# CSF EXOSOME AB42 AND TAU/AB42 RATIO ASSOCIATED WITH COGNITIVE DISORDERS IN OLDER PWH

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Poster # 00407

## Background

• HIV-associated neurocognitive disorders (HAND) remain prevalent despite viral suppression on antiretroviral therapy (ART).

• Older people with HIV (PWH) are also at risk for amnestic mild cognitive impairment (aMCI) and Alzheimer's disease (AD), but biomarkers to distinguish these disorders from HAND are unavailable.

• β-amyloid (Aβ42) and Tau are biomarkers associated with cognitive decline in aMCI/AD, but their relationship to mild forms of cognitive impairment in older PWH is unclear.

• Given evidence suggesting that exosomes and other types of extracellular vesicles (EVs) play a role in several age-related cognitive disorders (e.g., Alzheimer's Disease, Parkinson's Disease, amyotrophic lateral sclerosis) and may carry Tau, β-amyloid (Aβ42), and other biomarkers of these disorders, we investigated the relationship of plasma and CSF soluble and EV-associated markers of CNS injury (Aβ42, total Tau, NFL), glial activation (GFAP), and vascular injury (ICAM-1, VCAM-1, CRP) to cognitive impairment in older PWH.

## Methods

• The study cohort included 184 participants (98 PWH virally suppressed on ART enrolled in National NeuroAIDS Tissue Consortium (NNTC) and CNS HIV Anti-Retroviral Therapy Effects Research (CHARTER) during 2006-2016, age 30-75 years, 84% male, 52% with HAND diagnoses of asymptomatic neurocognitive impairment (ANI) or mild neurocognitive disorder (MND) and 86 HIV- controls matched for age, gender, and race). Among 98 PWH, 66 had paired plasma and CSF samples.

• Inclusion criteria for PWH: on 3 or more ART drugs for at least 1 year and virally suppressed (plasma viral load <200 HIV RNA copies/ml).

• PWH with HIV-associated dementia (HAD) or neuropsychological impairment due to other causes (NPI-O) were excluded.

• PWH were administered a comprehensive neuropsychological test battery designed to assess 7 neurocognitive domains. Demographically corrected global neurocognitive T scores were generated from individual test T scores.

• Plasma and CSF EVs were isolated from 300 µl samples using Izon qEV 35 nM isolation columns and Automatic Fraction Collector or Systems Biosciences ExoQuick reagent, respectively, and characterized by transmission electron microscopy (EM), nanoparticle tracking analysis (NTA) (Particle Metrix), and immunoblotting.

• Biomarkers of CNS injury (Aβ42, total Tau, NFL), glial activation (GFAP), or vascular injury (ICAM-1, VCAM-1, CRP) were measured in plasma and CSF samples or lysed plasma and CSF EVs using the Meso Scale Discovery platform.

**Table 1: Demographic and clinical characteristics of the study cohort**

| | HIV- (n=86) | HIV+ (n=98) | No HAND (n=47) | HAND* (n=51) | p-values |
|---|---|---|---|---|---|
| **Age (years)** | 54 (46 - 61) | 52.6 (47- 59) | 52.1 (47 - 57) | 52.6 (49 - 61) | 0.55 |
| **Gender (Male, n, %)** | 60 (70) | 82 (84) | 47 (100) | 40 (78) | **0.0006** |
| **Race (n, %)#** | | | | | 0.68 |
| **Black** | 18 (27.7) | 27 (27.6) | 15 (31.9) | 12 (23.5) | |
| **White** | 46 (70.8) | 68 (69.4) | 31 (65.9) | 37 (72.5) | |
| **Other** | 1 (1.5) | 3 (3.1) | 1 (2.1) | 2 (3.9) | |
| **Current Smoking (n, %)** | | 48 (48.9) | 19 (40.4) | 29 (56.9) | 0.11 |
| **Current Alcohol use (n, %)** | | 45 (45.9) | 23 (48.9) | 22 (43.1) | 0.69 |
| **Diabetes (n, %)** | | 16 (16.3) | 6 (12.8) | 10 (19.6) | 0.42 |
| **Hypertension (n, %)** | | 50 (51.0) | 22 (46.8) | 28 (54.9) | 0.54 |
| **Hyperlipidemia (n, %)** | | 28 (28.6) | 10 (21.3) | 18 (35.3) | 0.18 |
| **Cerebrovascular disease/CVD (n, %)** | | 23 (23.5) | 5 (10.6) | 18 (35.3) | 0.005 |
| **Neurocognitive T score** | | 48 (41 - 53) | 52.8 (49.5 - 56.7) | 41.2 (36.6 - 44.8) | **<0.0001** |
| **Global clinical rating** | | 4 (3 - 5) | 3 (2 - 4) | 5 (5 - 6) | **<0.0001** |
| **CSF protein** | | 37 (31 - 47) | 42 (32.5 - 51.5) | 35.1 (27.4 - 43.8) | **0.04** |
| **CSF WBC (cells/µl)** | | 1 (1- 3) | 1 (0 – 3) | 1 (1 -3) | 0.77 |
| **Duration of HIV infection (years)** | | 15 (11 - 21) | 15 (11 - 20) | 15 (11 - 22) | 0.89 |
| **Plasma viral load (<200 copies/ml)** | | 93 (96) | 45 (96) | 48 (94) | >0.99 |
| **CSF viral load (<50 copies/ml)** | | 87 (96) | 43 (98) | 44 (94) | 0.62 |
| **CD4 count (cells/µl)** | | 540 (372 - 738) | 589 (382 - 845) | 537 (343 - 679) | 0.41 |
| **Nadir CD4 count (cells/µl)** | | 84 (14 - 223) | 88 (12 - 262) | 75 (16 - 183) | 0.62 |
| **ART use (n, %)** | | 100 (100) | 100 (100) | 100 (100) | >0.99 |
| **Protease inhibitors** | | 59 (60.2) | 29 (61.7) | 30 (58.8) | |
| **Integrase inhibitors** | | 12 (12.2) | 9 (19.1) | 10 (19.6) | |
| **Duration of ART (years)** | | 9 (5 - 14) | 9.2 (5 - 14) | 9.5 (5 - 14) | 0.84 |

Median (interquartile range) is shown unless otherwise indicated. P-values for two-group comparisons between no HAND and HAND groups were calculated using chi-square or Fisher exact tests for categorical variables and Mann-Whitney test for continuous variables. Bold font denotes p <0.05. *HAND diagnoses were asymptomatic neurocognitive impairment (ANI, n=27) or mild neurocognitive disorder (MND, n=24). # Race data were not available for 21 HIV- individuals
Abbreviations: ART, antiretroviral therapy; CVD, Cardiovascular disease; HAND, HIV-associated neurocognitive disorders, HIV, human immunodeficiency virus; WBC, white blood cells.

## Results

• Median age of virally suppressed PWH on ART was 53 years (IQR 47–59) and median CD4 count, nadir, duration of HIV infection were 540, 84 cells/µl, and 15 years, respectively. 52% of PWH were diagnosed with mild forms of HAND (53% ANI, 47% MND).
• HIV infection was associated with increased plasma soluble NFL (p=0.04) and CSF soluble Aβ42 (p=0.0003), while plasma and CSF soluble Tau showed no significant difference by HIV status.
• CSF EV-associated Aβ42 was decreased (p=0.0002) and CSF EV-associated Tau/Aβ42 ratio was increased (p=0.001) in PWH with HAND vs. no HAND.
• CSF EV Aβ42 was associated with higher global neurocognitive T scores (p<0.0001) and CSF EV Tau/Aβ42 ratio and plasma soluble NFL were associated with lower global neurocognitive T scores (p=0.0003 and p=0.098, respectively) in linear regression models adjusted for age.
• Cerebrovascular disease was more prevalent among PWH with HAND vs. no HAND (p=0.005) and was associated with increased plasma soluble NFL (p=0.03) and CSF EV Tau/Aβ42 ratio (p=0.10).
• HIV infection and HAND were associated with increased plasma ICAM-1 (p=0.005 and 0.02, respectively), VCAM-1 (p=0.02 and 0.004, respectively) and CRP (both p=0.02). PWH had higher levels of plasma EV ICAM-1 and VCAM-1 compared with HIV- controls (p<0.0001), but these EV biomarkers had no significant association with HAND. Thus, soluble forms of these plasma vascular injury markers were more closely related to HAND than EV-associated forms of these biomarkers

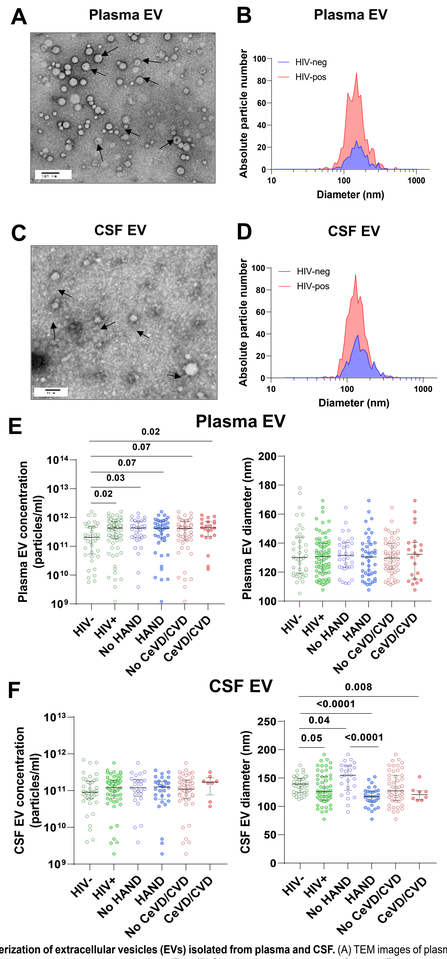



**Figure 1. Characterization of extracellular vesicles (EVs) isolated from plasma and CSF.** (A) TEM images of plasma EVs isolated from a representative HIV-negative individual. Arrows indicate EVs. (B) Size distribution histograms of plasma EVs by nanoparticle tracking analysis (NTA) of representative HIV-negative and HIV-positive individuals. (C) TEM images of CSF EVs isolated from a representative HIV-negative individual. Arrows indicate EVs. (D) Size distribution histograms of CSF EVs by NTA of representative HIV-negative and HIV-positive individuals. (E) Association of plasma EV concentration and size with HIV infection, HAND, and vascular disease status. (F) Association of CSF EV concentration and size with HIV infection, HAND, and vascular disease status. Exosome markers CD63, CD9, and Flotillin-1 were detected by immunoblotting of plasma and CSF EVs (not shown). Medians and IQRs are indicated as horizontal and vertical lines, respectively. Statistical significance calculated using Mann–Whitney U test; significant differences (p<0.05) are indicated.

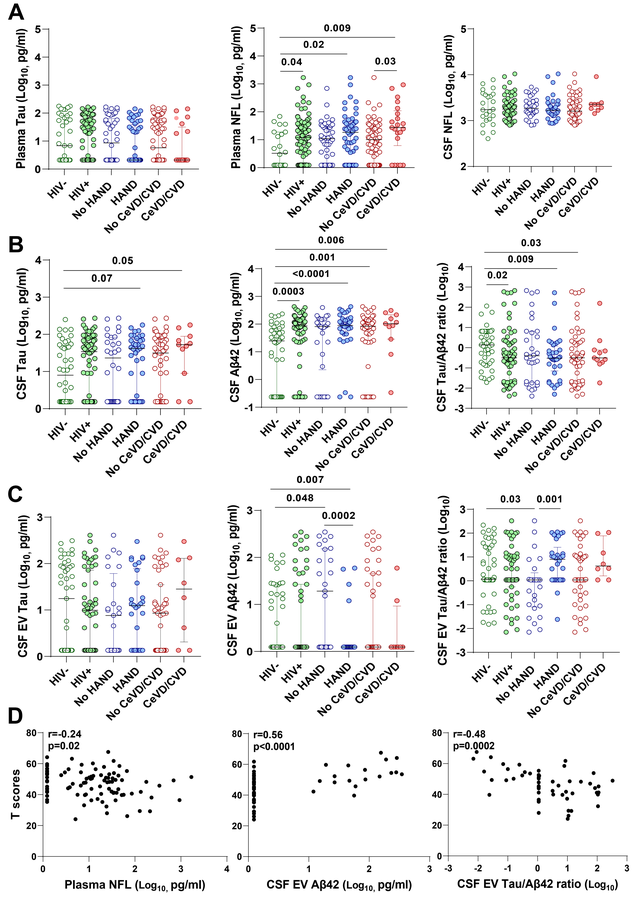



**Figure 2. CSF extracellular vesicle (EV) Aβ42 and Tau/Aβ42 ratio are associated with HAND diagnoses of asymptomatic neurocognitive impairment or mild neurocognitive disorder in virally suppressed HIV-positive individuals on ART.** (A) Association of plasma total Tau, plasma NFL, and CSF NFL with HIV infection, HAND, and vascular disease status. (B) Association of CSF total Tau, Aβ42, and Tau/Aβ42 ratio with HIV infection, HAND, and vascular disease status. (C) Association of CSF EV-associated Tau, Aβ42, and Tau/Aβ42 ratio with HIV infection, HAND, and vascular disease status. Medians and IQRs are indicated as horizontal and vertical lines, respectively. Statistical significance was calculated using Mann–Whitney U test; significant differences (p<0.05) are indicated. Presence of Tau and Aβ42 in CSF EVs was confirmed by immunoblotting following proteinase K treatment of CSF EVs (not shown). (D) Plasma NFL and CSF EV Tau/Aβ42 ratio correlate negatively and CSF EV Aβ42 correlates positively with global neurocognitive T scores.

## Results

**Table 2: Association of CSF EV-associated Aβ42 and Tau/Aβ42 ratio, plasma NFL, and plasma GFAP with global neurocognitive T scores in multiple linear regression analysis**

| Variable | β-coefficient | SE | 95% CI | p-value |
|---|---|---|---|---|
| Intercept | 43.71 | 1.976 | 39.74 - 47.67 | <0.0001 |
| Age | -0.1641 | 0.1245 | -0.414 - 0.086 | 0.19 |
| CSF EV Aβ42 | 1.851 | 0.3873 | 1.074 - 2.628 | <0.0001 |
| Intercept | 49.52 | 1.832 | 45.85 - 53.20 | <0.0001 |
| Age | -0.204 | 0.1309 | -0.467 - 0.0586 | 0.13 |
| CSF EV Tau/Aβ42 ratio | -1.218 | 0.3116 | -1.843 to -0.593 | 0.0003 |
| Intercept | 50.77 | 1.721 | 47.36 - 54.19 | <0.0001 |
| Age | -0.1201 | 0.1076 | -0.3338 - 0.09355 | 0.26 |
| Plasma NFL | -0.6464 | 0.3862 | -1.413 - 0.1206 | 0.098 |
| Intercept | 55.4 | 4.401 | 46.66 - 64.14 | <0.0001 |
| Age | -0.1439 | 0.1044 | -0.3512 - 0.06340 | 0.17 |
| Plasma GFAP | -0.9767 | 0.6751 | -2.318 - 0.3641 | 0.15 |

Multiple linear regression models were fit with T scores as the dependent variable in ART-treated PWH. Independent variables CSF EV Aβ42 (n=55), CSF EV Tau/Aβ42 ratio (n=55), plasma NFL (n=95), and plasma GFAP (n=95) were log2 transformed.
Abbreviations: SE, standard error; CI, confidence interval

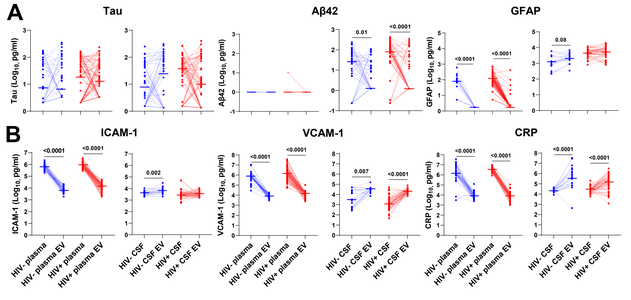



**Figure 3. Comparative abundance of biomarkers in plasma and CSF soluble and extracellular vesicle (EV) fractions.** (A) Comparison of plasma and CSF CNS injury and glial activation marker levels (Tau, Aβ42, GFAP) with those of equivalent volumes of corresponding plasma and CSF EV fractions among HIV-negative and HIV-positive participants. (B) Comparison of plasma and CSF vascular injury marker levels (ICAM-1, VCAM-1, CRP) with those in corresponding EV fractions. Significance by Wilcoxon matched-pairs signed rank test.

## Conclusions

• Decreased CSF EV Aβ42 and increased CSF EV Tau/Aβ42 ratio are associated with cognitive impairment in a subgroup of older PWH on suppressive ART.

• These CSF EV-associated biomarkers may help to distinguish age-related aMCI and AD from HIV-related neurocognitive disorders in older PWH with viral suppression on ART.

## Acknowledgements

We thank the Harvard Medical School Electron Microscopy Facility for TEM imaging.

This work was supported by National Institutes of Health grants to D.G. (R01 MH110259, R01 DA040391, R01 DA046203).

Financial support for the NNTC was provided through the following cooperative agreements from the National Institutes of Health: U24MH100930; U24MH100931; U24MH100928; U24MH100929; U24MH100925. CHARTER sites were supported by Award Number HHS-N-271-2010-00036C) and HHSN271201000030C from NIMH/NINDS.