD8+) ($r = -0.25$, $p = 0.02$)), and with some monocyte subsets during ART ($r = 0.25$, $p = 0.02$). Higher interleukin-6 and percent CD38+/HLA-DR+(CD8+) were independently associated with worse severity of HIV-associated neurocognitive disorders (HAND) ($p = 0.04$ and 0.01 , respectively). More studies to identify HAND biomarkers are needed.

Keywords

Biomarker; Neurocognitive performance; Monocytes; Inflammation; Antiretroviral therapy

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Conflict of interest A.L. has served on the scientific advisory board for Merck and has provided scientific consultation to GlaxoSmithKline, Bristol-Myers Squibb, and Tobira.

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Introduction

HIV-associated dementia (HAD) has become rare with the advent of effective combination antiretroviral therapy (ART), but asymptomatic to milder forms of HIV-associated neurocognitive disorders (HAND) still affect 18 to 55% of HIV+ individuals on ART (Heaton et al. 2010; Antinori et al. 2007; Ellis et al. 2007; McArthur and Brew 2010; Spudich 2013; Zayyad and Spudich 2015). The proposed mechanisms for HAND during ART include irreversible brain injury prior to initiating ART or ongoing injury caused by HIV persistence in the central nervous system (CNS) with residual inflammation and immune activation (Ellis et al. 2002; Liner 2nd et al. 2010; Marra et al. 2009; Hagberg et al. 2010). While HAND negatively affects adherence, learning, behavior, employment, and quality of life (Benedict et al. 2000; Heaton et al. 2004; Tozzi et al. 2007; Heaton et al. 2011), there is no treatment besides ART. Identification of HAND biomarkers could improve the understanding of HAND pathogenesis and lead to targeted interventions.

Several cerebrospinal fluid (CSF), plasma, and peripheral blood mononuclear cell (PBMC) markers have been investigated as potential biomarkers for HAND. These include measures of macrophage-mediated disease such as soluble CD163, soluble CD14 and CCL2, HIV RNA, neopterin, and neurofilament light chain protein (NFL). These biomarkers have been found to have different degrees of correlation with HAND in treatment-naïve and ART-suppressed individuals, as well as with mild versus severe manifestations of HAND (Burdo et al. 2013; Lyons et al. 2011; Seigny et al. 2004; Price et al. 2007; Peluso et al. 2012; McGuire et al. 2015). Overall, the evidence is limited and there is no consensus biomarker. To address this, we investigated selected systemic biomarkers in the AIDS Clinical Trials Group (ACTG) study A5303. This study showed that neurocognitive functioning improved with ART, without differential improvement based on antiretroviral regimens (i.e., maraviroc versus tenofovir disoproxil fumarate (TDF)). We present the results of secondary and exploratory investigation of associations between neuropsychological performance (NP) and systemic markers of immune activation, inflammation, and coagulation to determine their potential to serve as HAND biomarkers.

Methods

Study design

The A5303 study population, randomization schedule, neurocognitive evaluations, safety monitoring, and primary results have been published (Robertson et al. 2016). Briefly, A5303 was a phase 2, prospective, double-blind, placebo-controlled, randomized clinical trial conducted at 33 ACTG and 4 Adolescent Trials Network clinical research sites funded by the National Institutes of Health ([Clinicaltrials.gov](https://clinicaltrials.gov) identifier). Individuals whom the site investigator felt could not complete the neurocognitive protocol due to HIV or other illness were excluded. As the largest US neurocognitive antiretroviral clinical trial to date, 262 participants received maraviroc 150 mg or TDF 300 mg, each combined darunavir 800 mg, ritonavir 100 mg, and emtricitabine 200 mg once daily. Data from the 230 participants who remained on their randomized treatment through study week 48 were included in this analysis.

Neurocognitive testing and analysis

Neurocognitive performance (NP) and Activities of Daily Living were assessed at study entry and week 48 (Robertson et al. 2016). The neurocognitive battery included 15 tests: WRAT-4 Reading (Casaletto et al. 2014), Hopkins Verbal Learning Test Revised learning trials, WAIS-III Symbol Search, Stroop Word, Digit Symbol, Trailmaking A, Stroop color naming, Trailmaking B, Stroop Interference, Letter Fluency, Semantic Verbal Fluency, Grooved Pegboard bilateral, and Delayed Recall—HVLt-R, Recognition—HVLt-R.

A total NP *z* score (Blackstone et al. 2012) was computed from the average of the 15 test *z* scores. We focused on the total *z* score, as a summary NP measure for these analyses. Individual test *z* scores were computed by subtracting the test raw score from the demographically corrected normative mean score adjusted for age, education, gender, and race where appropriate, then dividing by the normative standard deviation (Heaton and Heaton 2004). For the total *z* score, zero reflects average performance, positive scores denote above average performance, and negative scores reflect below average performance. The primary NP outcomes were assessed at baseline (i.e., pre-ART) and week 48 of ART.

Impairment was defined according to conventional HAND categorization from the Frascati criteria (Blackstone et al. 2012): normal, asymptomatic neurocognitive impairment (ANI), mild neurocognitive disorder (MND), and HIV-associated dementia (HAD). For each domain, *z* score and deficit scores were computed. Deficit scores were computed as follows: 0 ($z > -1$); 1 ($-1.5 \leq z < -1$); 2 ($-2.0 \leq z < -1.5$); 3 ($-2.5 \leq z < -2.0$); 4 ($-3.0 \leq z < -2.5$); and 5 ($z \leq -3.0$); missing if *z* score is missing. The total ADL score is the sum of 16 ADL scores. These scores were combined and interpreted as follows: normal (DS < 1 for all domains, ADL = 0); ANI (DS = 1 for at least 2 domains, ADL = 0); MND (DS = 1 for at least 2 domains and ADL = 1, or DS = 2 for 2 domains and ADL 0–3); HAD (DS = 2 for 2 domains, ADL = 4).

Health care personnel responsible for administering the tests participated in standardized training and certification under the supervision of Dr. Kevin Robertson, a neuropsychologist. This ensured consistency across the study sites. After the initial training and completion of a web-based certification test, subsequent review of the training materials and recertification of research staff occurred at least annually.

Laboratory methods

The laboratory testing took place in a single Immunology Specialty Laboratory at Rush University that operates under strict Quality Control/Quality Assurance standards. Polychromatic flow cytometry was performed in batch on week 0 and 48 cryopreserved PBMC. Cells were stained for viability with Aqua Live/Dead (Life Technologies) followed by cell staining using fluorochrome-conjugated monoclonal antibodies (BD Biosciences, Biolegend, eBiosciences). PBMC were stained for T cell subsets (CD3/CD4, CD3/CD8) and associated markers of immune activation (CD38/HLA-DR), senescence (CD57/CD28), and T regulatory (CD25/FOXP3) cells. In a separate tube, PBMC were stained for markers of monocyte subsets (CD14/CD16/CCR2/CX3CR1), B cells (CD19/FcRL4), and NK cell subsets (CD56/CD16). All tubes were fixed in 1% formaldehyde and analyzed within 24 h

on an LSRFortessa™ flow cytometer (BD) using BD FACSDiva software v7.0. Analysis of flow cytometry data was performed using FlowJo software (Tree Star Inc).

Frozen/thawed week 0 and 48 EDTA plasma samples were analyzed using ELISA kits for quantification of soluble CD14, CD163, interferon gamma-induced protein (IP)-10, soluble tumor necrosis factor receptor II (sTNFRII), and high-sensitivity interleukin-6 (hsIL-6) (Quantikine, R&D Systems). D-Dimer was measured in frozen/thawed sodium citrate plasma using an ELISA kit from Diagnostica Stago and performed according to the manufacturers' protocols.

Statistical methods

Spearman correlation coefficients were calculated to examine correlations between (1) total NP *z* score and biomarkers at baseline, (2) total NP *z* score and biomarkers at week 48, and (3) 48-week changes in total NP *z* score and biomarkers. If participants were unable to perform a test because of a reason unrelated to HAND, their individual test *z* scores were treated as missing. The association between the baseline HAND diagnosis status and the biomarkers at baseline and 48-week changes were examined by the Jonckheere-Terpstra test. The Benjamini-Hochberg method was applied to adjust *p* values for multiple comparisons. All statistical tests were two-sided and interpreted at the 5% nominal level of significance. Analyses were conducted using SAS statistical software 9.4.

Results

Study population

The 230 ART-naïve participants included in this analysis had a median age of 33 years, most attended some college (70%), and the majority were male (91%). Pre-ART, median plasma HIV RNA was 4.5 log₁₀ copies/mL (IQR 4.0, 5.0) and median CD4 count was 389 cells/mm³ (IQR 293, 508) (Table 1). Of 229 participants with HAND diagnosis status at baseline (1 participant excluded due to a missing Activities of Daily Living score), a HAND diagnosis was found in 45%, with further categorization demonstrating 12.2% with ANI, 30.6% with MND, and 2.2% with HAD. The participants initiated maraviroc (*N* = 119) or tenofovir (*N* = 111) containing ART, and a similar proportion in both arms (92% and 93%) was virologically suppressed (HIV RNA < 50 copies/mL) at week 48.

Total NP *z* score and biomarkers

Correlations between total NP *z* score and biomarkers pre-ART and after week 48 of ART are shown in Table 2. There was evidence of associations between total NP *z* score and some biomarkers, although the magnitude was small and only a few reached statistical significance after adjusting for multiple comparisons. Pre-ART, higher levels of lymphocyte activation correlated with worse NP as shown with percent CD38+/HLA-DR+ (CD4+) (*r* = −0.22, *p* = 0.02) and percent CD38+/HLA-DR+(CD8+) (*r* = −0.25, *p* = 0.02). In contrast, at week 48, there were associations between the total NP *z* score with monocyte subsets: a positive correlation with percent CD14++CD16− (classical) monocytes (*r* = 0.25, *p* = 0.02) and a negative correlation with percent CD14+CD16++ (non-classical) monocytes (*r* = −

0.26, $p = 0.02$). No significant correlations were detected between NP and inflammation, lymphocyte activation, and other biomarkers at week 48.

Next, we conducted analyses focused on trajectories of the biomarkers and NP over 48 weeks of ART. There was no correlation between changes in the total NP z score from pre-ART to week 48 of ART and changes in any biomarker during the same period. As a sensitivity analysis, we have examined the 48-week changes relative to the baseline value between the total NP z score and each biomarker, and the conclusion remained the same. Also, there were no substantial correlations between 48-week change in the total NP z score and the pre-ART levels of biomarkers and no significant correlation between pre-ART NP z score and 48-week changes in biomarkers.

HAND diagnosis and biomarkers

A positive relationship between pre-ART interleukin-6 (IL-6) level and the severity of HAND at baseline was found. The neurocognitively unimpaired participants had the lowest pre-ART IL-6 level (median, 1.4 ng/mL; IQR 1.0, 2.0), in contrast to the HAD group with the highest (median, 2.3 ng/mL; IQR 1.8, 2.3) ($p = 0.04$) (Table 3). There was a positive relationship between the pre-ART %CD38+/HLA-DR+(CD8+) and the severity of HAND. The neurocognitively unimpaired participants had the lowest pre-ART %CD38+/HLA-DR+(CD8+) value (median, 19.1; IQR 13.7, 26.4) and the HAD group had the highest (median, 38.1; IQR 34.1, 43.8) ($p = 0.01$). No significant correlations between the 48-week changes in biomarkers and HAND status were reported.

Discussion

Persistent neurocognitive impairment during ART is a barrier to the overall well-being of persons living with HIV (PLWH). We investigated associations between neurocognitive functioning as measured by total NP z score and 31 systemic biomarkers pre-ART and after 48 weeks of ART. While very few significant associations were found, our results provide two important insights into the quest for HAND biomarkers. First, the systemic biomarkers associated with NP pre-ART were different from those at week 48. Specifically, higher levels of the inflammation biomarker (IL-6) and T cell activation (as reflected by the percentage of cells expressing CD38 and HLA-DR) were independently associated with worse severity HAND pre-ART, while a different pattern was seen at week 48 at which point NP correlated with some monocyte subsets.

finding that a higher percentage of CD14++CD16– monocytes (i.e., classical monocytes) correlated with better neurocognition during ART while the percentage of CD14+ CD16++ (non-classical) monocytes demonstrated an opposite association. Classical monocytes are the most abundant monocyte subtype and are scavenger cells (Ziegler-Heitbrock et al. 2010). On the other hand, non-classical monocytes are pro-inflammatory, being preferentially mobilized in disease and with secretion of inflammatory cytokines (Belge et al. 2002; Schlitt et al. 2004; Grip et al. 2008). We did not detect a correlation with CD14++CD16+ (intermediate) monocytes, which are also pro-inflammatory (Wong et al. 2012), suggesting that non-classical monocytes may be a more attractive subset for further investigation. A significant role for non-classical monocytes in HAND during ART is plausible because this

monocyte subset has been implicated in other chronic diseases in PLWH on stable ART, including cardiovascular disease (Chow et al. 2016) and renal disease (Zungsontiporn et al. 2016; Mitchell et al. 2016).

One of the strengths of our study is that we analyzed data from the largest clinical trial of HAND conducted to date. Detection of few associations may indicate that blood biomarkers poorly reflect CNS functioning, and CSF biomarkers could be of greater value. Although not addressed here, CNS inflammation is thought to be key in our current understanding of HAND pathogenesis. Unlike some other studies (Burdo et al. 2013; Lyons et al. 2011), we did not detect a correlation between neurocognitive performance and soluble markers of monocyte-macrophage-mediated disease. Our study is limited by the lack of a control group, and we cannot completely rule out the impact of potential practice or learning effects on the observed correlations, although we would expect this effect to be uniform on a test by biomarker correlation basis. It is possible that the observed correlations were limited because only about half of the participants had baseline impairment, and a small percentage had dementia.

In conclusion we found few associations between evaluated biomarkers and neurocognitive functioning. Biomarkers associated with NP differed pre-ART and during ART. Some markers of lymphocyte activation and inflammation modestly correlated with neurocognition pre-ART while percentage of non-classical monocytes was found to modestly correlate with neurocognition during ART. Although there were limited significant correlations here, the quest for HAND biomarkers should continue in novel studies, particularly focused on CSF biomarkers of neuronal injury and inflammation markers.

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References

- Heaton RK, Clifford DB, Franklin DR Jr, Woods SP, Ake C, Vaida F et al. (2010) HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER Study. *Neurology* 75(23): 2087–2096 [PubMed: 21135382]
- Antinori A, Arendt G, Becker JT, Brew BJ, Byrd DA, Cherner M, Clifford DB, Cinque P, Epstein LG, Goodkin K, Gisslen M, Grant I, Heaton RK, Joseph J, Marder K, Marra CM, McArthur JC, Nunn M, Price RW, Pulliam L, Robertson KR, Sacktor N, Valcour V, Wojna VE (2007) Updated research nosology for HIV-associated neurocognitive disorders. *Neurology* 69(18):1789–1799 [PubMed: 17914061]
- Ellis R, Langford D, Masliah E (2007) HIV and antiretroviral therapy in the brain: neuronal injury and repair. *Nat Rev Neurosci* 8(1):33–44 [PubMed: 17180161]
- McArthur JC, Brew BJ (2010) HIV-associated neurocognitive disorders: is there a hidden epidemic? *AIDS* 24(9):1367–1370 [PubMed: 20559041]
- Spudich S (2013) HIV and neurocognitive dysfunction. *Curr HIV/AIDS Rep* 10(3):235–243 [PubMed: 23860944]
- Zayyad Z, Spudich S (2015) Neuropathogenesis of HIV: from initial neuroinvasion to HIV-associated neurocognitive disorder (HAND). *Curr HIV/AIDS Rep* 12(1):16–24 [PubMed: 25604237]
- Ellis RJ, Moore DJ, Childers ME, Letendre S, McCutchan JA, Wolfson T, Spector SA, Hsia K, Heaton RK, Grant I (2002) Progression to neuropsychological impairment in human immunodeficiency virus infection predicted by elevated cerebrospinal fluid levels of human immunodeficiency virus RNA. *Arch Neurol* 59(6):923–928 [PubMed: 12056927]
- Liner KJ 2nd, Ro MJ, Robertson KR (2010) HIV, antiretroviral therapies, and the brain. *Curr HIV/AIDS Rep* 7(2):85–91 [PubMed: 20425562]
- Marra CM, Zhao Y, Clifford DB, Letendre S, Evans S, Henry K, Ellis RJ, Rodriguez B, Coombs RW, Schifitto G, McArthur J, Robertson K, AIDS Clinical Trials Group 736 Study Team (2009) Impact of combination antiretroviral therapy on cerebrospinal fluid HIV RNA and neurocognitive performance. *AIDS* 23(11):1359–1366 [PubMed: 19424052]
- Hagberg L, Cinque P, Gisslen M, Brew BJ, Spudich S, Bestetti A, Price RW, Fuchs D (2010) Cerebrospinal fluid neopterin: an informative biomarker of central nervous system immune activation in HIV-1 infection. *AIDS Res Ther* 7:15 [PubMed: 20525234]
- Benedict RH, Mezhr JJ, Walsh K, Hewitt RG (2000) Impact of human immunodeficiency virus type-1-associated cognitive dysfunction on activities of daily living and quality of life. *Arch Clin Neuropsychol* 15(6):535–544 [PubMed: 14590207]
- Heaton RK, Marcotte TD, Mindt MR, Sadek J, Moore DJ, Bentley H, McCutchan J, Reicks C, Grant I, HNRC Group (2004) The impact of HIV-associated neuropsychological impairment on everyday functioning. *J Int Neuropsychol Soc* 10(3):317–331 [PubMed: 15147590]
- Tozzi V, Balestra P, Bellagamba R, Corpolongo A, Salvatori MF, Visco-Comandini U, Vlassi C, Giulianelli M, Galgani S, Antinori A, Narciso P (2007) Persistence of neuropsychologic deficits despite long-term highly active antiretroviral therapy in patients with HIV-related neurocognitive impairment: prevalence and risk factors. *J Acquir Immune Defic Syndr* 45(2):174–182 [PubMed: 17356465]
- Heaton RK, Franklin DR, Ellis RJ, McCutchan JA, Letendre SL, Leblanc S et al. (2011) HIV-associated neurocognitive disorders before and during the era of combination antiretroviral therapy: differences in rates, nature, and predictors. *J Neuro-Oncol* 17(1):3–16
- Burdo TH, Weiffenbach A, Woods SP, Letendre S, Ellis RJ, Williams KC (2013) Elevated sCD163 in plasma but not cerebrospinal fluid is a marker of neurocognitive impairment in HIV infection. *AIDS* 27(9): 1387–1395 [PubMed: 23435298]
- Lyons JL, Uno H, Ancuta P, Kamat A, Moore DJ, Singer EJ, Morgello S, Gabuzda D (2011) Plasma sCD14 is a biomarker associated with impaired neurocognitive test performance in attention and learning domains in HIVinfection. *J Acquir Immune Defic Syndr* 57(5):371–379 [PubMed: 21646912]

- Sevigny JJ, Albert SM, McDermott MP, McArthur JC, Sacktor N, Conant K et al. (2004) Evaluation of HIV RNA and markers of immune activation as predictors of HIV-associated dementia. *Neurology* 63(11):2084–2090 [PubMed: 15596754]
- Price RW, Epstein LG, Becker JT, Cinque P, Gisslen M, Pulliam L, McArthur JC (2007) Biomarkers of HIV-1 CNS infection and injury. *Neurology* 69(18):1781–1788 [PubMed: 17967994]
- Peluso MJ, Ferretti F, Peterson J, Lee E, Fuchs D, Boschini A, Gisslén M, Angoff N, Price RW, Cinque P, Spudich S (2012) Cerebrospinal fluid HIVescape associated with progressive neurologic dysfunction in patients on antiretroviral therapy with well controlled plasma viral load. *AIDS* 26(14):1765–1774 [PubMed: 22614889]
- McGuire JL, Gill AJ, Douglas SD, Kolson DL, group CHA-RTER (2015) Central and peripheral markers of neurodegeneration and monocyte activation in HIV-associated neurocognitive disorders. *J Neuro-Oncol* 21(4):439–448
- Robertson KR, Miyahara S, Lee A, Brown TT, Chan ES, Berzins B, Rusin D, Eron JJ, Taiwo BO, AIDS Clinical Trials Group (ACTG) 5303 team (2016) Neurocognition with maraviroc compared with tenofovir in HIV. *AIDS* 30(15):2315–2321 [PubMed: 27333088]
- Casaletto KB, Cattie J, Franklin DR, Moore DJ, Woods SP, Grant I, Heaton RK, HNRG Group (2014) The Wide Range Achievement Test-4 Reading subtest “holds” in HIV-infected individuals. *J Clin Exp Neuropsychol* 36(9):992–1001 [PubMed: 25283135]
- Blackstone K, Moore DJ, Franklin DR, Clifford DB, Collier AC, Marra CM, Gelman BB, McArthur JC, Morgello S, Simpson DM, Ellis RJ, Atkinson JH, Grant I, Heaton RK (2012) Defining neurocognitive impairment in HIV: deficit scores versus clinical ratings. *Clin Neuropsychol* 26(6):894–908 [PubMed: 22708483]
- Heaton RK, Heaton RK (2004) Psychological Assessment Resources Inc Revised comprehensive norms for an expanded Halstead-Reitan battery: demographically adjusted neuropsychological norms for African American and Caucasian adults, professional manual. Updated ed Lutz, Fla.: Psychological Assessment Resources
- Ziegler-Heitbrock L, Ancuta P, Crowe S, Dalod M, Grau V, Hart DN, Leenen PJM, Liu YJ, MacPherson G, Randolph GJ, Scherberich J, Schmitz J, Shortman K, Sozzani S, Strobl H, Zembala M, Austyn JM, Lutz MB (2010) Nomenclature of monocytes and dendritic cells in blood. *Blood* 116(16):e74–e80 [PubMed: 20628149]
- Belge KU, Dayyani F, Horelt A, Siedlar M, Frankenberger M, Frankenberger B, Espevik T, Ziegler-Heitbrock L (2002) The pro-inflammatory CD14+CD16+DR++ monocytes are a major source of TNF. *J Immunol* 168(7):3536–3542 [PubMed: 11907116]
- Schlitt A, Heine GH, Blankenberg S, Espinola-Klein C, Doppeide JF, Bickel C et al. (2004) CD14+CD16+ monocytes in coronary artery disease and their relationship to serum TNF-alpha levels. *Thromb Haemost* 92(2):419–424 [PubMed: 15269840]
- Grip O, Janciauskiene S, Bredberg A (2008) Use of atorvastatin as an anti-inflammatory treatment in Crohn's disease. *Br J Pharmacol* 155(7):1085–1092 [PubMed: 18806816]
- Wong KL, Yeap WH, Tai JJ, Ong SM, Dang TM, Wong SC (2012) The three human monocyte subsets: implications for health and disease. *Immunol Res* 53(1–3):41–57 [PubMed: 22430559]
- Chow DC, Kagihara JM, Zhang G, Souza SA, Hodis HN, Li Y, Mitchell BI, Nakamoto BK, Kallianpur KJ, Keating SM, Norris PJ, Kohorn LB, Ndhlovu LC, Shikuma CM (2016) Non-classical monocytes predict progression of carotid artery bifurcation intima-media thickness in HIV-infected individuals on stable antiretroviral therapy. *HIV Clin Trials* 17(3):114–122 [PubMed: 27125366]
- Zungontiporn N, Tello RR, Zhang G, Mitchell BI, Budoff M, Kallianpur KJ, Nakamoto BK, Keating SM, Norris PJ, Ndhlovu LC, Souza SA, Shikuma CM, Chow DC (2016) Non-classical monocytes and monocyte chemoattractant protein-1 (MCP-1) correlate with coronary artery calcium progression in chronically HIV-1 infected adults on stable antiretroviral therapy. *PLoS One* 11(2):e0149143 [PubMed: 26867220]
- Mitchell BI, Byron MM, Ng RC, Chow DC, Ndhlovu LC, Shikuma CM (2016) Elevation of non-classical (CD14+/lowCD16++) monocytes is associated with increased albuminuria and urine TGF-beta1 in HIV-infected individuals on stable antiretroviral therapy. *PLoS One* 11(4):e0153758 [PubMed: 27097224]

Table 1

Participant characteristics at study entry

Participants (<i>n</i> = 230)	
Demographics—number of participants (%)	
Male	209 (91%)
White non-Hispanic	102 (44%)
Black non-Hispanic	72 (31%)
Hispanic (regardless of race)	50 (22%)
Asian, Pacific Islander	3 (1%)
American Indian, Alaskan Native	1 (0%)
More than one race	2 (1%)
English as primary language	188 (82%)
Spanish as primary language	21 (9%)
Unknown primary language	18(8%)
Less than 12 years of education	18(9%)
Baseline viral characteristics—median (IQR)	
Plasma HIV RNA	4.5 log ₁₀ copies/mL (IQR 4.0, 5.0)
CD4 count	89 cells/mm ³ (IQR 293, 508)

Table 2

Spearman correlations between total NP Z score and biomarkers at baseline and at week 48

Biomarker	Baseline		At 48 weeks	
	r	p value [*]	r	p value [*]
IL-6 (pg/mL)	-0.14	0.14	-0.15	0.14
Ip-10 (pg/mL)	-0.19	0.05	-0.04	0.68
sTNF- α I (pg/mL)	-0.09	0.35	-0.03	0.72
sCD14 (ng/mL)	-0.00	0.99	0.00	0.99
D-Dimer (ng/mL)	-0.15	0.14	-0.12	0.21
sCD163 (ng/mL)	-0.05	0.63	-0.07	0.50
CD4 count (cells/mm ³)	0.07	0.44	0.00	0.99
CD8 count (cells/mm ³)	-0.10	0.33	-0.08	0.37
CD4:CD8 ratio	0.14	0.14	0.10	0.33
Percent CD38+/HLA-DR+(CD4+) (%)	-0.22	0.02 ^{**}	-0.16	0.14
Percent CD28-/CD57+ (CD4+) (%)	0.05	0.66	-0.03	0.72
Percent CD57+(CD4+) (%)	-0.09	0.35	-0.13	0.17
Percent CD28+(CD4+) (%)	-0.07	0.45	-0.04	0.68
Percent Treg+(CD4+) (%)	0.02	0.85	-0.04	0.68
Percent CD4 (%)	0.08	0.37	0.11	0.27
Percent CD38+/HLA-DR-(CD8+) (%)	-0.25	0.02 ^{**}	-0.15	0.14
Percent CD28-/CD57+ (CD8+) (%)	-0.02	0.81	-0.06	0.59
Percent CD57+(CD8+) (%)	-0.10	0.33	-0.09	0.37
Percent CD28+(CD8+) (%)	0.05	0.63	0.05	0.63
Percent CD8 (%)	-0.09	0.37	-0.11	0.30
Percent CD14++CD16- (total monocytes) (%)	0.14	0.14	0.25	0.02 ^{**}
Percent CD14++CD16+ (total monocytes) (%)	-0.13	0.16	-0.13	0.17
Percent CD14+CD16++ (total monocytes) (%)	-0.10	0.34	-0.26	0.02 ^{**}
Percent CCR2+CD14++CD16+ (%)	0.05	0.65	0.08	0.41
Percent CX3CR1+(CD14++CD16+) (%)	-0.05	0.66	-0.03	0.72
Percent total CD19+ (%)	-0.08	0.44	-0.14	0.14

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Biomarker	Baseline		At 48 weeks	
	<i>r</i>	<i>p</i> value *	<i>r</i>	<i>p</i> value *
Percent FcRL4+(CD19+) (%)	− 0.01	0.99	0.04	0.71
Percent CD56HI/CD16− (NK cells) (%)	0.14	0.14	0.16	0.14
Percent CD56dim/CD16− (NK cells) (%)	0.00	0.99	0.10	0.35
Percent CD56+/CD16+ (NK cells) (%)	0.13	0.16	0.03	0.72
Percent CD56−/CD16+ (NK cells) (%)	− 0.14	0.14	− 0.09	0.56

* The Benjamini-Hochberg method was applied to adjust *p* values for multiple comparisons

** *p* < 0.05

Table 3

Baseline biomarkers by HAND diagnosis

Biomarker	Normal (<i>n</i> = 126) Median (IQR)	ANI (<i>n</i> = 28) Median (IQR)	MND (<i>n</i> = 70) Median (IQR)	HAD (<i>n</i> = 5) Median (IQR)	<i>p</i> value*
IL-6 (pg/mL)	1.4 (1.0, 2.0)	1.3 (0.8, 2.3)	2.0 (1.3, 3.1)	2.3 (1.8, 2.3)	0.04**
Ip-10 (pg/mL)	342.2 (220.5, 510.5)	330.3 (234.8, 468.1)	403.5 (273.6, 652.3)	518.6 (362.9, 703.1)	0.13
sTNF- α I (pg/mL)	3727.6 (2906, 4209)	3706.0 (3117, 4193)	3681.7 (3004, 4875)	3255.3 (3121, 4084)	0.70
sCD14 (ng/mL)	1674.0 (1429, 1901)	1607.2 (1459, 1832)	1721.3 (1526, 1953)	1572.6 (1540, 2057)	0.70
D-dimer (ng/mL)	235.9 (145.6, 368.8)	325.2 (153.9, 615.2)	262.5 (161.1, 434.1)	516.6 (251.6, 525.9)	0.23
sCD163 (ng/mL)	820.7 (613, 1046)	724.1 (547, 1058)	848.9 (630, 1113)	944.9 (939, 1229)	0.65
CD4 count (cells/mm ³)	389 (316, 493)	403.5 (287.0, 489.5)	383 (271, 555)	262 (210, 431)	0.79
CD8 count (cells/mm ³)	841 (626, 1036)	831.5 (628.5, 1249)	945.5 (686, 1340)	1093 (942, 1275)	0.14
CD4:CD8 ratio	0.5 (0.4, 0.7)	0.4 (0.3, 0.7)	0.4 (0.3, 0.6)	0.4 (0.2, 0.5)	0.13
Percent CD38+/HLA-DR+(CD4+) (%)	5.5 (3.6, 8.5)	8.7 (5.3, 10.3)	6.4 (4.0, 10.9)	10.1 (8.2, 10.1)	0.13
Percent CD28-/CD57+(CD4+) (%)	5.9 (2.5, 11.2)	6.3 (3.8, 15.5)	5.6 (2.7, 10.4)	2.0 (1.5, 9.0)	0.92
Percent CD57+(CD4+) (%)	11.7 (7.3, 18.9)	14.9 (9.7, 20.2)	13.2 (9.5, 17.8)	17.9 (11.0, 20.6)	0.25
Percent CD28+(CD4+) (%)	87.2 (82.2, 92.4)	88.7 (78.8, 91.2)	87.8 (81.8, 93.4)	88.3 (86.4, 96.3)	0.70
Percent Treg+(CD4+) (%)	9.7 (8.2, 12.0)	10.5 (7.4, 13.7)	9.5 (7.8, 12.1)	11.9 (10.7, 13.7)	0.99
Percent CD4 (%)	39.8 (29.8, 46.3)	36.2 (23.1, 46.7)	35.1 (24.7, 45.1)	29.8 (11.9, 33.1)	0.16
Percent CD38+/HLA-DR+(CD8+) (%)	19.1 (13.7, 26.4)	25.1 (14.4, 30.2)	23.8 (17.3, 33.4)	38.1 (34.1, 43.8)	0.01**
Percent CD28-/CD57+(CD8+) (%)	48.0 (38.8, 55.2)	43.1 (32.1, 52.2)	49.9 (41.7, 56.0)	44.6 (43.1, 48.5)	0.84
Percent CD57+(CD8+) (%)	60.5 (51.1, 68.3)	60.1 (40.6, 66.4)	60.8 (55.9, 70.3)	64.5 (60.9, 68.6)	0.65
Percent CD28+(CD8+) (%)	41.9 (32.3, 50.7)	44.6 (30.6, 53.9)	37.2 (31.0, 45.1)	39.7 (37.9, 46.9)	0.47
Percent CD8 (%)	52.0 (45.1, 63.1)	53.8 (42.8, 67.7)	56.6 (48.5, 68.2)	64.2 (62.7, 84.1)	0.17
Percent CD14++CD16- (total monocytes) (%)	87.4 (81.8, 92.5)	80.1 (72.1, 88.1)	85.2 (79.2, 90.6)	75.2 (73.0, 88.3)	0.13
Percent CD14++CD16+ (total monocytes) (%)	6.2 (3.5, 9.3)	9.7 (6.7, 11.9)	6.6 (4.6, 10.4)	7.0 (4.7, 13.9)	0.25
Percent CD14+CD16++ (total monocytes) (%)	6.0 (3.4, 9.3)	7.5 (5.1, 16.4)	6.6 (3.4, 11.4)	10.9 (7.0, 12.7)	0.25
Percent CCR2+CD14++CD16+ (%)	0.2 (0.1, 0.5)	0.1 (0.0, 0.3)	0.2 (0.1, 0.3)	0.2 (0.1, 0.3)	0.65
Percent CX3CR1+(CD14++CD16+) (%)	99.6 (99.1, 99.8)	99.7 (99.5, 99.9)	99.7 (99.3, 99.8)	99.6 (99.3, 99.8)	0.49
Percent total CD19+ (%)	5.5 (3.6, 8.4)	4.7 (3.1, 9.7)	6.5 (4.0, 9.3)	4.7 (4.0, 6.7)	0.72
Percent FoRL4+(CD19+) (%)	6.1 (4.5, 8.1)	4.3 (3.4, 6.7)	6.0 (4.7, 8.4)	5.9 (4.8, 12.1)	0.84

Biomarker	Normal (<i>n</i> = 126) Median (IQR)	ANI (<i>n</i> = 28) Median (IQR)	MND (<i>n</i> = 70) Median (IQR)	HAD (<i>n</i> = 5) Median (IQR)	<i>p</i> value [*]
Percent CD56HI/CD16– (NK cells) (%)	4.1 (2.5, 6.8)	3.0 (1.7, 5.1)	3.9 (2.1, 7.0)	3.2 (1.1, 4.0)	0.50
Percent CD56dim/CD16– (NK cells) (%)	5.8 (3.6, 8.7)	4.8 (3.6, 7.0)	6.3 (4.3, 11.5)	13.2 (6.8, 13.5)	0.47
Percent CD56+/CD16+ (NK cells) (%)	63.1 (52.1, 74.8)	71.7 (63.4, 77.7)	57.0 (44.7, 71.8)	64.3 (30.8, 70.0)	0.35
Percent CD56–/CD16+ (NK cells) (%)	20.6 (12.9, 34.9)	17.8 (12.4, 26.0)	25.7 (14.3, 40.2)	22.2 (15.5, 57.6)	0.47

ANI, asymptomatic neurocognitive impairment; MND, mild neurocognitive disorder; HAD, HIV-associated dementia; IQR, interquartile range

^{*} The Benjamini-Hochberg method was applied to adjust *p* values for multiple comparisons

^{**} *p* < 0.05