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Plasma Extracellular Vesicles and Cell-Free Mitochondrial DNA are Associated with Cognitive Dysfunction in Treated Older Adults with HIV

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BACKGROUND

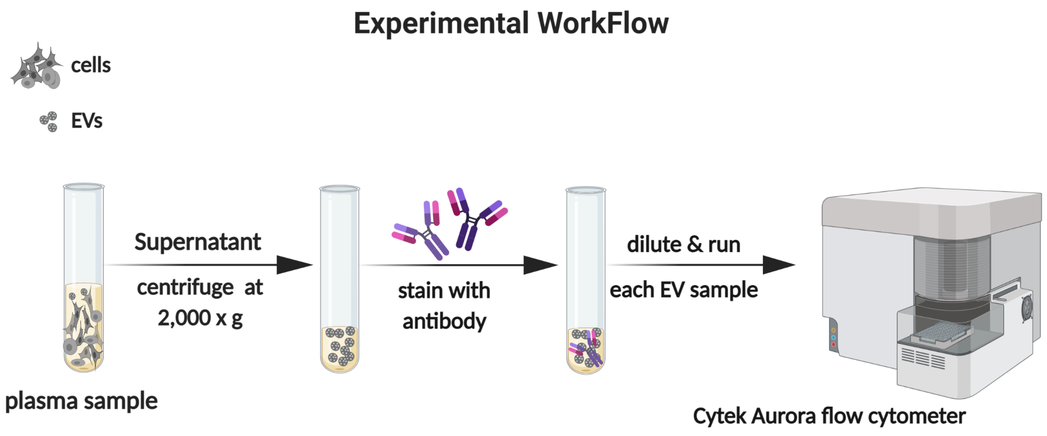
Extracellular vesicles (EVs) are small structures with a range of functions including cell-to-cell communication and inflammation.

- Neurons and microglia can secrete EVs that cross the blood brain barrier.[1]
- Plasma cell-free mitochondrial DNA (cfmtDNA) has been associated with cognitive dysfunction in older adults with HIV (OAH). [2]
- Specific EVs have been associated with inflammation in the setting of HIV, and some EVs carry HIV genetic material.[3]
- EVs can provide diagnostic and predictive value for CNS injury in HIV; EVs from the CSF have been shown to serve as such biomarkers however it remains unclear the role of EVs in OAH.[4]

We hypothesized that plasma EVs would be associated with cognitive dysfunction in OAH.

METHODS

- A nested case-control study compared OAH age ≥55 with cognitive dysfunction (Montreal Cognitive Assessment [MoCA] score <23) to demographically similar OAH controls (MoCA >26).
- Frailty testing was conducted using the Fried Frailty Index. Participants with HIV viral load >40 copies/ml were excluded.
- Plasma and urine cfmtDNA measured by PCR for NADH dehydrogenase 1
- Plasma EVs were isolated by centrifugation → Flow cytometry designed method to phenotype EVs.[5]



Dye	Panel 1	Panel 2
PE	**CD4** (T cell MHC II co-R)	**CCR5** (Trafficking)
APC	**CD14** (Mono LPS R)	**CD62p** (P-selectin)
PerCP-Cy5.5	**CD16** (Mono FcR, NK cells)	**CD41a** (Platelet GPIIb)
PE-Cy7	**CD19** (B cells)	**CD163** (Mono/Mac Hgb R)
BV421	**CX3CR1** (Fractalkine R)	**CCR2** (Trafficking)
FITC	**WGA** (Sialic acid)	**MAL-1** (Maackia amurensis lectin 1)

Dye	Panel 3	Panel 4	Panel 5
FITC	**MAP2** (Neurons)	**CD9** (Exosomes tetraspanin)	**CD68** (Mono/Mac lectin R)
BV421	**GFAP** (Astrocytes)	**MHC-Class 2** (Antigen presentation, microglial)	**CD73** (B and T cell subset)
PerCP-Cy5.5	**CD200** (Microglia cells)	**CD63** (Exosomes tetraspanin)	**CD66b** (Neutrophils, granulocytes)
PE	**S100B** (Astrocytes)	**GLUT-1** (Glucose transport 1)	**CD36** (oxLDL R)
APC	**NFL** (Neurons)		
PE-Cy7	**CD11b** (Granulocytes, mac)		

STATISTICAL METHODS

A support vector machine learning-based model using recursive feature elimination and 10-fold cross validation was employed and area under the curve of the receiver operating characteristic (AUC-ROC) assessed the probability of discriminating cognitive function.

RESULTS: PARTICIPANT DEMOGRAPHICS

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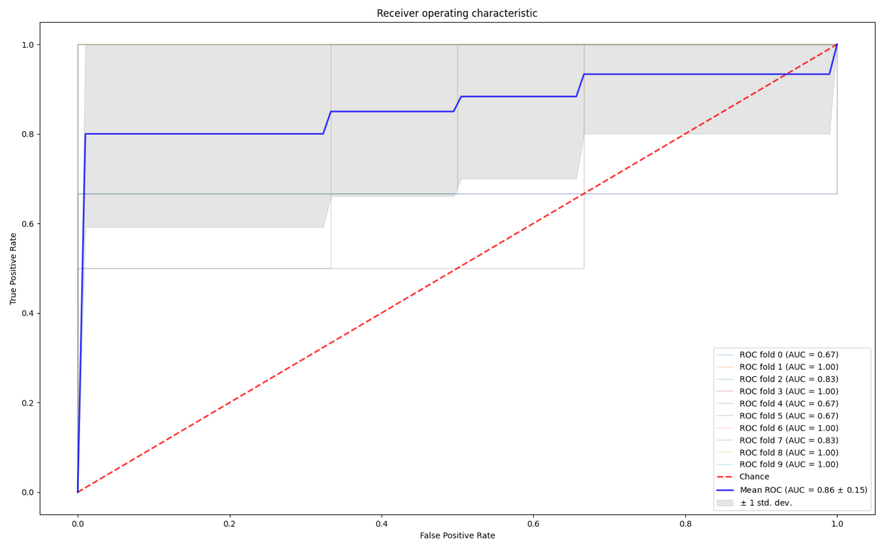
	Total (n=50)	Cognitively Impaired (n=25)	Cognitively Non-Impaired (n=25)	p-value
Age, years, median [IQR]	60.0 [57.0, 64.8]	61.0 [57.0, 66.0]	58.0 [57.0, 63.0]	0.263
Sex, Male, n (%)	31 (62.0%)	15 (60.0%)	16 (64.0%)	1
HIV Duration, years, median [IQR]	24.0 [21.3, 27.8]	23.0 [22.0, 27.0]	24.0 [20.0, 29.0]	0.861
CD4 T cell count, median [IQR]	596 [479, 859]	593 [498, 750]	703 [476, 962]	0.541
Plasma cell-free mitochondrial DNA, Copies/µL, geometric mean [IQR]	5.51 [5.08, 5.89]	5.75 [5.32, 6.30]	5.28 [4.92, 5.77]	0.051
Urine cell-free mitochondrial DNA, Copies/g of urine creatinine, geometric mean [IQR]	19.34 [18.34, 20.25]	19.29 [18.59, 20.16]	19.40 [18.11, 20.27]	0.846
Frailty, n (%)				0.132
Not Frail	17 (35.1%)	6 (24.0%)	11 (47.8%)*	
Pre-Frail/Frail	31 (64.6%)	19 (76.0%)	12 (52.2%)*	
4m Walk Time, seconds, median [IQR]	4.84 [3.90, 5.63]	5.21 [4.44, 5.87]	4.21 [3.58, 5.13]	0.013

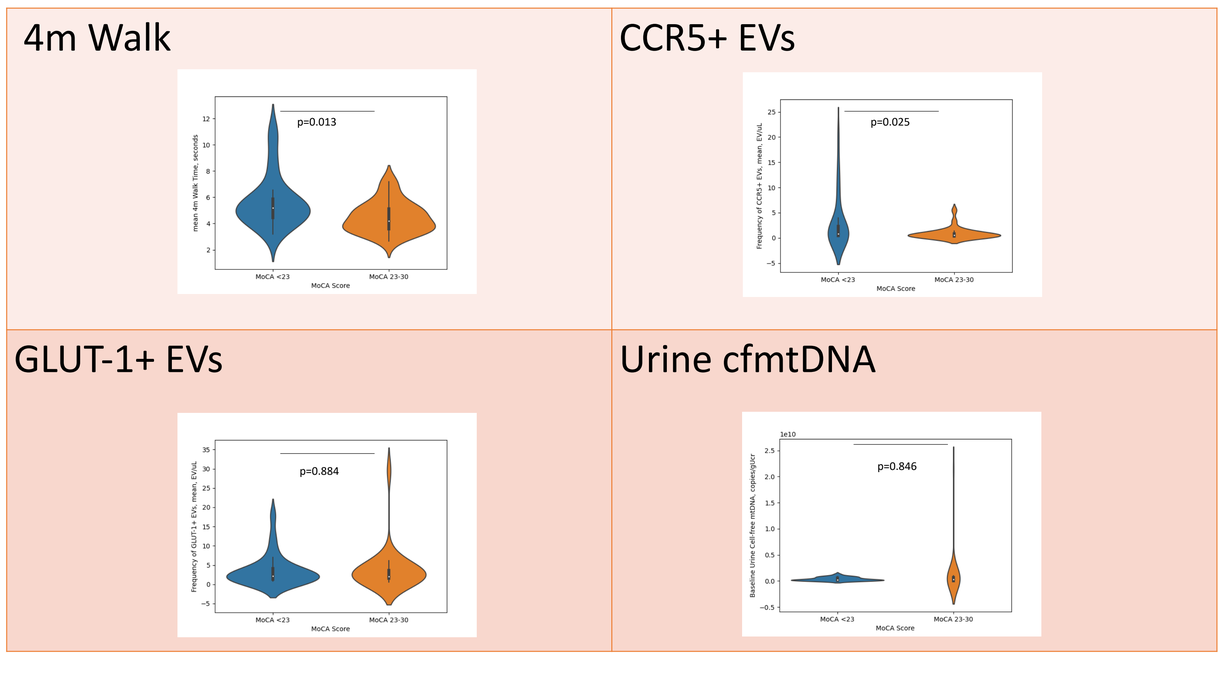
** N=23. Continuous variables compared by Mann-Whitney U test*

RESULTS:

Recursive feature elimination identified 4-meter walk time, CCR5+ EVs, GLUT-1+ EVS, and urine cfmtDNA as predictive of cognitive dysfunction in older adults with HIV



Receiver operating characteristic curve illustrating the probability of discriminating cognitive function using 4-meter walk time, CCR5+ and GLUT-1+ EVS, and urine cfmtDNA



CONCLUSIONS

- Our machine learning model predicted cognitive dysfunction with 86% certainty (± 15%) using a combination of 4-meter walk time, CCR5+ and GLUT-1+ EVS, and urine cfmtDNA which may reflect inflammatory and metabolic activity in the central nervous system.
- These findings suggest a role of EVs and cfmtDNA as potential biomarkers of cognitive dysfunction and warrant further investigation.

4-meter walk time, CCR5+ EVs, GLUT-1+ EVs and Urine cfmtDNA predicted cognitive dysfunction in older adults with HIV with 86% (± 15%) certainty in a machine learning model

ADDITIONAL INFORMATION

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