

# ARV-mediated changes in viral levels and neuroaxonal function precede improvements in cognition in chronically HIV-infected subjects.

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**Introduction:** While the use of antiretroviral (ARV) therapy has reduced the incidence of frank dementia in patients with AIDS, increased patient survival has been associated with increased prevalence of protracted forms of HIV-associated neurological disorder (HAND)[1]. Many studies that examine the effects of combined ARV on brain metabolism and its relationship to cognitive change have suffered from small cohort size, the use of ratios, the lack of congruency or insufficient duration in the study timeline, preventing clear observation of ARV-mediated metabolic and cognitive changes [2]. Recently we reported the use of factor analysis on magnetic resonance spectroscopic imaging (MRSI) data of chronically HIV-infected subjects. That study generated a N-acetylaspartate (NAA) factor capable of showing improvements in neuronal metabolism across white matter regions three months after initiation of a new ARV regimen [3]. However, no improvement in Memorial Sloan-Kettering (MSK) staging criteria for HIV-associated dementia was observed after 10 months. It has been proposed that more sensitive and continuous scales of measuring cognitive impairment, such as global deficit scoring (GDS), may be necessary to evaluate properly the protracted forms of HAND in the ARV era than those like the MSK score, which were originally used for frank dementia. [4]. The following study used a mixed model regression analysis to examine the hypothesis that the early reduction of viral levels in the cerebrospinal fluid (CSF) provides an early reprieve from neuronal dysfunction, which manifests itself later in cognition.

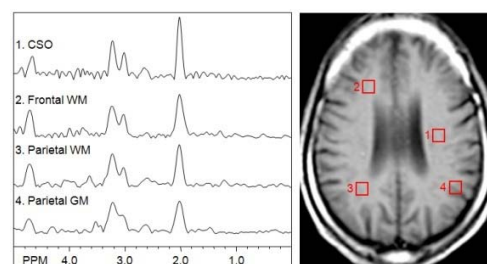
**Methods:** Fifty-one chronically HIV-infected subjects who were about to undergo a change in ARV regimen due to failing health, complications, or a lack of adherence to their current regimen, were enrolled in this study. Subjects underwent MRSI of seven brain regions at study entry and three and 10 months afterwards. MRSI used a spin-echo (SE) sequence with two-dimensional phase-encoding, CHES pulse water suppression, and eight outer-volume saturation (OVS) pulses arranged in an octangular pattern for lipid suppression. Three 15-mm thick slices (2.5 mm gap) were recorded parallel to the AC/PC line. Previous factor analysis of the MRSI data generated an "NAA" factor, which produces subject scores that are heavily influenced by NAA concentrations in white matter and parietal gray matter regions (Figure 1). Blood and CSF samples were obtained at each study visit for HIV RNA and CD4+ T cell quantification. At each study visit, neuropsychological (NP) testing covering five domains was performed and MSK scores were determined. GDS was calculated by first transforming raw NP test z-scores into T-scores and then to deficit scores (0: T-score  $\geq 40$ , 1:  $35 \leq$  T-score  $< 40$ , 2:  $30 \leq$  T-score  $< 35$ , 3:  $25 \leq$  T-score  $< 30$ , 4:  $20 \leq$  T-score  $< 25$ , 5: T-score  $< 20$ ). Deficit scores were averaged for a GDS determination for the battery. Impairment status was determined based on the GDS (no impairment: GDS  $< 0.5$ , mild impairment:  $0.5 \leq$  GDS  $< 1$ , moderate impairment:  $1 \leq$  GDS  $< 1.5$ , severe impairment: GDS  $\geq 1.5$ ). Repeated-measures analysis of variance (RM ANOVA) was performed to identify significant changes over time, while matched pairs t-tests were used to isolate changes between time points. Mixed-effects model testing was used to test the dependence of GDS and MSK changes between three and 10 months on early changes in NAA factor scores and CSF or plasma viral loads (between baseline and three months).

**Results:** Upon initiation of a new ARV therapy regimen, viral loads in the blood and CSF decreased after three months and remained decreased after 10 months (RM ANOVA:  $P < 10^{-11}$  and  $P < 10^{-4}$  respectively). CD4+ T cell counts increased after three months and remained increased after 10 months (RM ANOVA:  $P = 0.0006$ ). As previously reported, NAA factor scores were increased after three months and remained increased after 10 months (RM ANOVA:  $P = 0.0001$ ). These scores were found to be most influenced by NAA concentrations in the white matter and parietal gray matter regions (Figure 2). Using MSK, four subjects had no cognitive impairment (MSK=0), 22 as MSK=0.5, and 25 had dementia (19 as MSK=1, and six as MSK=2) at baseline. However, using the GDS method, only two subjects were found to lack cognitive impairment, 10 had mild impairment, eight had moderate impairment, and 31 had severe impairment. MSK scores of the cohort were not found to change over time (RM ANOVA:  $P = 0.45$ ), but GDS showed strong trends towards improvements (RM ANOVA:  $P < 0.06$ ). In particular, improved global dementia scores were observed between three and 10 months ( $P = 0.03$ ). The change in GDS scores between three and 10 months ( $\Delta$ GDS<sub>10,3</sub>) was discovered to be associated with improvements in neuroaxonal function ( $\Delta$ NAA Factor Scores<sub>3,0</sub>) and CSF viral load ( $\Delta$ CSF Viral Loads<sub>3,0</sub>) observed at three months. The overall model indicated that the  $\Delta$ CSF Viral Loads<sub>3,0</sub> and  $\Delta$ NAA Factor Scores<sub>3,0</sub> significantly affects the change in GDS ( $P = 0.009$ ). Specifically, the change in GDS between three and 10 months did not directly depend on  $\Delta$ CSF Viral Loads<sub>3,0</sub> and had trends for dependence on the  $\Delta$ NAA Factor Scores<sub>3,0</sub> ( $P = 0.70$  and  $P = 0.09$ , respectively). However, the  $\Delta$ CSF Viral Loads<sub>3,0</sub> and the  $\Delta$ NAA Factor Scores<sub>3,0</sub> were shown to interact significantly with each other ( $P = 0.004$ ). When this analysis was performed using MSK or plasma viral loads, the overall models were not significant.

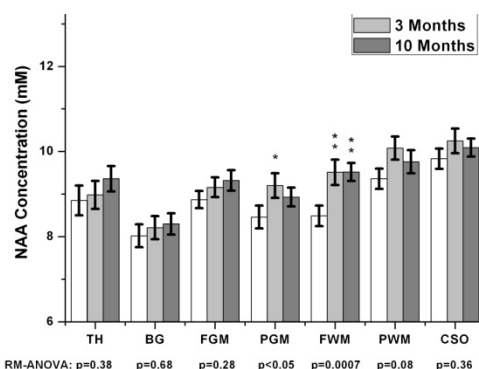
**Conclusions:** This study provides support for the use of continuous measure scales such as GDS to complement current neurologic methods for determination of subtle improvements in cognitive functioning in the ARV era. Interestingly, the improved GDS between three and 10 months reinforces the proposal that the recovery of cognitive function is slower than that of reversible neuroaxonal injury, perhaps due to persistent/latent infection and an activated glial response. ARV therapy has been shown to change viral and immune signaling kinetics[5], indicating that correlations between these and MR measures at single time points in cross-sectional studies may not last. Results from this analysis imply that early ARV-mediated changes in CSF viral levels and neuroaxonal metabolism affect later improvements in cognitive functioning as measured by GDS. The significant interaction effect between changes in neuronal metabolism and CSF viral levels imply that a continued relationship persists even if it is not directly observed by traditional linear regression methods. Interestingly, similar models were not significant using the changes in MSK or plasma HIV RNA. A recent SIV-macaque study indicated that complete suppression of viral load in the plasma was not as important to controlling neuronal injury as the suppression of activated and potentially infected, monocyte populations [6]. While suppressing viral levels throughout the body is important, it is perhaps key to focus on the mechanisms of viral entry into the brain and viral levels in the CNS regarding models of cognitive impairment and improvement.

1. Nath, A., et al., *Int Rev Psychiatry*, 2008. 20(1): p. 25-31.
2. Chang, L. and T. Ernst, *The Neurology of AIDS*, H.E. Gendelman, et al., Editors. 2005. p. 585-600.
3. Lentz, M. et al. Abstract #1402, *ISMRM 2009*. 2009. Honolulu, HI.

4. Carey, C.L., et al., *J Clin Exp Neuropsychol*, 2004. 26(3): p. 307-19.
5. Perelson, A.S., et al., *Nature*, 1997. 387(6629): p. 188-91.
6. Williams, K., et al., *J Clin Invest*, 2005. 115(9): p. 2534-45.



**Figure 1.** Sample spectra (left) and voxel locations (right) of white matter and parietal gray matter regions. NAA concentrations of these regions are heavily reflected in each subject's calculated NAA factor scores.



**Figure 2.** Average NAA concentrations in seven brain regions of HIV+ subjects at baseline, three and 10 months after the start of the new ART regimen (n = 51). Significant increases from baseline are indicated by asterisks (t-tests; \* :  $P < 0.05$ ; \*\*  $P \leq 0.002$ ).