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Immunologic Predictors of Bone Loss in a Contemporary HIV Cohort

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Background

- HIV infection is an independent risk factor for Osteoporosis and Fragility Fractures.
- Traditional Risk factors include
  - Older age, lower BMI, menopausal state, tobacco, alcohol
- HIV-related factors
  - Duration of HIV, lower CD4 count, HCV co-infection, initiation of ART, certain ART medications (e.g., tenofovir DF)
- Recent work has demonstrated the key role of persistent inflammation and innate immune dysfunction as key contributors to end organ disease in HIV.
  - IL6 is a soluble inflammatory biomarker that is released by many different cell types and has been linked to end-organ disease and mortality.
  - sCD14 is released by monocytes in response to bacterial translocation products and is another important soluble marker that has been associated with relevant outcomes in HIV disease.
- In this analysis, we utilized data from the SUN Study to evaluate the association between bone loss over time with inflammation and T cell and monocyte phenotypes, among persons with chronic HIV disease at low risk for AIDS complications.

Methods

- Study to Understand the Natural History of HIV/AIDS in the Era of Effective Therapy ('SUN' Study) is a prospective cohort-designed to study clinical complications of treated HIV infection.
- Participants were followed from 7 clinics in 4 U.S. cities (Denver, Minneapolis, Providence, and St. Louis) from 2004 to 2006.
  - At baseline, comprehensive clinical and behavioral data were collected .
  - At enrollment and annually, whole-body dual X-ray absorptiometry (DXA) scans were obtained at-study sites and transmitted to central reading centers.
  - Fasting whole blood and plasma specimens were collected. PBMCs were cryopreserved within 30 hours of collection.
  - Soluble biomarkers were measured at the Diabetes and Training Center Radioimmunoassay Core Laboratory (Washington University School of Medicine).
  - Immunophenotyping of cryopreserved PBMCs was performed using multicolor flow cytometry using validated panels of fluorochrome-conjugated antibodies for cell surface markers at the NIH/NIAID HIV Pathogenesis Laboratory.
- The primary objective of this analysis was to identify potential immunologic mechanisms that may accelerate bone loss in HIV infection.
- For this analysis, we focused on the total hip BMD as the primary outcome of interest.
- Statistical Analysis:
  - Baseline characteristics were summarized with frequencies or medians and interquartile ranges.
  - Spearman rank correlations and linear regression were used to explore associations between inflammatory biomarkers and T cell and monocyte and BMD at baseline.
  - The change in total hip BMD was evaluated with longitudinal mixed models that account for the within person correlation of measurements over time.
  - Fully adjusted models included the following baseline covariates: age, gender, race, smoking, hepatitis B or C co-infection, cholesterol and blood pressure lowering treatment, CD4 cell count, HIV viral load, prior AIDS diagnosis, current tenofovir use, and time since HIV diagnosis.
  - Regression coefficient represents an absolute percent change from baseline in total hip BMD per doubling of given biomarker or cellular phenotype.

NOTE: The findings and conclusions in this poster presentation are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

Table 1. Baseline Characteristics

	Hip T-Score Normal (T > -1.0)	Hip T-Score Not Normal (≤ -1.0)	P-value[1]
Number of Participants	460 (72%)	177 (28%)	
Age, median (IQR) years	**40 [34, 46]**	**44 [38, 50]**	**<0.001**
Male, N (%)	**342 (74%)**	**150 (85%)**	**<0.01**
Race, N (%)			0.54
White	274 (60%)	104 (59%)	
Black	135 (29%)	48 (27%)	
Other	51 (11%)	25 (14%)	
BMI, median (IQR) kg/m²	**26.6 [24.1, 30.1]**	**23.5 [21.6, 25.9]**	**<0.001**
Current smoker, N (%)	194 (43%)	83 (47%)	0.38
IV drug use, N (%)	**54 (12%)**	**33 (19%)**	**0.03**
Diabetes, N (%)	39 (8%)	15 (8%)	>0.99
Hypertension, N (%)	134 (29%)	64 (36%)	0.09
Treatment for HTN, N (%)	77 (17%)	41 (23%)	0.06
Lipid lowering therapy, N (%)	40 (9%)	16 (9%)	0.89
HBV/HCV co-infection, N (%)	**64 (14%)**	**41 (24%)**	**<0.01**
Previous AIDS diagnosis, N (%)	107 (23%)	48 (27%)	0.31
Current HAART use, N (%)	353 (77%)	143 (81%)	0.27
Time on HAART, median (IQR) months	**29 [11, 60]**	**40 [16, 73]**	**<0.01**
HIV RNA <400c/mL, N (%)			
Overall	341 (74%)	126 (71%)	0.40
Among those on HAART	**321 (91%)**	**121 (85%)**	**0.02**
CD4 cell count, median (IQR) cells/mm³	471 [338, 691]	462 [320, 669]	0.72
Nadir CD4 cell count, median (IQR) cells/mm³	**216 [98, 324]**	**185 [65, 309]**	**0.03**
Time Since HIV Diagnosis, median (IQR) years	**4.6 [2.1, 7.7]**	**5.8 [2.6, 9.3]**	**0.01**
Current use of PI, N (%)	164 (36%)	61 (34%)	0.78
Current use of NNRTI, N (%)	172 (37%)	74 (42%)	0.31
Tenofovir use (ever), N (%)	198 (43%)	90 (51%)	0.08
Tenofovir use (current), N (%)	**164 (36%)**	**80 (45%)**	**0.03**
Duration of Tenofovir use, median (IQR) months[2]	**12 [5, 21]**	**16 [10, 25]**	**0.01**

[1]Summaries are N (%) or median (IQR). P-values are from Chi-square or Kruskall Wallis tests.
[2]Among those ever on Tenofovir.

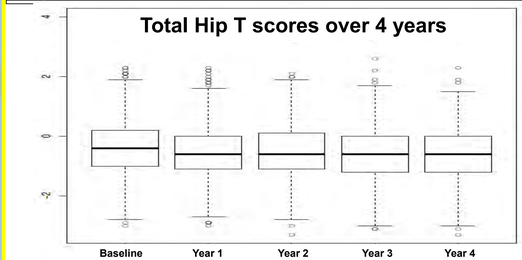
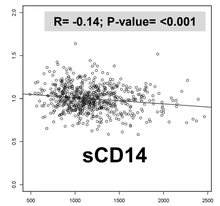
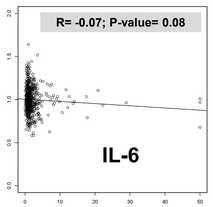


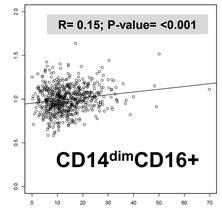
Table 2. Correlations With Baseline Total Hip BMD.

Soluble Biomarker	Spearman Correlation	P-value
IL-6 (pg/mL)	-0.069	0.08
sCD14 g/mL	**-0.142**	**<0.001**
sCD163 (pg/L)	0.040	0.34
D-Dimer	-0.019	0.63
hsCRP	0.074	0.06
Monocyte Phenotypes		
CD14+/CD16+	0.022	0.58
CD14^{dim}/CD16+	**0.150**	**<0.001**
CCR2+	**-0.120**	**<0.01**
CCR5+	0.025	0.54
Tissue Factor (TF)+	0.022	0.58
T-Cell Phenotypes		
CD4+/HLADR+/CD38+	0.006	0.89
CD4+/CD57+/CD38+	-0.019	0.64
Memory CD4+/CD28+	-0.009	0.82
CD8+/HLADR+/CD38+	-0.030	0.45
CD8+/CD57+/CD38+	0.009	0.66
Memory CD8+/CD28+	0.009	0.82

Spearman Correlation Plots Specific Markers versus Total Hip BMD







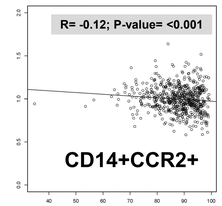


Table 3. Immunologic Predictors of Total Hip BMD Change.

Soluble Biomarker	Model 1* Regression Coefficient# (95% CI)	P-value	Model 2* Regression Coefficient# (95% CI)	P-value	Model 3* Regression Coefficient# (95% CI)	P-value
IL-6 (pg/mL)	**-0.77 (-1.13, -0.41)**	**<0.001**	**-0.60 (-0.98, -0.22)**	**<0.01**	**-0.52 (-0.91,-0.12)**	**<0.01**
sCD14 g/mL	**-1.37 (-2.26, -0.49)**	**<0.01**	-0.80 (-1.75, 0.14)	0.10	-0.86 (-1.83, 0.11)	0.08
sCD163 (pg/L)	-0.13 (-0.49, 0.22)	0.46	-0.34 (-0.75, 0.07)	0.11	-0.22 (-0.64, 0.20)	0.31
Monocyte Phenotypes						
CD14+/CD16+	-0.09 (-0.48, 0.30)	0.66	-0.20 (-0.59, 0.20)	0.33	-0.08 (-0.48, 0.32)	0.68
CD14^{dim}/CD16+	0.06 (-0.33, 0.46)	0.76	-0.14 (-0.54, 0.27)	0.51	-0.07 (-0.47, 0.34)	0.75
CCR2+	-1.34 (-4.04, 1.36)	0.33	0.21 (-2.64, 3.06)	0.89	0.36 (-2.48, 3.20)	0.80
CCR5+	**-0.24 (-0.48, -0.00)**	**0.05**	-0.23 (-0.47, 0.01)	0.06	**-0.24 (-0.49, -0.00)**	**0.05**
Tissue Factor (TF)+	**-0.30 (-0.58, -0.01)**	**0.04**	-0.27 (-0.56, 0.02)	0.07	-0.27 (-0.56, 0.02)	0.07
T-Cell Phenotypes						
CD4+/HLADR+/CD38+	-0.12 (-0.43, 0.20)	0.48	-0.18 (-0.50, 0.14)	0.28	-0.08 (-0.41, 0.25)	0.62
CD4+/CD57+/CD38+	-0.20 (-0.46, 0.06)	0.14	-0.22 (-0.49, 0.05)	0.11	-0.22 (-0.49, 0.05)	0.11
Memory CD4+/CD28+	**0.75 (0.29, 1.22)**	**<0.01**	**0.75 (0.29, 1.21)**	**<0.01**	**0.72 (0.26, 1.18)**	**<0.01**
CD8+/HLADR+/CD38+	0.01 (-0.32, 0.35)	0.93	0.01 (-0.33, 0.35)	0.96	0.14 (-0.21, 0.49)	0.44
CD8+/CD57+/CD38+	-0.07 (-0.43, 0.30)	0.71	-0.11 (-0.49, 0.27)	0.57	-0.04 (-0.43, 0.35)	0.84
Memory CD8+/CD28+	**0.52 (0.18, 0.86)**	**<0.01**	**0.69 (0.35, 1.03)**	**<0.001**	**0.65 (0.30, 0.99)**	**<0.001**

*All models are adjusted for DXA type (Hologic or Lunar). Model 1 is otherwise unadjusted. Model 2 is adjusted for age, gender, race, smoking, hepatitis B or C co-infection, cholesterol and blood pressure lowering treatment. Model 3 is additionally adjusted for CD4 cell count, HIV viral load, prior AIDS diagnosis, current tenofovir use, and time since HIV diagnosis.
#Regression coefficient represents an absolute % change from baseline in total hip BMD per doubling of given biomarker or cellular phenotype.

Conclusions

- Despite that SUN is a largely ART-treated HIV cohort with viral suppression, there was an ongoing modest decline in BMD over 4 years of follow up.
- Low BMD was associated with traditional (older age, lower BMI) and HIV-specific (IVDA, hepatitis co-infection, detectable viremia, nadir CD4 count, duration of HIV, and TDF exposure).
- At baseline, lower total hip BMD was associated with higher sCD14, and CCR2+ monocytes, but was positively correlated with CD14^{dim} CD16+ monocytes.
- Longitudinally, greater bone loss was independently associated with: a) higher baseline levels of IL-6 and CCR5+ monocytes, and b) inversely with the proportion of CD28+ memory CD4 and CD8 lymphocytes.
  - These relationships are independent of traditional and HIV specific factors.
- These data suggest that activated innate immunity, indicated by both soluble and cellular markers, is associated with ongoing bone loss despite ART.
- Furthermore, the relationship between higher BMD was independently and T lymphocyte memory populations indicates that functional adaptive immunity can potentially protect against bone loss.

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