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L-FERRITIN AND TIM-1 ARE ASSOCIATED WITH FRAILTY MEASURES IN PEOPLE WITH HIV

Harpreet Kaur[1], Robert C. Kalayjian[2], Kunling Wu[3], Katherine K. Tassiopoulos[3], Frank J. Palella[4], Babafemi O. Taiwo[4], William S. Bush[5], Corrilynn O. Hileman[2], Roger J. Bedimo[6], Susan L. Koletar[7], Ronald J. Ellis[8], Kristine M. Erlandson[9], and **Asha R. Kallianpur[10]** for the AIDS Clinical Trials Group A5322 (HAILO) Study Team.

[1]Cleveland Clinic/Lerner Research Institute, Cleveland, OH, USA; [2]MetroHealth Medical Center & Case Western Reserve University School of Medicine, Cleveland, OH, USA; [3]Harvard T. H. Chan School of Public Health, Boston, MA, USA; [4]Northwestern University Feinberg School of Medicine, Chicago, IL, USA; [5]Cleveland Institute of Computational Biology and Case Western Reserve University, Cleveland, OH, USA; [6]VA North Texas Health Care System, Dallas, TX, USA; [7]The Ohio State University, Columbus, OH, USA; [8]University of California-San Diego, CA, USA; [9]University of Colorado-Anschutz Medical Campus, Aurora, CO, USA; [10]Lerner Research Institute, Cleveland Clinic & Cleveland Clinic Lerner College of Medicine of Case Western Reserve University, Cleveland, OH, USA.

BACKGROUND

- People with HIV (PWH) are at risk for premature physical function impairment and frailty.[1]
- Iron is important for energy production in brain, skeletal muscles, *etc*., and is dysregulated by HIV infection and inflammation.[2, 3]
- Higher cerebrospinal fluid (CSF) levels of **heavy-chain ferritin (FTH1)**, an antioxidant iron-transporter, predict better cognitive function in PWH over time.[4] **Light-chain ferritin (FTL)** is another major circulating ferritin.
- T-cell-immunoglobulin-and-mucin-domain **(TIM)-1** is an **FTH1 receptor**[5] in brain, which is also expressed systemically and is measurable in urine.
- Reasons for **sex-specific differences** in frailty risk are largely unknown.[6]

HYPOTHESES

- ***Higher* FTL & FTH1 levels in serum & *lower* TIM-1 levels in urine are associated with *better* physical function measures and *less* pre-frailty/frailty.**

METHODS

- **Participants**: 324 virally suppressed PWH from the ACTG HIV Infection, Aging, and Immune Function Long-Term Observational Study (A5322: HAILO), all >40 years of age.
- **Exposures:** **Serum FTH1**, **FTL**, and **urine TIM-1** were quantified at entry by Enzyme-Linked Immunosorbent Assay (ELISA). Isoprostanes (**F_2-IsoPs**), measures of oxidative stress, were measured in urine at entry (gas chromatography/mass spectroscopy at Vanderbilt University).
- **Outcomes:** 1) **Pre-Frail or Frail** status, defined by the Fried criteria[1];
- 2) **Average 4-Meter Walk Time** (secs) *OR* **Slowness**[1]; 3) **Average Grip Strength** (kg, measured by dynamometer) *OR* **Weak Grip**[1].
- **Covariates:** Included in multivariable analyses were variables with $p \leq 0.10$ in univariate analyses of each outcome, including global cognitive function (assessed by Neuroscreen, NPZ-4 score).[7] *Note: No participants in this analysis had active substance use.*
- **Analyses:** 1) Pearson's Chi-Square or Fisher's exact test, non-parametric tests for trend, and Spearman's correlations (biomarker relationships to each other or clinical variables); 2) Biomarker levels were dichotomized (above/below median or top/bottom quartile) for key analyses; 3) Log-transformed biomarker levels as needed; 3) Multiple Linear Regression or Logistic Regression analysis at entry; 4) Maximum Likelihood Estimation with survival-time models and Cox Proportional Hazards Regression to assess multivariable-adjusted associations and estimate hazard ratios; 5) Each outcome was explored separately in females/males.

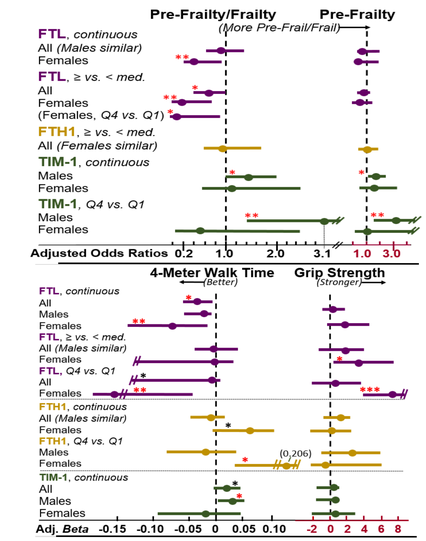
RESULTS

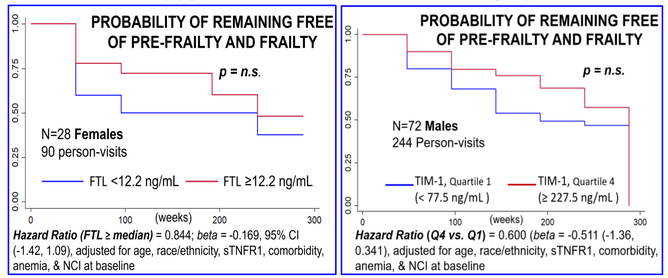
BIOMARKER LEVELS AT ENTRY (Median, IQR)

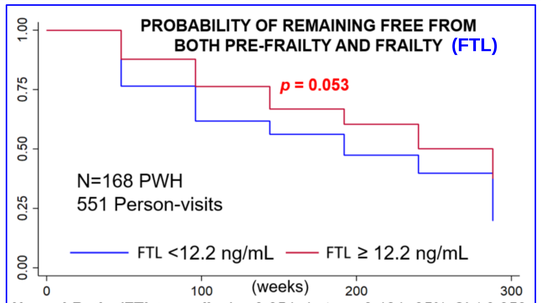
Biomarker	All PWH (N=324)	Males (N=263)	Females (N=61)
FTH1, ng/mL	289 (199, 499)	311 (197, 497)	277 (136, 526)
FTL, ng/mL	12.2 (7.7, 22.1)	12.4 (7.7, 22.4)	11 (7.2, 19.6)
TIM-1, ng/mL	152.5 (77.5, 227.5)	154 (78, 232)	147 (74, 213)
F_2-IsoPs*, ng/mg Creatinine	57 (32, 84)	56 (31, 84)	60 (43, 78)

PWH, people with HIV; *FTH1,* heavy-chain ferritin; *FTL,* light-chain ferritin; *F_2-IsoPs,* F_2-Isoprostanes (marker of *in vivo* oxidative stress); *TIM-1,* T-cell immunoglobulin and mucin domain-1; *IQR,* interquartile range. **Notes: 1) There were no significant differences in any biomarker by sex; 2) F_2-IsoPs were undetectable in 14 PWH.*

ADJUSTED BIOMARKER ASSOCIATIONS WITH FRAILTY MEASURES







Covariates (above): 1) Combined Pre-Frailty/Frailty: age, Hispanic ethnicity, IL-6, TNFR1, comorbidity, anemia, NCI at entry; 2) Pre-Frailty: *same* but dropped IL-6; 3) Walk Time: age, race, Hispanic ethnicity, sTNFR2, NPZ-4, IL-6, hemoglobin, comorbidity, (sex); 4) Grip Strength: age, ethnicity, sTNFR2, (sex). ***p* < 0.10; **p* < 0.05; ***p* < 0.01;****p* < 0.001**

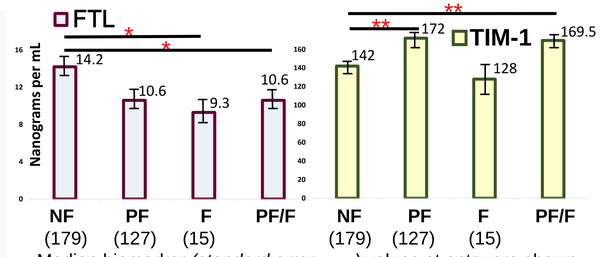
RESULTS... *(Figures above left, and at right)*

- *Higher* **TIM-1** (**p_{trend}=0.06**), *lower* **FTL** (**p_{trend}=0.02**) across non-frail/pre-frail/frail groups of PWH
- *Higher* **TIM-1** associated with pre-frailty (**OR 1.5, *p*<0.01**) & combined pre-frailty/frailty (**OR 2.3, *p*=0.01**) *vs.* non-frailty.
- *Higher* **FTL** associated with *faster* 4-meter walk (***p*<0.01**), *less* weak grip (**OR 0.49, *p*<0.01**), *less* frailty (especially in females)
- Females in top quartile of **FTH1** had *worse* average walk time.

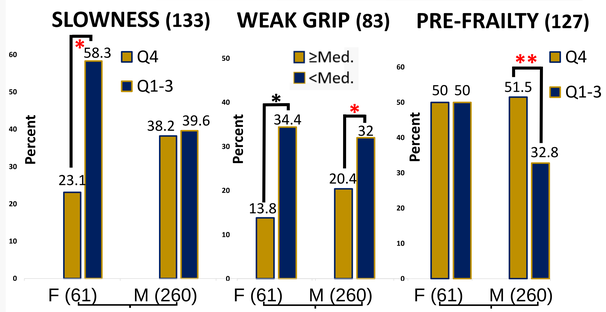
STUDY POPULATION*

Variable at Study Entry	Non-Frail (n=179)	Pre-Frail (n=127)	Frail (n=15)
Age, mean (SD)	50.7 (6.8)	53.4 (7.9)	53.8 (5.4)
Sex, % female	15.6	22.8	26.7
Non-Hispanic Black**, %	29.6	26.0	40.0
Hispanic**, %	19.0	33.1	20.0 #
BMI >30, %	22.3	27.6	13.3
Nadir CD4+ T-cells/μL, median (IQR)	205 (55, 306)	216 (78, 338)	220 (87, 377)
HIV RNA <200 copies/mL, %	95.0	97.6	93.3
HCV seropositive, %	2.2	5.5	13.3
Anemia, %	35.3	50.0	14.7 Ψ
≥ 2 Comorbidities***, %	29.0	41.7	73.3 #
Cognitively Impaired, %	18.1	33.6	46.7 #

SD, standard deviation; *BMI,* body mass index; *HCV,* hepatitis C virus.
**3 participants lacked complete frailty assessment data*
**Self-report; #*p* < 0.05; Ψ*p* < 0.01; ***Significant non-HIV comorbidities, *e.g.,* cancer, diabetes, cardiovascular or chronic kidney disease, HCV, fractures.



Median biomarker (*standard error$_{median}$*) values at entry are shown. *N*=Non-Frail; *PF*=Pre-Frail; *F*=Frail (Ns); ***p* < 0.05, ****p* < 0.01**



F=female/*M*=male (N); *Med.*= median; *Q4* = 4th quartile; *Q1-3* = 2nd, 3rd quartiles; **p*=0.079 but $p_{\text{continuous grip strength}} < 0.05$); ***p* < 0.05**; ****p* < 0.01**

MECHANISTIC INSIGHTS

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)

UNIVARIATE ASSOCIATIONS WITH 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)

STRENGTHS & LIMITATIONS

- First study of serum **FTL, FTH1,** and **TIM-1** (an **FTH1** receptor) relationships with pre-frail/frail status and component measures in PWH
- Identification of two new serum biomarkers of pre-frailty and combined pre-frailty/frailty: **TIM-1** and **FTL**
- Key sex-specific associations observed for **FTL** & possibly **TIM-1** with pre-frailty, combined pre-frailty/frailty overall, and with its components.
- *Time-updated biomarker levels, information on menopause, and gender identity as covariates were not available for these analyses.*
- *Analyses (especially in females) were underpowered.*

SUMMARY, IMPLICATIONS, & FUTURE DIRECTIONS

- In this study of mostly virally suppressed middle-aged & older PWH:
  1) Higher serum **FTL** was associated with *reduced* pre-frailty/frailty & frailty, particularly in females, and *predicted less* pre-frailty/frailty.
  2) Higher **FTL** was associated with *faster* walk time (& *less* slowness), especially in females, and *stronger* grip strength (*less* weak grip).
  3) The **highest quartile of TIM-1** was associated with *more* pre-frailty and combined pre-frailty/frailty, particularly in males.
- These effects are **independent** of inflammation, anemia, baseline cognitive status and comorbidity, but mechanisms may involve reduced anemia and oxidative stress in females with higher FTL levels.
- **FTH1** had no detectable effects on pre-frailty/frailty, but gait speed and grip strength may be reduced in females in the highest quartile.
- *Findings implicate iron-related mechanisms in frailty and may help explain sex-specific vulnerabilities in PWH. Larger studies are needed.*

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