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FECAL CALPROTECTIN IS ELEVATED IN HIV AND RELATED TO SYSTEMIC INFLAMMATION

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ABSTRACT

Background: FCP, a biomarker of gastrointestinal inflammation, is used in the diagnosis and management of inflammatory bowel disease. HIV infection severely damages gut-associated lymphoid and epithelial tissues leading to gut inflammation, microbial translocation and systemic inflammation/immune activation. We sought to investigate FCP in HIV for the first time and determine its relationship to HIV-specific factors and systemic inflammation/immune activation.

Methods: HIV+ naive to ART, ART-treated and uninfected controls were prospectively enrolled. Stool samples were collected and FCP was measured by ELISA. Plasma biomarkers of inflammation/immune activation were also measured. FCP was evaluated as a continuous variable and by thresholds. Spearman correlations were used to investigate associations with FCP.

Results: 101 HIV+ (83 ART-treated, 18 naive) and 89 uninfected controls were enrolled. ART-treated were older than naive (51 vs 31 yrs; P=0.006), but sex and race were similar (overall 78% males, 66% blacks). All but one ART-treated had HIV RNA <200 copies/mL. CD4 counts for treated and naive were 683 and 410 cells/uL, resp. Controls had a median age of 37 yrs (78% males, 22% blacks). There was a difference (P=0.001) in FCP among the 3 groups with the highest median (25th, 75th%ile) FCP in ART-naive [144 (33, 262) ug/g] followed by ART-treated [78 (36, 141) ug/g] and then controls [41 (21, 89) ug/g] (Fig). 56% of ART-naive had FCP >100 ug/g vs. 37% in treated and 19% in controls (P=0.0003). In HIV, high-sensitivity C-reactive protein (R=0.30; P=0.008), soluble tumor necrosis factor-II (R=0.28; P=0.006), and soluble vascular cellular adhesion molecule (R=0.21; P=0.04) were positively associated with FCP. Interleukin-6 (R=0.29; P=0.01) and soluble CD163 (R=0.54; P=0.04) were also positively associated with FCP in treated and naive, resp. FCP was inversely associated with CD4 in HIV+ (R=-0.24; P=0.02), but not with other HIV variables, nor age, sex, or race.

Conclusions: Stool concentrations of FCP are elevated in HIV. ART appears to reduce FCP but not to concentrations seen in uninfected controls. FCP concentrations are positively correlated with several markers of systemic inflammation/immune activation, and negatively with CD4. FCP may serve as a useful biomarker to monitor gastrointestinal inflammation and associated systemic inflammation/immune activation in HIV.

BACKGROUND

- HIV infection severely damages gut-associated lymphoid and epithelial tissues and preferentially depletes CD4+ T-lymphocytes in the gastrointestinal (GI) tract, the body's largest immunological site and predominant location of HIV replication.
- These gut changes begin in the very early phases of HIV infection and cause impairment of GI tract integrity, leading to gut inflammation, intestinal permeability, microbial translocation and systemic inflammation/immune activation.
- While antiretroviral therapy (ART) improves peripheral lymphocyte reconstitution, chronic GI inflammation and gut integrity disruption are important contributors to persistent systemic inflammation in the HIV population.
- Calprotectin is a calcium- and zinc-binding protein that is abundant in neutrophil cytoplasm. It has direct antimicrobial effects, functions as part of the innate immune system, and is stable in feces.
- Fecal calprotectin (FCP) is a biomarker of GI inflammation and is routinely used in the diagnosis and management of inflammatory bowel disease (IBD).
- Little is known about FCP in HIV or its utility as a potential biomarker of gut inflammation in people with HIV (PWH).

OBJECTIVES

- To assess FCP concentrations in both ART-naïve and ART-treated PWH
- To compare FCP concentrations in PWH (both ART-naïve and ART-treated) vs. people without HIV
- To investigate the relationship between FCP and markers of systemic inflammation and immune activation

METHODS

STUDY DESIGN / STUDY POPULATION

- Cross-sectional analysis comprised of participants enrolled at the Metabolic Research Center at the University Hospitals Cleveland Medical Center in Cleveland, OH, USA who were able to provide stool for FCP measurement.
- Participants were stratified by HIV status: HIV-infected/ART-treated (≥12 months cumulative duration of ART), HIV-infected/ART-naïve (no prior receipt of ART), and HIV-uninfected controls.
- All participants were ≥18 years of age and not currently pregnant or lactating.

STUDY ASSESSMENTS

- **Clinical and Laboratory Evaluation:** questionnaires, extensive chart review, weight/height (for calculation of body mass index (BMI)), HIV-1 RNA, CD4 count
- **Fecal Calprotectin:** quantitative sandwich ELISAs were used to measure stool concentrations in duplicate with an assay range of 32.5-2,100 µg/g. Intra- and inter-assay coefficients of variance were ≤5.6% and ≤11.6%, respectively
- **Soluble Inflammation/Immune Activation:** measured in plasma
  - **Interleukin-6 (IL-6)**: ELISA
  - **High-sensitivity C-reactive protein (hsCRP):** nephelometry
  - **Soluble tumor necrosis factor receptor-I (sTNFR-I):** ELISA
  - **Soluble tumor necrosis factor receptor-II (sTNFR-II):** ELISA
  - **Soluble vascular cell adhesion molecule-1 (sVCAM-1):** ELISA
  - **D-dimer:** immunoturbidometric methods
  - **Soluble CD163 (sCD163):** ELISA
  - **Soluble CD14 (sCD14):** ELISA
- **Gut Integrity Markers:** measured in plasma by ELISA
  - **Intestinal fatty acid binding proteins (I-FABP):** marker of enterocyte damage
  - **Lipopolysaccharide-binding protein (LBP):** marker of microbial (bacterial) translocation

STATISTICAL ANALYSIS

- **Group comparisons:** appropriate two-sample and three-sample tests were used.
- **Associations with FCP:** Spearman correlation coefficients for continuous variables and 2-sample tests for dichotomous variables.
- **Multivariable regressions:** variables were selected for inclusion based on clinical significance and results of bivariate analyses.
- P<0.05 considered significant for all analyses.

RESULTS

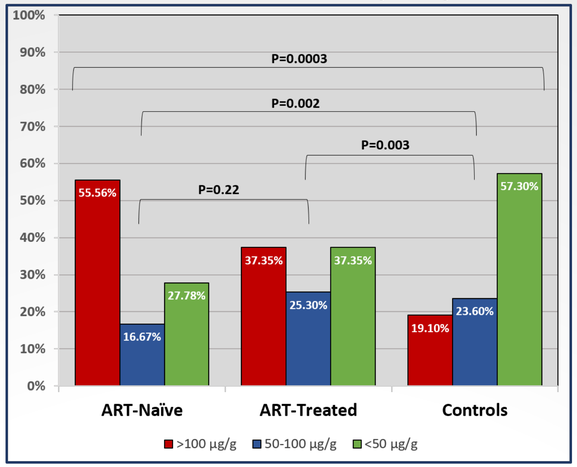
Characteristics of Participants

Median (quartile 1, quartile 3) or no. (%)	ART-Naïve (N=18)	ART-Treated (N=83)	Controls (N=89)	P Value: ART-Treated vs. ART-Naïve	P Value: ART-Naïve vs. Controls	P Value: ART-Treated vs. Controls
Sociodemographics and Clinical Parameters						
Age, years	31.4 (24.0, 44.1)	50.9 (44.4, 57.5)	36.7 (27.4, 51.0)	**0.0006**	0.20	**<0.0001**
Male sex	16 (89%)	63 (76%)	69 (78%)	0.35	0.35	0.80
Black race	14 (78%)	53 (64%)	20 (22%)	0.26	**<0.0001**	**<0.0001**
MSM status	8 (44%)	37 (45%)	2 (2%)	0.99	**<0.0001**	**<0.0001**
BMI, kg/m²	22.9 (20.4, 34.6)	26.0 (23.2, 30.5)	25.2 (23.1, 28.7)	0.26	0.46	0.41
Current smoking	7 (39%)	43 (52%)	20 (22%)	0.32	0.15	**<0.0001**
Current alcohol use	12 (67%)	62 (76%)	72 (81%)	0.55	0.21	0.46
Current marijuana use	10 (56%)	32 (39%)	87 (98%)	0.20	**0.01**	0.07
HIV Variables						
Current CD4, cells/mm³	410 (237, 704)	683 (480, 861)	-	**0.004**	-	-
Nadir CD4, cells/mm³	410 (237, 664)	190 (90, 291)	-	**0.0003**	-	-
HIV duration, years	0.31 (0.12, 0.40)	15.3 (9.4, 23.6)	-	**<0.0001**	-	-
HIV RNA <200 copies/mL	0 (0%)	82 (99%)	-	-	-	-
HIV RNA, copies/mL	72,506 (5,937, 115,100)	49,077 (N=1)	-	-	-	-
ARV duration, years	-	10.9 (7.2, 15.9)	-	-	-	-
PI duration, years	-	4.3 (0.0, 10.4)	-	-	-	-
INSTI duration, years	-	1.3 (0.0, 3.6)	-	-	-	-
Current PI use	-	20 (24%)	-	-	-	-
Current INSTI use	-	54 (66%)	-	-	-	-
Inflammatory Markers						
IL-6, pg/mL	2.4 (1.4, 3.9)	2.6 (1.8, 4.2)	1.2 (0.9, 2.1)	0.68	**0.004**	**<0.0001**
hsCRP, µg/mL	2.7 (1.1, 8.7)	2.1 (1.0, 9.8)	0.7 (0.4, 2.2)	0.43	**0.003**	**0.0003**
sTNFR-I, ng/mL	1.1 (0.9, 1.2)	1.0 (0.9, 1.2)	1.0 (0.7, 1.2)	0.51	0.06	**0.04**
sTNFR-II, ng/mL	4.1 (3.2, 5.0)	2.9 (2.3, 3.9)	2.2 (1.9, 2.7)	**0.009**	**<0.0001**	**<0.0001**
sCD163, ng/mL	1,145 (692, 1,762)	709 (492, 1,019)	483 (358, 645)	**0.004**	**<0.0001**	**<0.0001**
sCD14, ng/mL	1,696 (1,403, 2,056)	1,708 (1,476, 2,122)	1,415 (1,218, 1,586)	0.99	**0.001**	**<0.0001**
sVCAM-1, ng/mL	1,509 (1,012, 1,689)	843 (691, 1,006)	718 (580, 860)	**<0.0001**	**<0.0001**	**0.0001**
D-dimer, µg/mL	0.4 (0.3, 0.7)	0.4 (0.3, 0.7)	0.3 (0.2, 0.6)	0.94	0.11	**0.0006**
Gut Integrity Markers						
IFAB, pg/mL	2,795 (1,780, 3,861)	2,621 (1,635, 4,138)	939 (557, 1,706)	0.99	**<0.0001**	**<0.0001**
LBP, µg/mL	16.7 (14.1, 19.7)	14.5 (11.4, 19.7)	13.4 (11.4, 15.7)	0.33	**0.02**	0.07

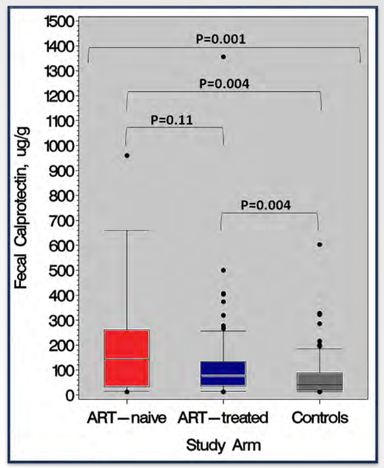
N.B. P-values with bolded font designate those <0.05; ART, antiretroviral therapy; MSM, men who have sex with men; BMI, body mass index; ARV, antiretroviral; PI, protease inhibitor; INSTI, integrase strand transfer inhibitor; IL-6, interleukin 6; hsCRP, high-sensitivity C-reactive protein; sTNFR-I, soluble tumor necrosis factor receptor-I; sTNFR-II, soluble tumor necrosis factor receptor-II; sCD163, soluble CD163, sCD14, soluble CD14; sVCAM-1, soluble vascular cell adhesion molecule-1; IFAB, intestinal fatty acid binding protein; LBP, lipopolysaccharide binding protein

RESULTS

Breakdown of FCP Values by Study Arm



FCP Concentrations by Study Arm

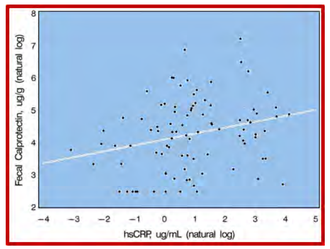


Median (Q1, Q3)	144 (33, 262)	78 (36, 141)	41 (21, 89)

Correlations Between Fecal Calprotectin and Variables of Interest

Variable	ART-Naïve	ART-Treated	All PWH	Controls
IL-6	-	R=0.29 P=0.01	R=0.20 P=0.06	R=0.26 P=0.02
hsCRP	-	R=0.34 P=0.003	R=0.28 P=0.008	-
sTNFR-II	R=0.56 P=0.03	-	R=0.28 P=0.006	-
sVCAM-1	R=0.66 P=0.006	-	R=0.21 P=0.04	-
sCD163	R=0.54 P=0.04	-	-	-
LBP	-	-	-	R=0.19 P=0.08
CD4 count	-	-	R=-0.24 P=0.02	-
Nadir CD4	R=-0.41 P=0.10	-	-	-

N.B. Variables presented in table include those with a P value ≤0.10 in any group; R = Spearman Correlation Coefficient; Variables tested but were not significant in any groups at P≤0.10 included age, BMI, sTNFR-I, sCD14, D-dimer, IFAB, HIV duration, ARV duration, PI duration, INSTI duration.



Multivariable Regression Models for Variables Associated with Fecal Calprotectin

Selected Sociodemographic and Clinical Variables

Variable	ß	ß (SE)	P
HIV-infected status*	75.08	34.80	**0.032**
Age	0.69	1.15	0.548
Black race	41.84	34.97	0.233
Male sex	64.51	36.62	0.080
Body mass index	-3.34	2.66	0.210
Current smoking	-60.11	33.84	0.077
Current alcohol use	-91.17	36.31	**0.013**
	R^2 = 0.11		

Selected Variables from Above Model + hsCRP

Variable	ß	ß (SE)	P
HIV-infected status*	53.13	27.74	0.057
Age	0.31	0.90	0.730
Black race	16.44	27.47	0.550
Male sex	58.20	29.59	0.051
Body mass index	-3.11	2.35	0.187
Current smoking	-42.45	26.73	0.114
Current alcohol use	-63.82	29.39	**0.031**
hsCRP	<0.01	<0.01	0.292
	R^2 = 0.10		

*ART-treated PWH vs. uninfected controls
N.B. P-values with bolded font designate those <0.05

SUMMARY OF RESULTS

- PWH, both ART-naïve and ART-treated, had higher concentrations of FCP compared to uninfected controls. This finding was consistent when FCP was analyzed as a continuous variable or dichotomized based on established cut-offs used in IBD (<50, 50-100, ≥100 µg/g).
- 56% of ART-naïve PWH had very high FCP concentrations (≥100 µg/g) compared to 57% of uninfected control with low FCP concentrations (<50 µg/g). In the ART-treated group, 37% had very high FCP concentrations which was not significantly different than the ART-naïve group (but was still significantly higher than the controls).
- There were a number of systemic inflammation and immune activation markers that were significantly positively associated with FCP concentrations in PWH. Similarly, current and nadir CD4 cell counts were inversely associated with FCP concentrations.
- HIV-infected status was independently associated with higher FCP concentrations even after adjusting for a number of sociodemographic and clinical confounders. When hsCRP was included in the model, however, this association was attenuated.

CONCLUSIONS

- This is the first study to evaluate FCP concentrations in ART-treated PWH, and we showed that PWH, even with virologic suppression, have high concentrations of FCP relative to people without HIV. These results demonstrate the degree of on-going GI inflammation in HIV despite successful treatment.
- We also investigated associations between FCP concentrations and systemic markers of inflammation and immune activation for the first time and showed significant correlations. This finding demonstrates the relationship between GI and systemic inflammation.
- Our results suggest a role for FCP as a non-invasive surrogate measure of GI inflammation and associated systemic inflammation in HIV. Further studies are warranted.

ACKNOWLEDGEMENTS

This work was made possible by the National Institutes of Health grants (R01DK121619 to GAM and ARE), Case Western Reserve University's Center for AIDS Research (P30 AI36219), and University Hospitals Cleveland Medical Center (UHCMC) and the Clinical and Translational Science Collaborative of Cleveland (UL1TR000439) from the National Center for Advancing Translational Sciences (NCATS) component of the National Institutes of Health and NIH roadmap for Medical Research. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of UHCMC or the NIH.

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