Circulating LOXL2 Levels Reflect Severity of Intestinal Fibrosis and CD4^{+} T Lymphocyte Depletion in Treated HIV Infection

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Disclosures

- No relevant conflicts of interest

Background

- Gut-associated lymphoid tissue (GALT) fibrosis occurs early in HIV infection and persists despite antiretroviral therapy (ART).
- Incomplete immune reconstitution may also persist despite successful ART, and is associated with multiple diseases of aging.
- GALT fibrosis may contribute to incomplete immune reconstitution (via local $CD4^+$ T lymphocyte depletion), disruption of the intestinal barrier and subsequent microbial translocation.
- Currently, the gold standard for quantification of GALT fibrosis is biopsy, which is not reasonable in clinical practice.
- We investigated relationships between circulating fibrosis biomarkers and GALT fibrosis in treated HIV infection.

Design & Methods

Objective: To determine associations between circulating fibrosis biomarker levels and severity of GALT fibrosis in SCOPE participants

Inclusion criteria

- Treated HIV infection, HIV-1 RNA <50 copies/mL with GALT biopsies

Measurement

- Fibrosis quantification in tissue: % Collagen deposition on biopsy by Masson trichrome staining
- Circulating plasma biomarkers of fibrosis measured by ELISA /multiplex assay

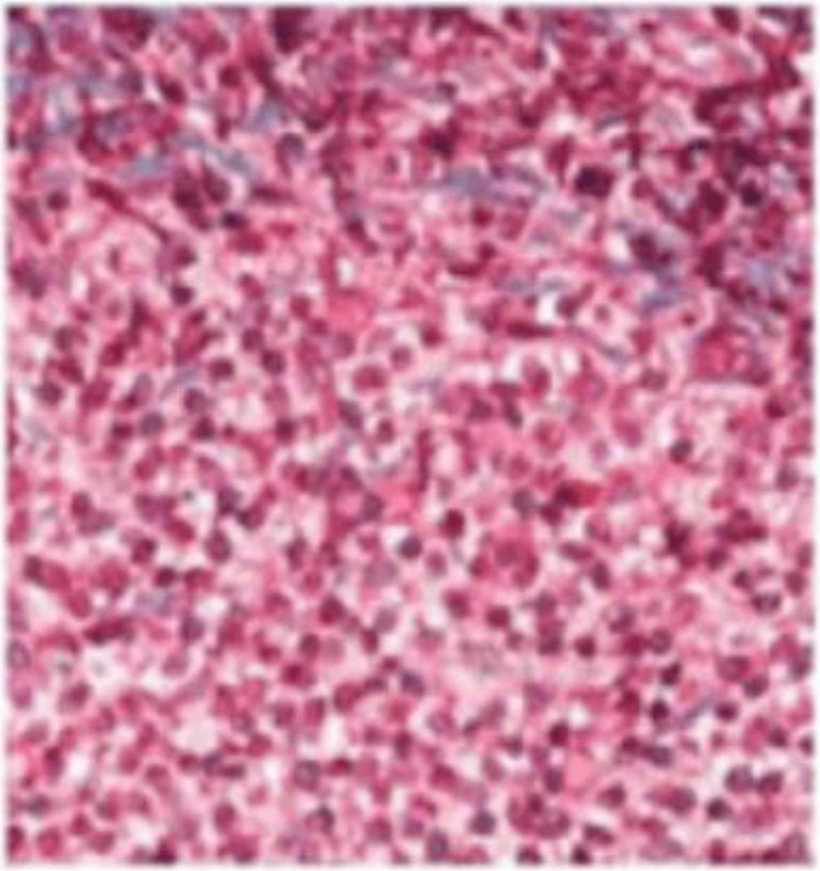
Statistical analysis:

- Cross-sectional analysis, pilot study
- Regression analysis between biomarker levels & collagen deposition lymphoid aggregate (LA) collagen deposition & LA $CD4^+$ T lymphocyte density
- Significance was assessed using a 2-sided $\alpha=0.05$

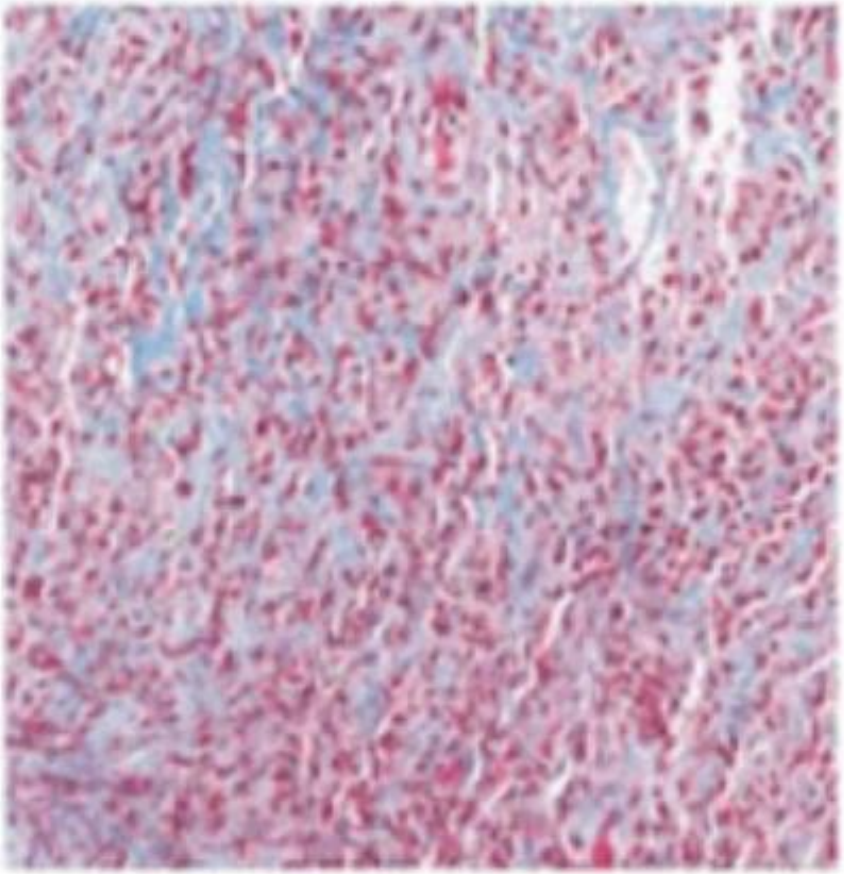
*Observational Study of the Consequences of the Protease Inhibitor Era

Background: SCOPE Cohort Data

Trichrome stain (blue) for collagen in GALT Lymphoid Aggregate



HIV-
(mean 4% fibrosis)

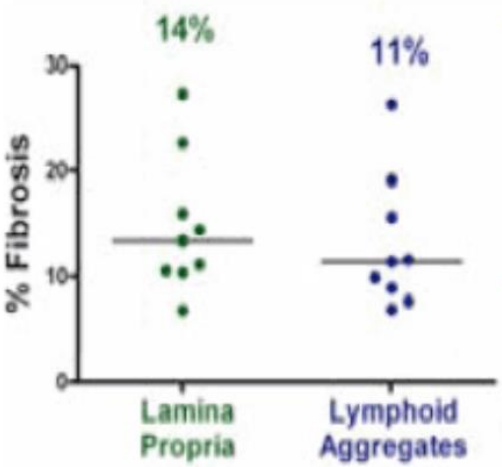


Untreated HIV+
(mean 16% fibrosis)

Hunt PW, et al. CROI 2011.

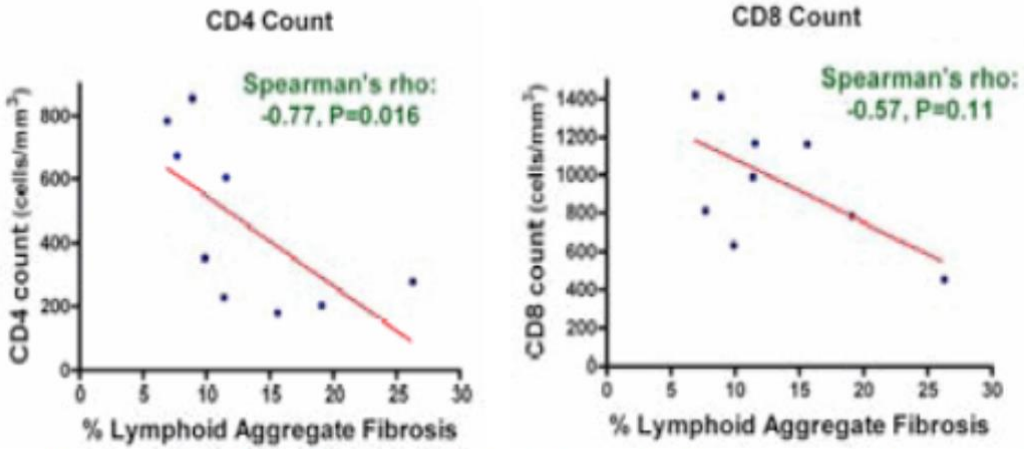
Background: SCOPE Cohort Data

% Fibrosis in Rectal Tissue among ART-suppressed Participants



- % Fibrosis in lymphoid aggregates comparable to mean level previously observed in Peyer's patches of untreated HIV+ individuals (16%) and much higher than that observed in HIV-negatives (4%, Estes, JID, 2008).
- While there were comparable levels of fibrosis in lamina propria, there was no evidence for a relationship between lamina propria and lymphoid aggregate fibrosis (rho: 0.12, P=0.77).

Greater Lymphoid Aggregate Fibrosis Associated with Lower Peripheral Blood T cell Counts



However, no evidence for an association between the % lamina propria fibrosis and CD4 counts (rho: -0.40, P=0.29) or CD8 counts (rho: -0.10, P=0.80).

Hunt PW, et al. CROI 2011.

Demographic and Clinical Characteristics

Percent or median (interquartile range)	
Number of Participants	39
Age (years)	48 (45, 55)
White race	59%
Male Sex	92%
Body Mass Index (kg/m2)	26 (24, 28)
Chronic viral hepatitis co-infection	33%
Time from HIV diagnosis (years)	17 (15, 21)
Current $CD4^+$ T lymphocyte count (cells/mm^3)	277 (177, 483)
Nadir $CD4^+$ T lymphocyte count (cells/mm^3)	66 (18, 108)
PI-Based ART	61%

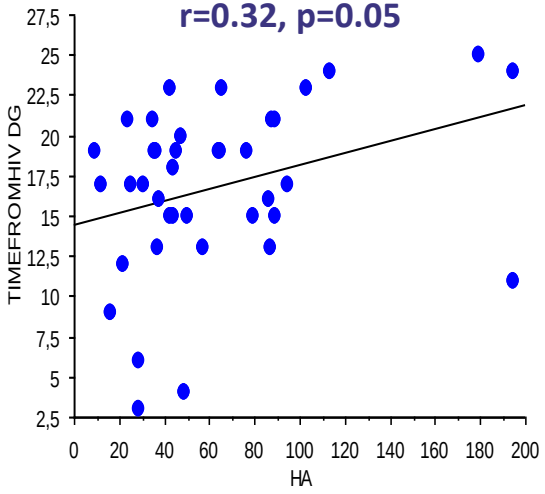
Median Biomarker Values

	Overall N=39	CD4 <350/mm^3 N= 27	CD4 ≥350/mm^3 N=12	P value
Transforming Growth Factor (TGF)-ß$_1$ (pg/mL)	12,699 (6,421, 17,930)	15,336 (5,661, 20,719)	12,316 (9,787, 14,038)	0.83
TGF-ß$_2$ (pg/mL)	996 (839, 1,124)	993 (709, 1,085)	1,099 (937, 1,146)	0.11
TGF-ß$_3$ (pg/mL)	300 (150, 404)	255 (99, 365)	351 (215, 410)	0.17
Matrix Metalloproteinase-2 (MMP-2, pg/mL)	151,129 (124,433, 203,864)	179,776 (134,654, 258,128)	121,898 (105,718, 143,495)	0.004
MMP-9 (pg/mL)	4.2 (3.5, 6.8)	4.7 (3.5, 6.8)	3.2 (2.3, 4.7)	0.04
Tissue Inhibitor of MMP-1 (TIMP-1, pg/mL)	7,823 (5,034, 12,473)	6878 (4814, 11,054)	7,984 (7,081, 16,910)	0.20
MMP-2:TIMP-1 ratio	0.2 (0.1, 0.4)	0.2 (0.1, 0.4)	0.3 (0.2, 0.4)	0.25
MMP-9:TIMP-1 ratio	42,589 (37,567, 47,792)	42,340 (37,567, 47,912)	42,780 (38,850, 45,310)	0.95
Chitinase-3-Like Protein 1 (CHI-3L1, pg/mL)	42,900 (26,144, 66,183)	39,939 (24,215, 64,226)	50,330 (28,614, 75,949)	0.42
Hyaluronic Acid (HA, ng/mL)	47 (35, 87)	49 (28, 89)	46 (37, 83)	0.86
Lysyl Oxidase-Like Protein 2 (LOXL2, ng/mL)	0.2 (0.2, 11.6)	0.2 (0.2, 11.0)	2.1 (0.2, 13.7)	0.16
type I C-terminal collagen pro-peptide (CICP, ng/ml)	106 (85, 149)	111 (90, 160)	98 (62, 146)	0.39
Circulating Immune Complexes of Complement 1q (CIC C1Q, µg Eq/mL)	147 (96, 200)	129 (96, 193)	177 (91, 233)	0.63
plasminogen activator inhibitor-1 (PAI-1, pg/mL)	32,239 (17,623, 45,084)	32,239 (13,741, 47,196)	30,706 (25,244, 40,171)	0.93
CXC chemokine ligand 4 (CXCL4, pg/mL)	1,561,900	1,232,500	2,347,450	0.12

Relationships Between Fibrosis Biomarkers and Duration of HIV Infection

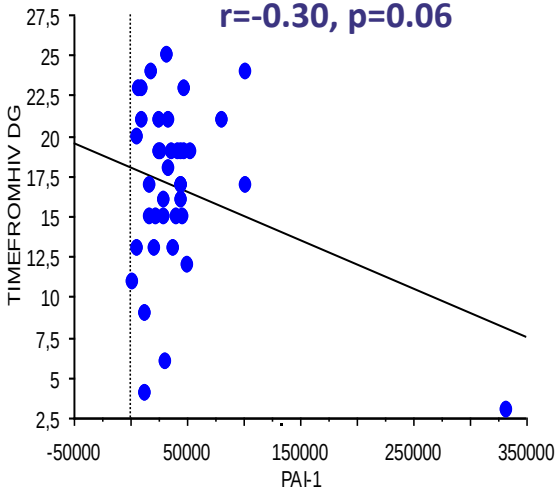
Hyaluronic Acid

r=0.32, p=0.05



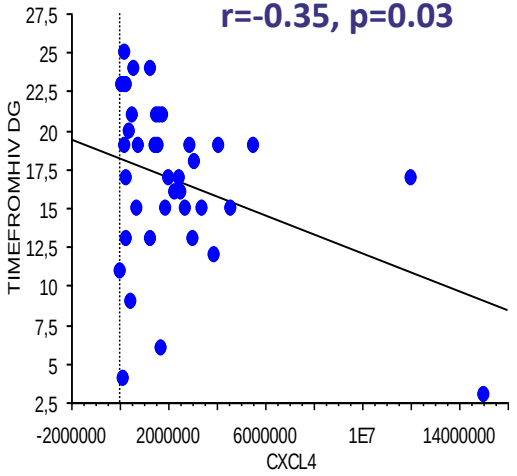
PAI-1

r=-0.30, p=0.06



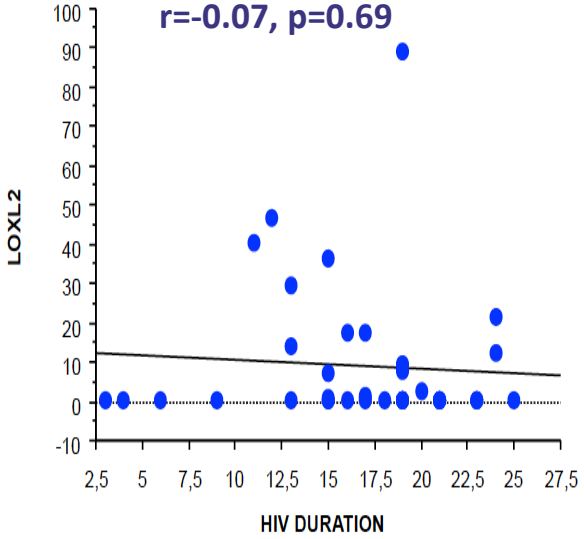
CXCL4

r=-0.35, p=0.03



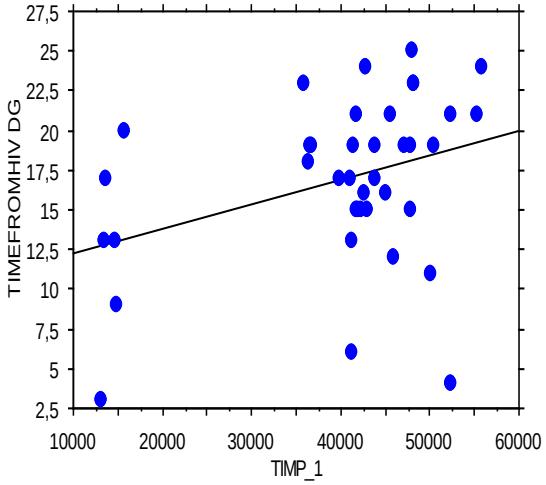
LOXL2

r=-0.07, p=0.69



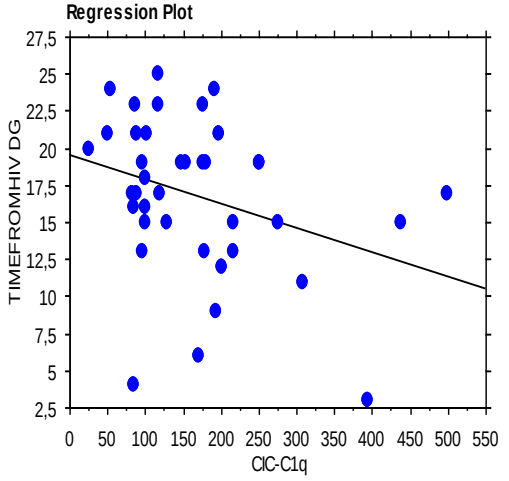
TIMP-1

r=-0.35, =0.03



CIC C1q

r=-0.32, p=0.04



Relationships Between Fibrosis Biomarkers and Duration of HIV Infection

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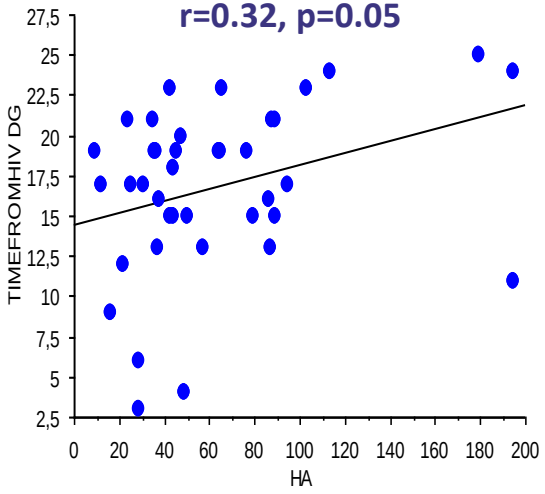

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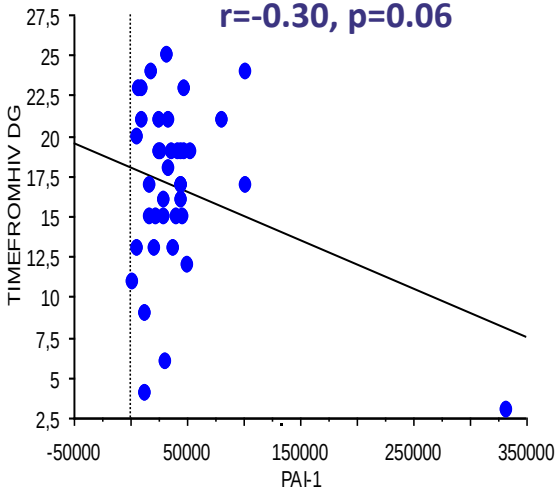
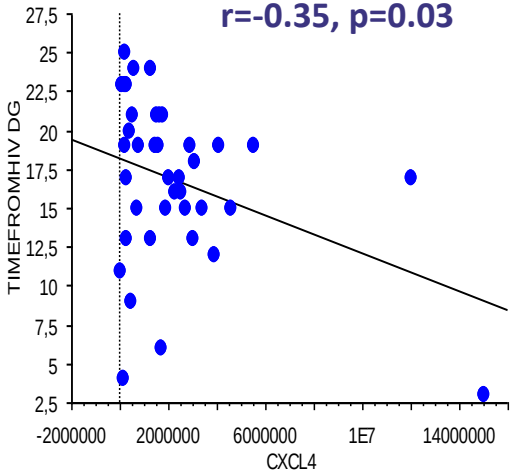


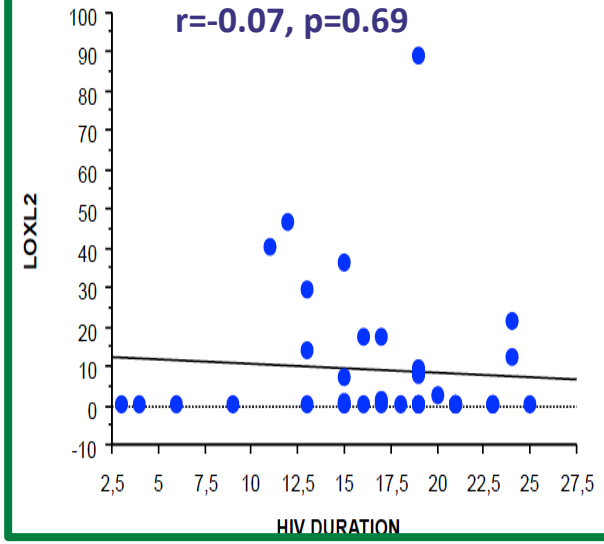
CXCL4

r=-0.35, p=0.03



LOXL2

r=-0.07, p=0.69



TIMP-1

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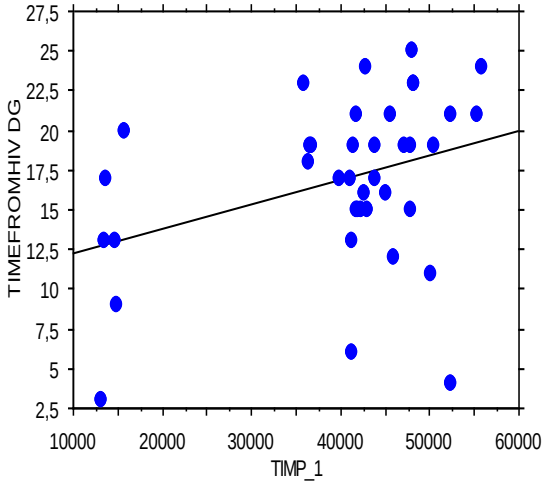
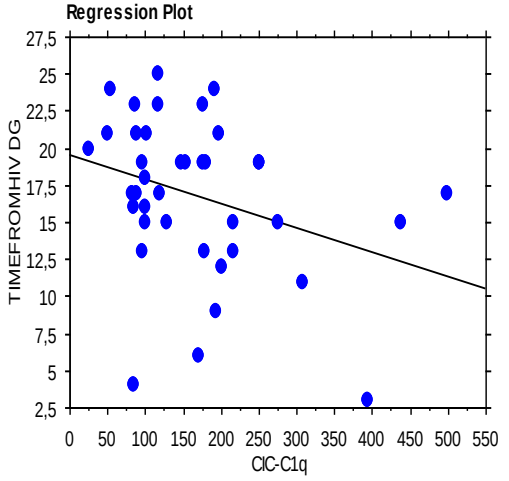
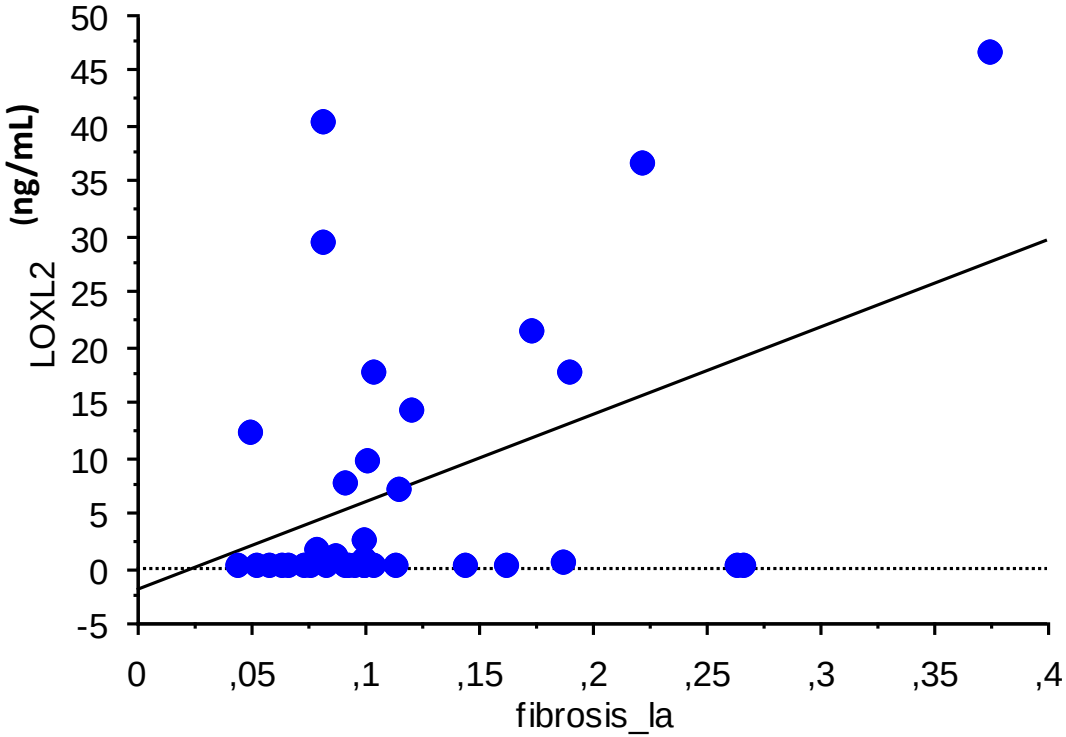


CIC C1q

r=-0.32, p=0.04

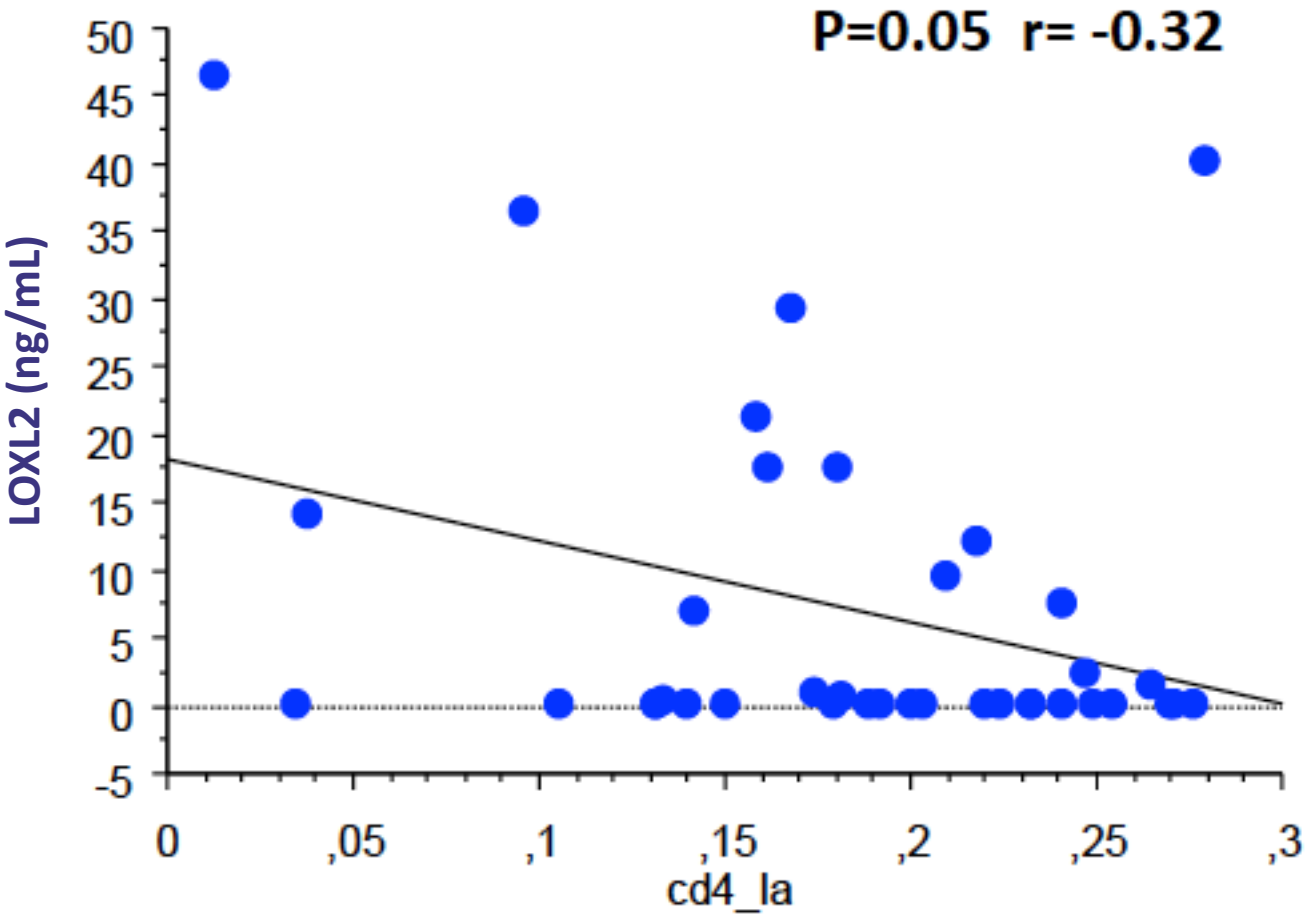


LOXL2 Correlates with GALT Lymphoid Aggregate Collagen Deposition



r= 0.44, p=0.007

LOXL2 Inversely Correlates with CD4 T Cell Count in Lymphoid Aggregate



Conclusions

In this pilot, cross-sectional study

- HA, CXCL4, PAI-1, TIMP-1 and CIC C1q levels correlated with duration of HIV infection, likely reflecting disease severity in this cohort
- Only LOXL2 levels corrected with histologic GALT disease burden, as measured by quantity of collagen deposition and severity of CD4+ T lymphocyte depletion
- Future, larger studies are needed to certify the utility of circulating LOXL2 levels as a non-invasive marker of fibrotic tissue burden in treated HIV infection
- As LOXL2 is only upregulated in pathologic states, future studies should investigate the utility of LOXL2 inhibition for the treatment of HIV-associated fibrotic disease

Thank You!

Thanks to the SCOPE Study participants, staff and investigators.