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Peripheral Blood Cytokines as Markers of Longitudinal Change in White Matter Microstructure Following Inpatient Treatment for Opioid Use Disorders

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ABSTRACT

BACKGROUND: Opioid use disorder (OUD) causes major public health morbidity and mortality. Although standard-of-care treatment with medications for OUD (MOUDs) is available, there are few biological markers of the clinical process of recovery. Neurobiological aspects of recovery can include normalization of brain white matter (WM) microstructure, which is sensitive to cytokine signaling. Here, we determined whether blood-based cytokines can be markers of change in WM microstructure following MOUD.

METHODS: Inpatient individuals with heroin use disorder (iHUDs) (n = 21) with methadone or buprenorphine MOUD underwent magnetic resonance imaging (MRI) scans with diffusion tensor imaging (DTI) and provided ratings of drug cue–induced craving, arousal, and valence earlier in treatment (MRI1) and ≈14 weeks thereafter (MRI2). Healthy control participants (HCs) (n = 24) also underwent 2 MRI scans during a similar time interval. At MRI2, participants provided a peripheral blood sample for multiplex quantification of serum cytokines. We analyzed the correlation of a multitarget biomarker score (from a principal component analysis of 19 cytokines that differed significantly between iHUDs and HCs) with treatment-related change in DTI metrics (ΔDTI; MRI2 − MRI1).

RESULTS: The cytokine biomarker score was negatively correlated with ΔDTI metrics in frontal, frontoparietal, and corticolimbic WM tracts in iHUDs but not in HCs. Also, serum levels of specific cytokines in the cytokine biomarker score, including the interleukin-related oncostatin M (OSM), similarly correlated with ΔDTI metrics in iHUDs but not in HCs. Serum levels of other specific cytokines were negatively correlated with changes in cue-induced craving and arousal in the iHUDs.

CONCLUSIONS: Specific serum cytokines, studied alone or as a group, may serve as accessible biomarkers of WM microstructure changes and potential recovery in iHUDs undergoing treatment with MOUD.

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Opioid use disorder (OUD) causes major morbidity and mortality (1,2) and constitutes a national epidemic. While treatment with medications for OUD (MOUDs) (e.g., methadone or buprenorphine) is the effective standard of care (2,3), many individuals with heroin use disorder (iHUDs) either do not receive treatment, drop out, or relapse (3,4). Insufficient knowledge about potential biomarkers of treatment outcomes (inclusive of recovery) of people receiving MOUD severely limits the development of personalized approaches and clinical stratification (5,6). Therefore, there is a critical unmet need for the identification of new and accessible biologically based markers to monitor change during treatment.

Chronic exposure to mu opioid receptor (MOR) agonists such as heroin, prescription opioids, morphine or fentanyl causes dysregulation of several peripheral and central targets, including cytokine systems (7–12). A small number of studies have reported potential differences in cytokine profiles based on the type of MOR agonist exposure (for example, with multiple daily use of short-acting compounds such as heroin vs. stable daily dosing with an MOUD such as methadone) (13,14). Likely based on pharmacodynamic or pharmacokinetic factors, such differences need to be examined more fully for a broader set of cytokine targets. Cytokines are a pleiotropic group of signaling molecules that act on cognate receptors in peripheral blood mononuclear cells, other leukocytes (15), and central neurons and glia (8,16,17). For example, several studies have shown that specific cytokines affect glial and white matter (WM) functions and responses to different insults (18,19). Specific leukocyte subsets or cytokines from blood can also affect central neuroglial processes by migration through the blood-brain barrier (20–22). The major canonical cytokine families include interleukins (ILs), chemokines, growth factors,

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and tumor necrosis factor–related proteins. We recently reported that serum analysis of a multiplex cytokine and inflammatory panel of proteins revealed significant differences in iHUDs (n = 21) receiving MOUD compared with healthy control participants (HCs) (n = 24) (23). Specifically, of the 19 cytokines (from different canonical families) that differed significantly between the groups, all but 3 exhibited iHUD > HC levels, and a multitarget cytokine biomarker score based on a principal component analysis (PCA) of these 19 cytokines robustly differentiated iHUDs from HCs, while adjusting for major demographic and clinical factors (23). Other studies have similarly shown changes in blood levels of several cytokines in iHUDs compared with HCs and changes in messenger RNA (mRNA) expression of several cytokine system genes, both in peripheral blood mononuclear cells and in the brain (9,15). These central and peripheral mechanisms may be broadly conserved because levels of several cytokines are altered in the blood of rats with extended-access fentanyl self-administration (24), and there are alterations in expression of several cytokine-related genes in the striatum of mice that chronically self-administered oxycodone (8). Importantly, preclinical studies have shown that the blood-brain barrier can also become functionally compromised after chronic MOR agonist exposure or withdrawal, potentially exacerbating neuroinflammatory effects (17,25). The brain's WM is composed primarily of myelinated neuronal tracts (axonal bundles), which underlie functional connectivity networks (26,27). Myelination is an active regulatory process based on interactions between glia, especially oligodendrocytes and oligodendrocyte progenitor cells (as well as microglia and astrocytes), and neurons (28,29) that affect neurotransmission and neuroplasticity (29). Crucially, myelination and neuroinflammation are governed by complex signaling networks, including cytokines and cognate receptors, as well as other proteins (30,31). Of relevance to HUD, a recent preclinical study showed that mu opioid agonist–mediated reward depended in part on interactions between neurons and oligodendrocytes (32). Also, there is robust mRNA expression of *OPRM1* (the gene that encodes MORs) in brain glia of humans post mortem (11). Chronic exposure to MOR agonists causes transcriptional and functional changes not only in molecular targets in central nervous system neurons (11,33,34) but also in oligodendrocytes and other glia (11), especially for diverse cytokine and neuroinflammatory genes (11).

WM microstructure can be examined in living humans with diffusion tensor imaging (DTI) and related techniques (35,36). We and others have documented widespread deficits in WM microstructure in individuals with substance use disorders including HUD (encompassing reduced fractional anisotropy [FA]) (37–39) being correlated with enhanced disease severity (e.g., increased craving and longer periods of regular use) (37). FA a measure of restriction in water molecule diffusion to a primary direction, often considered as a measure of normal WM microstructural organization. Importantly, some of these WM microstructure impairments appear to be dynamic, recovering in time with treatment. Specifically, we showed that following inpatient treatment of approximately 14 weeks with an MOUD (daily maintenance with methadone or buprenorphine), FA increased, especially in frontal callosal projections, association tracts, and the genu and body of the corpus callosum, and this increase was associated with reduced craving in iHUDs (40). Another recent longitudinal study of iHUDs (with 2 DTI scans separated by 8 months) found that prolonged abstinence was associated with an increase in mean FA in specific frontostriatal tracts, and this was correlated with a decrease in craving scores (41).

Brain levels of cytokines cannot be measured directly in living humans with currently available technologies. Furthermore, the passage of specific cytokines between the periphery and the brain depends on different mechanisms (42,43) and may vary dynamically during neuroinflammatory states (44). Therefore, it cannot be assumed that levels of cytokines from blood are directly reflective of their levels in the brain (or across different brain areas), and therefore research is needed to examine associations among blood levels of specific cytokines and change in brain WM. Here, we examined whether the longitudinal changes in WM microstructure found in iHUDs receiving MOUD would be correlated with readily accessible blood-based cytokine biomarkers. We hypothesized that levels of serum cytokines from a multiplex assay would be correlated with changes in WM microstructure and drug cue–induced subjective ratings (e.g., of craving, arousal, and valence) during MOUD treatment. The study was also intended to illuminate specific cytokine targets for future mechanistic evaluation of WM microstructure integrity in the context of OUD.

METHODS AND MATERIALS

Participants and Diagnostic Procedures

iHUDs (n = 21) were recruited from an inpatient treatment facility in New York City, and the HCs (n = 24) were recruited from the surrounding community. The study was approved by the Institutional Review Board of the Icahn School of Medicine at Mount Sinai; participants gave written informed consent. A highly trained study team supervised by clinical psychologists conducted participant evaluations that included the Mini-International Neuropsychiatric Interview (version 7.0.0 for DSM-5) (45) and the Addiction Severity Index (46). Medication exposure was recorded for all participants and did not include compounds with major immune effects (23).

Inclusion criteria for all participants included ages 18 to 65 years and having the capacity to understand and provide informed consent in English. Inclusion criteria for iHUDs included meeting DSM-5 criteria for OUD, with heroin as the drug of choice and the main reason for treatment. The iHUDs were inpatients receiving methadone (n = 17) or buprenorphine (n = 4) MOUD daily maintenance. Exclusion criteria for all participants included the following: 1) present or past history of a DSM-5 diagnosis of psychotic disorder or neurodevelopmental disorder; 2) history of head trauma with loss of consciousness (>30 minutes); 3) history of neurological disorders including seizures; 4) current use of any medication (with the exception of MOUD in the iHUDs) that may affect neurological functions; 5) current medical illness and/or evident infection including cardiovascular disease, as well as metabolic, endocrine, oncological, or autoimmune diseases and infectious diseases common in individuals with substance use disorders including Hepatitis B and C or HIV/AIDS (we did not exclude iHUDs for a history of other substance use

disorders [e.g., alcohol, cannabis, cocaine] or other psychiatric disorders with high rates of comorbidity with substance use disorders [e.g., depression, anxiety, posttraumatic stress disorder]); 6) magnetic resonance imaging (MRI) contraindications including any metallic implants, pacemaker device, or pregnancy; 7) a positive breathalyzer test for alcohol; and 8) MRI quality assurance, including the presence of incidental findings in the WM as indicated by a radiologist, insufficient quality of diffusion data, or an MRI session that did not include a diffusion sequence. Exclusion criteria for HCs included a lifetime history of any substance use disorders, including for alcohol, or a positive urine test for any drug of abuse. The HCs were recruited and assessed by the same study team with the same tools, but HCs did not complete scales specific to persons with HUD.

Behavioral Variables

Demographic (age, sex, race, body mass index) and clinical variables (sleep and drug use measures) are shown in Table 1. Because depression, anxiety, and stress exposure are comorbid with OUD and can be associated with change in cytokine mechanisms (22,47,48), participants completed the Beck Depression Inventory-II (49,50), Beck Anxiety Inventory (51), and Perceived Stress Scale (52). In the iHUDs, we also examined methadone/buprenorphine dose (either from documented report or self-report) and duration of current heroin abstinence from the daily interview. Age of first heroin use, age of onset of regular heroin use, and number of years of regular heroin use (excluding abstinence periods) were also assessed (Table 1). Following each of the 2 imaging scans (described below), we quantified self-reported ratings of cue-induced craving, valence, and arousal in response to drug-related pictures using a 5-point scale (with increasing numbers indicating greater ratings for these measures), as described previously (53).

Cytokine Assays

Blood samples were obtained by venipuncture on the day of the second MRI scan (MRI2) or on the nearest practical date (mean interval between MRI2 and blood sample = 1.3 days across all participants; $n = 45$). The blood sample was obtained during the time range of 09:00 AM to 5:00 PM (i.e., at least 1 hour after the daily MOUD administration in the iHUDs). Samples were centrifuged (10 minutes; 1200*g*) within ≈1 hour of collection, and serum was stored at −80 °C. Serum samples were analyzed with the Olink Target 96 Inflammation panel (Olink), following manufacturer's instructions (34), by the Human Immune Monitoring Center at the Icahn School of Medicine at Mount Sinai. This validated panel measures 92 targets, including cytokines from different canonical families and other inflammation-related proteins (full target list at https://olink.com/products-services/target/inflammation/). The assay provides relative quantification in normalized protein expression (NPX) separately for each target on a $\log_2$ scale; therefore a 1-unit NPX change indicates a 2-fold difference in protein levels. Targets with ≥50% of samples below the limit of detection within either group were not analyzed (47). Of 92 targets in the multiplex, 14 were excluded for this reason. The remaining 78 targets, including individual values below the limit of detection as in previous studies (54), were analyzed (23). Also, individual outliers (>±3 SDs from the group mean) were removed from the analyses and were replaced by imputation for the PCA (using missMDA in R; see below) (55).

MRI Acquisition and DTI

All participants were scanned twice (MRI1 and MRI2), as part of a larger study, as recently described (40). Briefly, MRI acquisition was performed using a Siemens 3T Skyra scanner (Siemens Healthineers AG) with a 32-channel head coil. Diffusion echo-planar sequence was acquired with opposite phase encoding along the left-right axis, monopolar diffusion encoding with 128 diffusion-weighted images (2×64 for each encoding phase) at single-shell maximum $b = 1500$ s/mm^2, 13 reference images at $b = 0$ s/mm^2, field of view (FOV) = 882 × 1044 mm, repetition time (TR) = 3650 ms, echo time (TE) = 87 ms, bandwidth = 1485 Hz/px, 80° flip angle, multiband = 3, no in-plane acceleration, and 1.8-mm isometric voxel size, with a total acquisition time of approximately 7 minutes. Anatomical T1-weighted structural images were acquired using the following parameters: 3-dimensional magnetization-prepared rapid acquisition gradient-echo (MPRAGE) sequence with FOV = 256 × 256 × 179 mm^3, 0.8-mm isotropic resolution, TR/TE/inversion time = 2400/2.07/1000 ms, 8° flip angle with binomial (1, −1) fat saturation, 240-Hz/px bandwidth, 7.6 ms echo spacing, and in-plane acceleration (generalized autocalibrating partially parallel acquisitions) factor of 2.

Statistical Analyses for Phenotypic Variables and Cue-Induced Subjective Ratings

Table 1 shows the distribution of sex and race across groups, analyzed with Fisher's exact tests. Demographic/clinical data (body mass index, age, sleep hours, depression, anxiety, and perceived stress) were compared across groups with Mann-Whitney *U* tests. Drug cue–induced subjective craving, arousal and valence ratings were analyzed as Δvalues (i.e., MRI2 − MRI1), where a negative Δvalue indicates a decrease in a specific rating from MRI1 to MRI2. Pearson correlations between levels of 19 cytokines (see below) and these Δdrug cue–induced ratings were examined (53). Normality of variables was examined with the D'Agostino and Pearson test, followed by examination of quantile-quantile plots, if necessary. The overall alpha level of significance was set at the $p = .05$ level.

Quantification of Individual Cytokines and PCA

NPX ($\log_2$ scale) were analyzed with Wilcoxon's rank-sum tests for group differences; *p* values were corrected for multiple comparisons with the false discovery rate (FDR) (5% cutoff level) approach (56). After identifying 29 targets that showed significant group differences from the previous step (including FDR correction), data from the 19 targets from canonical cytokine families that differed significantly between the 2 groups (these were primarily elevated in iHUDs vs. HCs) were entered into a PCA for dimensionality reduction (47,57), using *z* scores to standardize values. Because PCA requires complete data, individual outliers (i.e., greater than ±3 SDs of each group's mean) were replaced by multiple imputation (missMDA procedure in R) (55). The frequency of imputed values was

Table 1. Demographic and Clinical Variables

Variable	iHUD, n = 21	HC, n = 24	Group Differences, Mann-Whitney U or Fisher's Exact Test
Age, Years	42.0 (38.5–45.5)	40.7 (36.0–45.4)	NS
Sex, Female/Male	4/17	9/15	Fisher's exact test, NS
Race, Black/Other/White	0/3/18	8/4/12	Fisher's exact test, p = .006
Education, Years	12.1 (11.2–12.9)	16.6 (15.3–17.9)	U = 45.5, $p < .0001$
Body Mass Index at MRI2	32.3 (29.4–35.3)	26.7 (24.9–28.6)	U = 110, p = .0009
Sleep Hours in Night Prior to MRI2	6.54 (5.6–7.5)	6.51 (5.9–7.2)	NS
PSS-10 Perceived Stress Scores at MRI2, Scale Range: 0–40	20.5 (17.7–23.3), n = 20	12.3 (9.5–16.0)	U = 104, p = .001
BDI-II Depression Scores at MRI2, Scale Range: 0–63	15.5 (9.9–21.2), n = 19	3.8 (1.8–5.7)	U = 78, p = .0001
BAI Anxiety Scores at MRI2, Scale Range: 0–63	14.1 (8.8–19.4), n = 20	2.8 (1.1–4.4)	U = 85.5, p = .0001
Age of Onset of First Use of Heroin, Years	23.6 (17.4–27.8), n = 20	NA	NA
Age of Onset of Regular Use of Heroin, Years	25.1 (21.2–28.9), n = 20	NA	NA
Years of Regular Heroin Use	8.5 (5.9–11.2), n = 19	NA	NA
Heroin Abstinence Duration, Days	198 (130–268)	NA	NA
Methadone Daily Dose, mg	91.9 (63–121), n = 17	NA	NA
Years of Regular Alcohol Use	12.3 (5.9–18.6), n = 17	13.2 (6.6–19.8), n = 18	NS

Values are presented as n or mean (95% CI). Differing ns reflect missing data for specific variables.

BAI, Beck Anxiety Inventory; BDI-II, Beck Depression Inventory-II; HC, healthy control participant; iHUD, individual with heroin use disorder; MRI2, second magnetic resonance imaging scan; NA, not applicable; NS, nonsignificant; PSS, Perceived Stress Scale.

relatively low. Specifically, 3 targets in the HC group (TNFRSF9, CCL19, and CXCL9) each had 1 imputed data point (of a total n per target = 24). In the iHUD group, 3 targets (IL-6, CCL19, and CXCL9) also each had 1 imputed value (of a total n per target = 21). Nineteen principal components were calculated in the PCA algorithm, using a 95% threshold for significance and 1000 Monte Carlo simulations (GraphPad Prism). The complete results for these participants were reported recently (23). Scores for the first PC (PC1) accounted for the greatest proportion of variability (40.9%), and the individual PC1 scores were regressed against the whole-brain WM changes (i.e., ΔDTI for the 4 metrics; see below).

Analyses of Change in WM Metrics During Treatment

The preprocessing of the diffusion MRI data was conducted according to established pipelines with MRtrix3 (58) and FSL 6.0 toolboxes, as fully described (37,40). Tensor-based quantitative maps of diffusion metrics including FA, mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) were generated and used to perform tract-based spatial statistics (39). Whole-brain voxelwise analyses across all participants were conducted by first creating a WM skeleton and registered to the MNI152 standard space. Separate whole-brain correlation analyses were then performed between the 4 ΔDTI metrics (i.e., FA, MD, AD, RD) and the mean-centered cytokine PC1 scores (Figure S1) to test for slope differences between the iHUD and HC groups. We also tested the correlations between cytokine PC1 scores and the 4 DTI metrics at MRI1 and MRI2 separately for completeness. Within-group correlations were performed when significant group differences in slopes were detected. Analysis was performed via the FSL tool "Randomise," a general linear model for nonparametric permutation inferences via 10,000 permutations. In a follow-up, we repeated the same analyses with NPX (on a $\log_2$ scale) from the individual cytokines from each canonical family with the highest PC1 loadings (CCL7, OSM, CSF1, TNFRSF9) (23) (Figure S1). We also performed correlation analyses between the Δmaps of the 4 DTI metrics and intracranial volume (estimated by FreeSurfer from the individual T1-weighted structural scan), education, age, sex, and body mass index to rule out confounding factors. All DTI analyses accounted for multiple comparisons by using the threshold-free cluster enhancement correction (59), which enhances areas of signal that exhibit spatial contiguity, to better discriminate clusters.

RESULTS

As shown in Table 1, the groups were well matched on age, sex, sleep hours the night before the second scan, and years of alcohol use but different on racial distribution, education (iHUD < HC), body mass index, depression, anxiety, and perceived stress scores (iHUD > HC).

Cytokine Data

As recently reported (23), 19 cytokines were significantly different between the iHUD and HC groups, and these were analyzed with PCA (also see the Supplement, Table S1 and Figure S1). Of these 19 cytokines, 16 had iHUD > HC levels, and 3 had the opposite profile (HC > iHUD).

Correlations of Cytokine PC1 Scores With Longitudinal Changes in DTI Metrics

We found significant group differences only in correlations between cytokine PC1 scores and the changes in DTI metrics (i.e., MRI2 − MRI1 Δ scores) (Figure 1) but not with the absolute DTI metrics measured at either MRI1 or MRI2. Specifically, lower PC1 scores were correlated with overall increases in FA, MD, and AD following inpatient treatment across distributed WM anatomical connectivity networks that represent primarily frontal, frontoparietal, and corticolimbic tracts

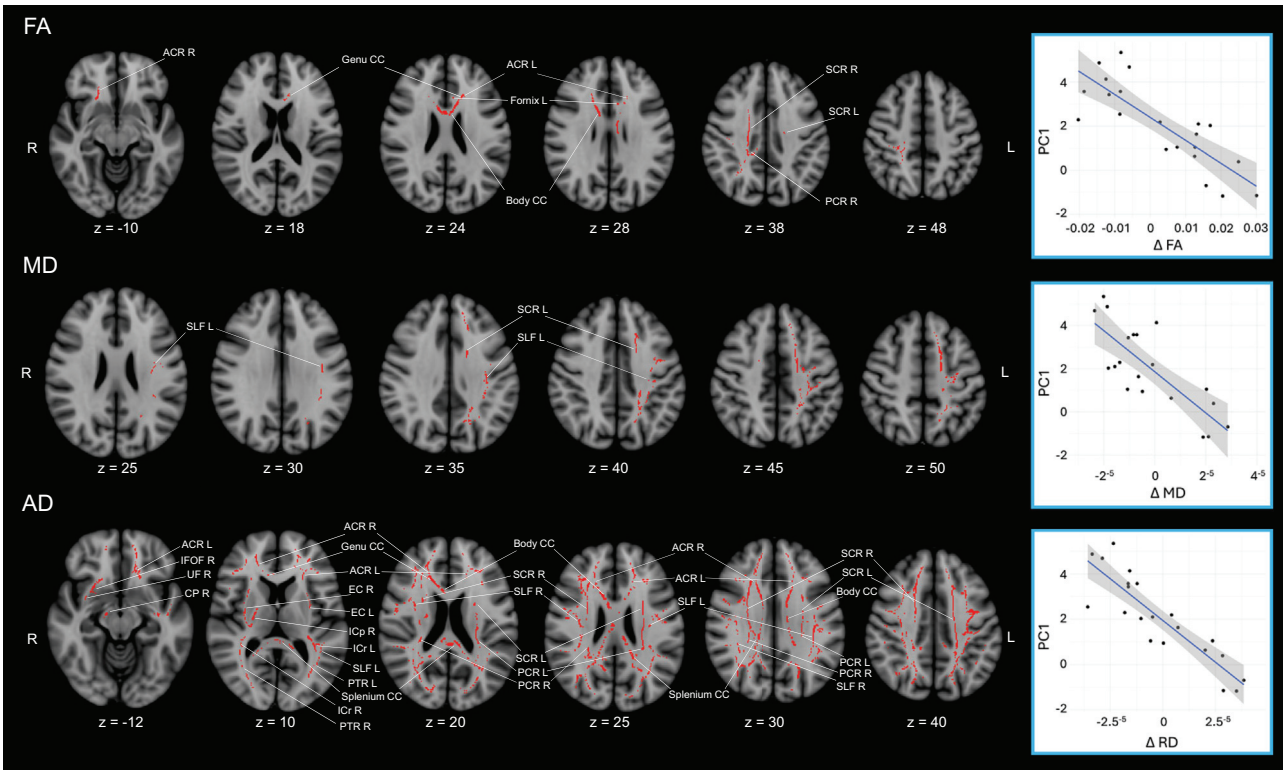

Figure 1. Cytokine first principal component (PC1) score correlations with change in diffusion tensor imaging (DTI) metrics across longitudinal scans (ΔDTI) (i.e., the second magnetic resonance imaging [MRI] scan – the first MRI scan). Binarized tracts that overlay the significant correlations between the PC1 score (of the 19 cytokines that showed group differences in individuals with heroin use disorder [iHUDs] vs. healthy control participants) and the changes in DTI metrics (fractional anisotropy [FA], mean diffusivity [MD], axial diffusivity [AD]; nonsignificant with radial diffusivity [RD]) (axial images). The negative correlations in the iHUD group are shown on the right (examining all significant voxels). ACR, anterior corona radiata; CC, corpus callosum; CP, midbrain cerebral peduncle; EC, external capsule; ICp, posterior limb of the internal capsule; ICr, retrolenticular part of the internal capsule; IFOF, inferior fronto-occipital fasciculus; L, left; PCR, posterior corona radiata; PTR, posterior thalamic radiation; R, right; SCR, superior corona radiata; SLF, superior longitudinal fasciculus; UF, uncinate fasciculus.

(encompassing the genu and body of the corpus callosum, fornix, anterior and posterior corona radiata, and superior longitudinal fasciculus for ΔFA and in the superior longitudinal fasciculus and superior corona radiata for ΔMD; correlations with ΔAD were observed in similar regions, as well as some more posterior cortical tracts). No correlation was found between cytokine PC1 scores and the other DTI metric, ΔRD (the maximum $1 - p$ value was .58). In a follow-up, we found that cytokine PC1 scores were significantly correlated with ΔMD in 3187 voxels, 2700 (84.7%) of which overlapped with the voxels with significant correlations with ΔAD (not shown). Therefore, this shows considerable spatial congruence for the correlation of cytokine PC1 scores and ΔMD or ΔAD. Other follow-up analyses within the HC and iHUD groups revealed that the slope differences mentioned above were driven by significant negative correlations in the iHUD group and null results in the HC group.

To explore possible specific targets involved in the findings mentioned above (as opposed to an overall cytokine PC1 score), we examined levels of the cytokines with the greatest PC1 loading from each canonical family for their correlations with ΔDTI metrics (Figures 2–4; also see Table S1 and Figure S1). Among these representative targets, levels of OSM were negatively correlated with ΔFA and ΔAD (Figure 2), levels of CSF1 were negatively correlated with ΔMD and ΔAD (Figure 3), and levels of MP3/CCL7 were negatively correlated with ΔAD (Figure 4) in iHUDs only. These correlations occurred over largely overlapping regions. No significant correlations were observed with TNFRSF9 (not shown).

Change in Cue-Induced Ratings (MRI2 − MRI1)

First, we examined whether changes in drug cue–induced ratings of craving, arousal, and valence were correlated with the overall cytokine PC1 score. Only cue-induced arousal showed a significant negative correlation with PC1 scores (Pearson's $r = -0.46$, uncorrected $p = .034$, did not survive FDR correction). However, we found that change in some cue-induced ratings was negatively correlated with levels of specific cytokines. Thus, change in cue-induced craving was negatively correlated with levels of CCL19 (Pearson's $r = -0.67$, uncorrected $p = .001$, FDR-corrected $p = .019$) (Figure 5A) such that a decrease in cue-induced craving (i.e., MRI2 − MRI1) was correlated with greater CCL19 levels. Also, change in cue-induced arousal was negatively correlated with levels of CCL2 (Pearson's $r = -0.68$, uncorrected $p = .001$, FDR-corrected $p = .011$) (Figure 5B) such that a decrease in cue-induced arousal was correlated with greater CCL2 levels. None of the correlations with changes in cue-induced valence

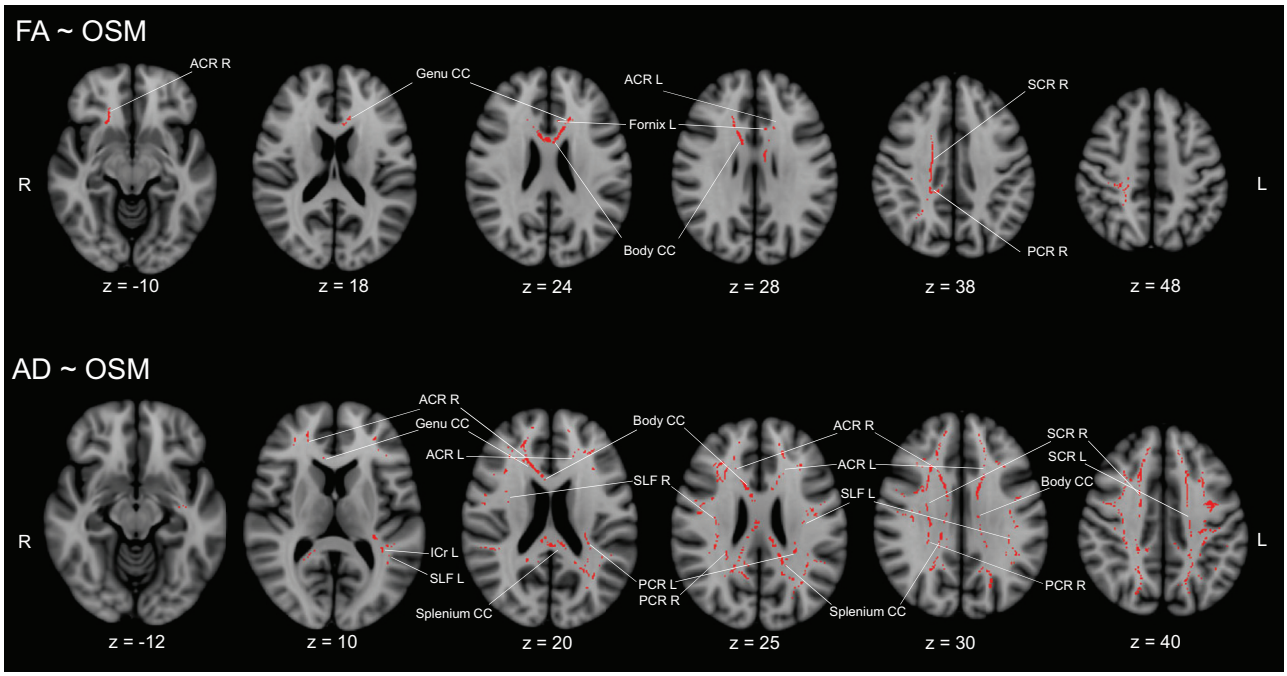

Figure 2. Correlation of serum OSM levels with change in diffusion tensor imaging metrics across longitudinal scans in the individuals with heroin use disorder (iHUDs) group. Binarized tracts for within-iHUD group negative correlations with serum OSM levels and ΔFA (top row) and ΔAD (bottom row). ACR, anterior corona radiata; AD, axial diffusivity; CC, corpus callosum; FA, fractional anisotropy; ICp, posterior limb of the internal capsule; L, left; PCR, posterior corona radiata; R, right; SCR, superior corona radiata; SLF, superior longitudinal fasciculus.

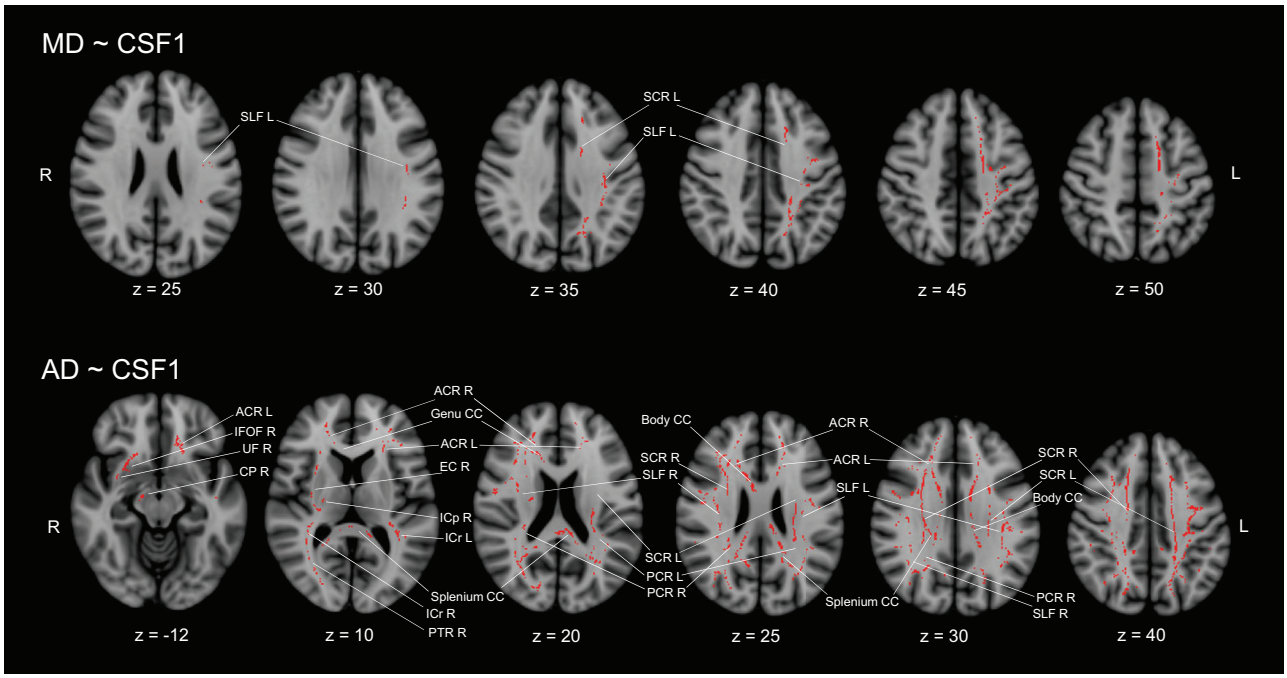

Figure 3. Correlation of serum CSF1 levels with change in diffusion tensor imaging metrics across longitudinal scans in the individuals with heroin use disorder (iHUDs) group. Binarized tracts for within-iHUD group negative correlations with serum CSF1 levels and ΔMD (top row) and ΔAD (bottom row). ACR, anterior corona radiata; AD, axial diffusivity; CC, corpus callosum; EC, external capsule; ICp, posterior limb of the internal capsule; ICr, retrolenticular part of the internal capsule; L, left; MD, mean diffusivity; PCR, posterior corona radiata; PTR, posterior thalamic radiation; R, right; SCR, superior corona radiata; SLF, superior longitudinal fasciculus.

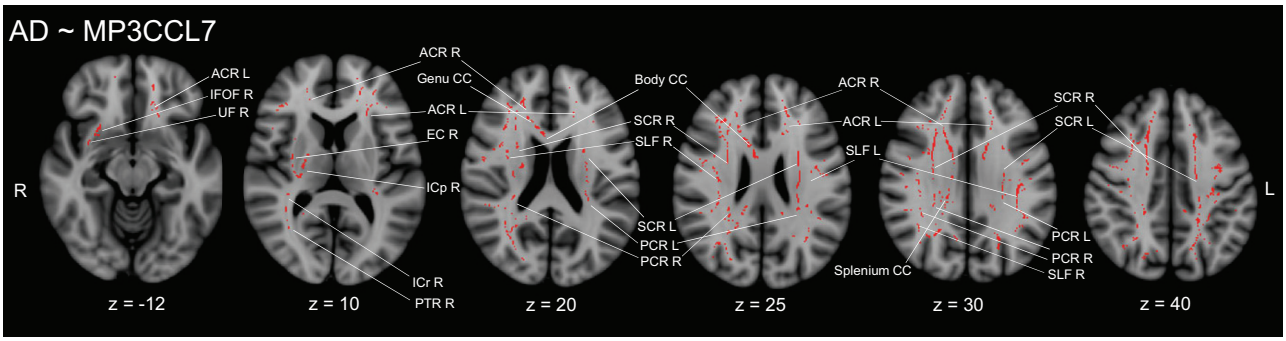

Figure 4. Correlation of MP3/CCL7 with change in diffusion tensor imaging metrics across longitudinal scans in the individuals with heroin use disorder (iHUDs) group. Binarized tracts for within-iHUD group negative correlations with serum MP3/CCL7 levels and ΔAD are shown. ACR, anterior corona radiata; AD, axial diffusivity; CC, corpus callosum; EC, external capsule; ICp, posterior limb of the internal capsule; ICr, retrolenticular part of the internal capsule; IFOF, inferior fronto-occipital fasciculus; L, left; PCR, posterior corona radiata; PTR, posterior thalamic radiation; R, right; SCR, superior corona radiata; SLF, superior longitudinal fasciculus; UF, uncinate fasciculus.

ratings survived FDR correction (full data are provided in Table S2). Neither PC1 scores nor levels of any of these 19 cytokines were correlated with cue-induced craving, arousal, or valence measured separately at either MRI1 or MRI2 after FDR correction (not shown).

DISCUSSION

The goal of this study was to examine the association between serum cytokine biomarkers and change in WM microstructure, together with drug cue–induced ratings, following ≈14 weeks of inpatient MOUD treatment in iHUDs compared with a similar interval in HCs. The main finding of this study is that a recently developed multitarget cytokine biomarker score (23) was correlated with ΔDTI metrics in several WM tracts, especially in the iHUDs. To our knowledge, this is the first study to examine cytokine blood levels as biomarkers of WM change in iHUDs, thereby expanding approaches that have been used for some other neuropsychiatric disorders (60–62).

Serum Levels of Specific Cytokines Are Correlated With WM Change With Treatment

We recently reported that, compared with HCs, iHUDs receiving chronic methadone or buprenorphine had robust dysregulation of serum cytokine levels (23). The current study shows that levels of specific cytokines (examined together as a multitarget score) were correlated with WM change following inpatient treatment. Thus, the cytokine PC1 score was negatively correlated with metrics of WM change with treatment in iHUDs, as quantified by higher FA, MD, and AD (but not RD), even with a stringent whole-brain approach (59,63), in anterior, posterior, and superior corona radiata and genu of the corpus callosum (Figure 1).

The different DTI measures studied here are interrelated (64,65), and it is informative to interpret them together. In this study, results of the correlations with ΔFA and ΔAD were in the expected direction; however, the direction of correlations with ΔMD were not (because higher FA and lower MD are commonly interpreted as representing more WM integrity and lower neuroinflammation) (66). Given the absence of correlations between the cytokine PC1 scores and RD, we interpret the correlations with MD (the average diffusivity of all directions, i.e., AD [λ1] and RD [λ2, λ3]) as being driven by the correlation with AD, consistent with the direction of this correlation with FA. The follow-up analyses of representative cytokines from different canonical families included in the PCA (i.e., the IL-6 family member OSM, the growth factor CSF1, and the chemokine CCL7) largely recapitulated these results (Figures 2–4). Overall, the similar pattern of results for ΔFA and ΔMD (and ΔAD) that we report here is an indicator of the caution needed when interpreting the levels of cytokine or group of cytokines (especially from serum), which are not necessarily indicative of overall neuroinflammation. Because different cytokines function in interdependent signaling networks (20,26) to support homeostasis and adaptation to change (allostasis), the direct impact of chronic drug use and/or MOUD treatment on these targets needs to be further examined. Other factors that may impact interpretation are

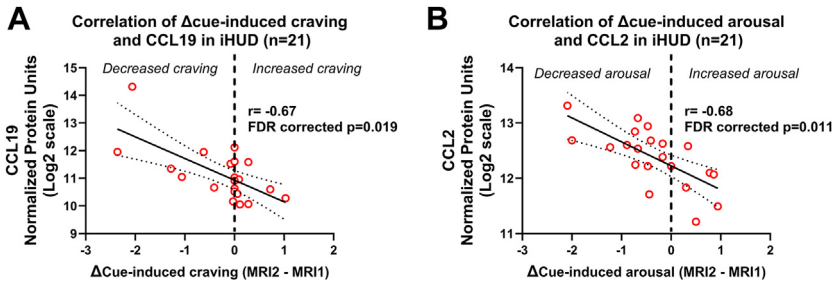

Figure 5. Correlations of changes in cue-induced craving or arousal (the second magnetic resonance imaging scan [MRI2] − the first MRI scan [MRI1]) with levels of specific serum cytokines in individuals with heroin use disorder (iHUDs), measured at the MRI2 stage. Pearson correlations are shown, with linear regression and 95% CI bands. Panel **(A)** shows change in cue-induced craving and CCL19 levels. Panel **(B)** shows change in cue-induced arousal and CCL2 levels. FDR, false discovery rate.

technical [e.g., the effect of crossing fibers or other heterogeneities on DTI metrics (67)], although commissural tracts such as the corpus callosum that were prominent in these correlations have predominantly parallel fiber organization, which makes them less vulnerable to such complexities (67).

Changes in WM microstructure in these and other tracts have previously been associated with neurocognitive dysfunction and disease severity in iHUDs (37,68,69), but the mechanisms that underlie WM impairment or recovery are not well understood. Although we cannot address the causality of the association between specific serum cytokines and changes in WM microstructure, some ancillary evidence may guide future studies. First, there is converging evidence that OSM and CSF1 mediate glial functions including myelination (70–73). Also, the transcription of genes encoding OSM and CSF1 (or that of cognate receptors) was elevated in postmortem ventral midbrain of iHUDs compared with HCs (11). Second, the chemokine CCL7 is a chemoattractant for macrophages and can promote neuroinflammation after trauma (74). More broadly, several studies have shown that central and peripheral cytokine/immune mechanisms can be causally linked (17,75,76). Future studies should examine whether specific cytokines (functioning individually or in networks) are causally involved in changes in WM microstructure during treatment with MOUD and the process of recovery or whether they can function as biomarkers for personalized therapeutic approaches.

Change in Cue-Induced Ratings (MRI2 - MRI1) Is Related to Cytokine Levels in the iHUDs

In exploratory analyses, we found that change in drug cue–induced subjective ratings in the iHUDs over the course of treatment (i.e., MRI2 − MRI1) was negatively correlated with serum levels of specific cytokines at the time of MRI2, suggesting that levels of these immune targets may be used as biomarkers of neurocognitive/emotional/motivational symptoms in OUD. Notably, we found a negative correlation between change in cue-induced craving and levels of the chemokine CCL19 (Figure 3A), a ligand for chemokine receptor CCR7 (77), which mediates heterologous desensitization of MORs in vitro (78). There was also a negative correlation between change in cue-induced arousal and levels of CCL2 (Figure 3B), the ligand for chemokine receptors CCR2 and CCR4 (79). Relatedly, a CCL2-induced mechanism has recently been implicated in the development of opioid withdrawal in a rodent model (17), and increased CCL2 blood levels have been causally associated with decreased FA in humans (76). Intriguingly, we recently reported that in these participants, serum levels of both CCL19 and CCL2 were greater in iHUDs than in HCs (23). Therefore, while these findings are apparently counterintuitive, two considerations may guide follow-up studies. First, it is unknown whether the timeline of specific cytokine serum levels is linear across the duration of MOUD treatment or recovery. Second, for simplicity, we only examined 4 representative members of canonical cytokine families for their correlations with ΔDTI measures. As mentioned above, cytokines work in complex functional networks (20). Therefore, other targets not examined here may more fully show how patterns of cytokine dysregulation in the iHUDs underlie these findings. Future multimodal longitudinal studies should examine larger serum cytokine networks together with cue-induced craving/arousal and DTI measures in iHUDs.

Limitations and Methodological Considerations

This relatively small (low *N*) observational study should be replicated and expanded in a larger sample of iHUDs and HCs. Furthermore, obtaining blood samples several times, including at treatment onset, would allow future studies to examine whether cytokine/immune changes in iHUDs (15,23,80) could contribute to the long-term changes observed with treatment in WM microstructure and drug cue–induced craving or arousal. Also, as a means of dimensionality reduction, we focused here on the 19 cytokines in this multiplex assay that differed significantly between iHUDs and HCs (23); larger longitudinal studies could examine a broader array of cytokines at different stages in the OUD trajectory. Furthermore, the serum samples were obtained during daytime hours, but further circadian precision should be implemented in future studies (81). Future studies could also examine the influence of broader health features (e.g., diet and hydration) associated with inpatient treatment for substance use disorders, in addition to MOUD treatment or disease trajectory per se.

Conclusions

This is the first study to examine serum cytokine multiplex levels as biomarkers of longitudinal change in WM microstructure in iHUDs receiving chronic methadone or buprenorphine treatment. We found that cytokine PC1 scores, based on 19 cytokines that differed significantly between iHUDs and HCs (23), were negatively correlated with change in WM microstructure in specific tracts across ≈14 weeks of treatment with these MOUDs. This result was also observed when we examined representative cytokines (OSM, CSF1, and CCL7), thus implicating specific molecular targets in this association. Furthermore, exploratory analyses showed that levels of other cytokines (i.e., CCL19 and CCL2) were negatively correlated with change in cue-induced craving or arousal. Future studies should explore how specific cytokine targets (including those identified here) are functionally involved in changes in WM microstructure during recovery in iHUDs, potentially by using pharmacological manipulation (82–86) or neuroimaging studies of immune and inflammatory processes (76,87–89) in humans with OUD (82–86) or in preclinical models (90,91).

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