HHS Public Access

Author manuscript

J Acquir Immune Defic Syndr. Author manuscript; available in PMC 2017 August 01.

Published in final edited form as:

J Acquir Immune Defic Syndr. 2016 August 1; 72(4): 372–375. doi:10.1097/QAI.0000000000000953.

Macrophage Activation in HIV-infected Adolescent Males Contributes to Differential Bone Loss by Sex: Adolescent Trials Network Study 021

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Abstract

Accumulating evidence suggests that rates of low bone mass are greater in HIV-infected males than females. Of eleven biomarkers assessed by sex and HIV-status, HIV-infected males had increased levels of soluble CD14 which inversely correlated with bone mineral content and bone mineral density measures, suggesting macrophage activation as a possible mechanism of differential bone loss.

Keywords

HIV; Bone; Macrophage Activation; sCD14; sVCAM; IL-6

Background

In the era of effective antiretroviral therapy (ART), low bone mass among HIV-infected individuals has emerged as a major co-morbidity of HIV infection and its treatment. In HIV-infected adults, combined rates of osteopenia and osteoporosis are as high as 90% in men and 60% in women [1], and osteoporosis-associated fractures are 60% higher than in the general population [2]. A recent study in HIV-infected adults reported lower bone mineral density (BMD) Z-scores, measured by dual-energy X-ray absorptiometry (DXA), in men vs. women, even after adjusting for other contributing factors [3]. In perinatally-infected children, low BMD was more pronounced in boys than girls and the effect was more pronounced as Tanner Stage increased [4]. Low BMD has also been observed in behaviorally-infected adolescent males [5]. Similarly, low BMD has been seen in boys receiving multiple courses of steroids for asthma, but not girls [6]. Low BMD has been linked to increased risk of fracture in HIV [7, 8].

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Bone loss in HIV infection is attributed to immune dysregulation, chronic inflammation, and antiretroviral therapy, as well as increased bone turnover from HIV-infection itself [8, 9]. The pathogenic mechanisms that account for the difference in bone loss by sex remain elusive, but are critical for intervention studies in youth, since this is the time that bone mass peaks [10]. In this study, biomarkers associated with inflammation, bone loss and/or bone formation in HIV-infected and uninfected adolescents were compared by sex and infection status to assess differences that may explain the differential bone loss.

Methods

Demographic data and blood samples were obtained from the Adolescent Trials Network for HIV/AIDS (ATN) studies 021A & B, which were cross sectional surveys of Tanner stage 5 behaviorally HIV-infected young women [11] and men [5], age 14–25, and seronegative controls from the same clinic populations. Participants were recruited from 18 sites in the US and Puerto Rico. Relevant demographic and laboratory data were collected at time of enrollment. Body composition, bone mineral content (BMC), and BMD were assessed by DXA scans of the whole body and spine and analyzed centrally. Total body BMC Z scores (TotalZ) were calculated using Baylor norms which allowed calculation only for subjects aged <23.0 years at time of scan [12]. The institutional review boards at each clinical site approved the study, and appropriate written informed consent/assent was obtained.

Four-hundred sixty frozen plasma samples were obtained and 457 were analyzed for the biomarkers listed in Table 1. Three samples were rejected; two were duplicates from subjects already included in the investigation and one subject was misidentified as HIV-negative. Seven HIV-positive and four HIV-negative males from the ATN 021B cohort [5] did not have available plasma samples for inclusion in this study. Soluble CD14 (sCD14) levels were measured with the Quantikine ELISA (R&D Systems, Minnesota). The remaining 10 analytes were measured using MagPix Assays (Millipore, Germany). Adiponectin and sVCAM were run in a duplex assay, while the remaining 8 analytes were run together. All samples were run in triplicate, and coefficients of variation (CVs) were calculated; all analytes with a CV >10% were rerun with or without dilution as needed.

Statistical analyses were performed using JMP10 (SAS, North Carolina) and R version 3.1.2 (http://www.r-project.org/). Standard curves were generated for each analyte and compared for variability. Analytes that were undetectable were assigned a value 0.01 less than the first detectable value for that specific analyte. Significance was assessed using Wilcoxon rank sum with multiple testing correction using the Benjamini-Hochberg FDR method. For potential confounding effects, linear regression analyses were performed using BMC and BMD z-scores and analyte values as the outcome variable and HIV status (Positive/Negative), body mass index (BMI) (continuous), total lean body mass (continuous) and sex (Male/Female) as covariates, based on demonstrated relationships with bone mass [3]. BMI and total lean body mass were added as covariates because BMD as well as some of the analytes (e.g. IL-6) have been shown to increase with obesity.

Results

The study population was stratified by sex and HIV status for analysis. Demographic, laboratory and clinical characteristics are summarized (see Table, Supplementary Digital Content 1): the median age was 21 and the majority of the participants were African American, ranging from 57.4–76.5%, depending on the subgroup. Approximately 50% of HIV-infected subjects were on antiretroviral therapy (48% of males and 52% of females). More than half of the individuals in each group had a BMI <25 kg/m^2, except HIV-positive females where more than 50% had a BMI of 25 kg/m^2 or greater. HIV-positive males had a significantly lower median BMI (22.7 kg/m^2) and total body fat (6.0 kg/m) than HIV-negative males (24.2 kg/m^2; p=0.02 and 7.9 kg/m; p=0.02) or HIV-positive females (26.3 kg/m^2; p<0.001 and 14.9 kg/m; p<0.001). Viral load and current and nadir CD4+ T-cell counts did not differ between HIV-positive males and females. High-sensitivity C-reactive protein (hsCRP) was elevated in the HIV-positive females (1.4 mg/L) compared to HIV-negative females (0.7 mg/L; p=0.015) and HIV-positive males (0.8 mg/L; p<0.001). The mean TotalZ was 0.333 vs. −0.557 for HIV-negative vs. HIV-positive males (p=0.002) and −0.127 vs. −0.232 for HIV-negative vs. HIV-positive females (p=0.73). The spine L1–L4 BMD Z score (SpineZ) was −0.493 vs. −0.912 for HIV-negative vs. HIV-positive males (p=0.02) and 0.092 vs. −0.268 for HIV-negative vs. HIV-positive females (p=0.18). The SpineZ and TotalZ scores were significantly lower in HIV-positive males than HIV-positive females (−0.912 vs. −0.268; p<0.001 and −0.557 vs. −0.232; p=0.005), despite a longer mean time since diagnosis in HIV-positive females (2.6 vs. 2.0 years; p=0.05). In regression analyses including BMI, total lean body mass, and sex as confounding variables, the negative effect of HIV-infection on the TotalZ of the males remained significant (p=0.005), whereas no difference by HIV status was observed in TotalZ for the female subjects (p=0.488)

Median values for the analytes and their statistical comparisons are presented in Table 1. Two analytes differed significantly between HIV-negative and HIV-positive females (IL-7 and TNFα were greater in the HIV-infected females), while four analytes differed between HIV-negative and HIV-positive males: sCD14 and sVCAM were greater in HIV-infected males, while IL-1b and IL-6 were significantly lower. There was a significant negative correlation between sCD14 and bone mass: in males, the Pearson correlation for TotalZ vs. sCD14 was −0.22 (p=0.006) and for SpineZ score vs. sCD14 it was −0.14 (p=0.031). In females, the correlation was only seen in the spine and not the total body, SpineZ score vs. sCD14 was −0.14 (p=0.048) and TotalZ vs. sCD14 was r=−0.08 (p=0.273) (see Figure, Supplementary Digital Content 2). There was no correlation between sVCAM and bone mass. In regression analyses of males to look for potential confounding effects, sCD14 and sVCAM remained positively associated with HIV-infection, but were not associated with BMI. The negative effect of HIV-infection on IL-1b and IL-6 values remained (r=−2.54 for IL-1b, p=0.005; and r=−5.44 for IL-6, p=0.014).

Discussion

The association between lower bone mass and higher sCD14 levels in HIV-infected males suggests that chronic macrophage activation may, in part, account for the differential bone

loss. Significant differences in sCD14, sVCAM, IL-1b, and Il-6 were observed between HIV-negative and HIV-positive males that were not seen between HIV-negative and HIV-positive females, despite higher levels of general inflammation (hsCRP) in HIV-positive females. This suggests that a physiologic difference in females, likely increased levels of estrogen, may be protective against some of the inflammation of chronic HIV. Estrogen is known to repress macrophage function and have a salutary effect on bone.

Soluble CD14, a biomarker of macrophage activation, is part of the TLR4 receptor complex for LPS and is associated with microbial translocation [13]. In the bone, osteoclasts, the cells responsible for the resorptive processes associated with continuous bone remodeling, are derived from the macrophage-monocyte system and require the presence of the receptor activator of nuclear factor KB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF) to form [9]. Therefore, the elevated macrophage activation seen in the HIV-positive subjects may result in increased osteoclast activity, leading to increased bone resorption. The inverse correlation between sCD14 and measures of bone mass (Total and spine Z scores) supports this hypothesis, as does a recent publication that found that upregulation of B cell expression of RANKL and decreased expression of osteoprotegrin correlated with total hip and femoral neck BMD Z-scores, but not lumbar spine [14].

Soluble VCAM is a marker of endothelial dysfunction which has been associated with cardiovascular disease [15] and elevated in HIV-infected adolescents [16]. However, there was no correlation between sVCAM levels and BMD in this cohort.

Given that HIV-infection is associated with chronic immune activation, the finding of lower levels of IL-1b and IL-6 in our HIV-infected males was unexpected. However, the IL-1b and IL-6 values of the HIV-infected males are similar to healthy males in other studies [17, 18]. It appears that IL-6 may not be a very useful marker of inflammation for HIV-infected youth who are at an early stage of infection. The reason for the relative elevation of IL-1b and IL-6 in the HIV-uninfected males in our study remains unknown.

Limitations of our study include differences between study groups in BMI and duration of infection, which may have influenced the findings. The HIV-positive males had significantly lower BMI than either the HIV-negative males or the HIV-positive females, which may have attenuated or altered differences in the biomarkers between these groups. Additionally, as bone mass increases with BMI, the average BMI of 26.3 kg/m^2 in the HIV-infected females may have increased the BMD in this cohort. Z-scores, which adjust for differences in weight, sex and race, were used to limit confounders in bone outcomes in HIV-infected females. HIV-infected females had on average 6 months longer time since HIV diagnosis than males, a difference that may have reduced the difference in some of the biomarkers between the two groups. Furthermore, the study was performed relatively early in infection (2.0 and 2.6 years for males and females, respectively) so that some important differences may not yet be detectable. Despite these limitations, this hypothesis generating study suggests that HIV-associated macrophage activation may be one mechanism responsible for HIV-associated bone loss.

In summary, the elevated levels of sCD14, indicating increased monocyte-macrophage activation, specifically in HIV-infected males, may lead to increased bone resorption via osteoclast activity. Interventions to decrease monocyte-macrophage activation early in HIV-infection may be useful to decrease HIV-associated morbidity and mortality.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We acknowledge the contribution of the investigators and staff at the following sites that participated and enrolled subjects into this study: Children's Diagnostic and Treatment Center, Fort Lauderdale, FL (Puga, Leonard, Eysallanne, Inman); Children's Hospital of Los Angeles, CA (Belzer, Tucker, Salata); Children's Hospital of Philadelphia, PA (Rudy, Tanney, DiBenedetto, Seth), Children's Memorial Hospital, Chicago, IL (Garofolo, Cagwin, Kershaw); Children's National Medical Center, Washington, DC (D'Angelo, Trexler, Hagler, Klamberg); John H. Stroger Jr. Hospital of Cook County and the Ruth M. Rothstein CORE Center, Chicago, IL (Martinez, Henry-Reid, Bojan, Jackson); Montefiore Medical Center, Bronx, NY (Futterman, Enriquez-Bruce, Campos); Mount Sinai Medical Center, New York, NY (Levin-Carmine, Geiger, Lee); St. Jude Children's Research Hospital, Memphis, TN (Flynn, Stender, Dillard, McKinley); Tulane University Health Sciences Center, New Orleans, LA (Abdalian, Baker, Kozina); University of California at San Francisco (Moscicki, Auerswald, Molaghan); University of Maryland, Baltimore (Peralta, Flores, Gorle); University of Miami School of Medicine (Friedman, Maturo, Major-Wilson); University of Puerto Rico, San Juan (Febo, Ayala-Flores, Rivera); and University of South Florida, Tampa (Em-manuel, Lujan-Zilbermann, Straub, Callejas, Julian). We thank Justin Wheeler and Andrea Miller (Desilets) at the Body Composition Analysis Center at Tufts University, who read the scans and tabulated the data.

The investigators are grateful to the members of the local youth Community Advisory Boards for their insight and counsel and are particularly indebted to the youth who participated in this study.

The study was scientifically reviewed by the ATN's Therapeutic Leadership Group. Network, scientific and logistical support was provided by the ATN Coordinating Center (C. Wilson, C. Partlow) at the University of Alabama at Birmingham. Network operations and analytic support was provided by the ATN Data and Operations Center at Westat, Inc (J. Korelitz, B. Driver, N. Liu, L. Cuasay, Jiahong Xu and R. Harris).

Funding:

This work was supported by The Adolescent Medicine Trials Network for HIV/AIDS Interventions (ATN) from the National Institutes of Health [NIH U01 HD 040533 and U01 HD 040474] through the Eunice Kennedy Shriver National Institute of Child Health and Human Development (B. Kapogiannis, L. Serchuck, R. Hazra), with supplemental funding from the National Institutes on Drug Abuse (N. Borek) and Mental Health (P. Brouwers, S. Allison). The following sites utilized their General Clinical Research Center/Pediatric Clinical Research Centers, which were supported by grants from the General Clinical Research Center Program of the National Center for Research Resources, National Institutes of Health, Department of Health and Human Services: Children's Hospital of Los Angeles, M01 RR00043; Mt. Sinai Medical Center, M01 RR00071; University of California San Francisco, M01 RR001271/ UL1 RR024131; University of Maryland, M01 RR165001; University of Pennsylvania/Children's Hospital of Philadelphia, M01 RR00240/ NCRRUL1-RR-024134; University of South Florida/All Children's Hospital Clinical Research Center, R60 MC00003; and Children's National Medical Center (M01RR020359); The Tulane University Health Sciences Center utilized its Clinical and Translational Research Center (CTRC) for the study; the center was supported in whole or in part by funds provided through the Louisiana Board of Regents RC/EEP (RC/EEP-06).

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Table 1

Analytes by Sex and HIV Status

Analytes by Sex									
	All Subjects			*HIV Negative*			*HIV Positive*		
Analyte[*]	*Female (N=216)*	*Male (N=241)*	*P-value*[**]	*Female (N=54)*	*Male (N=49)*	*P-value*	*Female (N=162)*	*Male (N=192)*	*P-value*
sCD14	**1,758,352**	**1,616,812**	**0.0486**	**1,573,290**	**1,380,489**	**0.0301**	1,820,780	1,710,030	0.1308
sVCAM	855,715	868,248	0.8044	809,486	794,478	0.8941	880,357	910,042	0.8851
Adiponectin	**13,232,027**	**9,994,291**	**0.0011**	**12,996,140**	**8,344,274**	**0.0208**	**13,301,509**	**10,347,777**	**0.0181**
IL-1b	1.52	1.44	0.8941	1.52	2.76	0.3605	1.47	1.22	0.6161
IL-6	1.53	1.60	0.9015	1.67	4.24	0.1071	1.46	1.07	0.6161
IL-7	**7.85**	**10.49**	**0.0151**	**6.07**	**9.52**	**0.0197**	8.45	10.54	0.1308
IL-12 (p40)	12.03	12.69	0.9468	10.37	13.47	0.6220	13.01	12.51	0.6634
IL-17a	4.41	5.10	0.0897	4.20	5.99	0.1108	4.41	5.07	0.2934
sCD40L	**1,221**	**857**	**0.0181**	1,273	657	0.1308	1,221	898	0.0581
TNFα	12.17	12.06	0.8372	**6.84**	**9.50**	**0.0181**	13.76	13.03	0.4285
IFNγ	8.52	9.18	0.4774	9.03	10.96	0.1656	8.39	8.78	0.8941
Analytes by HIV Status									
	All Subjects			*Female*			*Male*		
Analyte	*HIV Neg (N=103)*	*HIV Pos (N=354)*	*P-value*	*HIV Neg (N=54)*	*HIV Pos (N=162)*	*P-value*	*HIV Neg (N=49)*	*HIV Pos (N=192)*	*P-value*
sCD14	**1,520,620**	**1,758,352**	**0.0001**	1,573,290	1,820,780	0.0581	**1,380,489**	**1,710,030**	**0.0001**
sVCAM	**795,818**	**897,612**	**0.0038**	809,486	880,357	0.0667	**794,478**	**910,042**	**0.0208**
Adiponectin	10,747,455	11,310,470	0.6041	12,996,140	13,301,509	0.9736	8,344,274	10,347,777	0.2033
IL-1b	1.84	1.33	0.1108	1.52	1.47	0.8304	**2.76**	**1.22**	**0.0486**
IL-6	**2.74**	**1.27**	**0.0486**	1.67	1.46	0.8509	**4.24**	**1.07**	**0.0181**
IL-7	7.50	9.35	0.0899	**6.07**	**8.45**	**0.0308**	9.52	10.54	0.9214
IL-12 (p40)	11.13	12.65	0.4545	10.37	13.01	0.2586	13.47	12.51	1.0000
IL-17a	4.72	4.69	0.5988	4.20	4.41	0.8941	5.99	5.07	0.2009
sCD40L	812	1,032	0.1179	1,273	1,221	0.6041	657	898	0.1071
TNFα	**8.49**	**13.38**	**0.0000**	**6.84**	**13.76**	**0.0000**	9.50	13.03	0.0581
IFNγ	9.80	8.60	0.5117	9.03	8.39	0.8372	10.96	8.78	0.1308

sCD14 = soluble CD14, sVCAM = soluble vascular adhesion molecule, IL = interleukin, TNFα = tumor necrosis factor α, IFNγ = interferon γ.

* All analytes are measured in picograms/milliliter

** P-value determined by Wilcoxon Rank Sum with multiple testing correction using the Benjamimi-Hochberg FDR method.