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H/L-FERRITINS AND TIM-1 ARE ASSOCIATED WITH NEUROCOGNITIVE FUNCTION IN PEOPLE WITH HIV

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BACKGROUND

- People with HIV (PWH) are at heightened risk for neurocognitive impairment (NCI) despite antiretroviral therapy.
- Iron is essential for energy production and brain health[1].
- HIV infection and inflammation alter iron homeostasis[2].
- We previously reported that cerebrospinal fluid (CSF) levels of **ferritin heavy-chain** (**FTH1**), an antioxidant iron-delivery protein, predict better neurocognitive (NC) function in people with HIV (PWH)[2]. **T-cell-immunoglobulin-and-mucin-domain (TIM)-1** is an **FTH1 receptor** in the brain[3], which is also systemically expressed.
- **Light-chain ferritin** (**FTL**) is another major circulating ferritin subunit.

Hypotheses: ***Higher FTL & FTH1 levels in serum, and lower TIM-1 levels in urine, will be associated with less NCI and higher NC domain scores in PWH cross-sectionally and over time.***

METHODS

Study Population and Outcome Ascertainment:

- ACTG Protocol A5322 is a prospective cohort study of 1035 PWH >40 years of age. This analysis included 324 PWH with NC assessments and existing inflammatory biomarker data.
- **NC function –** assessed by Neuroscreen[4] at entry & every 48 weeks for up to 288 weeks, with assessment in **key ability domains** – z-scores for Trail-making A (**TMA**), Trail-making B (**TMB**), Wechsler Adult Intelligence Scale-Revised Digit Symbol (**DSY**), & Hopkins Verbal Learning tests (**HVLT**). Domain scores were averaged to give the **NPZ-4 (global) NC score**.
- **NCI** – defined by ≥2 z-scores ≥1 SD below the mean, or ≥1 score ≥2 SD below the mean.

Biomarker Quantification:

- Serum FTH1, FTL, and urine TIM-1 quantified by Enzyme-Linked Immunosorbent Assay.
- Urine F_2-Isoprostanes (F_2-IsoPs) quantified in urine at entry by gas chromatography/mass spectroscopy at Vanderbilt University.

Statistical Methods:

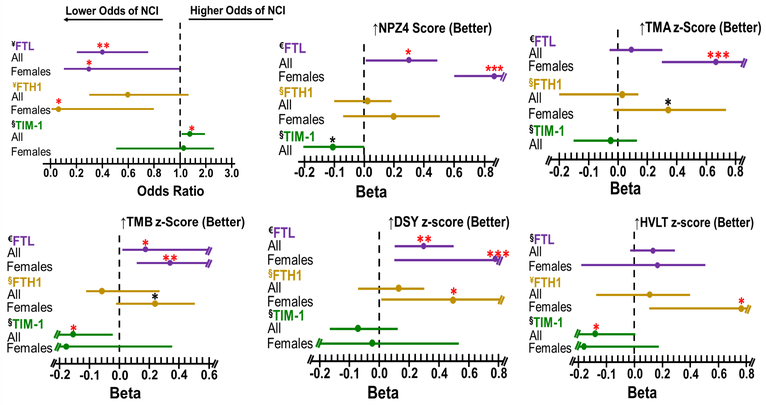
- **Outcomes: NCI; Global NC Function (NPZ-4); 4 Domain Scores (TMA, TMB, DSY, HVLT)**
- **Exposures: Serum FTH1, FTL** and **urine TIM-1** levels, collected at entry
- **Baseline Analyses**: *Univariate Analyses-* Spearman's correlations (biomarkers); Pearson's Chi-square and Wilcoxon Rank Sum tests (potential confounders). *Multivariable Analyses-* Logistic Regression and Multiple Linear Regression models to assess FTH1, FTL, and TIM-1 associations with NCI and with NPZ-4, TMA, TMB, DSY, and HVLT scores, respectively.
- **Longitudinal Analyses:** Generalized Estimating Equations (ability to predict NC function)
- **Covariates:** Multivariable models included variables with p≤0.10 in univariate analyses.

Note: No participants in this sample reported active substance abuse.

RESULTS

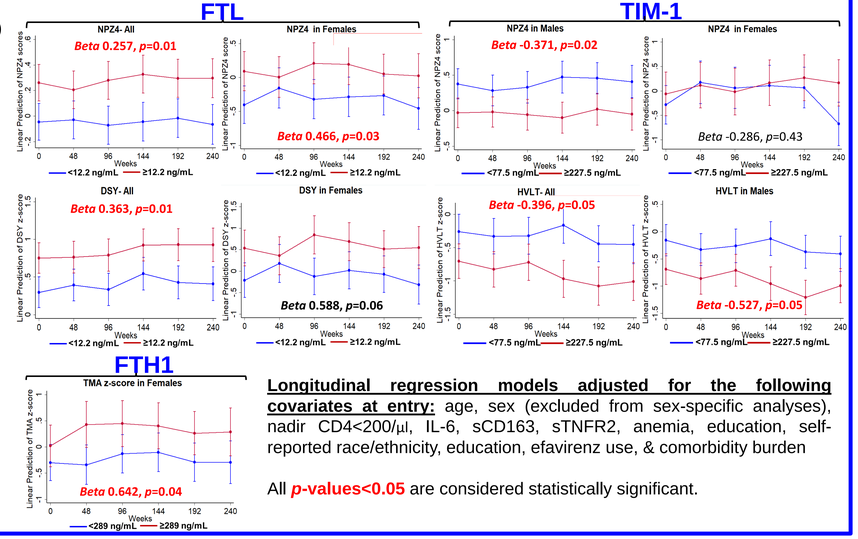
Multivariable Associations of Global or Domain-Specific Neurocognitive Function Scores with FTL, FTH1, and TIM-1

Associations at Entry



Regression models adjusted for the following covariates at entry: age, self-reported Hispanic ethnicity, anemia, nadir CD4<200/µl, sCD163, sTNFR2, IL-6, comorbidity burden, hepatitis C virus seropositivity, and efavirenz use.
$continuous, log-transformed; ¥binary (≥ *vs.*<median); €binary (Quartile 4 *vs.* Quartile 1)
***p*<0.10, **p*<0.05, ***p*<0.005, ****p*<0.0005**

Predicted Adjusted Changes in NC Scores



Longitudinal regression models adjusted for the following covariates at entry: age, sex (excluded from sex-specific analyses), nadir CD4<200/µl, IL-6, sCD163, sTNFR2, anemia, education, self-reported race/ethnicity, education, efavirenz use, & comorbidity burden

All ***p*-values<0.05** are considered statistically significant.

STUDY POPULATION

Variable at Entry	Not Impaired (N=237)	Impaired* (N=82)
Age, mean (SD)	51.4 (6.9)	53.7 (8.3)
Sex, % female	17.3	24.4
Non-Hispanic Black[1], %	31.6	20.7 Ψ
Hispanic[1], %	20.7	36.6 **
Efavirenz use, %	51.5	39.0 #
Nadir CD4⁺ T-cells/µL, median (IQR)	192 (67, 314)	237 (80, 347)
HIV RNA < 200 copies/mL, %	51.5	36.6 *
Anemia, %	10.5	12.7
HCV seropositive, %	2.53	8.54 *
≥ 2 Comorbidities[2], %	32.5	47.6 *
Pre-Frail or Frail, %	38.6	60.5 **

SD, standard deviation; *HCV*, hepatitis C virus; [1]Self-report
***p* < 0.01; **p* < 0.05; #*p* = 0.050; Ψ*p* = 0.060; [2]Clinically significant comorbidities, *e.g.,* cancer, diabetes mellitus, cardiovascular or chronic kidney disease, HCV, fractures, prior transient ischemic attack, *etc.*
**NCI status was missing for 5 PWH due to incomplete score data.*

BIOMARKER LEVELS AT ENTRY (Median, IQR)

Biomarker*	All PWH (N=324)	Males (n=263)	Females (n=61)
FTH1, ng/mL	289 (199, 499)	311 (197, 497)	277 (136, 526)
FTL, ng/mL	12.2 (7.7, 22.1)	12.4 (7.7, 22.4)	11 (7.2, 19.6)
TIM-1, ng/mL	152.5 (77.5, 227.5)	154 (78, 232)	147 (74, 213)
F_2-IsoPs, ng/mg Creat.	57 (32, 84)	56 (31, 84)	60 (43, 78)

PWH, people with HIV; *FTH1,* heavy-chain ferritin; *FTL,* light-chain ferritin; *F_2-IsoPs,* F_2-Isoprostanes; *TIM-1,* T-cell immunoglobulin and mucin domain-1; *Creat,* creatinine; *IQR,* interquartile range. ***Note:* No significant differences by sex; 2) F_2-IsoPs were undetectable in 5 PWH.**

CORRELATIONS AT ENTRY

Biomarker	Hemoglobin (N=312) rho (*p*)	IL-6 (N=324) rho (*p*)	sTNFR2 (N=324) rho (*p*)	sCD163 (N=324) rho (*p*)	TIM-1 (N=324) rho (*p*)	F_2-IsoPs (N=310) rho (*p*)
FTH1 (All)	0.175 (<0.01)	-0.007 (0.91)	-0.043 (0.45)	<0.001 (0.99)	-0.050 (0.37)	-0.061 (0.28)
(Female)	0.391 (<0.01)					-0.290 (0.02)
FTL (All)	0.053 (0.35)	-0.026 (0.64)	-0.078 (0.16)	-0.050 (0.37)	-0.011 (0.84)	0.006 (0.92)
(Female)	0.215 (0.10)					-0.471 (<0.001)
TIM-1 (All)	0.018 (0.76)	0.218 (<0.01)	0.234 (<0.01)	0.165 (0.01)	---	-0.028 (0.61)
(Female)	-0.194 (0.14)	0.196 (0.13)	0.191 (0.14)	0.114 (0.38)		-0.045 (0.73)

MECHANISTIC INSIGHTS:

HIGHER FTH1 & FTL ARE ASSOCIATED WITH LESS ANEMIA

FTH1	-0.836 (*p*<0.01; 95% CI -1.088 to -0.583)
FTL	(All) -0.160 (*p*=0.254; 95% CI -0.436 to 0.116)
	(Female) -0.456 (*p*=0.086; 95% CI -0.980 to 0.067)
TIM-1	0.047 (*p*=0.788; 95% CI -0.296 to 0.389)

MULTIVARIABLE ASSOCIATIONS OF HIGHER F_2-ISOPs WITH LOWER NC TEST SCORES

NPZ-4	All (n=285)[1]	-0.170 (-0.298, -0.041); *p*=0.010
	Females (n=59)[2]	-0.425 (-0.797, -0.054); *p*=0.026
TMA	All (n=292)[1]	-0.164 (-0.330, 0.002); *p*=0.052
	Females (n=60)[2]	-0.576 (-1.07, -0.083); *p*=0.023
DSY	All (n=292)[1]	-0.283 (-0.412, -0.053); *p*=0.011
	Females (n=60)[2]	-0.543 (-1.12, 0.034); *p*=0.065

[1]Models in all PWH adjusted for age, Hispanic ethnicity, sTNFR2, nadir CD4<200/µl, efavirenz use, anemia, comorbidity
[2]Models in females adjusted for age, Hispanic ethnicity, sTNFR2, comorbidity

CONCLUSIONS

- In virally suppressed PWH, *higher* serum **FTH1** and **FTL** levels are associated with *less* NCI, *better* global NC function, and *better* performance in multiple NC ability domains, particularly in females.
- *Higher* urine **TIM-1** levels are associated with *higher* odds of NCI, *worse* global NC scores and *worse* performance on domain-specific (TMB, DSY, and HVLT) tests.
- Results for ***serum* FTH1** replicate our reported association of higher ***CSF* FTH1** with *better* NC function in PWH but suggest that the effect is more pronounced in females.
- Our findings suggest differential impacts of altered iron homeostasis on NC performance by sex.
- Such sex-based differences may be adaptive, providing protection against anemia and oxidative stress.

Strengths and Limitations

- To our knowledge, first study to show associations of *serum* FTL and FTH1, and urine TIM-1, with NCI / NC domains in PWH
- Key sex differences implicate iron homeostasis as a pathway underlying sex-specific vulnerabilities
- *Analyses were underpowered, particularly in females and did not include information on time-updated biomarker levels or account for information on gender identity and anti-depressant medication use.*

Implications/Future Directions

- Iron-modulating interventions might have a role to play in preventing NCI or halting its progression.
- Studies with more females are needed to refine estimates, identify interaction effects, & explore mechanisms.

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