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Plasma and cerebrospinal fluid biomarkers predict cerebral injury in HIV-infected individuals on stable combination antiretroviral therapy

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Abstract

Objectives—HIV-associated brain injury persists despite antiretroviral therapy (cART), but contributing factors remain poorly understood. We postulated that inflammation-associated biomarkers will be associated with cerebral injury on proton magnetic resonance spectroscopy (MRS) in chronically HIV-infected subjects.

Methods—Five biomarkers were measured in 197 HIV-infected subjects: soluble CD14, MCP-1, IP-10, MIP-1β, and fractalkine. Levels of N-acetyl aspartate (NAA), Choline (Cho), Myoinositol (MI), Glutamate+Glutamine (Glx), and Creatine (Cr) were acquired in the midfrontal cortex (MFC), frontal white matter (FWM), and basal ganglia (BG). Predictive models were built via linear regression and the best models were chosen using the Akaike Information Criterion.

Results—Increases in plasma or CSF MCP-1 were associated with lower NAA/Cr in the MFC and BG while metabolite changes in the FWM for NAA/Cr, GlxCr and Cho/Cr were explained almost exclusively by a single factor, sCD14. Plasma and CSF levels of this factor were also significantly associated with Glx/Cr in MFC and BG. Higher CSF FKN was associated with higher NAA/Cr in BG. Best predictors for higher Cho/Cr in BG and MFC were CSF sCD14 and CSF MIP-1β. Plasma and CSF IP-10 were only associated with Cho/Cr in MFC. Of the three models that simultaneously accounted for both plasma and CSF, there were more associations between CSF biomarkers and MRS metabolites.

Conclusions—Markers of inflammation and immune activation, in particular MCP-1 and sCD14, predominantly reflecting CNS sources, contribute to the persistence of brain injury in a metabolite and region dependent manner in chronically HIV-infected patients on stable cART.

Keywords

HIV; AIDS; HIV-associated neurocognitive disorder; cerebrospinal fluid

Introduction

HIV disease is associated with pathological changes in the brain resulting in neurocognitive, motor, and behavioral disturbances, including syndromes that were previously termed AIDS Dementia Complex (ADC), known now as HIV-associated neurocognitive disorder (HAND).[1] While the incidence of severe dementia has decreased in the combination antiretroviral (cART) era, the prevalence of milder disease has remained stable or perhaps even increased.[2–5] Several studies have shown that cognitive impairment and brain injury persist in chronically infected patients on stable cART, though the underlying mechanism is poorly understood.[6,7] Proton magnetic resonance spectroscopy (MRS) provides a sensitive

and noninvasive *in vivo* method to detect metabolite changes in the brain.[8] Specific metabolites that have been identified include N-acetyl aspartate (NAA), a neuronal and axonal marker of integrity; choline (Cho), derived from a complex of transmembrane markers whose presence reflects membrane remodeling after injury; glutamate+glutamine, an excitatory neurotransmitter plus its precursor (together referred to as Glx), which are elevated in encephalopathic states and may reflect damage to neuronal glial cell environment; myo-inositol (MI), a carbohydrate synthesized primarily by glial cells, generally considered a marker of glial cell proliferation in response to neuronal injury; and creatine (Cr), a marker of energy production that is often used as a reference in ratios with other metabolites. Several studies in HIV-infected individuals and SIV-infected macaques have found decreased levels of NAA/Cr in the frontal white matter, basal ganglia and occasionally in the mesial frontal gray matter.[7,9–11] High levels of Cho/Cr and MI/Cr have also been found in these regions, consistent with a pattern of neuronal injury and inflammation.

Multiple biomarkers found in cerebrospinal fluid have been associated with HAND. These include markers of monocyte/macrophage activation such as soluble CD14 (sCD14) and chemotactic cytokines such as monocyte chemotactic protein-1 (MCP-1), interferon gamma inducible protein-10 (IP-10), macrophage inflammatory protein-1β (MIP-1β), and fractalkine (FKN).[12–17] One small study found that lower NAA/Cr is associated with higher MCP-1, suggesting a link between monocyte chemotaxis and neuronal injury during HIV infection.[18] The HIV Neuroimaging Consortium (HIVNC), a 12-center collaborative group was formed to investigate the patterns and correlates of brain injury and cognitive impairment in over 300 subjects with chronic HIV infection on cART.[7,19] Recent studies suggest than chronic immune activation plays an important role in systemic complications observed in HIV-infected patients on cART.[20] The contribution of inflammatory factors to the persistence of brain injury in this setting, however, remains relatively unexplored. We therefore hypothesized that markers of immune activation would contribute to the persistence of brain injury in these patients in a metabolite and region dependent manner.

Methods

Design

This cross-sectional project included 197 HIV-infected subjects from 7 sites: UC-San Diego, UC-Los Angeles, Harbor-UCLA, Stanford University, University of Colorado, University of Pittsburgh, and University of Rochester. The study was conducted after approval by all local Institutional Review Boards (IRBs) and protections for subjects followed the Helsinki Declaration. Inclusion criteria included: Nadir CD4 count ≤ 200 cells/μl and stable cART regimen for at least 12 consecutive weeks prior to screening. Exclusion criteria included severe premorbid or comorbid psychiatric disorders, chronic seizures, stroke, head trauma resulting in loss of consciousness > 30 minutes, multiple sclerosis, non-HIV brain infection, brain neoplasms, active alcohol or drug abuse within 6 months of study; hemoglobin ≤ 9.0 g/dL; > 3 x upper limit of normal (ULN) of creatinine, AST, ALT, or alkaline phosphatase; or diabetes mellitus with a fasting glucose > 140. Subjects were enrolled for this study between the years of 2005 and 2008.

Magnetic Resonance Spectroscopy

The ^{1}H-MRS protocol has been previously described.[6,7] Briefly, levels of cerebral metabolites NAA, MI, Cho, Glx, and Cr were measured by single-voxel ^{1}H spectra using a PRESS pulse sequence. Voxels 6 ml in volume were obtained in three regions: gray matter in mid-frontal cortex (MFC), right or left mid-frontal white matter (FWM), and right or left basal ganglia (BG). Field homogeneity and water suppression were adjusted using automated algorithms. Water-suppressed spectra were collected with echo time/repetition time (TE/TR) of 35/3000 ms, bandwidth 2500 Hz, 128 averages. MRS data were acquired using matched pulse sequence parameters on GE and Siemens MRI units. In addition to the water-suppressed MRS acquisition, single-scan fully relaxed unsuppressed water FIDs were acquired from each voxel at 7 different echo times (TE = 30, 45, 65, 100, 200, 500, and 1500 ms; TR = 15 s). These data were used to infer the voxel's CSF content. To control for instrument bias, we collected phantom MRS data concurrently with the subject evaluation, using the same protocol described above.

The time domain spectral data were transferred to a central MRS processing site at the University of Hawaii. MR spectra were evaluated for quality based on visual inspection and the %SD (Cramer-Rao lower bounds) and FWHM outputs from the LC Model spectral analysis software.[21] Cerebral metabolite concentrations were computed using LC Model with unsuppressed water FID at TE=35ms used for eddy-current correction. The automated processing method yields metabolite concentration estimates with coefficient of variation < 15.[22] The variability in measurements of metabolite ratios over the group of human subjects was about 3-fold larger than the variability measured in the phantoms. The variability in the phantom metabolite ratio measurements between the seven sites did not exceed 7% of the overall mean, with most of the variation falling within 2–3%. Because the phantom agreement between sites (including between acquisitions) that used Siemens and GE scanners was within a few percent, we did not make scanner-specific corrections to the derived metabolite concentrations and ratios.

Assessment procedures

Neurocognitive status was assessed at each imaging time point using the AIDS Dementia Complex Scale as previously described[23] (much of the study was performed prior to publication of the most recent HAND nosology).[1] The ADC staging was performed by a trained clinician at each site and was based on the neurological examination, assessment of functional impairment, and neuropsychological performance.[7] Neuropsychological impairment was defined as performance of at least 1.0 standard deviation below normative values on two or more neuropsychological tests or at least 2.0 standard deviations below normative values on one or more tests. Participants were classified at baseline using the ADC staging as follows: ADC stage 0- neurologically asymptomatic with no evidence of cognitive, functional or neuropsychological impairment; ADC stage 0.5- subclinical impairment, with evidence of neuropsychological impairment only, ADC stage 1- mild neurocognitive impairment, with evidence of definite cognitive and functional impairment on activities of daily living but without loss of independent functioning, ADC stage 2 - moderate impairment, requiring assistance on ADLs, or ADC stage 3- severe impairment. CSF was collected by lumbar puncture from 99 subjects and was stored at −80C.

Laboratory Procedures

Soluble CD14 was measured using a commercial enzyme-linked immunosorbent assay (ELISA) with a sensitivity of 125 pg/mL (R&D Systems, Minneapolis, MN). Other biomarkers (MCP-1, IP-10, MIP-1β, FKN) were measured by multiplex bead suspension array (Millipore, Billerica, MA) on a BioPlex 100 platform (Bio-Rad, Hercules, CA) with sensitivities of 3.2 pg/mL. All results were reviewed for quality assurance and assays were repeated when coefficients of variation exceeded 20% or if concentration distributions revealed possible batch effects. HIV RNA levels were quantified in plasma and CSF by RT-PCR using commercial assays with lower limits of quantitation of either 400 or 50 c/mL. CD4+ T-cells were measured by flow cytometry.

Statistical Analyses

Biomarker concentrations were log-transformed to reduce skewness. Predictive models for cerebral metabolites as functions of biomarker concentrations in plasma and CSF were built via linear regression models. The best models were then chosen using the Akaike Information Criterion (AIC), which balances the parsimony and the fit of the regression models.[24] We prefer the AIC over other methods as a model selection criterion, since it balances the estimation error associated with including the additional covariates with the approximation error that associated with excluding the covariates. Analyses were performed in 3 groups: plasma biomarkers alone (N=186), CSF biomarkers alone (N=99), or both plasma and CSF markers (N=88). We deem the associations to be statistically significant if the test of the regression coefficients equal to zero results in a p-value less than 0.05.

Model Building Strategy—A stepwise variable selection algorithm using AIC as the selection criterion was used to choose the biomarkers that were associated with each of the four relative metabolite concentrations (NAA/Cr, Cho/Cr, MI/Cr and Glx/Cr) in three brain regions: frontal white matter (FWM), basal ganglia (BG) and mid-frontal cortex (MFC). For each relative metabolite concentration, the stepwise model selection procedure was repeated on 500 bootstrapped datasets, and only those biomarkers selected in at least 70% of the 500 bootstrapped model fits were used in the final model. To study the association of the metabolite concentrations and the biomarker levels in the presence of demographic, clinical and viral covariates (age, gender, race, education level, plasma HIV RNA, CD4+ count, CD4+ nadir, duration of HIV infection, and presence of ADC stage 1 or higher), we used the same model selection strategy as described above starting with the best metabolite-biomarker model chosen above.

Results

Cohort characteristics (Table 1)

The entire cohort of 197 subjects who had at least one cerebral metabolite and the panel of soluble biomarkers in at least one body fluid measured had a median age of 46 years (IQR: 41,52) with a median duration of HIV disease of 12 years (IQR: 8,17). Median nadir CD4+ count was 34 cells/μL (IQR: 12,81) while median current CD4+ count was 288 cells/μL (IQR: 179,461). Of 197 subjects with ADC assessment, 96 (48.7%) were stage “0”, 55 (27.9%) were stage “0.5”, 33 (16.8%) were stage “1”, 11 (5.6%) were stage “2–4”, and 2

were missing. Therefore, nearly 78% of subjects were cognitively normal or had only subclinical cognitive impairment. Tables 2 and 3 summarize associations between biomarkers (plasma available for n=186, CSF available for n=99, and plasma plus CSF available for n=88) and MRS organized by brain region.

Frontal White Matter Associations—In the analysis of all plasma biomarker data (n=186), no statistically significant associations were found between the panel of biomarkers and cerebral metabolites. In the analysis of all CSF biomarker data (n=99), higher sCD14 levels were associated with lower NAA/Cr values ($p = 0.001$) and with lower Glx/Cr values ($p=0.04$) (Figure 1). In the subgroup that had matched plasma and CSF biomarkers measured (n=88), higher CSF sCD14 levels were again associated with lower NAA/Cr values ($p = 0.001$) and higher plasma sCD14 levels were associated with higher Cho/Cr values ($p = 0.01$).

Basal Ganglia Associations—In the analysis of all plasma biomarker data, higher MCP-1 levels ($p=0.02$) and lower MIP-1β levels ($p=0.02$) were significantly associated with lower NAA/Cr while higher sCD14 levels were associated with lower Glx/Cr ($p = 0.05$). In the analysis of all CSF biomarker data, higher MCP-1 ($p = 0.01$) and lower FKN ($p = 0.02$) levels were associated with lower NAA/Cr values (figure 2). Higher MIP-1β levels were associated with higher Glx/Cr values ($p = 0.04$). In the subgroup that had matched plasma and CSF biomarkers measured, higher CSF MCP-1 ($p = 0.001$) and plasma MCP-1 ($p < 0.001$) as well as higher CSF MIP-1β levels ($p = 0.06$) were associated with lower NAA/Cr. Higher CSF sCD14 levels were associated with higher Cho/Cr ($p = 0.04$).

Mid Frontal Cortex Associations—In the analysis of all plasma biomarker data, higher MCP-1 levels were associated with lower NAA/Cr values ($p=0.04$) while higher sCD14 levels were related to increases in Glx/Cr ($p=0.01$). Higher IP-10 levels were associated with higher Cho/Cr ($p=0.02$). In the analysis of all CSF biomarker data, higher MCP-1 levels ($p = 0.04$) was associated with lower NAA/Cr. Higher MIP-1β levels ($p = 0.004$), higher sCD14 levels ($p = 0.02$), and lower IP-10 levels were associated with higher Cho/Cr values ($p = 0.047$). In the subgroup that had matched plasma and CSF biomarkers measured, higher MCP-1 levels in CSF ($p = 0.03$) were again associated with lower NAA/Cr values. Higher sCD14 levels in CSF ($p = 0.02$) and lower FKN levels in plasma ($p=0.01$) were associated with lower Glx/Cr values. Again, higher MIP-1β levels ($p = 0.04$) in CSF were associated with higher values Cho/Cr values.

Analyses accounting for demographic and disease covariates

All the associations remained statistically significant in the multivariable analysis with exceptions only in the basal ganglia (BG) region. In analyses of all plasma biomarker data, the p values for the associations of NAA/Cr with MCP-1 levels ($p=0.08$) and Glx/Cr with sCD14 levels ($p=0.10$) rose above 0.05. In analyses of all CSF biomarker data, the p value for the association between FKN and NAA/Cr ($p=0.11$) rose above 0.05. In the subgroup that had matched plasma and CSF biomarkers measured, the p values for the associations of CSF MIP-1β with NAA/Cr ($p=0.82$) or Cho/Cr ($p=0.07$) rose above 0.05.

Discussion

HAND and HIV- associated brain injury remain common complications in chronically infected patients despite cART. [25] Several recent studies including those from the HIVNC have shown persistence and progression of neuronal injury and atrophy as measured by MRS and MRI.[6,7,19,26,27] Risk factors such as the nadir CD4+ T-cell count, HCV co-infection, substance abuse, and cardiovascular disease may increase the risk for neurocognitive impairment and brain atrophy.[28] While the underlying mechanisms remain incompletely understood, one possible convergent mechanism is immune activation,[29,30] which in turn may lead to neuronal injury. The results from the current study point to certain inflammatory markers, in particular MCP-1 and sCD14, as important factors that contribute significantly to distinct patterns of brain injury in these patients.

A strength of the study is the use of robust multivariable analyses using AIC, which allowed for a more accurate characterization of individual biomarkers, given the likelihood of collinearity between those that may reflect similar immune processes. The only similar analysis that we found was for two completely different biomarkers (neopterin and β2-microglobulin) measured in only 38 adults.[31] The multivariable analyses in our study revealed that multiple biomarkers were simultaneously associated with distinct regional patterns, e.g. MCP-1 with NAA/Cr in the BG/MFC and sCD14 with metabolites in the FWM, suggesting that the pathogenesis of HIV-associated brain injury may be a multifactorial process due to the combined effects of several immune or inflammatory events.

Our study extends prior findings of this group in a different cohort where significant associations were found between CSF MCP-1 and decreases in NAA/Cr.[32] MCP-1 is a potently chemotactic protein expressed on brain macrophages of adults with HIV encephalitis.[33] Interestingly, CSF levels of this biomarker were elevated in adults with HIV-associated dementia even in the pre-CART era,[14] while polymorphisms in the genes encoding MCP-1 and its receptor, CCR2, have been associated with increased risk of this severe form of HAND.[30,34] Of note, the associations between MCP-1 and NAA/Cr reflected mostly CNS activity, where in the basal ganglia it accounted for 24 percent of the variance (Table 2), a finding that has not been previously reported. Given the importance of this region in HAND,[11] the in vivo observations in this study further support the pathogenic importance of this chemokine in neuronal injury and HAND among chronically HIV-infected patients.

More than half of the statistically significant models included sCD14, a marker of activated monocytes that has been linked to HIV disease progression and HAND.[13,17,35] Of note, sCD14 accounted for nearly all the associations with cerebral metabolites in FWM, including the decrease in NAA/Cr, suggesting this region may be particularly susceptible to the deleterious effects of monocyte activation and migration. As with the basal ganglia, evidence suggests that white matter plays a key role in cognitive function in HIV-infected patients and recent studies have shown significant disturbances in functional connectivity in this region in HIV-infected patients.[36] MIP-1β entered over one third of statistically significant models. Elevated CSF levels of MIP-1β have been found in adults with HAND

and decrease during treatment with cART.[16,37] However, in contrast to MCP-1 and sCD14, the associations between MIP-1β and cerebral metabolites did not exhibit a strong regional preference but did favor the importance of CSF over blood (5 of 7 models that included MIP-1β included the CSF level).

Other soluble biomarkers were included in relatively few models. IP-10 (CXCL10) is interesting because its involvement may indicate a pathogenic role for activated T cells in the CNS,[15,38] particularly in the MFC. IP-10 was associated with evidence of inflammation in this region (Cho/Cr) but not neuronal injury (NAA/Cr). The current analysis did not confirm our prior findings linking IP-10 to NAA/Cr, though the subject groups were somewhat different between the two studies, particularly in neurocognitive function (only 22% had ADC score ≥1 compared with 77% with ADC score ≥1 in the prior study).[32] FKN (CX3CL1) is produced by neurons and has neuroprotective properties that were evident in the BG.[12,39] Here, FKN was the only biomarker that was associated with better NAA/Cr levels. FKN is involved in crosstalk between neurons and glia and we acknowledge that it has been found to be neuroprotective in some settings but contributing to neuronal damage in others, possibly through mediation of chemotaxis.[40] Our finding supports that its predominant effect in our sample was neuroprotective.

The findings for the plasma biomarkers in our study are provocative since the direction of the associations with MRS metabolites were dissimilar in some cases from those for CSF biomarkers (sCD14 with Glx in MFC, IP-10 with Cho/Cr in MFC). These discordances may reflect the pleiotropic nature of biomarkers (e.g., MIP-1β can be produced by monocytes, B-lymphocytes, T-lymphocytes) or extraneural processes that can adversely affect the nervous system. For instance, some of these same biomarkers are also associated with vascular disease,[41,42] which may be an important determinant of neurocognitive functioning among adults taking suppressive cART.[43]

There were several limitations to this study. Reflecting when the neurocognitive data were generated, the neurocognitive assessments in our study were not based on the most recent criteria for HIV-associated neurocognitive disorder (HAND),[1] which was published subsequent to the inception of our study. Our study population had a history of advanced immunosuppression (median nadir CD4+ T-cell count < 50 cells/μL) and remained relatively immunocompromised (median CD4+ T-cell count < 300 cells/μL) at the time of assessment. Thus, the findings may not reflect HIV-infected adults who have started cART at higher CD4+ T-cell counts and have not progressed to AIDS. Additionally, our study had a relatively low percentage of black and female subjects compared to the HIV-infected population in the US and thus the findings may not generalize to these demographic groups. At the time that the study was designed, the CNS effects of hepatitis C virus (HCV) were not fully appreciated. As a result, HCV serostatus was available on very few subjects, which precluded HCV-focused analyses.

We acknowledge that many other biomarkers, including neopterin, neurofilament-light, beta-2-microglobulin and interferon-alpha have been found to reflect HIV neuropathogenesis.[44–46] Patients with HIV-associated dementia have particularly high concentrations of CSF neopterin and severity of ADC has been shown to correlate with CSF

beta-2-microglobulin levels.[45,46] However, the primary aim of our study was to focus on chemokines, and therefore we did not include an analysis of all biomarkers. We did consider the potential effect of all biomarkers on ADC stage and performed an exploratory analysis, finding that only plasma IP-10 (p=0.01) and CSF sCD14 (p=0.02) were significantly associated with ADC stage. Conclusions based on this ADC-biomarker analysis are limited due to the low number of subjects with significant neurocognitive impairment in this study. We also acknowledge that the extent to which variance was explained in this study was relatively modest. This limitation is consistent with prior analyses,[32] which also did not explain substantial variance in MRS metabolites, and is mitigated by the fact that only a small number of our observed associations were substantially weakened by inclusion of demographic and disease covariates. Additional research, particularly in the form of longitudinal studies, is needed to better understand the pathogenesis and evolution of HIV-associated brain injury despite cART.

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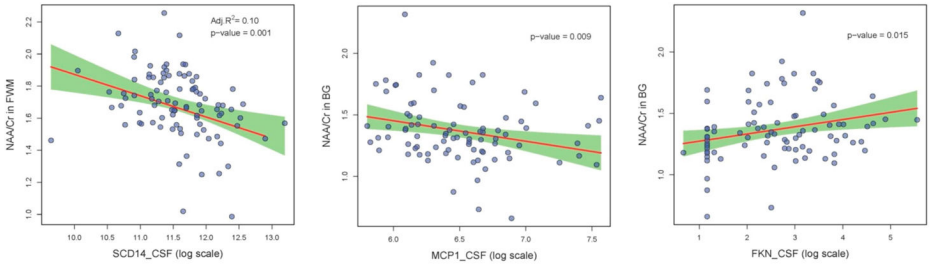

Figure 1.

In all figures, the red line shows the linear regression fit of the association between the soluble biomarker levels in CSF and cerebral metabolite values, the green shaded area indicates the 95% pointwise confidence interval, and the blue dots are the raw data. Figures 1A (left): Higher sCD14 levels in CSF correlate with lower NAA/Cr ratios is FWM. Figure 1B (center): Higher MCP-1 levels in CSF correlate with lower NAA/Cr ratios is BG. Figure 1C (right): Lower FKN levels in CSF correlate with lower NAA/Cr ratios in BG.

Table 1
Demographic and disease characteristics of the cohort

Values are either number (%) or median (IQR)

Characteristic	Total N=197 unless otherwise stated
Gender (Men)	173 (88%)
Years of age	46 (41,52)
Race (White)	135 (69%)
Education ≤12 years	82 (42%)
Cognition	n=195
Significant neurocognitive impairment (ADC Stage 1–3)	44 (22%)
Subclinical neurocognitive impairment (ADC stage 0.5)	55 (27.9%)
No neurocognitive impairment (ADC stage 0)	96 (48.7%)
Duration of HIV (Years)	12 (8,17)
Current CD4+ T-cells (/μL)	288 (179,461)
Nadir CD4+ T-cells (/μL)	34 (12,81)
Plasma HIV RNA ≤ 400 c/mL (% of n=186)	72%
CSF HIV RNA ≤ 75 c/mL (% of n=86)	69%
Current ART (%)	100%
ART Regimens Containing:	n=183
Protease Inhibitors (excluding ritonavir)	
Lopinavir	69 (37.7%)
Atazanavir	36 (19.7%)
Fosamprenavir	14 (7.7%)
Saquinavir	10 (5.5%)
Nelfinavir	7 (3.8%)
Darunavir	4 (2.2%)
Amprenavir	3 (1.6%)
Indinavir	2 (1.1%)
Tipranavir	1 (0.5%)
Non-nucleoside Reverse Transcriptase Inhibitors	
Efavirenz	49 (26.8%)

Characteristic	Total N=197 unless otherwise stated
Nevirapine	16 (5.6%)
Tenofovir	117 (64%)
Lamivudine	100 (54.6%)
Abacavir	69 (37.7%)
Emtricitabine	64 (35%)
Zidovudine	53 (29%)
Stavudine	15 (8.2%)
Didanosine	11 (6%)

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Table 2

Statistically significant associations between soluble biomarkers and MRS metabolites by brain region

Regression coefficients with the associated p-values are shown as well as the variation explained by the models resulting from the variable selection via AIC. Only the biomarkers that were present in the final models are reported below. Blank spaces indicate non-significant associations. Underlined associations became statistically non-significant when baseline demographic and disease characteristics were included in the models.

				Model 1: Plasma (N=186)			Model 2: CSF (N=99)			Model 3: CSF & Plasma (N=88)		
Region	**Metabolite**	**Biomarker***	**Fluid**	**β**	**p**	**Adj. R^2**	**β**	**P**	**Adj. R^2**	**β**	**P**	**Adj. R^2**
FWM	NAA/Cr	sCD14	CSF				−0.12	0.001	0.10	−0.16	0.001	0.11
		MIP-1β	CSF							−0.06	0.243	
	Cho/Cr	sCD14	Plasma							0.02	0.008	0.07
	Glx/Cr	sCD14	CSF				−0.13	0.042	0.04			
BG	NAA/Cr	MCP-1	CSF				−0.17	0.009	0.10	−0.24	0.001	0.24
		FKN	CSF				0.06	0.015				
		MCP-1	Plasma	−0.04	0.017	0.07				−0.10	<0.001	
		MIP-1β	Plasma	0.03	0.015							
		MIP-1β	CSF							−0.11	0.057	
	Cho/Cr	sCD14	CSF							0.017	0.037	0.05
		MIP-1β	Plasma							0.006	0.073	
	Glx/Cr	sCD14	Plasma	−0.06	0.048	0.02						
		MIP-1β	CSF				0.19	0.038	0.06			
MFC	NAA/Cr	MCP-1	CSF				−0.07	0.039	0.04	−0.08	0.034	0.04
		MCP-1	Plasma	−0.02	0.035	0.02						
	Cho/Cr	MIP-1β	CSF				0.03	0.004	0.08	0.02	0.039	0.04
		sCD14	CSF				0.02	0.015		0.01	0.104	
		IP-10	CSF				−0.01	0.047				
		IP-10	Plasma	0.01	0.021	0.03						
	Glx/Cr	sCD14	CSF							−0.16	0.020	0.10
		sCD14	Plasma	0.06	0.010	0.04						
		FKN	Plasma							0.06	0.006	

*Value log transformed

Table 3

Summary of statistically significant associations between biomarkers and MRS metabolites

Positive associations are denoted by up arrow (↑) while negative associations are denoted by down arrow (↓). (1 = plasma models, 2 = CSF models, 3 = combined CSF and plasma models).

Underlined associations became statistically non-significant when baseline demographic and disease characteristics were included in the models.

		Frontal White Matter			Basal Ganglia			Mid Frontal Cortex		
Biomarker	**Fluid**	**NAA/Cr**	**Cho/Cr**	**Glx/Cr**	**NAA/Cr**	**Cho/Cr**	**Glx/Cr**	**NAA/Cr**	**Cho/Cr**	**Glx/Cr**
FKN	CSF				**↑(2)**					
	Plasma									**↑(3)**
IP-10	CSF								**↓(2)**	
	Plasma								**↑(1)**	
MCP-1	CSF				**↓(2,3)**			**↓(2,3)**		
	Plasma				**↓(1,3)**			**↓(1)**		
MIP-1β	CSF	**↓(3)**			**↓(3)**		**↑(2)**		**↑(2,3)**	
	Plasma				**↑(1)**	**↑(3)**				
sCD14	CSF	**↓(2,3)**		**↓(2)**		**↑(3)**			**↑(2,3)**	**↓(3)**
	Plasma		**↑(3)**				**↓(1)**			**↑(1)**