Fractures Are Not Associated with CD8⁺ T Cell Activation: An Analysis of the ACTG ALLRT Study

Michelle A. Kendall,¹ Katherine Tassiopoulos,² Grace A. McComsey,³ and Michael T. Yin⁴

EDITOR: In HIV-1-infected individuals, chronic T cell activation despite antiretroviral therapy (ART) has been associated with AIDS-defining outcomes and surrogate markers for non-AIDS outcomes, such as cardiovascular disease.¹ Activated T cells promote bone loss in inflammatory diseases, such as rheumatoid arthritis and periodontitis, and play a role in postmenopausal osteoporosis. The role of HIV-associated T cell activation and low bone mineral density (BMD) by dual-energy X-ray absorptiometry (DXA) has been reported in recent studies with conflicting results.^{2,3} While previous studies have examined associations between traditional risk factors, frailty, CD4⁺ T cell count, and ART with fractures in HIV-infected individuals,^{4,5} none has examined associations between T cell activation and increased fracture.

An exploratory retrospective analysis of ACTG A5001 [ACTG Longitudinal Linked Randomized Trial (ALLRT)] examined whether higher levels of markers of CD8⁺ T cell activation were associated with incident fracture. ALLRT is a longitudinal cohort composed of ART-naïve or -experienced participants randomized into 26 ACTG clinical trials (parent studies) with specific interventions, which participants entered either while participating in or shortly after completing their parent study.⁴

Activated CD8⁺ T cells (CD8⁺CD38⁺HLA-DR⁺; “activated CD8%”) were measured on fresh peripheral blood mononuclear cell (PBMC) samples using ACTG consensus methods in a subset of the ALLRT cohort. Predictors of fracture were investigated in participants who had activated CD8% measured prior to fracture and after >8 weeks of ART. Associations between risk factors and time-to-first fracture were examined using multivariable Cox proportional hazards models before the addition of activated CD8% (dichotomized based on a threshold analysis). Because only 1,466/4,640 (31.5%) participants from the ALLRT fracture incidence study⁶ could be included, we

acknowledge that this study had very low power to detect associations.

Median (Q1, Q3) age and lifetime ART use were 40 (34, 46) and 4.7 (2.6, 7.1) years, respectively; 86% were men and 54% were white. Thirty-six separate fractures were reported in 34 participants. The median (Q1, Q3) time-to-first fracture was 4.6 (3.0, 8.9) years. The median (Q1, Q3) activated CD8% was 24% (15%, 38%), with no difference by fracture status ($p=0.71$). In the univariable analysis, activated CD8% <12.25% was associated with increased hazard of fracture [HR=2.04, 90% CI=(1.03, 4.00), $p=0.084$; Table 1], but continuous activated CD8% was not associated with fracture ($p=0.69$). In the adjusted multivariable analysis, however, activated CD8% <12.25% [HR=1.59, 90% CI=(0.68, 3.70)] was not associated with fractures ($p=0.37$) after adjusting for older age, HCV infection, and bisphosphonate use.

In 78 HIV-1-infected adults, Gazzola *et al.* found higher proportions of CD4⁺HLA-DR⁺ and CD8⁺HLA-DR⁺ T cells in subjects with low bone mineral density (BMD) (spine or hip T - or Z -score < -1) versus normal BMD, but no difference if both markers (CD38⁺HLA-DR⁺) were examined.³ Similarly, Erlandson *et al.* found no difference in median proportions of activated (CD38⁺HLA-DR⁺) CD4⁺ and CD8⁺ cells in 142 HIV-1-infected subjects on ART with low versus normal BMD at the hip or spine.² Our results indicate that elevation in markers of CD8⁺ activation (CD8⁺CD38⁺HLA-DR⁺) in HIV-1-infected individuals on ART was not associated with incident fracture. These data do not disprove the role of chronic immune activation with HIV infection in bone loss and fracture; association with other cellular markers, such as monocyte, B cell, or other T cell activation markers, or soluble markers should be investigated to test the hypothesis that chronic immune activation results in increased fractures.

¹Center for Biostatistics in AIDS Research, Harvard T.H. Chan School of Public Health, Boston, Massachusetts.

²Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, Massachusetts.

³Division of Infectious Diseases, Case Western Reserve University, Cleveland, Ohio.

⁴Division of Infectious Diseases, Columbia University Medical Center, New York, New York.

TABLE 1. UNIVARIABLE AND MULTIVARIABLE COX PROPORTIONAL HAZARDS REGRESSION MODELS OF TIME-TO-FIRST FRACTURE

	Univariable models ^a HR (90% CI)	p-value	Multivariable model ^b HR (90% CI)	p-value
Age (5 years)	1.30 (1.10, 1.52)	0.008	1.24 (1.03, 1.50)	0.064
Race				
White versus others	1.36 (0.75, 2.49)	0.40		
Black versus others	1.16 (0.58, 2.30)	0.72		
Hispanic versus others	0.59 (0.25, 1.44)	0.33		
Sex (men versus women)	1.20 (0.50, 2.90)	0.74		
Body mass index (kg/m ²)	1.03 (0.97, 1.09)	0.39		
Diabetes (yes versus no)	0.48 (0.09, 2.60)	0.48		
Renal insufficiency (yes versus no; ALLRT entry)	1.11 (0.33, 3.72)	0.89		
Bisphosphonate use (yes versus no; ALLRT entry)	14.59 (2.57, 82.75)	0.011	9.85 (1.39, 69.70)	0.055
HCV infection (yes versus no; first available)^b	2.68 (1.25, 5.73)	0.033	2.68 (1.24, 5.80)	0.035
HBV infection (yes versus no; first available)	1.64 (0.48, 5.64)	0.51		
Smoking (ever versus never)	1.69 (0.89, 3.22)	0.177		
IVDU (ever versus never)	1.37 (0.57, 3.31)	0.56		
Glucocorticoid use (yes versus no; ALLRT entry)	1.55 (0.29, 8.34)	0.67		
History of AIDS-defining illness (ever versus never)	1.43 (0.78, 2.61)	0.34		
CD4, baseline (50 cells/ μ l)	1.01 (0.93, 1.10)	0.87		
CD4, nadir (50 cells/ μ l)	1.01 (0.92, 1.10)	0.93		
HIV-1 RNA (log ₁₀ copies/ml)	0.90 (0.57, 1.42)	0.70		
Cumulative NRTI use (1 year)	1.02 (0.94, 1.14)	0.72		
Cumulative NNRTI use (1 year)	1.00 (0.88, 1.14)	0.98		
Cumulative PI use (1 year)	0.98 (0.85, 1.12)	0.78		
Cumulative TDF use (1 year)	0.98 (0.80, 1.21)	0.90		
Cumulative RTV-boosted PI use (1 year)	0.93 (0.75, 1.14)	0.54		
Activated CD8% <12.25% (yes versus no; first available)	2.04 (1.03, 4.00)	0.084	1.59 (0.68, 3.70)	0.37
Activated CD8% (10 points; first available)	0.95 (0.79, 1.16)	0.69		

^aAdditional baseline/entry variables considered in the regression models included height, BMI classification (underweight versus not), and diagnosed weight loss/wasting. In all analyses, univariable models containing these variables either had $p > 0.20$ or did not converge due to small numbers.

^bIncluded 1,349 participants (29 fractures) with complete data.

Time-to-first fracture excluded fractures of the face, skull, digits, and unknown body sites.

The renal insufficiency, bisphosphonate use, HCV infection, HBV infection, glucocorticoid use, activated CD8%, HIV-1 RNA measured nearest to activated CD8%, and all the cumulative antiretroviral variables were fit as time-updated covariates since they were measured at or after ALLRT entry and not at parent study entry. All *bolded variables* except activated CD8% were included in the multivariable model ($p \leq 0.20$) and eliminated if they did not meet the backward elimination selection criterion of $p \leq 0.10$. Parent study was forced into the models to control for any effect parent study may have had on the outcome.

ALLRT, ACTG Longitudinal-Linked Randomized Trial; HBV, hepatitis B virus; HCV, hepatitis C virus; IDVU, intravenous drug use; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; TDF, tenofovir; RTV, ritonavir.

Acknowledgments

We would like to thank the participants, ACTG study personnel, ALLRT team, and ALLRT parent study teams. This work was supported by Award Numbers U01 AI068636, AI38855, AI06534, AI69501, AI095089, K24 AI078884, and U01 AI068634 from the National Institute of Allergy and Infectious Diseases. The contents of this publication are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health.

Author Disclosure Statement

No competing financial interests exist.

References

- Kaplan RC, Sinclair E, Landay AL, *et al.*: T cell activation and senescence predict subclinical carotid artery disease in HIV-infected women. *J Infect Dis* 2011;203(4): 452–463.
- Erlanson KM, O’Riordan M, Labbato D, and McComsey GA: Relationships between inflammation, immune activation, and bone health among HIV-infected adults on stable antiretroviral therapy. *J Acquir Immune Defic Syndr* 2014; 65(3):290–298.
- Gazzola L, Bellistri GM, Tincati C, *et al.*: Association between peripheral T-lymphocyte activation and impaired bone mineral density in HIV-infected patients. *J Translation Med* 2013;11:51.

4. Womack JA, Goulet JL, Gibert C, *et al.*: Physiologic frailty and fragility fracture in HIV-infected male veterans. *Clin Infect Dis* 2013;56(10):1498–1504.
5. Shiao S, Lund E, Arpadi SM, and Yin MT: Incident fractures in HIV-infected individuals: A systematic review and meta-analysis. *AIDS* 2013;27:1949–1957.
6. Yin MT, Kendall MA, Wu X, *et al.*: Fractures after anti-retroviral initiation. *AIDS* 2012;26(17):2175–2184.

Address correspondence to:
Michael T. Yin
Division of Infectious Diseases
Columbia University Medical Center
630 West 168th Street, PH8-876
New York, New York 10032
E-mail: mty4@columbia.edu