Mitochondrial DNA in CSF is Associated with Inflammation and Degree of Neurocognitive Deficits in HIV Infection
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Background
• HIV invades the central nervous system (CNS) early in infection, often resulting in impairment of neurocognitive function.
• Even with antiretroviral therapy (ART), some HIV-infected individuals can experience neurocognitive decline in part due to persistent inflammation.
• Persistent inflammation might result in cell death.1,3,4 Cell death would release into the cerebrospinal fluid (CSF) mitochondrial DNA (mtDNA), which itself can cause a potent inflammatory response.5

Objective
To characterize the relationship between mtDNA in the CSF, inflammation, and neurocognitive function.

Methods
• We quantified mtDNA levels in CSF of 25 HIV-infected patients by droplet digital PCR (ddPCR).
  ✓ A Global Deficit Score (GDS) reflecting demographically adjusted neuropsychological performance across ability domains was used as a measure of neurocognitive impairment (NCI), with GDS < 0.5 considered neurocognitively normal (NCN).
  ✓ We measured soluble markers (CD14, sCD10) in CSF by ELISA and cytokines (TNF-α, IL-6) and chemokines (MCP-1, IP-10) in CSF and blood plasma with a liquid bead suspension array system.
  ✓ Intracellular cytokine production of TNF-α, IL-2, IFN-γ, and CD107α in CD4+ and CD8+ T cells was quantified by flow cytometry.
• To investigate the relationship between pleocytosis and mtDNA levels, we also included 5 HIV-infected individuals, who had undergone a structured ART interruption.
• In these patients, we selected 8 samples for each subject: 2 prior to interruption of ART, 2 within 8 weeks of ART interruption and 2 after re-initiation of ART and quantified levels of mtDNA by ddPCR.
• All statistical analyses were performed in R statistical software.

Results
Figure 1. Higher mtDNA in CSF is positively associated with worse neurocognitive performance among individuals with NCI.

Figure 2. Higher mtDNA in CSF is associated with inflammation in individuals with NCI.

Figure 3. Higher mtDNA in CSF is associated with inflammation if CSF VL is detectable in both NCI and NCN groups.

Figure 4. mtDNA in CSF precedes pleocytosis.

Conclusions
✓ While mtDNA was not associated with the presence of NCI, higher levels of mtDNA were strongly associated with greater severity of impairment among those with NCI (Figure 1).
✓ Higher levels of mtDNA were associated with more inflammation in the CNS and blood (Figure 2, 3), and was the strongest predictor of NCI severity (Table 2).
✓ Levels of mtDNA in CSF were not a result of pleocytosis as mtDNA peaks levels preceded pleocytosis (Figure 4).
✓ Altogether these data support that mtDNA is a biomarker for severity of NCI. Given that mtDNA is pro-inflammatory and is strongly associated with other markers of inflammation in the CNS (IP-10, CD8+ T cells in CSF producing IFN-γ and IL-2), we hypothesize that either the source of mtDNA in individuals with NCI is from neurons or individuals with NCI have inflammatory mechanisms that are different than individuals who are NCN or both.

Table 1. Virologic, Immunologic and Neurocognitive Characteristics

Table 2. mtDNA in CSF is strongest correlate of NCI severity

Table 3. Association of clinical and soluble markers with GDS. Associations were tested with all participants and for those grouped into defined NCI and NCN levels of mtDNA. NCI vs. NCN levels were compared with NCI vs. NCN. All tests were two-tailed. *p<0.05. **p<0.01. ***p<0.001. All tests were two-tailed.

References