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Neurofilament light chain in blood is negatively associated with neuropsychological performance in HIV-infected adults and declines with initiation of antiretroviral therapy

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Abstract

HIV-associated neurocognitive disorder (HAND) persists in the combination antiretroviral therapy (cART) era and is associated with diminished quality of life and survival. However, the disorder remains challenging to diagnose given the requirement for comprehensive neuropsychological testing. There is a need for blood biomarkers to facilitate the diagnosis of HAND and to track neuronal damage during HIV. We performed a study of plasma neurofilament light chain (NFL) that included 37 HIV-infected individuals and 54 HIV-negative individuals. When including all HIV+ visits, NFL correlated significantly with worse neuropsychological composite (NPT-11) performance (rho= - 0.4, p= 0.005). In mixed effects multivariate modeling that accounted for multiple visits, there continued to be a significant negative relationship between plasma NFL and NPT-11 (slope p=0.01). In a subset of participants who had visits before and after at least 24 weeks after starting cART, there was a significant decrease in plasma NFL (visit 1 median= 22.7 pg/ml versus visit 2 median= 13.4 pg/ml, p= 0.02). In contrast, there was a trend towards increase in plasma NFL over time among the HIV-negative participants who had a second visit (median 10.3 pg/ml to 12.6 pg/ml, p=0.065). Plasma NFL appears to closely reflect neuronal damage and shows promise as a marker of neuropsychological performance during HIV. Larger studies are indicated to determine if this marker could be accurate as a diagnostic tool for HAND during suppressive cART.

Introduction

The prevalence of HIV-associated neurocognitive disorder (HAND) continues to be high in the combination antiretroviral therapy (cART) era. Even in large cohorts of individuals who have achieved virologic suppression, HAND prevalence is over 40% (Heaton *et al*, 2010). Health-related quality of life scores for individuals with HAND are significantly lower than for HIV-infected individuals without HAND (Tozzi *et al*, 2003). Even in the cART era, the hazard ratio for mortality is 3.1 for patients with HAND compared to HIV-infected patients without HAND (Vivithanaporn *et al*, 2010).

The diagnosis of HAND is made through comprehensive neuropsychological testing, which requires significant time and expertise. More research is needed on biomarkers that could facilitate the rapid diagnosis of HAND and at the same time provide a window into HIV neuropathogenesis. While multiple biomarkers have been identified, one of the most promising is neurofilament light chain (NFL). NFL is a major structural component of axons and is thus a marker of neuronal injury. Cerebrospinal fluid (CSF) concentrations of NFL are elevated in the setting of HIV-associated dementia (Abdulle *et al*, 2007).

While procurement of CSF is a safe procedure, it is more time consuming and associated with more side effects than venipuncture. A recently published study showed that NFL can be accurately measured in plasma and correlates strongly with CSF NFL in HIV-infected individuals (Gisslen *et al*, 2016). Plasma NFL concentrations were particularly high in individuals with HIV-associated dementia off cART. In the current study, our aim was to examine plasma NFL during milder HIV-associated neurocognitive impairment, as well as change with initiation of cART. For comparison, we also analyzed a cohort of HIV-negative individuals who had two visits over time.

Methods

HIV-positive adults were enrolled from 2011 through 2014 at the Emory University Center for AIDS Research (CFAR) clinical core site in Atlanta as part of ongoing studies on HIV and neurocognition. Individuals were excluded from the study for any of the following: 1) history of any other neurologic disease known to affect memory (including stroke, malignancy involving the brain, traumatic brain injury, and AIDS-related opportunistic infection of the central nervous system); 2) current ongoing substance use (marijuana use in the last 7 days OR cocaine, heroin, methamphetamine, or other non-marijuana illicit drug use in the last 30 days); 3) heavy alcohol consumption in the last 30 days (defined as >7 drinks per week for women and >14 drinks per week for men); or 4) serious mental illness including schizophrenia and bipolar disorder (depression was not excluded if participants were well controlled on treatment). Lastly, participants were excluded in the event that significant cognitive symptoms had occurred precipitously in the last 30 days in order for further medical workup to be undertaken.

In addition to the HIV Dementia Scale, a full neuropsychological (NP) battery was administered to the participants that included the following eleven tests used commonly in studies of cognition and HIV infection(Robertson and Yosief, 2014): 1) Trailmaking Part A; 2) Trailmaking Part B; 3) Hopkins Verbal Learning Test total recall; 4) Hopkins Verbal Learning Test delayed recall; 5) Grooved Pegboard (dominant) 6) Grooved Pegboard (non-dominant); 7) Stroop Color Naming, 8) Stroop Color-Word; 9) Symbol Digit Modalities Test; 10) Letter Fluency (Controlled Oral Word Association Test), and 11) Category fluency (animals). These tests were selected in order to examine at least five domains as recommended in the most recent nosology of HAND criteria (Antinori *et al*, 2007). Scores were adjusted for demographic characteristics using published norms (Heaton *et al*, 2004). A composite neuropsychological test

score (NPT-11) was then calculated by average of individual T scores. Global Deficit Score (GDS), a validated measure of neurocognitive impairment in HIV based on demographically corrected T scores, was calculated and neurocognitive impairment was judged to be present for scores of 0.5 or higher (Carey *et al*, 2004). A subset of participants who were off cART at baseline had a second visit 24-48 weeks after starting therapy, at which time the NP battery was repeated. Score adjustment for practice effects was made for longitudinal visits by using median practice effect data from previous work (Cysique *et al*, 2011).

A group of HIV-seronegative (HIV-negative) individuals were enrolled as part of ongoing projects from 2003 to 2013 at the HIV-Neurobehavioral Research Program (HNRP) at the University of California at San Diego. Seronegative HIV status was confirmed at study baseline using commercially available antibody tests. Individuals were excluded for any of the following characteristics: 1) Serious neuropsychiatric comorbidities that could affect cognition including traumatic brain injury, schizophrenia or other psychotic disorder, stroke, or seizure disorder, 2) Substance abuse or dependence in the previous five years, and 3) history of chronic hepatitis C virus (HCV) infection or a positive HCV antibody test at study entry (performed on all participants).

HIV RNA concentrations from plasma and CSF were performed at the Emory Center for AIDS Research virology core using the Abbott laboratories m2000 Real Time HIV-1 assay system (reverse transcriptase polymerase chain reaction, lowest limit of detection of 40 copies/ml). Plasma NFL concentrations (and CSF concentrations in a subset of participants) were measured using an in house digital enzyme-linked immunosorbent assay on a Single molecule array (Simoa) platform (Quanterix, Lexington, MA), as previously described in detail (refs: PMID: 26870824 + PMID: 27581216). The measurements were performed in one round of

experiments using one batch of reagents by board-certified laboratory technicians who were blinded to clinical information. Intra-assay coefficients of variation were below 10%. The univariate statistical analyses were performed with SAS JMP version 13 as well as Graphpad Prism 6. For mixed effects and multivariate analyses, SAS version 9.4 was used. Normality of continuous variables was assessed with the Shapiro-Wilk test. Correlations were performed using spearman's rho test, unless normality conditions were met, in which case Pearson's r test was used. The Wilcoxon signed rank test was used for paired continuous variables, unless normality conditions were met, in which case the paired t-test was used. Fisher's exact test was used to compare categorical results between groups. Degree of NPT-11 decline was obtained using a mixed-effects linear model specifying that NPT-11 follows a linear regression over NFL, with random intercept for each participant. The first model examined only $\log_{10}$ plasma NFL as a covariate for the outcome of NPT-11. Age, current CD4+, and plasma HIV RNA concentration have all been associated with HAND (McArthur *et al*, 1997; Robertson *et al*, 2007; Saylor *et al*, 2016; Valcour *et al*, 2006), and therefore were considered as covariates for the second model in addition to $\log_{10}$ plasma NFL. To account for collinearity with CD4+, plasma HIV RNA was categorized as a detectable/undetectable variable. The SAS MIXED Procedure was used to fit the models and estimate the degree of NPT-11 decline and its standard error and 95% confidence interval in the presence of other covariates. Goodness of fit was estimated by the second order Akaike Information Criterion (AICc). The study was approved by the Institutional Review Boards at both institutions and written consent was obtained from all participants.

Results

Thirty-seven HIV infected adults were analyzed (table 1). Participants were mostly male (78%) and African-American (62%). Median estimated duration of HIV infection was 11 years (25%-75% interquartile range [IQR]= 4-22 years). There were 21 participants (57% overall) who were on three drug cART with plasma HIV RNA < 40 copies/milliliter (ml) for at least six months at baseline. The remaining 16 participants were off antiretrovirals for at least six months (including seven who were antiretroviral naïve) and had a median plasma HIV RNA of $\log_{10}$ 5.0 (IQR= $\log_{10}$ 4.0-5.4). Four participants were hepatitis C virus (HCV) antibody positive with confirmatory positive HCV RNA, while two participants were positive for chronic hepatitis B virus (HBV) surface antigen (HbsAg). Rapid plasma regain (RPR) was negative on all participants. Median HIV Dementia Scale score at baseline was 14 (out of 16) with IQR= 11-16. Mean NPT-11 score at baseline was 45.0 (Standard deviation, SD= 8.0). Median GDS was 0.64 (IQR 0.14- 0.91). In the subset of participants with cerebrospinal fluid results available at baseline (n=34), $\log_{10}$ plasma NFL concentration correlated significantly with $\log_{10}$ CSF NFL concentration (rho= 0.78, $p<0.001$, see figure 1 with regression line and 95% confidence interval).

Examining the relationship between plasma NFL and NP performance, there was a trend towards a negative correlation between NFL and NPT-11 when only baseline visits (n=37) were analyzed (rho= - 0.23, p= 0.17). Similarly, there was a trend towards a negative correlation between plasma NFL and NPT-11 when only including participants (n=28) with a visit on cART for at least 24 weeks with plasma HIV RNA < 100 copies/ml (rho= - 0.22, p= 0.25). However, when all visits were included (total n=48), the negative association between increasing plasma NFL and decreasing NPT-11 was statistically significant (rho= - 0.4, p= 0.005). When using GDS $\geq$ 0.5 as the gold standard for neurocognitive impairment for all visits, the area under the

receiver operating curve for plasma NFL was 0.61 (p= 0.2). A Plasma NFL value of > 23.7 pg/ml was 95.5% specific for neurocognitive impairment.

In the univariate mixed effects model, there was a statistically significant linear relationship between NPT-11 and $\log_{10}$ plasma NFL (slope = 9.9; standard error = 3.0 with 95% confidence interval: 3.2 – 16.6 and p =0.008 when testing slope = 0). AICc for this model was 314.0. Similarly, in the multivariate mixed effects model, there was a significant linear relationship between NPT-11 and $\log_{10}$ plasma NFL (slope = -11.5 points (standard error = 3.3, 95% confidence interval: -19.0 to -3.7 and p=0.01 when testing slope = 0). AICc for this model was 317.1. The rate of decrease in NPT-11 was 5.8 points per 0.50 $\log_{10}$ increase in NFL. Age (p= 0.16), CD4 T-cell count (p = 0.67) and plasma HIV RNA concentration (p = 0.37 for detectable versus non-detectable) were not related to NPT-11 in the model.

Eleven of the 16 HIV+ participants who were off antiretrovirals at baseline had a second study visit at least 24 weeks after starting cART (median 28 weeks after therapy initiation, IQR= 25-30 weeks). Nine of eleven (82%) achieved a decrease of at least 1.0 $\log_{10}$ plasma HIV RNA at this second visit. NPT-11 significantly improved in these eleven participants (visit 1 mean= 41.6 versus visit 2 mean= 46.3, p= 0.001). Concurrently, plasma NFL concentration significantly decreased (see figure 2) among these eleven participants (visit 1 median= 22.7 pg/ml versus visit 2 median= 13.4 pg/ml, p= 0.02).

For the longitudinal HIV-negative analysis, samples from 54 randomly selected HIV-negative HNRP participants with two visits were analyzed. For this group, median age was 36.5 years (IQR= 24-48) and 78% were male. Race/ethnicity was 42.6% white, 27.8% Hispanic, 22.2% African-American, and 7.4% other races/ethnicities. Therefore, HIV-negative participants at baseline were younger (p=0.0002) and less likely to be black (p<0.0001) than HIV+

participants. Median plasma NFL at baseline was 10.3 pg/ml (IQR 6.9- 16.2) in the HIV-negatives, which was not significantly different (p= 0.45) than HIV+ participants at baseline (see figure 3). Median time between baseline and the second visit was 13 months (IQR 12-14 months) among the 54 HIV-negative participants. The second visit median NFL was 12.6 pg/ml (IQR 7.5-19.0), which resulted in a trend towards increase in NFL between first and second visits (see figure 4, p=0.065).

Discussion

With HAND remaining highly prevalent in the cART era, more tools are needed to diagnose and better understand this disorder. Given that venipuncture is more readily available and is associated with fewer side effects than lumbar puncture, a blood test that reflects neuropsychological performance during HIV would be a significant advance. In this exploratory study of well characterized HIV-infected adults, we found that plasma NFL concentrations correlated negatively with neuropsychological performance. We acknowledge that this correlation was only significant with the inclusion of repeated measures from a small number of participants with a second visit after initiation of cART. Therefore, these participants had a greater influence on the correlation than the other participants. However, after accounting for repeated measures with mixed modeling that included factors known to be associated with HAND, a significant negative relationship between NPT-11 and NFL remained.

Most HIV-infected individuals with HAND in the cART era have milder disease, and this was reflected in our study. Using the GDS criteria for severe impairment of 1.5, a small minority of participants were severely impaired. NFL is a sensitive marker of axonal injury and our results suggest that neuronal injury can be present also in mild HAND cases. Plasma NFL appears to be

a marker of not just HIV-dementia, but milder forms of HAND as well. Larger studies examining the use of this marker for the diagnosis of mild HAND (ANI/MND) are indicated. Plasma NFL concentrations also correlated closely with CSF NFL concentrations. This confirms the findings of a recently published study in which plasma and CSF NFL concentrations in the setting of HIV were found to be closely correlated (Gisslen *et al*, 2016). Therefore, despite the blood brain barrier, plasma NFL is a marker of CNS neuronal injury during HIV.

In a small subset of HIV+ participants who were followed longitudinally after starting cART, there was a significant decrease in plasma NFL. This extends the finding of decrease in NFL from CSF after cART initiation (Jessen Krut *et al*, 2014), and suggests that neuronal damage is at least in part ameliorated by cART, a finding which is concordant with studies that have consistently shown improvement in neuropsychological function after cART initiation (Cysique *et al*, 2009). In contrast, there was a trend towards longitudinal increase in plasma NFL in the larger group of HIV-negative participants. While a relationship between increasing age and increasing NFL has been found in other studies (Jessen Krut *et al*, 2014), the NFL increase over a relatively short period of time in this study is surprising. We did not find that plasma NFL concentrations differed significantly between HIV+ and HIV-negative groups, but the small nature of the study and the fact that many HIV+ participants did not have impairment may have played a role in this.

We acknowledge the limitations of this exploratory study, including its small sample size. We also acknowledge that the final multivariate model was comprised of a relatively small number of individuals, but results were similar to the univariate model and model diagnostics were not indicative of overfitting. Lastly, given that many of the study participants were not on cART, the results may not be generalizable to the HIV+ population on consistent treatment. A

larger study focusing on individuals with virologic suppression is needed to determine if plasma NFL is associated with neuropsychological performance during effective cART.

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