Persistent Inflammation Despite Effective ART in Children with HIV Infection

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Clinical Implications of Chronic Inflammation in General Population

- Cardiovascular diseases
  - Cardiomyopathy
  - Atherosclerosis
  - Stroke

- Neurodegenerative diseases
  - Alzheimer's
  - Parkinson's

- Metabolic disorders
  - Type 2 diabetes
  - Fatty liver disease
  - Sleep apnea

- Musculoskeletal disorders
  - Osteoarthritis
  - Osteoporosis
  - Sarcopenia

- Cancer
  - Gastric, liver, lung, gall bladder, colon, rectal, pancreatic, prostate, etc.

What about in HIV infection?

In children?

In children with HIV infection?

- 1,876 deaths among 39,727 patients
- Non-AIDS related deaths accounted for 50.5%

Morbidities associated with aging increased in treated HIV disease

- Cardiovascular disease [1-3]
- Cancer (non-AIDS) [4]
- Fractures / osteoporosis [5,6]
- Liver disease [7]
- Kidney disease [8]
- Cognitive decline [9]
- Frailty [10]

Predictors of CVD in General Population

- **Framingham Risk Score**\(^1\) estimates **10-year risk** of Major Adverse Cardiovascular Events
  - Heart attack, stroke, cardiovascular death
  - Risk levels: <10%, Low; 10-20%, Intermed; >20%, High
  - Validated only for *adults*

- Based on regression model which includes traditional cardiovascular risk factors
  - Age, sex, race, HDL/LDL, BP, diabetes, smoking

- Other CVD risk factors NOT included in Framingham Risk Score include: Family hx, Renal dz, BMI, *hsCRP* or any other biomarker of inflammation...

\(^1\) Wilson, PF. *Circulation*. 1998.
Inflammation Associated with CVD in General Population

*hsCRP independently associated with incident CVD but not yet recommended by USPSTF as additional contributor to global CVD risk score (USPSTF 2009)
Anti-inflammatory Nature of Interventions to Reduce CVD Risk in General Population

- ASA - ↓ MI in men (45-79 yo), ↓ ischemic stroke in women (55-79 yo) (USPSTF 2009)
- Statins
- Smoking cessation
- DM control
- Exercise: ↓ inflamm (Nicklas 2008), ↑ cogn function (Muscari 2010)
Additional Sources of Inflammation

Cardiovascular Risk Factors

Hypertension

Diabetes Mellitus

Dyslipidemia

Smoking

Obesity

Inflammatory Mediators

The Endothelium

Impaired regulation of Vasomotion

Hypertension

Disordered haemostasis

Thrombosis

Cardiovascular Diseases

Cerebrovascular Disease

Coronary Atherosclerosis

Peripheral Vascular Disease
SMART: Inflammatory Markers Strongly Associated with Mortality and CVD Events in Adults with HIV


<table>
<thead>
<tr>
<th>Biomarker</th>
<th>All-Cause Mortality (N=85)</th>
<th>Fatal or Non-fatal CVD (N=136)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR*</td>
<td>P-value</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>3.1</td>
<td>0.02</td>
</tr>
<tr>
<td>IL-6</td>
<td>12.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Amyloid A</td>
<td>3.1</td>
<td>0.05</td>
</tr>
<tr>
<td>Amyloid P</td>
<td>1.1</td>
<td>0.78</td>
</tr>
<tr>
<td>D-dimer</td>
<td>41.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>F1.2</td>
<td>1.3</td>
<td>0.64</td>
</tr>
</tbody>
</table>

Even after adjusting for CD4 count

*OR: 4th vs 1st quartile
Inflammatory markers: predictive value beyond usual CVD risk factors in HIV+ Adults (Duprez PlosOne 2012)

IL-6, hsCRP and D-dimer at baseline strong predictors of mortality, despite ART

ROC Curves 29-Month CVD Risk
- “Basic” model: age, sex, race, ART use, VL, CD4, prior AIDS dx, smoking, BMI, prior CVD, DM, HTN, lipid rx, total/HDL cholesterol, major ECG abnl, HBV/HCV, SMART treatment group.
- “extended” model: basic model covariates + hsCRP, IL-6 and D-dimer
Decreased but persistent (1) defects in T cell regenerative potential, (2) loss of immunoregulatory function, (3) CMV and other copathogen levels, and (4) microbial translocation

T cell activation declines during long-term ART, but remains elevated, even after many years of viral suppression.

Hunt, JID, 2003, 2008
Microbial Translocation Persists Despite Viral Suppression

Enterocyte loss, microbial translocation, and monocyte activation are increased in HIV infection, even with suppressive ART. Sandler, JID, 2011
Microbial Translocation Persists Despite Viral Suppression

Lipopolysaccharide and soluble CD14 decrease during ART but remain elevated despite years of viral suppression.

Rajasuriar, JID, 2010
CMV Contributes to Immune Activation

Copathogens such as CMV may help drive immune activation in patients with viral suppression.

Adults with VL<50 for at least 2 years on consistent cART regimen

Sandler-Utay

Wittkop, JID, 2013
Innate Immune Markers Predict Mortality Independent of Nadir AND Current CD4 count

Gut Epithelial Barrier Dysfunction

IDO-1 Induction

Monocyte Activation (LPS receptor, microbial translocation)

Inflammation / Coagulation

Current CD4 count no longer predictive of mortality after adjusting for innate markers

COURTESY: Peter Hunt

SOCA: Study of Ocular Complications of AIDS

Adults all VL < 400 on cART

Hunt JID 2014; Hunt, CROI 2012, Abstr #278 (see also: Tenorio, CROI 2013, Abstr# 790)
Adult HIV -> Gut (despite ART) -> Inflammation -> Mortality

• HIV infects gut lymphoid cells leading to:  CD4 T cell loss -> enteropathy -> increased permeability -> microbial translocation -> systemic immune activation

• Increased mortality risk despite ART linked to....
  – Impaired gut integrity & bacterial translocation: I-FABP, (zonulin), IDO-1, sCD14
  – Systemic inflammation and coagulation: TNFR1, CRP, D-dimer, IL6
  – Independent of CD4 and duration of VL suppression
  – Possible viral copathogen (CMV) potentiation

• HIV-related enteropathy may not be reversed by ART and remains a persistent trigger of systemic inflammation that is independently associated with mortality risk
Enteropathy, Microbial Translocation

Inflammatory Mediators

Cardiovascular Risk Factors

Hypertension
Diabetes Mellitus
Dyslipidemia
Smoking
Obesity

The Endothelium

Impaired regulation of Vasomotion
Hypertension

Disordered haemostasis
Thrombosis

Cardiovascular Diseases

Cerebrovascular Disease
Coronary Atherosclerosis
Peripheral Vascular Disease
Cardiovascular Risk Assessment in Healthy Children & Youth

• CV events rare in children but...
  – Atherosclerosis begins in childhood  Berenson Bogalusa NEJM 1998
  – CV risk factors associated with atherosclerosis in youth (PDAY)
    • Berenson Bogalusa NEJM 1998. PDAY JAMA 1990
  – CV Risk factors in childhood persist into adulthood
  – High BMI in childhood associated with increased CV risk in adulthood Baker NEJM 2007
  – Obesity, glucose intolerance, and hypertension in youth associated with premature death Franks NEJM 2010, Saydah Ped 2013

• So cardiovascular risk factors matter in childhood and cardiovascular disease has onset early in life
Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Score  McMahan AIM 2005

• PDAY score: estimates risk of currently having advanced atherosclerotic lesion in the coronary arteries (CA) or the abdominal aorta (AA) relative to an individual of the same age and sex without any CVD risk factors
• Based on autopsy data from over 1100 15-34 year-olds who died of external causes
• Lipids (TC, HDL), glucose, smoking, blood pressure, and obesity
• Like adult Framingham score, no inflammatory markers
• ≥ 1 -> ↑risk by 18% of CA, by 29% of AA
As for HIV+ adults, evidence of ↑ CVD risk in youth with HIV
• Strong effect of long-term PI use driving dyslipidemia
• Inflammatory biomarkers not included
Biomarkers of Systemic Inflammation, Coagulation, and Vascular Dysfunction Elevated in PHIV vs Controls, PHACS Cohort (US)

<table>
<thead>
<tr>
<th>Log-transformed biomarker</th>
<th>HIV-uninfected Controls (n= 55)</th>
<th>PHIV (n= 105) (&lt;50% VL&lt;400)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inflammation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>0.54 (0.34, 0.86)</td>
<td>0.92 (0.64, 1.34)</td>
<td>0.08</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.88 (0.68, 1.14)</td>
<td>1.26 (1.06, 1.51)</td>
<td>0.029</td>
</tr>
<tr>
<td>MCP-1</td>
<td>106 (92, 123)</td>
<td>154 (139, 171)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Coagulant dysfunction</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>324 (299, 350)</td>
<td>376 (356, 399)</td>
<td>0.006</td>
</tr>
<tr>
<td><strong>Endothelial dysfunction</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sICAM</td>
<td>185 (154, 222)</td>
<td>240 (210, 271)</td>
<td>0.032</td>
</tr>
<tr>
<td>sVCAM</td>
<td>685 (589, 793)</td>
<td>1141 (1026, 1263)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Means and p-values are adjusted for correlation between siblings, sex, race, age and BMI z-scores. Data were analyzed on the log scale.*

Miller TL, et al  JAIDS 2010
Microbial Translocation and Monocyte Activation Persist Despite 44 Weeks of ART in Children

- Children treated with cART for 44 weeks
- No reduction in LPS or sCD14 at week 44
- Whether VL suppressed (VR) or not (VF) (not shown for LPS)
- LPS and sCD14 remained higher than in HIV-uninfected controls through week 44

Pilakka-Kanthikeel, PIDJ 2012
### Sustained Elevation of Immune Activation Markers Despite Long-term ART-mediated Virologic Suppression

Persaud/PHACS CROI2014, Abs# 72

<table>
<thead>
<tr>
<th>Plasma Markers</th>
<th>Age HAART Initiated</th>
<th>p-value</th>
<th>PHEU (N=10)</th>
<th>p-value (PHIV+ vs. PHEU)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;1 year (N=14)</td>
<td>1-5 years (N=53)</td>
<td>&gt;5 years (N=77)</td>
<td></td>
</tr>
<tr>
<td>sCD14 (x 10⁶ pg/ml)</td>
<td><strong>1.9</strong> (1.6, 2.3)</td>
<td><strong>1.8</strong> (1.5, 2.0)</td>
<td><strong>1.8</strong> (1.5, 2.1)</td>
<td>0.41</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td><strong>19.6</strong> (17.2, 25.7)</td>
<td><strong>21.9</strong> (15.2, 26.7)</td>
<td><strong>22.9</strong> (16.3, 29.3)</td>
<td>0.56</td>
</tr>
<tr>
<td>GMCSF (pg/mL)</td>
<td><strong>2.6</strong> (2.5, 2.6)</td>
<td><strong>2.6</strong> (2.4, 2.9)</td>
<td><strong>2.5</strong> (2.4, 2.9)</td>
<td>0.93</td>
</tr>
<tr>
<td>IL-β (pg/mL)</td>
<td><strong>2.0</strong> (0.9, 3.7)</td>
<td><strong>3.3</strong> (1.1, 6.9)</td>
<td><strong>3.7</strong> (1.8, 9.2)</td>
<td>0.17</td>
</tr>
<tr>
<td>IL-8 (pg/mL)</td>
<td><strong>18.4</strong> (13.2, 29.2)</td>
<td><strong>18.6</strong> (9.6, 27.9)</td>
<td><strong>18.2</strong> (12.2, 23.4)</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Higher inflammatory marker levels (compared to HEU) even in the group started on ART at age < 1 year and suppressed for at least 10 years.
Evidence of persistent inflammation in children and adults with HIV infection despite ART

Evidence of ↑mortality and CVD for adults with HIV despite ART….

But what about clinically concerning outcomes for children?
Non-invasive Assessments of Subclinical Vascular Disease
Urbina Hypertension 2009

• PDAY score - CA or AA atherosclerotic lesions (≥ 15 yrs old)
• Carotid intimal-medial thickness (cIMT) – atherosclerosis
• Pulse wave velocity (PWV) – vascular stiffness
• Flow-mediated dilation (FMD) – vascular reactivity, endothelial function
• Coronary artery calcifications – coronary atherosclerosis
Clinical Correlations of Inflammatory Markers in Adults with Treated HIV Infection, Adjusted for CVD Risk Factors

- Monocyte activation - coronary artery calcification (CAC) progression (Baker, CROI 2013, Abstract #66LB)
- sCD14 - presence and severity of CAC (Longnecker AIDS 2014)
- *sCD14, hs-CRP, fibrinogen – combined risk of CAC, cIMT, brachial artery reactivity (FMD) (Longnecker AIDS 2014)
- sVCAM-1, activated monocytes - higher cIMT (Barbour Athero 2014)
- sCD163 – aortic inflammation by PET scan (Subramaniam JAMA 2012)

*NOTE:: Adults on ART but not all virologically suppressed
Subclinical atherosclerosis (cIMT) higher in Children with HIV

DiBiagio JUltrasoundMed 2013

- 40 participants with PHIV, mean age: 16 years
  - 37 cART, 2 3TC, 1 naïve
  - 8 (20%) with VL > 50
  - For VL<50: median duration 34 mos
- 27 healthy controls (18 yrs)
- IMT thickness higher
  - PHIV vs controls
  - Age, BMI
  - Higher insulin
  - Higher HgbA1C
  - Not by cART or specific ARV
  - Not by CD4 or VL
  - Not by CRP or D-dimer

P=NS

P=0.038
A, IMT was increased by age ($P=0.04$) (solid) in HIV+ children but not controls (dotted).

B, IMT significantly higher with ART ($*P=0.04$) and PI > nonPI ART ($**P=0.01$).

A, FMD significantly ↓ (poorer endothelial function) in HIV+ children, $*P=0.02$.

B, FMD reduced in children on ART, $**P=0.0001$; PI > nonPI ART, $*P=0.006$.

hsCRP - significant inverse association with FMD
SubClinical Atherosclerosis (IMT) in PHIV even with Virologic Suppression

Sainz JAIDS 2014

- Measured cIMT in 150 HIV+ adolescents and 150 age- and sex-matched HIV-uninfected controls (age 14.6 years; 63% F)
- HIV: 97% perinatal; 97% on ART; 76% VL<50; 17% smokers
- IMT higher in HIV+ vs HIV- overall and HIV w/VL < 50
- NO assoc of IMT with CRP or T cell activation/senescence
- MV Analysis: Shorter PI duration -> Lower cIMT (?)
Arterial Stiffness (PWV) is Increased in HIV Children with HIV

83 HIV+ (mean age, 11y) vs. 59 controls (mean age, 12y)
- 39 no ART, 23 PI-ART, 25 nonPI-ART
- Mean VL = 302 if on ART

PWV higher (stiffer) in HIV vs controls.

Among HIV, PWV higher with ART
- No diff between PI and non-PI ART
- Age, Sex, Cholesterol, BP, HR, ART, CDC stage were independently associated with PWV

hsCRP
- Higher in HIV vs controls
- Higher in HIV on ART than untreated
- ?Relation to PWV not reported

Problematic mix of those with and without virologic suppression

Charakida, Antivir Ther 2009
Coronary Artery Abnormalities (CAA) in HIV+ Youth

• Asymptomatic 27 HIV+ subjects (93% perinatal)
• Mean age 18.9 years (range 13-29)
• All on ART but only 70% undetectable
• 14/27 had CAA with luminal narrowing detected on MR angiography
• CAA linked to longer duration of TDF and FTC and trend for higher CRP and smoking.
• On multivariate regression analysis, only smoking was a significant predictor of CAA (p=0.03).

Mikhail, Peds IDJ 2012
CVD is not the Only Relevant Clinical Consequence of Chronic Inflammation

• sCD163 (monocyte activation) - cognitive impairment in adults (Burdo AIDS 2013)

• Higher p-selectin (marker of monocyte activation) associated with lower ND test scores in children (Kapetanovic AIDS 2010)
Summary: Persistent inflammation and clinical consequences in well-treated HIV infection in youth

• Despite prolonged virologic suppression in HIV+ children
  – Persistent microbial translocation (16s rRNA, LPS)
  – Almost no reduction in sCD14
  – Abnormal markers of systemic inflammation, coagulation, endothelial function

• HIV+ children have higher IMT, more coronary artery abnormalities, stiffness (PWV), endothelial dysfunction (FMD) and subclinical atherosclerosis (IMT) (than HIV uninfected controls)
  – Despite ART
  – Inconsistent findings re associations with inflammatory biomarkers and ARV treatment type (PI)

• Higher p-selectin (monocyte marker) associated with lower ND test scores (like monocyte marker and HAND in adults)

• Overall patterns and associations similar to those in adults

Grace McComsey
Evidence of persistent inflammation in children and adults with HIV infection despite ART

Evidence of ↑mortality and CVD for adults with HIV despite ART...

And evidence of subclinical atherosclerosis and vascular dysfunction in children on ART
Interventions targeting persistent inflammation/immune activation in ADULTS with well-treated HIV infection

Peter Hunt, MD

- Agents under active study in adults: rifaximin, sevelamer, meselamine (all for microbial translocation)
- Failed
  - IL-2 upped CD4 but no change in mortality (Abrams NEJM 2009)
  - MVC increased CD8 activation (Hunt Blood 2013)
- ↓ immune activation with hydrochloroquine (Piconi Blood 2011-YES; Paton JAMA 2012-NO) and valganciclovir (Hunt JID 2011)
- Statins reduce monocyte activation and non AIDS malignancy (McComsey CROI 2013; Ganesan JID 2011; Overton CID 2013)
- Pilot study showed ASA reduced sCD14 (O’Brien JAIDS 2013)
- Omega -3 fatty acids reduced sTNFR (Hileman ARHR 2012)
- ARV choice: ↑ hsCRP, IL-6 with ABC/3TC v TDF/FTC; ↑hsCRP with EFV vs ATVr (McComsey A5224s/A5202 AIDS 2012)
Future Directions for Children & Youth

• Prevent or eliminate modifiable risk factors
  – Smoking, BMI

• Maintain suppressive ART – regimen modification?
  – PI? Abacavir?
  – Management of hyperlipidemia in children

• Epidemiologic studies of adult outcomes (CVD, neuro, organ) of perinatal HIV infection

• Longitudinal studies of CVD/atherosclerosis, neuropsych and other outcomes as children/youth with well-controlled HIV age
  – Contribution of traditional CVD risk factors vs. HIV-related chronic inflammation

• Trials of anti-inflammatory interventions in children and youth
  – Primary prevention
  – In presence of subclinical disease
  – Targeting “enteropathy”
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- 81: Vikram (19)