Neuronal metabolism in HIV+ subjects lacking immune control correlates to M-CSF levels in cerebrospinal fluid

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Introduction: The prevalence of HIV-associated dementia (HAD) in the anti-retroviral therapy (ART) era is increasing [1, 2]. Macrophages are possibly the initial cell type infected and are key to the establishment of viral reservoirs such as the brain early during infection [3]. Current theories suggest infected monocyte-derived macrophages from the periphery permeate the blood-brain barrier (BBB) to bring the virus into the brain [4]. Magnetic resonance spectroscopic imaging (MRSI) allows for non-invasive measurement of N-acetylaspartate (NAA), which is located predominantly in neurons and is believed to be a surrogate marker of neuronal health [5]. A cascade of events including a host of cytokines, immune cells, and signaling molecules are believed to be involved in mediating neuronal injury. HIV studies indicate the extent of neuronal injury is best correlated with the number of infected macrophages in the cellular environment [6]. In lieu of pathology, macrophage colony-stimulating factor (M-CSF) may provide a surrogate marker indicating monocyte/macrophage activity within the central nervous system. M-CSF has been shown to play an important role in HIV pathogenesis [3]. It is responsible for the maturation and proliferation of monocytes into macrophages, the increased prevalence of receptors required for HIV infection on these cells, and can cause amplification of virus production in the brain. Thus, it is likely that this cytokine may be related to neuronal integrity in neuroAIDS. The purpose of this study was to explore the relationship between cognition, M-CSF levels and NAA concentrations in HIV+ subjects who lacked immune control.

Brain Region	Baseline (N=49)		3 Months (N=29)		10 Months (N=27)	
	R_S	P-value	R_S	P-value	R_S	P-value
Thalamus (TH)	-0.54	**<0.00007**	-0.35	0.07	-0.10	0.6
Basal Ganglia (BG)	-0.55	**0.00004**	-0.47	**0.01**	0.00	1.0
Centrum Semiovale (CSO)	-0.51	**0.0002**	-0.14	0.5	-0.26	0.2
Parietal Gray Matter (PGM)	-0.41	**<0.004**	-0.42	**0.02**	-0.18	0.4
Parietal White Matter (PWM)	-0.35	**0.01**	-0.06	0.7	-0.02	0.9
Frontal White Matter (FWM)	-0.35	**0.01**	-0.18	0.4	-0.14	0.5
Frontal Gray Matter (FGM)	-0.19	0.2	0.03	0.9	-0.24	0.2

Methods: Forty-nine HIV+ subjects (24 with HAD, 25 without) underwent MRSI at study entry, and at 3 and 10 months after initiating ART. Multi-slice, proton 2D-MRSI (TR/TE = 2000/280 msec) was performed at 1.5T. MRSI used a spin-echo (SE) sequence with two-dimensional phase-encoding, CHESS water suppression, and outer-volume lipid suppression [7]. NAA concentrations were calculated in 7 brain regions using phantom replacement methodology. Blood and cerebrospinal fluid (CSF) samples were obtained at each time point. Subjects had lack of immune control at baseline (CD4+ T cells<200, CSF or plasma viral RNA>50,000 copies/mL). CSF samples were obtained to determine levels of M-CSF. ANOVA was used to determine differences in NAA concentrations between HIV+ subjects with and without HAD. Nonparametric Spearman rank correlation coefficients were used to explore associations between M-CSF and regional NAA levels (N=49). Repeated-measures ANOVA was used to determine changes in NAA levels (N=36) and immunologic markers at 3 and 10 months post ART initiation.
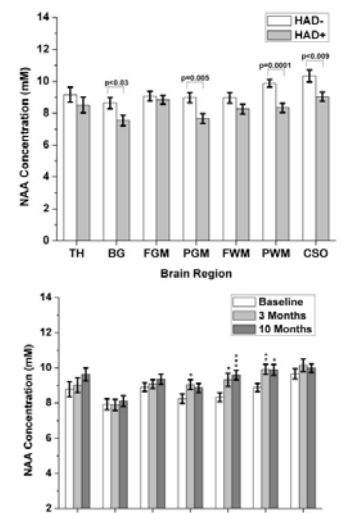
Results: Lower baseline NAA levels were measured in HAD+ subjects (compared to HAD-) in all brain regions, but were only significant in the centrum semiovale, basal ganglia, parietal gray and white matter (Figure 1, top). Spearman rank correlations indicated M-CSF levels were negatively correlated to NAA levels in 6 of the 7 brain regions at baseline. The strongest correlations were seen in the thalamus, basal ganglia, and centrum semiovale (Table). Outside of this disease model, M-CSF and NAA are two markers that should have no biological connection, and upon therapy adherence, the association was found to have dissolved. This occurred slowly at first, with the correlation lasting longest in the deeper gray matter regions, but all correlations were lost at 10 months after new therapy initiation. After therapy initiation, CD4+ T cell levels improved (p<0.002), while M-CSF (p=0.003) and plasma and CSF viral levels of the subject declined (p = 2×10^{-8}, p<0.0003) indicating the overall health of the subjects' improved, and consequently neuronal dysfunction receded (Figure 1, bottom). NAA levels began to recover with therapy, with the significant increases seen in the PGM, FWM, and PWM (p<0.02, p=0.001, p=0.001, respectively). Matched pairs t-tests show significant increases in these areas at the two timepoints as compared to initial baseline levels (*: p<0.05; **: p<0.01; ***: p=0.005). Cognitive status did not improve over the 10 months (p=0.50).

Discussion & Conclusions: These results suggest that NAA is a marker of neuronal dysfunction that is capable of recovery before improvements in cognitive function. As observed in other studies, both NAA (in some regions) and M-CSF were able to distinguish between HIV+ subjects with and without dementia. Furthermore, the M-CSF cytokine marker was negatively associated with NAA concentrations in almost every region of the brain at baseline. After therapy initiation, this association decreased gradually over time. These data suggest that higher expression of M-CSF (the cytokine required for maturation and survival of macrophages) facilitates widespread neuronal injury across the brain and is controlled with initiation of ART. Interestingly, zidovudine (AZT), the first drug approved for treatment of HIV, was found to reduce both the levels of M-CSF and viral production from macrophages, consequently providing some explanation for the observed declines in neuroAIDS early in the ART era [8]. Therefore, M-CSF's role as a potential therapeutic target for reducing macrophage infection and accumulation has been proposed [3]. These data support the development of M-CSF antagonists as potential targets for therapeutic intervention and MRSI as a means of monitoring efficacy.

Figure 1. NAA levels in seven brain regions between HAD+/- groups at baseline (**top**) and among all subjects over 10 months of anti-retroviral therapy (**bottom**).

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