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## Cerebrospinal Fluid Interferon alpha Levels Correlate with Neurocognitive Impairment in Ambulatory HIV-infected Individuals

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### Abstract

HIV-associated neurocognitive disorders (HAND) continue to be common and are associated with increased morbidity and mortality. However, the underlying mechanisms in the combination antiretroviral therapy (cART) era are not fully understood. Interferon alpha (IFN $\alpha$ ) is an antiviral cytokine found to be elevated in the cerebrospinal fluid (CSF) of individuals with advanced HIV-associated dementia in the pre-cART era. In this cross-sectional study, we investigated the association between IFN $\alpha$  and neurocognitive performance in ambulatory HIV-infected individuals with milder impairment. An eight test neuropsychological battery representing six cognitive domains was administered. Individual scores were adjusted for demographic characteristics and a composite neuropsychological score (NPT-8) was calculated. IFN $\alpha$  and CSF neurofilament light chain (NFL) levels were measured using enzyme linked immunosorbent assay

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(ELISA). There were 15 chronically infected participants with a history of significant immunocompromise (median nadir CD4+ of 49 cells/microliter). Most participants were neurocognitively impaired (mean Global Deficit Score of 0.86). CSF IFN $\alpha$  negatively correlated with three individual tests (Trailmaking A, Trailmaking B, and Stroop Color-word) as well as the composite NPT-8 score ( $r = -0.67$ ,  $p = 0.006$ ). These negative correlations persisted in multivariable analyses adjusting for chronic hepatitis B and C. Additionally, CSF IFN $\alpha$  correlated strongly with CSF NFL, a marker of neuronal damage ( $\rho = 0.748$ ,  $p = 0.0013$ ). These results extend findings from individuals with severe HIV-associated dementia in the pre-cART era and suggest that IFN $\alpha$  may continue to play a role in HAND pathogenesis during the cART era. Further investigation into the role of IFN $\alpha$  is indicated.

## Keywords

HIV; AIDS; memory disorders; interferon alpha

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## Background

HIV-associated neurocognitive disorders (HAND) continue to be a major problem in the era of combination antiretroviral therapy (cART). Studies in mostly cART-treated populations have shown that HAND is present in 25–50% of HIV-infected individuals (Heaton *et al*, 2010; Robertson *et al*, 2007). In addition to being highly prevalent, HAND is associated with multiple adverse clinical outcomes, including decreased quality of life and increased mortality (Tozzi *et al*, 2003; Vivithanaporn *et al*, 2010). Yet, the pathogenesis of HAND, particularly in the setting of cART, has not been fully elucidated. A number of soluble biomarkers have been studied and are associated with the presence of HAND. Interestingly, many of these biomarkers appear to represent diverse pathological pathways (neopterin reflecting monocyte activation, neurofilament light chain reflecting neuronal degeneration, and others) (Hagberg *et al*, 2010; Marcotte *et al*, 2013; Peterson *et al*, 2014). Therefore, more research is needed to identify biomarkers that could contribute to a more comprehensive picture of HAND pathogenesis. Additionally, given that no treatments other than cART have been found to ameliorate HAND (Sacktor *et al*, 2011) (and even with cART the disorder is often not completely eradicated), new therapeutic targets are needed.

Interferon-alpha (IFN $\alpha$ ) is an antiviral cytokine found to be the only type I interferon that is elevated in the plasma of HIV-infected individuals compared to uninfected controls (Hardy *et al*, 2013). IFN $\alpha$  has been shown to have a direct neurotoxic role in animal models as well as in humans. In a murine model of HIV encephalitis, mice develop altered behavior and express increased IFN $\alpha$  in the brain (Sas *et al*, 2009). Blockage of IFN $\alpha$  in the setting of this model has been shown to ameliorate encephalitis (Fritz-French *et al*, 2014). In humans, IFN $\alpha$  has been associated with significant neuropsychiatric toxicity when used for the treatment of chronic hepatitis C infection and other diseases (Fritz-French and Tyor, 2012). While found to be undetectable in >90% of normal individuals (Abbott *et al*, 1987), CSF IFN $\alpha$  levels were shown to be significantly elevated among individuals with HIV-associated dementia in the pre-cART era (Rho *et al*, 1995). However, given that HAND during the cART era is mostly comprised of milder impairment (Heaton *et al*, 2010), further research

on the role of IFN $\alpha$  is indicated. Therefore, we sought to measure CSF IFN $\alpha$  in a cohort of predominantly cART experienced individuals to determine its relationship with neurocognitive performance.

## Methods

### Participants

HIV-infected adult outpatients who reported changes in memory were enrolled between March 2011 and November 2011 at the Emory University Center for AIDS Research (CFAR) clinical core site in Atlanta, Georgia. In order to minimize confounding comorbidities, potential participants were excluded from the study for any of the following: 1) history of any neurologic disease known to effect memory (including stroke, malignancy involving the central nervous system, traumatic brain injury, and AIDS-related opportunistic infection of the central nervous system); 2) 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). Participants were also screened for hypothyroidism (with serum TSH level), vitamin B12 deficiency (with serum B12 level), and cryptococcal disease if CD4+ T-cell count was <100 cells/ $\mu$ l (with serum cryptococcal antigen) and excluded if any were found to be abnormal. Participants with a history of treated syphilis and a persistently positive rapid plasma reagin (RPR) titer of 1:4 or less were eligible for the study if there was a decrease in RPR of at least fourfold at six months after treatment and there were no neurological symptoms at initial syphilis presentation. Lastly, participants were excluded in the event that severe cognitive symptoms had occurred precipitously in the last 30 days in order for further medical workup to be undertaken.

### Neuropsychological Testing

The neuropsychological (NP) battery included the HIV dementia scale as well as the following eight tests that are used commonly in studies of cognition during 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 hand); 6) Stroop Color/Word; 7) Letter Fluency (Controlled Oral Word Association Test) and 8) Animal Fluency. 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). The tests were administered by study staff after intensive training and supervision by an experienced, board certified neuropsychologist (DL). Test scores were adjusted for demographic characteristics using published norms (Heaton *et al*) that account for age, sex, and race (with the exception of HVLT and Stroop tests, for which adjusted values were not available) to arrive at individual T scores. A composite neuropsychological test score (NPT-8) was then calculated by averaging the eight individual T scores, as performed in other studies of neurocognition in HIV. Global Deficit Score (GDS), a validated measure of neurocognitive impairment in HIV based on demographically corrected T scores, was also calculated and as per published literature a score of  $\geq 0.5$  was

indicative of neurocognitive impairment (Carey *et al*, 2004). The study was approved by the Emory University Institutional Review Board and written consent was obtained from all subjects.

### Blood and Cerebrospinal Fluid Evaluations

On the same day of NP testing, paired blood and CSF samples were obtained, processed and stored at  $-80$  degrees centigrade. Plasma and CSF HIV RNA levels were quantified with the Abbott laboratories Real Time HIV-1 assay (reverse transcriptase polymerase chain reaction) using the Abbott m2000 system. Lowest limit of HIV detection was 40 copies/ml. IFN $\alpha$  levels were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits (PBL Assay Science, Piscataway NJ, USA) according to the manufacturer's instructions and technicians were blinded to clinical data. All samples and standards were measured in triplicates as picograms/ milliter (pg/ml). CSF neurofilament light chain (NFL), an established marker of neuronal injury (Peterson *et al*, 2014), was also measured by ELISA (UmanDiagnostics, Umea, Sweden). The NFL assay is most specific for myelinated axons and has intra-assay coefficients of variation below 10%. All NFL samples were analyzed as single samples in one round of experiments using one batch of reagents by board-certified laboratory technicians who were blinded to clinical data. Other studies including CD4+ T-lymphocyte counts were performed by the hospital clinical laboratories. In addition to the HIV-infected participant samples, the cerebrospinal fluid samples from four HIV-seronegative individuals were included for IFN $\alpha$  testing.

### Statistical analyses

Statistical analyses were performed with SAS software (version 9.4, SAS institute, Cary, NC) as well as SAS JMP (version 12.0, SAS institute, Cary, NC). Normality was assessed with the Shapiro-Wilk test. Differences between non-normal groups were calculated with the Wilcoxon rank-sum test. For correlations, non-normal variables were log $_{10}$  transformed for the Pearson product moment correlation coefficient ( $r$ ). If log $_{10}$  transformation did not yield values that met criteria for normality, the Spearman correlation test ( $\rho$ ) was used. Alpha levels were two-tailed and a  $p$  value of  $<0.05$  was considered statistically significant. Analyses with multiple  $p$  values of  $<0.05$  were corrected for multiple comparisons using the Benjamini-Hochberg false discovery rate (FDR) procedure. For correlations between IFN $\alpha$  and NP measures that remained statistically significant after FDR, linear regression analysis (Analysis of covariance, ANCOVA) was then used to generate adjusted Pearson correlation coefficients accounting for the presence of chronic hepatitis B or C.

### Results

A total of 15 HIV-infected participants were enrolled. Median age (table 1) was 49 years, 80% were men, and 73.3% were African-American. Most subjects had a long history (median 8 years) of HIV and a history of significant immunocompromise (median CD4+ nadir of 49 cells/ $\mu$ l and median current CD4+ of 62 cells/ $\mu$ l). The majority of subjects (60%) were cART experienced and 20% were currently on cART with undetectable plasma and CSF HIV RNA  $<40$  copies/ml. Mean NPT-8 score was 43.7 (standard deviation 9.34) and mean GDS of 0.86 (standard deviation 0.995). 9 of 15 subjects (60%) met criteria for

neurocognitive impairment by GDS  $\geq 0.5$  while 4 of 15 (26.7%) had more severe impairment with GDS  $\geq 1.5$ . Median CSF IFN $\alpha$  result was 7.86 pg/ml (range 2.62–45.95) and median CSF NFL result was 563 pg/ml (range 293–12233). CSF IFN $\alpha$  correlated significantly with CSF NFL ( $\rho = 0.748$ ,  $p = 0.0013$ ).

There were two healthy HIV-seronegative controls (one 57 year old female with CSF IFN $\alpha$  level of 2.14 pg/ml and one 52 year old female with CSF IFN $\alpha$  1.19 pg/ml) as well as two HIV-seronegative individuals with Alzheimer's disease (one 64 year old male with CSF IFN $\alpha$  5.95 pg/ml and one 73 year old female with CSF IFN $\alpha$  3.1 pg/ml). CSF IFN $\alpha$  level was significantly higher ( $p=0.012$ ) in the participants with HIV (median 7.86 pg/ml, overall range 2.62–45.95) compared to the participants without HIV. When limiting the comparison to the two Alzheimer's disease participants, HIV-infected participants tended to have higher CSF IFN $\alpha$  but this difference was not statistically significant ( $p = 0.13$ ).

In the HIV-infected participants, there was no significant difference ( $p=0.94$ ) in plasma IFN $\alpha$  levels between participants on cART with undetectable plasma/CSF HIV RNA (median 34.1 pg/ml, interquartile range [IQR] 3.07 – 98.73) and participants off cART (median 18.9 pg/ml, IQR 2.07–115.9). Similarly, CSF IFN $\alpha$  levels did not significantly differ ( $p=0.31$ ) between participants on cART with undetectable plasma/CSF HIV RNA (median 7.38 pg/ml, IQR 4.52 – 7.86) and participants off cART (median 8.34 pg/ml, IQR 6.67 – 16.55). There was no statistical difference ( $p=0.28$ ) in plasma IFN $\alpha$  among participants with either active hepatitis B or C (median 39.9 pg/ml, IQR 18.9 – 209.73) versus participants without (15.9 pg/ml, IQR 2.07 – 98.73). Similarly, there was no statistical difference ( $p=0.69$ ) in CSF IFN $\alpha$  levels among participants with either active hepatitis B or C (median 9.5 pg/ml, IQR 6.67 – 37.38) versus participants without (median 7.86 pg/ml, IQR 4.52 – 12.62).

There were multiple negative correlations between CSF IFN $\alpha$  levels and neurocognitive performance that were statistically significant (table 2). CSF IFN $\alpha$  correlated negatively with composite NPT-8 score ( $r = -0.67$ ,  $p = 0.006$ ). In terms of individual NP tests, CSF IFN $\alpha$  correlated negatively with Trailmaking test A ( $r = -0.735$ ,  $p = 0.0018$ , see figure 1 with 95% density ellipse), Trailmaking test B ( $r = -0.677$ ,  $p = 0.006$ ), and Stroop color-word test ( $r = -0.67$ ,  $p = 0.009$ ). These three correlations remained statistically significant after false discovery rate correction ( $p$  values= 0.014, 0.022, and 0.025 respectively). There was only one significant correlation with plasma IFN $\alpha$  (Trailmaking test A,  $r = -0.561$ ,  $p = 0.037$ ), but this correlation became non-significant ( $p = 0.28$ ) after false discovery rate correction. Pearson correlation coefficients between CSF IFN $\alpha$  and the identified NP performance measures remained similarly significant in the multivariable analyses adjusting for the presence of chronic hepatitis B or C (see table 2). Figure two shows the linear regression analysis between CSF IFN $\alpha$  and NPT-8 ( $Y = 4.6564 - 0.05452X$ ; standard deviation = 0.5581).

When comparing participants with GDS  $\geq 0.5$  versus  $< 0.5$ , there was no significant difference in CSF IFN $\alpha$  levels (median 8.81 pg/ml [IQR 5.24–19.5] versus median 7.86 [IQR 5.95–9.05],  $p=0.59$ ) or in plasma IFN $\alpha$  levels (median 37 pg/ml [IQR 5.53–175.4] versus median 11.65 pg/ml [IQR 2.3–103.02],  $p=0.61$ ). However, when grouped by severe

impairment (GDS  $\geq 1.5$  versus  $< 1.5$ ), participants with severe impairment had significantly higher CSF IFN $\alpha$  levels (median 19.53 pg/ml [IQR 10.0–39.76] versus median 7.86 pg/ml [IQR 4.52–8.81],  $p=0.049$ ). Participants with GDS  $\geq 1.5$  versus  $< 1.5$  tended to have higher plasma IFN $\alpha$  levels (median 94.4 pg/ml [IQR 39.9–202.4] versus median 15.9 pg/ml [IQR 2.07–98.73]) but this difference was not statistically significant ( $p=0.16$ ).

## Discussion

Given the high prevalence of HANDs in the cART era, there is an ongoing need to better understand the mechanisms behind these disorders. CSF levels of IFN $\alpha$ , an antiviral cytokine with neurotoxic properties, were found in the pre-cART era by members of our group to be significantly elevated among individuals with HIV-associated dementia (Rho *et al*, 1995). However, it is unclear if this cytokine remains associated with HAND in the modern HIV treatment era, in which most affected individuals have milder forms of neurocognitive impairment. While the present study represents a small sample size, the study population was comprised of ambulatory outpatients who did not have advanced dementia. Our results show that CSF IFN $\alpha$  levels negatively correlate with multiple measures of neurocognitive function, including a composite score that reflects global performance. These correlations may have been driven by participants with more significant impairment (a GDS of  $\geq 1.5$  as suggested by other studies) (Cysique *et al*, 2015). Participants with GDS  $\geq 1.5$  had significantly higher CSF IFN $\alpha$  levels.

Of note, the individual tests (Trailmaking and Stroop) that were negatively associated with CSF IFN $\alpha$  and appeared to underpin the overall negative association with composite performance are generally regarded as tests of executive function and processing speed. These contrast with the other individual tests in our battery (which evaluated memory, fluency, and motor skill) that were not associated with CSF IFN $\alpha$ . Our findings suggest that the detrimental effects of IFN $\alpha$  may be most specific to brain regions that are linked to executive function (such as the frontal lobes and associated subcortical tracts) and processing speed (such as white matter). This is supported by the strong correlation between CSF IFN $\alpha$  and CSF NFL, an established marker of injury to large caliber myelinated axons in HIV and other CNS diseases (Peterson *et al*, 2014; Skillback *et al*, 2014; Zetterberg *et al*, 2016).

However, we acknowledge that the mechanism of IFN $\alpha$ -induced neurotoxicity is not fully understood at this time (Fritz-French and Tyor, 2012; Kessing and Tyor, 2015). IFN $\alpha$  initiates an antiviral response by binding to the type I IFN receptor (IFNAR) with subsequent activation of the JAK/STAT pathway. However, blocking the IFNAR only partially prevents neurotoxicity caused by IFN $\alpha$ , suggesting that the cellular signaling involved in IFN $\alpha$  neurotoxicity is more complex than binding to its own receptor. The NMDA receptor (NMDAR) has also been linked to IFN $\alpha$  toxicity in clinical and *in vitro* studies (Quarantini *et al*, 2006). Interestingly, other neurotoxins that have been implicated in the pathogenesis of HAND also appear to act through the NMDAR (Potter *et al*, 2013). Targeting NMDAR mediated excitotoxicity may be a common pathway and further research is needed to identify HAND therapeutic candidates that would complement cART.

We acknowledge the limitations of the study, including the small sample size. We had very few HIV-seronegative controls, though previous research has shown that CSF IFN $\alpha$  is undetectable or near undetectable in the vast majority of healthy individuals (Abbott *et al*, 1987). Two of these HIV seronegative subjects had Alzheimer's disease, and while these individuals appeared to have lower CSF IFN $\alpha$  levels than the HIV-infected individuals, we were not able to reject the null hypothesis that there was no statistical difference between Alzheimer's and HIV groups. It is very likely that our study was under-powered to effectively address this question. Larger studies that include more healthy HIV-seronegative individuals, more individuals with Alzheimer's disease, and more HIV-infected individuals with long term virologic suppression on cART will be needed to determine if CSF IFN $\alpha$  levels are different between these groups.

We also acknowledge that one third of study subjects had either chronic hepatitis B or C. Since the presence of chronic hepatitis has been shown to effect systemic interferon alpha levels (Kakumu *et al*, 1989), the inclusion of these individuals may have influenced our results. However, the multivariable analysis adjusting for the presence of chronic hepatitis revealed similar negative correlations between CSF IFN $\alpha$  and NP performance. A larger study that excludes individuals with chronic hepatitis B/C would be needed to completely rule out the influence of hepatitis on IFN $\alpha$ .

While the majority of subjects in the study were cART experienced, a minority were on cART at the time of the study with suppressed plasma and CSF HIV RNA levels. While we did not find that plasma or CSF IFN $\alpha$  levels were lower in the individuals on cART, the sample size of subjects on cART was too small to perform a separate analysis limited to this group. Therefore our results cannot necessarily be extrapolated to HIV-infected individuals who have achieved virologic suppression on cART. Ideally a larger study would be performed focusing on patients with neurocognitive impairment despite virologic suppression on cART. Given the persistence of HAND in the cART era, more research is needed to better understand the relationship between IFN $\alpha$  and neurocognition in HIV-infected individuals.

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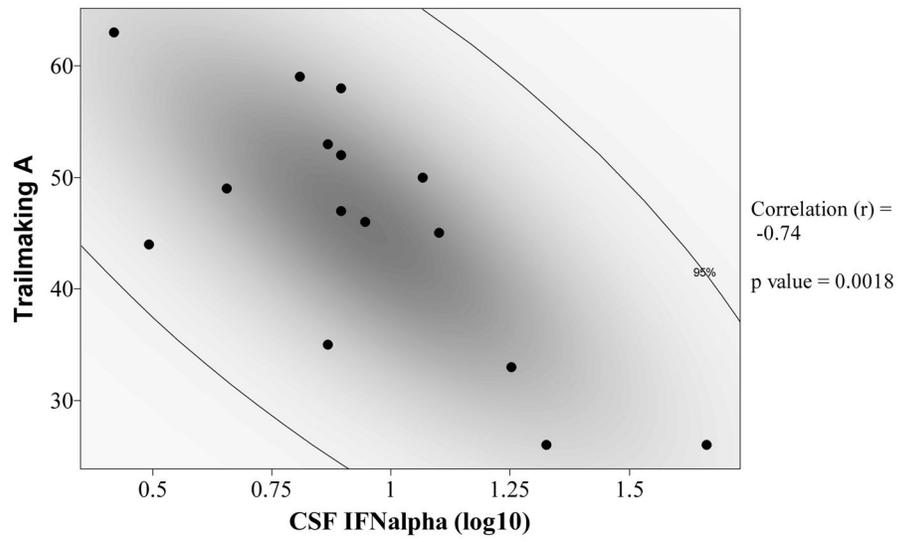
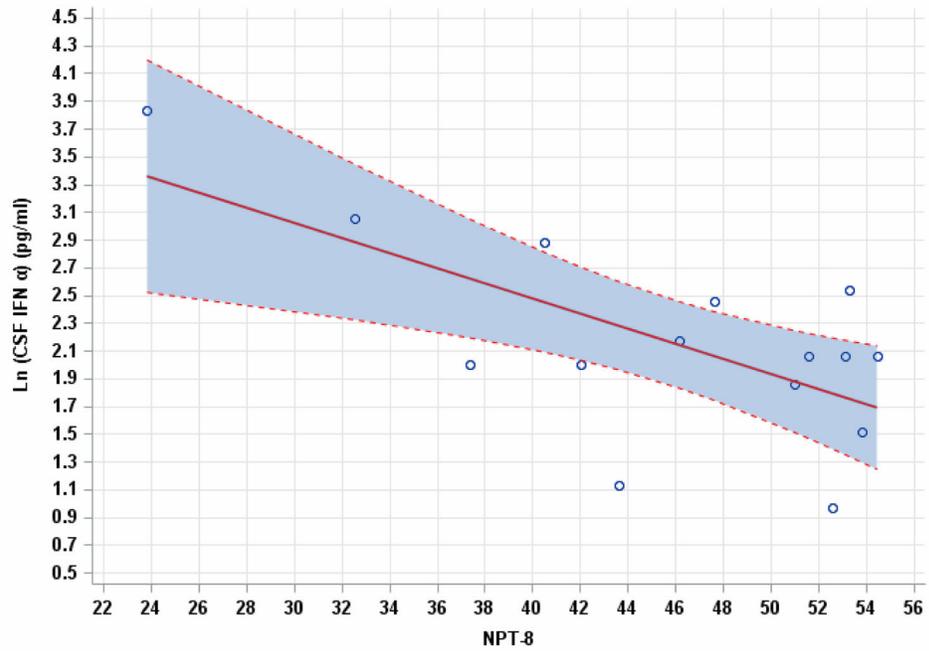


Figure 1

Figure 1.



**Figure 2.**  
Linear regression relationship between CSF IFN $\alpha$  and NPT-8 with 95% confidence interval in shaded blue

**Table 1**

Participant demographic and disease characteristics (see box for abbreviations)

<b>Variable</b>	<b>Frequency (%)</b>
<b>N = 15 unless otherwise stated</b>	<b>or median (IQR; range)</b>
	<b>or mean [Standard deviation]</b>
<b>Age in years</b>	49 (43–52; 35–59)
<b>Sex</b>	
Male	12 (80%)
Female	3 (20%)
<b>Race</b>	
African-American	11 (73.3%)
Caucasian	4 (26.7%)
<b>Years of HIV diagnosis</b>	8 (2–14; <1–25)
<b>Co-morbidities</b>	
Current cigarette smoker	9 (60%)
Hepatitis C positive	3 (20%)
Hypertension	2 (13.3%)
Depression	2 (13.3%)
Hepatitis B positive	1 (6.7%)
cART naïve	6 (40%)
cART experienced but off treatment	6 (40%)
Currently on cART	3 (20%)
<b>Laboratory results</b>	
CD4 count (cells/μl)	62 (29–201; 10–922)
CD4%	10 (4–21; 1–39)
CD4 nadir (n=13)	49 (20–102.5; 10–153)
Hemoglobin (g/dl)	12.8 (12–14.2; 9.4–15.7)
Creatinine (mg/dl)	1.0 (0.8–1.4; 0.7–1.6)
Plasma IFN-α (pg/ml, n=14)	26.5 (2.82–103.02; 0–209.73)
Log Plasma HIV (detectable n=12)	4.76 (3.92–5.34; 3.6–5.82)
CSF WBC	0 (0–4; 0–12)
CSF RBC	0 (0–1; 0–58)
CSF Protein	38.5 (33.25–52.75; 15–95)
Log CSF HIV (detectable n= 12)	3.09 (2.70–4.16; 1.61–5.25)
CSF IFN-α (pg/ml)	7.86 (6.43–12.62; 2.62–45.95)
CSF NFL (pg/ml)	563 (487–826; 293–12233)
<b>Neuropsychological testing</b>	
HIV dementia scale score	11.47 [3.91]
NPT-8	43.7 [9.34]

<b>Variable</b>	<b>Frequency (%)</b>
<b>N = 15 unless otherwise stated</b>	<b>or median (IQR; range)</b> <b>or mean [Standard deviation]</b>
<b>GDS</b>	0.86 [0.995]

IQR = interquartile range; HIV = human immunodeficiency virus; cART = combination antiretroviral therapy; CD = cluster of differentiation;  $\mu$ l = microliters; g = grams; dl = deciliter; mg = milligrams; pg = picograms; CSF = cerebrospinal fluid; WBC = white blood cell count; RBC = red blood cell count; IFN $\alpha$  = interferon alpha; NFL = Neurofilament light chain; NPT = composite neuropsychological test score; GDS = global deficit score

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**Table 2**Pearson correlation coefficients (r) between IFN $\alpha$  (Log10 transformation) and Neurocognitive Performance

NC performance measure	CSF IFN $\alpha$ (p value)	Plasma IFN $\alpha$ (p value)	* CSF IFN $\alpha$ adjusted r (p value)
<b>NPT-8</b>	-0.67 (0.006)	NS	-0.68 (0.02)
<b>Trailmaking A</b>	-0.735 (0.002)	-0.561 (0.037)	-0.76 (0.003)
<b>Trailmaking B</b>	-0.677 (0.006)	NS	-0.69 (0.01)
<b>HVLT total</b>	NS	NS	
<b>HVLT delay</b>	NS	NS	
<b>Pegboard dominant</b>	NS	NS	
<b>Stroop Color word</b>	-0.67 (0.009)	NS	-0.69 (0.02)
<b>Letter Fluency</b>	NS	NS	
<b>Animal Fluency</b>	NS	NS	

Abbreviations: CSF = cerebrospinal fluid; IFN $\alpha$  = interferon alpha; NPT = composite neuropsychological test score; NS = not significant; GDS = global deficit score; HVLT = Hopkins Verbal Learning Test

\* Adjusted for the presence of chronic hepatitis B or C