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)

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).

### Demographic and Clinical Characteristics

<table>
<thead>
<tr>
<th>Percent or median (interquartile range)</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Number of Participants</td>
<td>39</td>
</tr>
<tr>
<td>Age (years)</td>
<td>48 (45, 55)</td>
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<tr>
<td>White race</td>
<td>59%</td>
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<tr>
<td>Male Sex</td>
<td>92%</td>
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<tr>
<td>Body Mass Index (kg/m²)</td>
<td>26 (24, 28)</td>
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<tr>
<td>Chronic viral hepatitis co-infection</td>
<td>33%</td>
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<tr>
<td>Time from HIV diagnosis (years)</td>
<td>17 (15, 21)</td>
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<tr>
<td>Current CD4⁺ T lymphocyte count (cells/mm³)</td>
<td>277 (177, 483)</td>
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<tr>
<td>Nadir CD4⁺ T lymphocyte count (cells/mm³)</td>
<td>66 (18, 108)</td>
</tr>
<tr>
<td>PI-Based ART</td>
<td>61%</td>
</tr>
<tr>
<td>Biomarker</td>
<td>Overall N=39</td>
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<td>-----------------------------------------------</td>
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<tr>
<td>Transforming Growth Factor (TGF)-β₁ (pg/mL)</td>
<td>12,699 (6,421, 17,930)</td>
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<td>TGF-β₂ (pg/mL)</td>
<td>996 (839, 1,124)</td>
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<tr>
<td>TGF-β₃ (pg/mL)</td>
<td>300 (150, 404)</td>
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<tr>
<td>Matrix Metalloproteinase-2 (MMP-2, pg/mL)</td>
<td>151,129 (124,433, 203,864)</td>
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<tr>
<td>MMP-9 (pg/mL)</td>
<td>4.2 (3.5, 6.8)</td>
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<tr>
<td>Tissue Inhibitor of MMP-1 (TIMP-1, pg/mL)</td>
<td>7,823 (5,034, 12,473)</td>
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<tr>
<td>MMP-2:TIMP-1 ratio</td>
<td>0.2 (0.1, 0.4)</td>
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<tr>
<td>MMP-9:TIMP-1 ratio</td>
<td>42,589 (37,567, 47,792)</td>
</tr>
<tr>
<td>Chitinase-3-Like Protein 1 (CHI-3L1, pg/mL)</td>
<td>42,900 (26,144, 66,183)</td>
</tr>
<tr>
<td>Hyaluronic Acid (HA, ng/mL)</td>
<td>47 (35, 87)</td>
</tr>
<tr>
<td>Lysyl Oxidase-Like Protein 2 (LOXL2, ng/mL)</td>
<td>0.2 (0.2, 11.6)</td>
</tr>
<tr>
<td>type I C-terminal collagen pro-peptide (CICP, ng/ml)</td>
<td>106 (85, 149)</td>
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<tr>
<td>Circulating Immune Complexes of Complement 1q (CIC C1Q, µg Eq/mL)</td>
<td>147 (96, 200)</td>
</tr>
<tr>
<td>plasminogen activator inhibitor-1 (PAI-1, pg/mL)</td>
<td>32,239 (17,623, 45,084)</td>
</tr>
<tr>
<td>CXC chemokine ligand 4 (CXCL4, pg/mL)</td>
<td>1,561,900</td>
</tr>
</tbody>
</table>
Relationships Between Fibrosis Biomarkers and Duration of HIV Infection

Hyaluronic Acid

PAI-1

CXCL4

LOXL2

TIMP-1

CIC C1q

Y = 19.567 - 0.016 * X; R^2 = 0.106

\[ r = 0.32, p = 0.05 \]

\[ r = -0.30, p = 0.06 \]

\[ r = -0.35, p = 0.03 \]

\[ r = -0.07, p = 0.69 \]

\[ r = -0.35, p = 0.03 \]

\[ r = -0.32, p = 0.04 \]
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**
- $r=-0.35$, $p=0.03$

**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

\[ P=0.05 \quad r=-0.32 \]
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 correlated 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.