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ORIGINAL ARTICLE

CCR2 on Peripheral Blood CD14^{+}CD16^{+} Monocytes Correlates with Neuronal Damage, HIV-Associated Neurocognitive Disorders, and Peripheral HIV DNA: reseeding of CNS reservoirs?

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Abstract

HIV-associated neurocognitive disorders (HAND) occur in ~50% of HIV infected individuals despite combined antiretroviral therapy. Transmigration into the CNS of CD14^{+}CD16^{+} monocytes, particularly those that are HIV infected and express increased surface chemokine receptor CCR2, contributes to neuroinflammation and HAND. To examine whether in HIV infected individuals CCR2 on CD14^{+}CD16^{+} monocytes serves as a potential peripheral blood biomarker of HAND, we examined a cohort of 45 HIV infected people. We correlated CCR2 on CD14^{+}CD16^{+} monocytes with cognitive status, proton magnetic resonance spectroscopy (^{1}H-MRS) measured neurometabolite levels, and peripheral blood mononuclear cell (PBMC) HIV DNA copies. We determined that CCR2 was increased specifically on CD14^{+}CD16^{+} monocytes from people with HAND (median [interquartile range (IQR)]) (63.3 [51.6, 79.0]), compared to those who were not cognitively impaired (38.8 [26.7, 56.4]) or those with neuropsychological impairment due to causes other than HIV (39.8 [30.2, 46.5]). CCR2 was associated with neuronal damage, based on the inverse correlation of CCR2 on CD14^{+}CD16^{+} monocytes with total N-Acetyl Aspartate (tNAA)/total Creatine (tCr) ($r^2 = 0.348$, $p = 0.01$) and Glutamine-Glutamate (Glx)/tCr ($r^2 = 0.356$, $p = 0.01$) in the right and left caudate nucleus, respectively. CCR2 on CD14^{+}CD16^{+} monocytes also correlated with PBMC HIV DNA copies ($\rho = 0.618$, $p = 0.02$) that has previously been associated with HAND. These findings suggest that CCR2 on CD14^{+}CD16^{+} monocytes may be a peripheral blood biomarker of HAND, indicative of increased HIV infected CD14^{+}CD16^{+} monocyte entry into the CNS that possibly increases the macrophage viral reservoir and contributes to HAND.

Keywords HAND · CD14^{+}CD16^{+} Monocytes · CCR2 · MRI/MRS · HIV · ddPCR

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Introduction

HIV-associated neurocognitive disorders (HAND) are present in up to 50% of HIV infected individuals in the combined antiretroviral therapy (cART) era, despite cART adherence and widespread suppression of plasma viral loads (Heaton et al. 2010; Cysique and Brew 2011; Saylor et al. 2016; Gott et al. 2017). On neuroimaging, low level neuroinflammation and progressive neuronal damage may be seen in cART treated cohorts. Neuronal changes are postulated to mediate cognitive dysfunction (Kaul et al. 2001; Ellis et al. 2007; Harezlak et al. 2011; Gongvatana et al. 2013). Central nervous system (CNS) inflammation and damage is mediated, in part, by HIV infection of the brain, which occurs within 4–8 days after peripheral infection, and by transmigration of monocytes into the CNS (Witwer et al. 2009; Burdo et al. 2010; Valcour et al. 2012; Campbell et al. 2014). More specifically, the mature $CD14^+CD16^+$ monocyte subset is strongly associated with HAND (Pulliam et al. 1997; Fischer-Smith et al. 2001; Valcour et al. 2010; Burdo et al. 2011; Williams et al. 2012; Williams et al. 2013). $CD14^+CD16^+$ monocytes can be infected by HIV and these cells preferentially transmigrate across the blood brain barrier (BBB), and some of these cells may differentiate into macrophages within the brain parenchyma and potentially contribute to continued viral (re-)seeding of the CNS viral reservoir (Veenstra et al. 2017). There are no biomarkers or therapeutics for HAND currently in clinical use. However, since neurocognitive testing requires specialized expertise, is laborious, and cannot definitively assign a causal mechanism for clinical deficits (Tedaldi et al. 2015), a peripheral biomarker that identifies those at risk of HAND would be invaluable.

The entry of mature $CD14^+CD16^+$ monocytes into the CNS is mediated by increased levels of chemokines in the brains of HIV infected individuals despite cART, including the chemokine (C-C motif) ligand 2 (CCL2) (Eugenin et al. 2006; Kamat et al. 2012; Williams et al. 2013). The chemokine receptor for CCL2 on monocytes is CCR2 (Williams et al. 2014). Our laboratory showed previously that CCR2 is increased on the $CD14^+CD16^+$ monocyte subset in the periphery from those with HAND, and that these cells from people with HAND transmigrate in much higher numbers across the blood brain barrier (BBB) to CCL2 than do cells from HIV infected people who are not cognitively impaired (NI) (Williams et al. 2014). In addition, we showed that HIV infected $CD14^+CD16^+$ monocyte transmigration is preferentially blocked by cenicriviroc, a CCR2/CCR5 dual antagonist (Veenstra et al. 2017). This suggests that HAND may be mediated, in part, by increased surface CCR2 on HIV infected $CD14^+CD16^+$ monocytes, providing a mechanism that promotes the entry of these cells into the CNS. This may result in an increase in HIV infected macrophages within the CNS, possibly contributing to the reseeding of viral reservoirs and the development of HAND.

To characterize further CCR2 on $CD14^+CD16^+$ monocytes as a potential biomarker specific for HAND, we examined CCR2 on $CD14^+CD16^+$ monocytes in the peripheral blood of HIV infected individuals from the Manhattan HIV Brain Bank (MHBB) cohort who were all prescribed cART, and were NI, had HAND, or had neuropsychological impairment due to causes other than HIV (NPI-O). We performed proton magnetic resonance spectroscopy (^{1}H-MRS) studies on a smaller group of individuals from the cohort in a pilot study to determine whether there were direct associations between neurometabolite levels and surface CCR2 on peripheral $CD14^+CD16^+$ monocytes. In addition, increased HIV DNA in peripheral blood mononuclear cells (PBMC) has been associated with HAND with some studies proposing that the primary source of HIV DNA in PBMC are $CD14^+CD16^+$ monocytes (Shiramizu et al. 2005; Kusao et al. 2012; Cysique et al. 2015). To examine the relationship between HAND, CCR2 on $CD14^+CD16^+$ monocytes, and PBMC HIV DNA, we performed association studies between the aforementioned variables that were characterized in our cohort.

Materials and Methods

Study Subjects and Sample Collection

All study participants were HIV infected, prescribed cART, and were part of the MHBB cohort (U24MH100931). Enrolled participants were older than 18 years of age and their demographic and virologic characteristics are presented in Table 1. Participant's cART regimen descriptions, as well as year of, and years from, diagnosis and commencement of cART are detailed in Table 2. All individuals gave written, informed consent for the provision of blood for the purposes of research. The Mount Sinai Program for the Protection of Human Subjects Institutional Review Board, and The Institutional Review Board at the Albert Einstein College of Medicine approved the protocols under which these samples were obtained and utilized. A subset of study participants underwent neuroimaging as part of a Mount Sinai Brain Imaging Center-sponsored pilot project, and gave written, informed consent for MRI/^{1}H-MRS procedures. Neuroimaging protocols were approved under a separate pilot protocol by The Mount Sinai Program for the Protection of Human Subjects Institutional Review Board. Blood samples from all individuals assessed for cognitive status were drawn on the same day as neuropsychological testing. Neuropsychological testing was performed as described previously (Byrd et al. 2013; Robbins et al. 2014). For the neuroimaging substudy, blood samples were drawn on the day of the radiologic procedure; the neuroimaging was performed within a one month period of the neuropsychological testing.

Table 1 Demographic and clinical characteristics

MHBB cohort characteristics	Overall (N = 45) Median [IQR] or n (%)
Age (yr)	56 [47, 65]
Gender	
Female	24 (53.3%)
Male	21 (46.7%)
Race	
Black	18 (40.0%)
Hispanic	18 (40.0%)
White	9 (20.0%)
Years from HIV diagnosis	21 [3, 30]
Years from starting cART	15 [0, 24]
Years between HIV diagnosis and starting cART	4 [0, 23]
HAND	21 (46.7%)
CD4 T cell count (cells/mm^3)	475 [0, 989]
Nadir CD4 T cell count (cells/mm^3)	67 [0, 261]
Plasma HIV RNA (log_{10} copies/ml)	1.4 [1.4, 2.1]
Viral load undetectable (<20 copies/ml)	19 (42.2%)
Viral load >20 - <400 copies/ml	20 (44.4%)
Viral load >400 copies/ml	6 (13.3%)

Sample Processing and Flow Cytometry

Blood samples collected in EDTA-coated collection tubes were processed within 2–4 h of blood draw. Whole blood was layered onto Ficoll-Paque PLUS (GE Healthcare, Uppsala, Sweden) for density gradient centrifugation after which PBMC were obtained. PBMC were immediately stained for flow cytometry, and if there were enough PBMC, 10^6 cells were pelleted and stored at −80 °C until HIV DNA analysis by droplet digital PCR (ddPCR) was performed. PBMC were stained using fluorochrome coupled antibodies to human CD14 (BD; clone M5E2), CD16 (BD; clone 3G8), and CCR2 (R&D; clone 48607) or isotype matched control antibodies. This staining was performed according to a protocol described previously (Williams et al. 2014). Briefly, PBMC (2×10^5 in 100 μl) were added to FACS tubes, spun, and media aspirated. CCR2 or isotype matched control antibody was added in 50 μl FACS buffer (1% BSA in PBS) for 5 min, prior to addition of antibodies to CD14 and CD16. Cells were stained for 30 min in the dark on ice, washed once, and fixed with 2% paraformaldehyde in PBS. At least 10,000 monocytes, or 1000 $CD14^+CD16^+$ monocytes, were acquired with the BD FACSCantoII flow cytometer. Analysis was performed using FlowJo software (v. 10.0.8, TreeStar, Ashland, OR). Forward and side scatter were used to determine monocyte gating. Monocytes were defined as CD14 positive, and monocyte subsets were then distinguished according to CD14 and CD16 expression. The monocyte subsets were identified as $CD14^+CD16^-$, $CD14^+CD16^+$, and $CD14^{low}CD16^+$. Mean fluorescence intensity (MFI) for CCR2 was calculated after subtracting the isotype matched control antibody MFI from the CCR2 MFI for each monocyte subset. An example of gating for monocyte subsets and quantification of CCR2 MFI on $CD14^+CD16^+$ monocytes from NI, HAND and NPI-O study participants is shown in Fig. 1a.

Neuropsychological Testing

Functional status questionnaires and neuropsychological tests sensitive to HIV-associated cognitive deficits that examine a broad range of cognitive domains were administered to study participants. Tests used and their normative data are detailed as published previously (Byrd et al. 2013). Scores for each test were converted to T-scores, after performance adjustment for the effects of age, education, sex, and ethnicity, where appropriate. Individuals were then assigned a cognitive diagnosis as NI or cognitively impaired, as previously described (Williams et al. 2014). Study participants who demonstrated cognitive impairment and had pre-existing or co-occurring conditions that accounted for cognitive dysfunction, for example, traumatic brain injury, severe depression, CNS opportunistic disease, or metabolic abnormality, were, after extensive examination, assigned the diagnosis of NPI-O. The remaining cognitively impaired participants were assigned a diagnosis of HAND (Antinori et al. 2007). In the current sample, low levels of literacy, CNS insult (history of cerebrovascular incident and traumatic brain injury), and recent illicit substance use accounted for the 10 cases of NPI-O. Participants with

Table 2 Description of ART regimens and HIV and HAND diagnoses

Study Participant	HAND Diagnosis	ART at Draw	Regimen Base	PI y/n	Yr Dx	Year Started Any ART	Time From Diagnosis	Time From Starting ART	Time From Diagnosis to Starting ART
1	No Impairment	truvada (viread, emtriva), raltegravir	INT	no	1996	1996	16	16	0
2	MCMD	epzicom (ziagen, epivir), rit darunavir, raltegravir	PI, INT	yes	1991	1992	21	20	1
3	No Impairment	atripla (viread, emtriva, efavirenz)	NNRTI	no	1984	1988	28	24	4
4	MCMD	epivir, abacavir, raltegravir	INT	no	1992	1997	20	15	5
5	HAD	epzicom (ziagen, epivir), atazanavir	PI	yes	1993	1998	19	14	5
6	ANI	tenofovir, rit darunavir, raltegravir	PI, INT	yes	1992	1993	20	19	1
7	MCMD	epzicom (ziagen, epivir), efavirenz	NNRTI	no	1994	1996	18	16	2
8	HAD	truvada (viread, emtriva), rit atazanavir	PI	yes	1991	2010	21	2	19
9	No Impairment	truvada (viread, emtriva), rit darunavir	PI	yes	1993	1997	19	15	4
10	MCMD	truvada (viread, emtriva), raltegravir	INT	no	2002	2003	10	9	1
11	NPI-O	non-compliant	none	no	1987	2010	25	2	23
12	NPI-O	non-compliant	none	no	1985	1990	27	22	5
13	MCMD	atripla (viread, emtriva, efavirenz)	NNRTI	no	1991	2003	21	9	12
14	NPI-O	truvada (viread, emtriva), rit atazanavir	PI	yes	1985	2004	27	8	19
15	ANI	epivir, abacavir, raltegravir	INT	no	1999	2000	14	13	1
16	MCMD	abacavir, kaletra (rit lopinivir)	PI	yes	1990	1993	23	20	3
17	MCMD	truvada (viread, emtriva), raltegravir, rit fosamprenavir	PI, INT	yes	1998	1999	15	14	1
18	No Impairment	epivir, rit darunavir, raltegravir	PI, INT	yes	1990	2005	23	8	15
19	NPI-O	epzicom (ziagen, epivir), rit darunavir	PI	yes	1997	2000	16	13	3
20	NPI-O	truvada (viread, emtriva), raltegravir	INT	no	2010	2011	3	2	1
21	HAD	epzicom (ziagen, epivir), rit darunavir	PI	yes	1994	1997	19	16	3
22	No Impairment	truvada (viread, emtriva), raltegravir	INT	no	1991	1995	22	18	4
23	NPI-O	combivir (azt, 3tc), kaletra (rit ritonivir), efavirenz	PI, NNRTI	yes	1985	2002	28	11	17
24	No Impairment	complera (emtricitabine, tenofovir, rilpivirine), rit darunavir, raltegravir	PI, INT, NNRTI	yes	1986	1991	27	22	5
25	No Impairment	atripla (viread, emtriva, efavirenz)	NNRTI	no	1999	1999	14	14	0
26	NPI-O	truvada (viread, emtriva), abacavir, rit darunavir	PI	yes	1999	2003	14	10	4
27	NPI-O	truvada (viread, emtriva), rit darunavir, raltegravir	PI, INT	yes	1998	1998	15	15	0
28	NPI-O	epzicom (ziagen, epivir), atazanavir	PI	yes	1985	1995	28	18	10
29	No Impairment	non-compliant	none	no	1991	2008	22	5	17
30	No Impairment	epivir, rit darunavir, raltegravir, maraviroc	PI, INT, fusion	yes	1988	1993	25	20	5
31	HAND/MCMD	combivir (azt, 3tc), rit atazanavir	PI	yes	1989	1997	26	18	8
32	HAND/MCMD	complera (emtricitabine, tenofovir, rilpivirine)	NNRTI	no	1992	1994	23	21	2
33	HAND/MCMD	3tc. Abacavir, efavirenz	NNRTI	no	1989	2000	26	15	11
34	No Impairment		PI	yes					

Table 2 (continued)

Study Participant	HAND Diagnosis	ART at Draw	Regimen Base	PI y/n	Yr Dx	Year Started Any ART	Time From Diagnosis	Time From Starting ART	Time From Diagnosis to Starting ART
		emtricitabine, tenofovir, rit atazanavir							
35	HAND/MCMD	truvada (viread, emtriva), rit atazanavir	PI	yes	2000	2001	15	14	1
36	HAND/HAD	truvada (viread, emtriva), raltegravir	INT	no	1999	2000	16	15	1
37	No Impairment	emtricitabine, tenofovir, raltegravir	INT	no					
38	HAND/HAD	complera (emtricitabine, tenofovir, rilpivirine), dolutegravir	NNRTI, INT	no	1994	2015	21	0	21
39	NPI-O	tenofovir, triumeq (abacavir, dolutegravir, 3tc)	INT	no	1990	1997	25	18	7
40	No Impairment	truvada (viread, emtriva), rit atazanavir	PI	yes	2000	2000	15	15	0
41	No Impairment	truvada (viread, emtriva), rit atazanavir, raltegravir	PI, INT	yes	1992	1992	23	23	0
42	No Impairment	epivir, rit darunavir, etravirine, raltegravir	PI, NNRTI, INT	yes	1991	1997	24	18	6
43	HAND/MCMD	truvada (viread, emtriva), kaletra (rit lopinivir)	PI	yes	1985	2000	30	15	15
44	HAND/ANI	epzicom (ziagen, epivir), dolutegravir	INT	no	1999	2000	16	15	1
45	HAND/HAD	epzicom (ziagen, epivir), rit atazanavir	PI	yes	1990	1997	25	18	7

cognitive impairment who had only HIV infection and not any potentially confounding condition were assigned a HAND diagnosis.

Neuroimaging

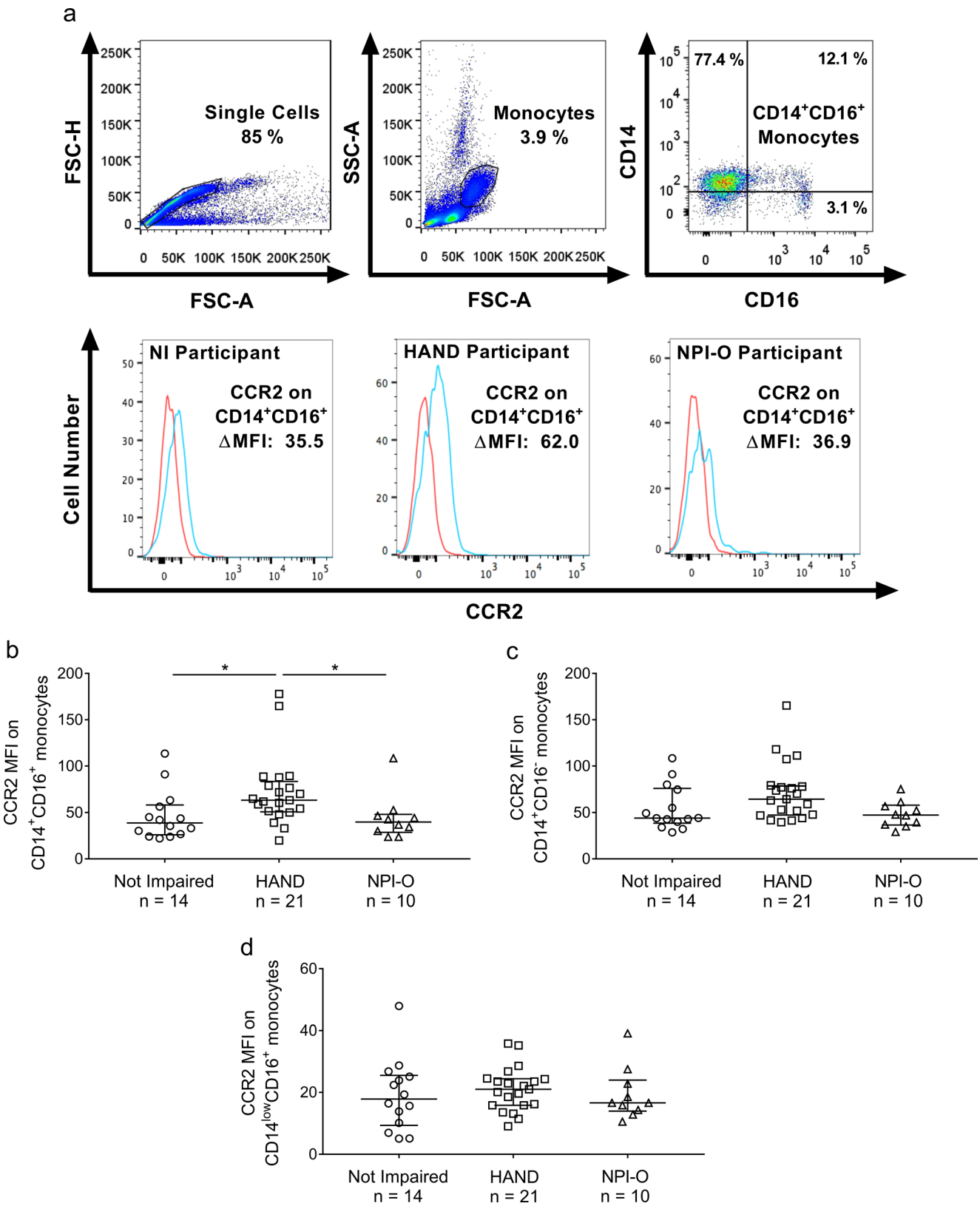
A subset of 18 study participants underwent MRI/^{1}H-MRS using a 3 T scanner (Skyra, Siemens Healthcare, Erlangen, Germany) using the vendor-provided head coil. The ^{1}H-MRS/MRI protocol included: a) Sagittal T1-weighted Magnetization-Prepared Rapid Gradient-Echo (MP-RAGE): (TE/TI/TR = 1.99/1000/2400 ms, voxel size: 1 mm^3); b) Sagittal 3D T2- weighted space sequence (TE/TR = 566/ /3200 ms, voxel size: 1 mm^3). T1 MPRAGE images were resliced in axial and coronal directions for chemical shift imaging (CSI) volume of interest (VOI) placement; c) Multivoxel 2D-CSI 1H-MRS was performed on an axial 15-mm thick slice at the level of the head of the caudate nucleus using a point-resolved spectroscopy (PRESS) sequence (TE/TR = 30/1700 ms, FOV: 160 × 160 mm2 matrix 16 × 16, voxel size 10 × 10 × 15 mm^3, 1024 data points, spectral width of 1200 Hz). Vendor provided semi-automatic shimming procedure was performed prior to the spectroscopic acquisition. The position of the CSI slice was carefully chosen to anatomically comparable position. Spectra were obtained from voxels of 1.5 cm^3 in the following regions: deep frontal white matter, head of caudate bilaterally; midline frontal gray matter.

Neuroimaging Post-Processing and Metabolite Quantification

For each participant undergoing neuroimaging, spectra from the above listed brain regions were fitted and metabolite amplitudes were evaluated using the time domain linear combination software LCModel (version 6.2) (Provencher 2001) and expressed in arbitrary units (a.u.). A quality control was applied to each voxel and the spectra were included in the analysis only if the signal-to-noise ratio (SNR) was equal to or greater than 5 and Cramer-Rao lower bounds (CRLBs) was equal to or smaller than 25% of the estimated concentration. The following metabolites were calculated per voxel: total N-Acetyl-Aspartate (tNAA), total choline (tCho), total creatine (tCr), myoinositol (mI), and glutamine and glutamate (Glx). Ratios of tNAA/tCr, tCho/tCr, mI/tCr, and Glx/tCr were measured and used for the correlative studies.

Droplet Digital PCR (ddPCR)

HIV DNA was quantified in PBMC. PBMC were isolated from whole blood as described in *Sample Processing*, after which DNA was extracted from 10^6 cells using the QIAamp


a
FSC-H
FSC-A
Single Cells
85 %
SSC-A
Monocytes
3.9 %
CD14
CD16
77.4 %
12.1 %
CD14+CD16+
Monocytes
3.1 %
Cell Number
CCR2
NI Participant
CCR2 on
CD14+CD16+
ΔMFI: 35.5
HAND Participant
CCR2 on
CD14+CD16+
ΔMFI: 62.0
NPI-O Participant
CCR2 on
CD14+CD16+
ΔMFI: 36.9
b
CCR2 MFI on
CD14+CD16+ monocytes
Not Impaired
n = 14
HAND
n = 21
NPI-O
n = 10
c
CCR2 MFI on
CD14+CD16- monocytes
Not Impaired
n = 14
HAND
n = 21
NPI-O
n = 10
d
CCR2 MFI on
CD14lowCD16+ monocytes
Not Impaired
n = 14
HAND
n = 21
NPI-O
n = 10

◀ **Fig. 1** HIV infected people with HAND have increased CCR2 on $CD14^+CD16^+$ monocytes. Surface CCR2 on three major monocyte subsets in PBMC from 45 subjects was determined by staining freshly isolated PBMC for human CD14, CD16, and CCR2, or with isotype matched control antibodies. Blood was drawn and PBMC isolated on the same day as neuropsychological assessment, which determined cognitive status. Cognitive status was determined as NI, HAND, or NPI-O. **a** Single cells were selected for analysis and forward- and side-scatter area (FSC-A and SSC-A) characteristics were used to determine monocyte gating. Monocyte subsets were then identified based on staining with isotype matched control, CD14 and CD16 antibodies. CD16 and the level of CD14 expression were used to gate for monocyte subsets, with high expression of both identifying $CD14^+CD16^+$ monocytes. CCR2 MFI was calculated by subtracting the MFI of the IgG2b isotype matched control antibody (red line) from the MFI of CCR2 (blue line) for each monocyte subset. **b** CCR2 MFI on $CD14^+CD16^+$ monocytes by cognitive status. **c** CCR2 MFI on $CD14^+CD16^-$ monocytes by cognitive status. **d** CCR2 MFI on $CD14^{low}CD16^+$ monocytes by cognitive status. Each data point is one individual subject, and data are represented as median ± IQR. Significance was determined by Kruskal Wallis test ($p < 0.01$) with multiple comparison's post-hoc testing. * $p < 0.05$

DNA Blood Mini Kit according to the manufacturer's protocol (Qiagen, Valencia, CA, USA). Analysis of HIV DNA was done using ddPCR. The ddPCR assay was performed in a Bio-Rad QX-100 system as described previously (Strain et al. 2013), using a primer/probe set for HIV Gag: HIV Gag F: '5-TCAGCCCAGAAGTAATACCCATGT-3', HIV Gag R: '5-CACTGTGTTTAGCATGGTGTTT-3', and HIV probe: 5'-ATTATCAGAAGGAGCCACCCCACAAGA-3′ (Integrated DNA Technologies, Coralville, Iowa, USA).

Statistical Analysis

Statistical analyses were performed using STATA/IC 14.2 for Mac (College Station, TX, USA). Kruskal-Wallis test was performed with Dunn's multiple comparison post hoc test to examine CCR2 on monocyte subsets between cognitive statuses, and race, and to examine differences in race between cognitive status. Kruskal-Wallis test with Dunn's multiple comparison post hoc test was also performed to examine differences between cognitive status and the following: percent of $CD14^+CD16^+$ monocytes that were CCR2+, years from HIV diagnosis, years from beginning cART, or years between HIV diagnosis and beginning cART. Mann Whitney U test was performed to examine CCR2 on $CD14^+CD16^+$ monocytes by sex, and detection of viral load, and to examine HIV DNA copies per 10^6 PBMC by cognitive status dichotomized in NI/NPI-O and HAND, as well as to examine cohort characteristics by cognitive status dichotomized in NI/NPI-O and HAND. Spearman's rank correlation coefficient was used to examine the relationship between CCR2 on $CD14^+CD16^+$ monocytes and plasma viral load (log_{10} copies/ml), current CD4+ T cell counts (cell/μl), nadir CD4+ T cell counts (cell/μl), HIV DNA copies per 10^6 PBMC, years from HIV diagnosis, year from beginning cART, and years between HIV diagnosis and beginning cART. Pearson's correlation coefficient was used to examine the relationship between MRI /^{1}H-MRS markers and CCR2 on $CD14^+CD16^+$ monocytes, $CD14^+CD16^-$ monocytes (%), and $CD14^+CD16^+$ monocytes (%).

Results

Characteristics of Study Participants

The demographic and clinical characteristics of HIV infected individuals from the MHBB cohort who participated in this study are in Table 1. The 45 study subjects had a median age of 56, with 24 participants (53.3%) being female and 21 being male (46.7%) with median years since HIV diagnosis of 21. All individuals in the cohort had longstanding histories of cART, with 96% on cART at the time of blood draw for this study (3 were non-compliant) with median years from beginning any cART treatment of 15 and median years between HIV diagnosis and time of beginning cART treatment of 4. Further information and descriptions of study participant's cART regimens, year of HIV diagnosis and year of beginning cART can be found in Table 2. The median plasma viral load was just above the limit of detection (19 copies/ml) at 24 copies per ml (log_{10} copies/ml: 1.4). While 59% of individuals had a detectable plasma viral load, only 28.9% had loads greater than 100 copies/ml. HAND was diagnosed in 21 of the participants (46.7%). We found no difference between cognitive status diagnosis and years from HIV diagnosis (data not shown), years from beginning cART (data not shown), or years between HIV diagnosis and beginning cART (data not shown).

CCR2 is Increased on CD14 + CD16+ Monocytes Specifically from HIV Infected Individuals with Cognitive Impairment Due to HIV

In a prior study, our laboratory demonstrated that surface CCR2 is increased on $CD14^+CD16^+$ monocytes in PBMC from HIV infected individuals with HAND, independent of cART status, viral load, nadir CD4+ T cell or current CD4+ T cell counts (Williams et al. 2014). To determine whether this effect was specific to impairment due to HIV related events, we examined CCR2 on $CD14^+CD16^+$ monocytes in PBMC from an additional 24 HIV infected individuals in the MHBB cohort that were NI, NPI-O, or had HAND for a total sample size of 45.

The gating strategy for monocytes and their subsets, as described in the Methods, is shown in Fig. 1a. We determined that there was a significant increase in CCR2 MFI on $CD14^+CD16^+$ monocytes (median [interquartile range (IQR)]) from those with HAND (63.3 [51.6, 79.0]) compared

to those that were NI (38.8 [26.7, 56.4]; Kruskal-Wallis test, $p < 0.01$; Dunn's multiple comparison post-hoc, $p < 0.05$), or those that were NPI-O (39.8 [30.2, 46.5]; Dunn's multiple comparison post-hoc, $p < 0.05$) (Fig. 1b). The CCR2 MFI on $CD14^+CD16^+$ monocytes was above 47 for 18 (85.7%) individuals with HAND, and below 47 for 10 (71.4%) individuals that were NI and 8 (80%) of the individuals with NPI-O. Thus, there was a "threshold of impairment" represented by the lowest CCR2 MFI on peripheral blood $CD14^+CD16^+$ monocytes that was indicative of HAND. CCR2 MFI's at, or above, this threshold level distinguish HIV infected individuals with HAND from those that are NI or NPI-O. In this study, CCR2 MFI levels at, or above, 47 on $CD14^+CD16^+$ monocytes was the "threshold of impairment". We did not find a significant difference among the three cognitive diagnoses with CCR2 on $CD14^+CD16^-$ monocytes (Fig. 1c), or CCR2 on $CD14^{low}CD16^+$ monocytes (Fig. 1d). We also did not find significance in the percentage of $CD14^+CD16^+$ monocytes that express CCR2 between the HAND, NI and NPI-O populations (data not shown).

The expression of CCR2 on $CD14^+CD16^+$ monocytes was not significantly associated with gender (Fig. 2a), ethnicity (Fig. 2b), detectable (>19 copies/ml) viral load (Fig. 2c), viral load quantification (Fig. 2d), CD4+ T cell counts (Fig. 2e), nadir CD4+ T cell counts (Fig. 2f), cART treatment (data not shown), years from HIV diagnosis to CCR2 analysis (data not shown), years from cART treatment to CCR2 analysis (data not shown), or years between HIV diagnosis and beginning of cART (data not shown). Thus, while CCR2 on $CD14^+CD16^+$ monocytes in PBMC from HIV infected individuals, specifically distinguished those with HAND from those who are NI or who are impaired due to causes other than HIV (NPI-O), it did not appear to be related to routinely measured immunovirologic indices. Moreover, the increase in CCR2 was independent of other demographic and clinical data assessed, providing additional support for the specificity of CCR2 on $CD14^+CD16^+$ monocytes as a biomarker for HAND.

Brain Imaging Cohort Characteristics

To characterize further CCR2 on $CD14^+CD16^+$ monocytes as a biomarker for neurobiologic processes associated with HAND, we performed additional analyses with 18 of the 45 individuals in this study who were also undergoing MRI/^{1}H-MRS in a pilot neuroimaging study. Thus, we were able to perform association studies between cognitive status, CCR2 on peripheral $CD14^+CD16^+$ monocytes, proton magnetic resonance spectroscopy (^{1}H-MRS) measured neurometabolite levels, and PBMC HIV DNA copies in a subgroup of the participants in our study. The demographic and clinical characteristics of this subgroup are shown in Table 3. As the NI and NPI-O group had very similar CCR2 MFI values on their $CD14^+CD16^+$ monocytes, data from participants from these groups were combined for further analyses. There were no significant differences in the demographic and clinical characteristics between the NI/NPI-O subjects and the subjects who had HAND. Of the 18 individuals participating in the neuroimaging pilot study, 9 (50%) were opioid users. These 9 individuals were relatively equally distributed across the NI/NPI-O and HAND groups (not shown, $p = 0.64$), and it did not affect the outcome of our additional analyses.

^{1}H-MRS-Derived Neuronal Damage is Greater in those with Increased CCR2 on CD14 + CD16+ Monocytes

Three regions of brain (deep frontal white matter, head of caudate bilaterally, midline frontal gray matter) were analyzed. We found, using Pearson's correlation coefficient, that there was an inverse association between CCR2 MFI on $CD14^+CD16^+$ monocytes and tNAA/tCr in the right caudate nucleus ($B = -32.16$, $r^2 = 0.348$, $p = 0.01$) (Table 4 and Fig. 3a). In the left caudate nucleus, we found an inverse association between CCR2 MFI on $CD14^+CD16^+$ monocytes and Glx/tCr ($B = -17.0$, $r^2 = 0.356$, $p = 0.01$) (Table 4 and Fig. 3b). This indicates that with higher CCR2 on peripheral blood $CD14^+CD16^+$ monocytes, there is increased impairment of ^{1}H-MRS metabolites specific for neuronal damage in the BG. For Glx/tCr we lack two measures in the left caudate nucleus ($n = 16$ versus $n = 18$ for tNAA/tCr and tCho/tCr in the right caudate nucleus) because we were unable to identify Glx measures reliably. There was also a significant inverse association between CCR2 on $CD14^+CD16^+$ monocytes and tCho/tCr in the right caudate nucleus ($B = -180.01$, $r^2 = 0.259$. $p = 0.03$) (Table 4 and Fig. 3c). As tCho/tCr is a marker indicative of cell proliferation and inflammation (Mohamed et al. 2010; Harezlak et al. 2014), and neuroinflammation is thought to be part of HAND (Campbell et al. 2014; Saylor et al. 2016), this association was opposite to what we expected. This may be related to longterm cART and the low level chronic inflammation that is more likely characteristic of this cohort. A more detailed analysis of these data is included in the Discussion. There were no other associations between ^{1}H-MRS data and CCR2 MFI on $CD14^+CD16^+$ monocytes (Table 4).

In addition, we found associations between decreased tNAA/tCr in the BG and the percentage of total peripheral monocytes that were $CD14^+CD16^+$ (Table 5); in contrast, the percentage of $CD14^+CD16^-$ monocytes was associated with increased tNAA/tCr in the caudate nucleus (not shown). $CD14^+CD16^+$ monocytes are important in HAND (Pulliam et al. 1997; Fischer-Smith et al. 2001; Valcour et al. 2010; Burdo et al. 2011; Williams et al. 2012; Williams et al. 2013), thus, these data indicate that an increased presence of these monocytes in the periphery may, in part, contribute to neuronal damage. As CCR2 on $CD14^+CD16^+$ monocytes was associated with neuronal damage as well, and with HAND, it

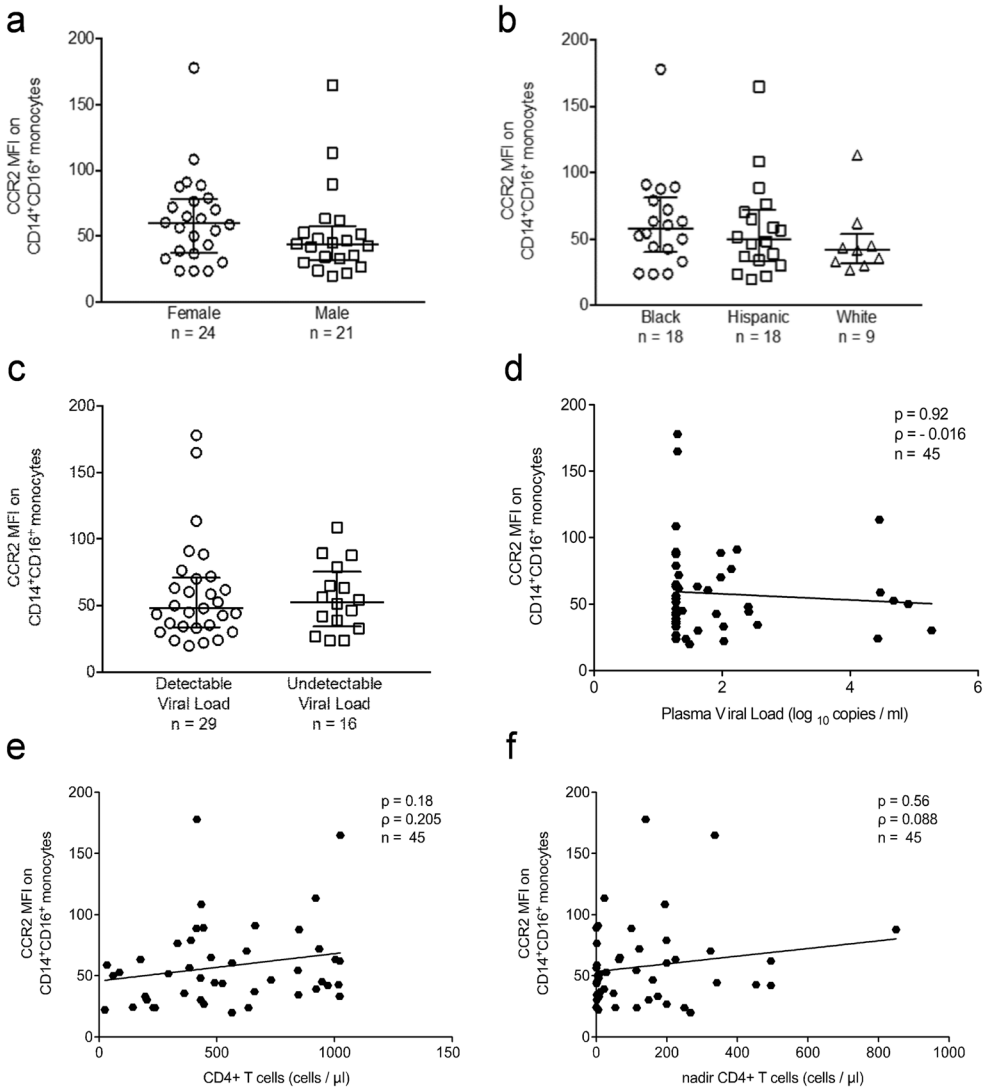


Fig. 2 CCR2 on $CD14^+CD16^+$ monocytes does not vary by demographic and HIV clinical variables. Surface CCR2 on $CD14^+CD16^+$ monocytes was determined by staining freshly isolated PBMC for human CD14, CD16, and CCR2, or with isotype matched control antibodies. CCR2 MFI was calculated by subtracting the MFI of the IgG2b isotype matched control staining on $CD14^+CD16^+$ monocytes from the MFI of CCR2 on $CD14^+CD16^+$ monocytes. Each data point is one individual subject. **a** CCR2 on $CD14^+CD16^+$ monocytes by sex (female: $n = 24$, male: $n = 21$). **b** CCR2 on $CD14^+CD16^+$ monocytes by ethnicity (Black: $n = 18$, Hispanic: $n = 18$, White: $n = 9$). **c** CCR2 on $CD14^+CD16^+$ monocytes by detection of viral load (detectable: $n = 29$, undetectable: $n = 16$). **d** Spearman's rank correlation between CCR2 on $CD14^+CD16^+$ monocytes and viral load ($\log_{10}$) ($n = 45$, $p = 0.92$, $\rho = -0.016$). **e** Spearman's rank correlation between CCR2 on $CD14^+CD16^+$ monocytes and current CD4+ T cell counts (cells/µl) ($n = 45$, $p = 0.18$, $\rho = 0.205$). **f** Spearman's rank correlation between CCR2 on $CD14^+CD16^+$ monocytes and nadir CD4+ T cell counts (cells/µl) ($n = 45$, $p = 0.56$, $\rho = 0.088$). (**a-c**). Data are represented as median ± IQR. Significance was determined by Mann Whitney U test. Data were not significant ($p > 0.05$)

suggests that this monocyte subset, and more specifically CCR2 on $CD14^+CD16^+$ monocytes, is related to the CNS pathology that is associated with HAND.

Increased PBMC HIV DNA is Associated with Increased CCR2 on CD14 + CD16+ Monocytes

Increased HIV DNA in PBMC has also been associated with HAND, with $CD14^+CD16^+$ monocytes proposed to be the major source of HIV DNA (Shiramizu et al. 2005; Kusao et al. 2012; Cysique et al. 2015). To determine whether in our study CCR2 on $CD14^+CD16^+$ monocytes was also associated with increased PBMC HIV DNA, we examined the number of HIV DNA copies per 10^6 PBMC from 14 of the 18 individuals from whom we were able to obtain sufficient cells for HIV DNA analyses. We performed a Spearman's rank correlation between CCR2 on $CD14^+CD16^+$ monocytes and the number of HIV DNA copies per 10^6 PBMC and found a significant association between the two variables ($\rho = 0.618$, $p = 0.02$) (Fig. 4a). In addition, we found that there was a trend to a higher number of HIV DNA copies per 10^6 PBMC (median [IQR]) in those with HAND (74 [39, 170]) as compared to

Table 3 Brain imaging cohort characteristics stratified by cognitive status

Cohort Characteristics	Overall (N = 18) Median [IQR] or n (%)	NI / NPI-O (*N* = 7) Median [IQR] or n (%)	HAND (*N* = 11) Median [IQR] or n (%)	*p* value
Age (yr)	56 [52, 59]	56 [53, 62]	56 [51, 59]	0.78
Gender				0.62
Female	14 (77.8%)	5 (71.4%)	9 (81.8%)	
Male	4 (22.2%)	2 (28.6%)	2 (18.2%)	
Race				0.31
Black	7 (38.9%)	2 (28.6%)	5 (45.5%)	
Hispanic	6 (32.3%)	2 (28.6%)	3 (36.4%)	
White	5 (27.8%)	3 (42.9%)	2 (18.2%)	
Years from HIV diagnosis	24 [15, 30]	24 [15, 30]	23 [16, 26]	0.84
cART	18 (100.0%)	7 (100.0%)	11 (100.0%)	>0.99
Years from starting cART	17 [0, 23]	18 [15, 23]	15 [14, 21]	0.19
Years between HIV diagnosis and starting cART	4 [0, 21]	6 [0, 10]	2 [1, 15]	0.52
CD4 T cell count (cells/μl)	662 [399, 922]	660 [399, 947]	702 [294, 922]	0.96
Nadir CD4 T cell count (cells/μl)	63 [8, 225]	12 [2, 77]	114 [10, 387]	0.09
Plasma viral load (log_{10} copies/ml)	1.3 [1.3, 1.8]	1.3 [1.3, 1.6]	1.3 [1.3, 2.0]	0.73
viral load undetectable	10 (55.6%)	4 (57.1%)	6 (54.5%)	0.92

those infected individuals that are NI/NPI-O (50 [8, 61]), but this was not statistically significant (Fig. 4b). These data suggest that in those that have increased CCR2 on $CD14^+CD16^+$ monocytes, and thus likely HAND, an increase in peripheral HIV DNA, proposed to correlate with the levels of infected peripheral $CD14^+CD16^+$ monocytes (Shiramizu et al. 2005; Kusao et al. 2012), may also contribute to the development of cognitive deficits.

Discussion

It is recommended for all HIV infected people to be screened for HAND at initial diagnosis and at subsequent appropriate intervals (Mind Exchange Working 2013). This is because HAND, even at subclinical presentation, could pose a risk for lower cART adherence, a decreased quality of life, and an increased risk of reduced life span (McArthur and Smith 2013). HAND is diagnosed by neuropsychologists after an extensive cognitive analysis consisting of a large battery of neuropsychological tests and examination of activities of daily living, as described in detail previously (Antinori et al. 2007). However, the identification of HAND in a different clinical setting may be difficult due to several reasons. The severity of HAND is decreased in the cART era as compared to pre-cART (Saylor et al. 2016), and symptoms may be subclinical, making it challenging to identify those with HAND. Also, there is a balance between the ease of screening measures and their sensitivity; in general, those measures readily applicable to clinic settings have reduced sensitivity, while more sensitive measures require specialized expertise in cognitive testing (McArthur and Smith 2013). A recent study showed that the majority of HIV infected people with long-term viral suppression experience subclinical slow cognitive decline in psychomotor speed and executive function, typical neurocognitive domains affected during HAND progression that could affect an individual's quality of life (Gott et al. 2017). Therefore, it would be valuable for practitioners to be able to test all HIV infected people for their risk of HAND. Screening tools for HAND that analyze cognitive function have been recently developed that shorten the time and cost of neuropsychological testing, but these depend on the test-taker's ability to use a smartphone/tablet, and rely on testing competence (Robbins et al. 2014). The development of an easy accessible biomarker that can be assessed at frequent intervals, in a fast and standardized manner, is therefore essential. In this study, we showed that CCR2 on $CD14^+CD16^+$ monocytes may be such a biomarker, as it is increased on this monocyte subpopulation in the peripheral blood specifically in infected individuals who have HAND, while CCR2 is lower in those with NPI-O or those who are not impaired (NI). In addition, we determined that increased CCR2 on $CD14^+CD16^+$ monocytes is associated with neuronal damage and PBMC HIV DNA that have both been associated with HAND in other studies.

We found that CCR2 on $CD14^+CD16^+$monocytes does not change with gender, ethnicity, viral load, current or nadir CD4+ T cell counts, cART, years from HIV diagnosis to CCR2 analysis, years from beginning cART to CCR2 analysis, and years between HIV diagnosis and beginning cART. In a prior study from our laboratory it was shown that increased CCR2 did not associate with other comorbid conditions, including substance use, diabetes, and liver disease (Williams et

Table 4 Pearson's correlation between ^{1}H-MRS measured metabolites and CCR2 on $CD14^{+}CD16^{+}$ monocytes

	tNAA/tCr	tCho/tCr	mI/tCr	Glx/tCr
Right Caudate Nucleus	B: −32.16	B: −180.01	B: −24.57	B: −0.66
	r^2: 0.348	r^2: 0.259	r^2: 0.082	r^2: 0.000
	P: 0.01	P: 0.03	P: 0.27	P: 0.95
Right Frontal White Matter	B: −2.83	B: −3.49	B: −25.94	B: −8.99
	r^2: 0.005	r^2: 0.000	r^2: 0.126	r^2: 0.073
	P: 0.80	P: 0.94	P: 0.18	P: 0.29
Midline Frontal Gray Matter	B: 19.44	B: −40.41	B: 12.70	B: 8.88
	r^2: 0.150	r^2: 0.019	r^2: 0.115	r^2: 0.080
	P: 0.17	P: 0.59	P: 0.22	P: 0.31
Left Caudate Nucleus	B: −13.16	B: 95.41	B: 3.87	B: −17.0
	r^2: 0.024	r^2: 0.035	r^2: 0.037	r^2: 0.356
	P: 0.54	P: 0.46	P: 0.45	P: 0.01
Left Frontal White Matter	B: 8.31	B: 27.80	B: 0.53	B: 0.98
	r^2: 0.013	r^2: 0.069	r^2: 0.000	r^2: 0.000
	P: 0.69	P: 0.31	P: 0.97	P: 0.93

al. 2014). Additionally, we now demonstrate that increased CCR2 is also not associated with neurocognitive deficits due to causes other than HIV (NPI-O). This suggests that CCR2 as a biomarker might specifically identify individuals at risk for cognitive deficits due to HAND. With professional neuropsychological assessment, the interrater reliability (IRR) for detecting cognitive impairment in people with HIV is very high, however, the IRR for distinguishing HAND from NPI-O is very low (Woods et al. 2004). Our study suggests that CCR2 may possibly be of use in the diagnosis of NPI-O, as we found that CCR2 remains below the threshold of impairment for 80% of individuals with NPI-O. As this study was performed in a cross-sectional manner, it is important to confirm this finding with longitudinal studies with a larger sample size that include subjects who are believed to have NPI-O. We examined one NPI-O subject twice over a period of 27 months, and found that at both time points CCR2 was below the CCR2 threshold of impairment (not shown). This suggests that CCR2 may assist in NPI-O diagnosis, and that it could be a reliable marker for frequent testing.

A prior study associated monocyte subsets with MRI/MRS neuroimaging during acute HIV infection. It showed a positive correlation between mI/Cr in the BG and absolute number of $CD14^{+}CD16^{-}$ monocytes (Lentz et al. 2011). This monocyte subset is not believed to be the major cell type to enter the CNS during HIV infection. They also found an inverse correlation between NAA in the BG and the $CD14^{+}CD16^{+}$ monocyte subset (Lentz et al. 2011). This monocyte population can

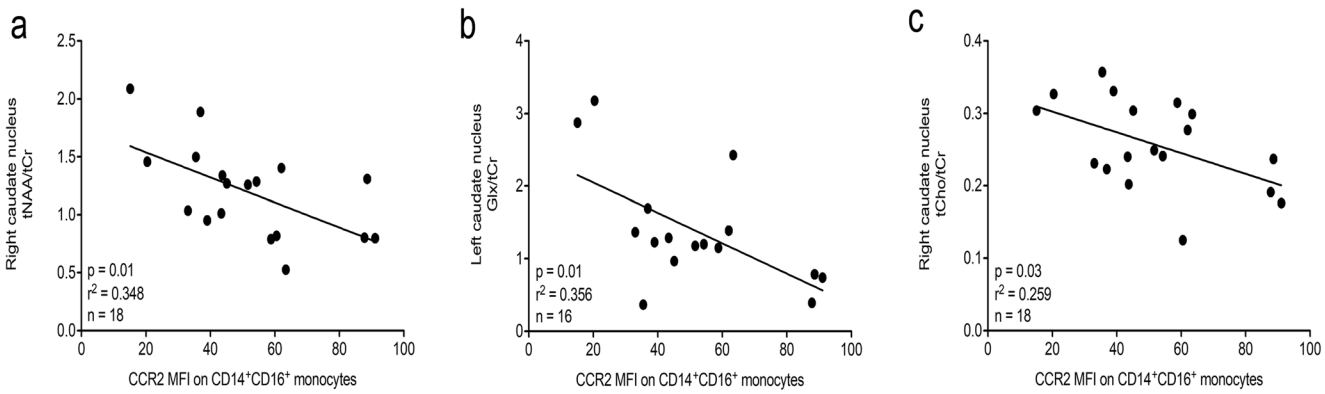


Fig. 3 ^{1}H-MRS markers of neuronal health correlate inversely with CCR2 on $CD14^{+}CD16^{+}$ monocytes. Freshly isolated PBMC were stained for human CD14, CD16, and CCR2, or with isotype matched control antibodies. CCR2 MFI was calculated by subtracting the MFI of the IgG2b isotype matched control staining on $CD14^{+}CD16^{+}$ monocytes from the MFI of CCR2 on $CD14^{+}CD16^{+}$ monocytes. MRI/^{1}H-MRS imaging was performed within 1–2 weeks of blood draw. Pearson's correlation coefficient was used to determine the coefficient of determination (r^2). Each data point is one individual subject. **a** Inverse correlation of tNAA/tCr in the right caudate nucleus with CCR2 on $CD14^{+}CD16^{+}$ monocytes (n = 18, $p = 0.01$, $r^2 = 0.348$). **b** Inverse correlation of Glx/tCr in the left caudate nucleus with CCR2 on $CD14^{+}CD16^{+}$ monocytes (n = 16, p = 0.01, $r^2 = 0.356$). c Inverse correlation of tCho/tCr in the right caudate nucleus with CCR2 on $CD14^{+}CD16^{+}$ monocytes (n = 18, $p = 0.03$, $r^2 = 0.259$)

Table 5 Pearson's correlation between ^{1}H-MRS measured metabolites and $CD14^{+}CD16^{+}$ monocytes (%, of total monocytes)

	tNAA/tCr	tCho/tCr	mI/tCr	Glx/tCr
Right Caudate Nucleus	B: −13.42	B: −19.83	B: 8.99	B: −1.42
	r^2: 0.324	r^2: 0.017	r^2: 0.066	r^2: 0.006
	P: 0.01	P: 0.61	P: 0.32	P: 0.75
Right Frontal White Matter	B: −0.95	B: −25.75	B: −0.77	B: −1.27
	r^2: 0.005	r^2: 0.106	r^2: 0.001	r^2: 0.011
	P: 0.80	P: 0.19	P: 0.91	P: 0.69
Midline Frontal Gray Matter	B: −2.18	B: 24.05	B: −0.68	B: 2.94
	r^2: 0.018	r^2: 0.052	r^2: 0.003	r^2: 0.081
	P: 0.65	P: 0.38	P: 0.85	P: 0.30
Left Caudate Nucleus	B: −17.33	B: −59.00	B: 4.00	B: 1.19
	r^2: 0.220	r^2: 0.072	r^2: 0.208	r^2: 0.013
	P: 0.05	P: 0.28	P: 0.06	P: 0.67
Left Frontal White Matter	B: −0.60	B: 16.57	B: 6.06	B: −1.41
	r^2: 0.000	r^2: 0.187	r^2: 0.110	r^2: 0.006
	P: 0.94	P: 0.08	P: 0.21	P: 0.77

be infected with HIV (Ellery et al. 2007), transmigrates preferentially across the BBB into the CNS (Buckner et al. 2011; Williams et al. 2013), and is found within the CNS of infected individuals (Fischer-Smith et al. 2001). This correlation between NAA/Cr and $CD14^{+}CD16^{+}$ monocytes was also shown in acute SIV infection, in the frontal white matter (Campbell et al. 2011). We now found, in a cohort with chronic HIV infection, that the percentage of $CD14^{+}CD16^{+}$ monocytes was inversely associated with tNAA/tCr and Glx/tCr in the right and left caudate nucleus, respectively, in agreement with the previous study. In contrast, we showed that the population of monocytes that is suggested to contribute only minimally to HAND, the $CD14^{+}CD16^{-}$ monocyte subset (Williams et al. 2012), associated positively with tNAA/tCr, meaning the higher the number of $CD14^{+}CD16^{-}$ monocytes the better the neuronal health. Thus, our data indicate that the $CD14^{+}CD16^{+}$ monocyte subset specifically is associated with neuronal damage in the BG, connecting the peripheral monocyte subset that has been shown to be key to HAND pathogenesis, to neuronal damage. Moreover, this is the first time to our knowledge, that a surface marker on peripheral immune cells isolated from HIV infected individuals is associated with neuronal damage detected by ^{1}H-MRS. The association between CCR2 on $CD14^{+}CD16^{+}$ monocytes and tNAA/tCr in the right caudate nucleus, and Glx/tCr in the left caudate nucleus, reinforces the potential of CCR2 as a biomarker for HAND, as it is not only associated with neuropsychological diagnosis of impairment, but also with neuronal damage.

While the inverse correlation of tCho/tCr with CCR2 on $CD14^{+}CD16^{+}$ monocytes in the right caudate nucleus was unexpected, it may be that an increase in tCho/tCr is associated more with acute rather than chronic neuroinflammation, as it is a well established marker in glioblastoma. Moreover, data from another group suggest that tCho may be decreased by cART, while it is increased in cART-naïve HIV infected individuals (Zahr et al. 2014). The majority of HIV infected individuals in our study were prescribed cART, and have been living with HIV for a median of 21 years. As such, the inverse correlation found in this study may reflect low level chronic neuroinflammation and cART.

An increase in peripheral HIV DNA content may indicate that a higher number of infected $CD14^{+}CD16^{+}$ monocytes will transmigrate across the BBB into the CNS. In the

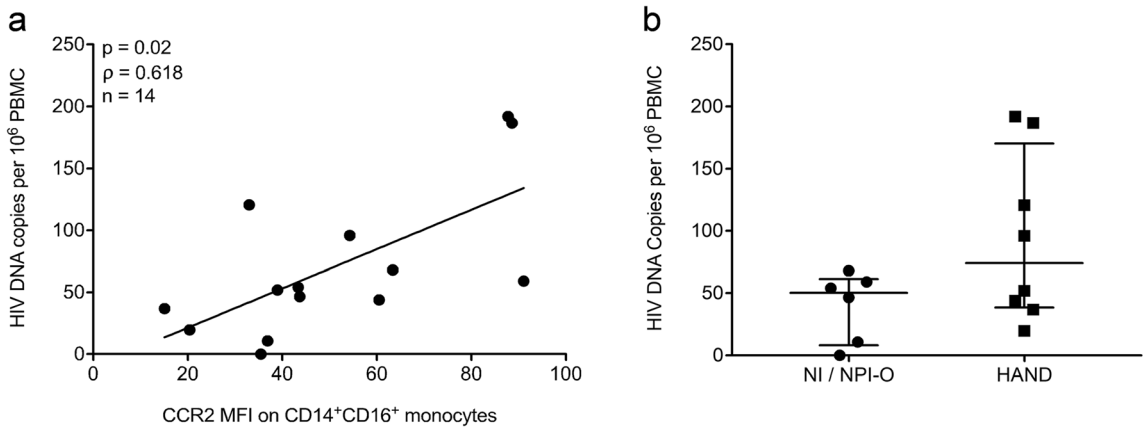


Fig. 4 PBMC HIV DNA is associated with CCR2 on $CD14^{+}CD16^{+}$ monocytes, and is increased in some people with HAND. PBMC were isolated from fresh peripheral blood samples, and 10^{6} cells collected, pelleted, and stored until DNA was isolated and HIV DNA was quantified. HIV DNA was quantified using ddPCR. CCR2 MFI on $CD14^{+}CD16^{+}$ monocytes was determined by subtracting the MFI of the IgG2b isotype matched control staining on $CD14^{+}CD16^{+}$ monocytes from the MFI of CCR2 on $CD14^{+}CD16^{+}$ monocytes in freshly isolated PBMC stained for human CD14, CD16, and CCR2, or with isotype matched control antibodies. Each data point is one individual subject. **a** Spearman's rank correlation associated HIV DNA copies per 10^{6} PBMC with CCR2 MFI on $CD14^{+}CD16^{+}$ monocytes ($n = 14$, $\rho = 0.618$, $p = 0.02$). **b** HIV DNA copies per 10^{6} PBMC from HIV infected people that are not impaired (NI) and NPI-O ($n = 6$), or are diagnosed with HAND ($n = 8$). Data are represented as median ± IQR. Significance was determined by Mann Whitney U test ($p > 0.05$)

presence of cART, cells with viral DNA may still produce early viral proteins, including Tat, that in the CNS lead to release of inflammatory and toxic mediators and subsequent neuronal damage (Weiss et al. 1999; Eugenin et al. 2003; Norman et al. 2008; Fields et al. 2015). The association between HAND and HIV DNA in PBMC, proposed to be localized predominately in $CD14^+CD16^+$ monocytes in some of these studies, has been established by several laboratories and in different cohorts (Shiramizu et al. 2005; Valcour et al. 2010; Kusao et al. 2012; Cysique et al. 2015). Therefore, the association we identified between PBMC HIV DNA and CCR2 on $CD14^+CD16^+$ monocytes is of high interest. CCR2 on $CD14^+CD16^+$ monocytes is associated with HAND and with increased $CD14^+CD16^+$ monocyte transmigration across the BBB. Thus, it suggests that a higher HIV DNA content in PBMC in those with increased CCR2 on $CD14^+CD16^+$ monocytes may result in increased infected macrophages within the CNS as a consequence of the transmigration of infected peripheral $CD14^+CD16^+$ monocytes across the BBB, perhaps contributing to the reseeding of CNS viral reservoirs and subsequent neuronal damage and HAND.

This study underscores that CCR2 on $CD14^+CD16^+$ monocytes may be a useful biomarker for HAND. The advantage of a biological biomarker in the peripheral blood is that it is easily accessible, and that it can be examined frequently. Moreover, it can be easily tested by flow cytometry, similar to assessment of CD4+ T cell counts. It may assist neuropsychologists in distinguishing NPI-O cases from HAND. Frequent evaluation of CCR2 may identify those who should be examined with a more thorough assessment by a neuropsychologist. Based on the findings of the neuropsychologist, HIV treatment and patient support may be adjusted. Although this study only examined individuals from one cohort, and performed neuroimaging and PBMC HIV DNA analysis on a small and heterogeneous subset of individuals, our study further reinforced that $CD14^+CD16^+$ monocytes are essential to HAND, due to the significant associations of this monocyte subset with neuronal damage detected by neuroimaging, and because of their potential to contribute to increased viral entry into the CNS (Veenstra et al. 2017). The association between PBMC HIV DNA and CCR2 on $CD14^+CD16^+$ monocytes, and PBMC HIV DNA and HAND in individuals prescribed cART further underscores that HIV infected $CD14^+CD16^+$ monocytes may contribute to HAND in the cART era. Future therapeutics that could reduce CNS entry of $CD14^+CD16^+$ monocytes may then assist in reducing and preventing HAND.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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