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Low Baseline CD4+ Count is Associated with Greater Bone Mineral Density Loss after ART Initiation

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40-word summary: Low pre-treatment CD4+ count is an independent risk factor for bone loss after ART initiation, providing further evidence for the benefits of early ART. Initiation of ART at higher CD4+ counts may reduce the burden of osteoporosis and fragility fracture.

Abstract

Background

Bone mineral density (BMD) decreases 2-6% in the two years after antiretroviral therapy (ART) initiation. Pre-ART immune deficiency and early immune recovery may contribute to this loss.

Methods

We pooled data from three treatment-naive ART initiation studies in which serial whole-body dual-energy X-ray absorptiometry scans were performed. We used linear regression to evaluate effects of baseline CD4+ and 16-week CD4+ change (both absolute and relative) on 96-week total BMD change from baseline. We performed multivariable linear regression to assess associations between baseline variables of age, sex, race/ethnicity, body mass index (BMI), hepatitis C status, parent study, HIV-1 RNA level, and assignment to a protease inhibitor (PI) or tenofovir-containing regimen on 96-week total BMD change.

Results

The included 796 subjects had mean 96-week total BMD loss of 2.0%. In multivariable analysis, baseline CD4+ count was significantly associated with 96-week BMD loss; individuals with baseline CD4+ <50 cells/μL lost significantly more BMD compared to those with CD4+ ≥500 cells/μL. A greater relative, but not absolute, 16-week increase in CD4+ count was significantly associated with greater declines in BMD, but not after controlling for baseline CD4+ count. In multivariable analysis, older age, female sex, lower BMI, higher HIV-1 RNA levels, and PI and tenofovir assignment were also associated with greater BMD decline.

Conclusions

Low pre-treatment CD4+ count, but not greater CD4+ count increase, is a strong and independent risk factor for bone loss after ART initiation. ART initiation at higher CD4+ counts may reduce the burden of osteoporosis and fragility fractures.

Background

With improvements in antiretroviral therapy (ART) and the accompanying aging of the HIV-infected population, HIV-associated non-AIDS (HANA) complications are increasingly impacting the health of HIV-infected individuals [1]. While the rates of other HANA complications appear to decrease with ART, bone mineral density (BMD) loss is unique in that it accelerates after ART initiation, at least over the short term [2]. Low BMD occurs in 40-90% of HIV-infected individuals [3], leading to a 50% increased risk of fragility fracture compared to the risk in uninfected individuals [4].

BMD loss appears most prominent during the first two years of ART, decreasing 2-6%, [5], although recent data suggest this loss may be less with some nucleoside reverse transcriptase inhibitor (NRTI)-sparing regimens [6]. In addition to traditional osteoporotic risk factors and antiretroviral medication effects, some data suggest that baseline immune deficiency or ART-associated immune reconstitution could contribute to BMD loss [7]. Here, we pooled data from three treatment-naive ART initiation studies where serial whole-body dual x-ray absorptiometry (DXA) were performed to examine the relationship between baseline CD4+ count and early CD4+ count change with BMD change two years after ART initiation.

Methods

Parent Studies

We accessed subject-level data from three AIDS Clinical Trials Group (ACTG) treatment-naive ART initiation studies in which serial whole-body dual-energy X-ray absorptiometry (DXA) were performed. We included data from ACTG A5005s (enrolled 1998-1999) [8], A5142

(enrolled 2003-2004) [9, 10], and A5224s (enrolled 2005-2007) [5].

ACTG A5005s (a substudy of ACTG 384 [11]) included subjects randomized to nelfinavir, efavirenz, or both combined with either didanosine/stavudine or lamivudine/zidovudine; ACTG A5142 randomized subjects to one of three class-sparing regimens: lopinavir/ritonavir and two NRTIs (lamivudine plus either zidovudine, stavudine or tenofovir disoproxil fumarate - TDF), efavirenz and two NRTIs, or lopinavir/ritonavir and efavirenz [12]; and ACTG A5224s (a substudy of ACTG A5202 [13]) included subjects randomized to atazanavir/ritonavir or efavirenz combined with either abacavir/lamivudine or TDF/emtricitabine.

We included participants from these studies who underwent whole-body DXA scan at baseline (allowable window from 30 days prior to parent study enrollment to 15 days after ART initiation) and 96 weeks (allowable window from 84 to 108 weeks after ART initiation). Because ACTG A5005s and ACTG A5142 did not perform site-specific hip and spine DXA, all assessments of BMD change are based on whole-body BMD, which was collected in all three parent studies. While site-specific DXA is more commonly used to screen for osteoporosis, both total BMD and site-specific BMD predict incident fractures [14, 15]. Additionally, studies in HIV-uninfected and HIV-infected individuals have shown good concordance between BMD measures obtained from whole-body and site-specific DXA (R^2=0.88 and 0.83, respectively) [16] [D.K., unpublished data from ACTG A5260]. All DXAs were standardized at the participating sites, then read at the central reading site (Tufts).

Statistics

We performed univariate and multivariable linear regression to determine the association between baseline CD4+ count and 16-week CD4+ count changes and 96-week BMD change. We considered CD4+ count as an ordinal variable, using relevant clinical cut-offs (<50, 50-199, 200-349, 350-499, and ≥500 cells/μL). We chose the 16-week timepoint for CD4+ count change as this was the earliest common post-ART timepoint where CD4+ count was assessed in the parent studies. We tested the associations of both absolute and relative (log_{10}[CD4+ count week 16/CD4+ count week 0]) CD4+ count changes in these models. We also evaluated the following baseline variables for their association with 96-week BMD change: age, sex, race/ethnicity, body mass index (BMI), hepatitis C virus infection status (not collected in ACTG A5005s), parent study, HIV-1 RNA level, randomization to a protease inhibitor (PI)-containing regimen, and receipt of an initial regimen containing TDF. Factors with a univariate p-value of <0.20 were included into the multivariable model which used backward selection and retained factors with a p-value of <0.05. We explored interactions between baseline CD4+ count and the above baseline covariates on 96-week BMD change. All analysis testing was two-sided with a type I error of 5%; thus p-values of <0.05 were considered statistically significant with no adjustment for multiple comparisons.

Results

Of the 1116 subjects enrolled in the parent studies, 796 subjects had baseline and 96-week DXA evaluations and are included in this analysis. **Table 1** displays the baseline characteristics of the included subjects by parent study. Median age was 39 years (IQR 32, 44); 83% were male. The median baseline BMI was 25 (IQR 22, 28). Median baseline CD4+ count and HIV-1 RNA level

were 208 cells/μL (IQR 68, 328) and 4.8 $\log_{10}$ copies/mL (IQR 4.4, 5.2), respectively. Sixty-two percent of subjects were randomized to a PI-containing regimen, and 27% received an initial regimen containing TDF.

The mean 96-week total BMD loss from baseline was 2.0% (standard deviation 3.3%). **Figure 1** displays the unadjusted relationship between baseline CD4+ count and BMD change 96 weeks after ART initiation. In the base model adjusting for parent study, and PI and TDF use, baseline CD4+ count was associated with BMD loss ($p<0.001$) with individuals initiating ART at a CD4+ count of <50 cells/μL losing 3.0% more BMD (95% CI:-4.0, -2.0) than those who initiated ART at a CD4+ count of ≥500 cells/μL. Greater relative, but not absolute, 16-week CD4+ count increase was associated with greater BMD decreases at 96 weeks (-2.3% per 10 fold higher; 95% CI: -3.0, -1.7; $p<0.001$), but this association was no longer significant after controlling for baseline CD4+ count (-0.7% per 10 fold higher; 95% CI: -1.8, 0.4; $p=0.21$).

In univariate analyses, we also found older age, lower BMI, parent study, higher baseline HIV-1 RNA levels, and assignment to a PI- or TDF-containing regimen each to be significantly associated with 96-week BMD loss (**Table 2)**.

Most associations found in univariate analyses were maintained in multivariable analysis (**Table 2)**. Baseline CD4+ count remained strongly associated with 96-week BMD loss ($p<0.001$) with individuals in the lowest CD4+ category (i.e., <50 cells/μL) losing a significantly larger BMD percentage than those with a CD4+ count of ≥500 cells/μL (-2.27%; 95% confidence interval: -3.30, -1.25). Additionally, older age, female sex, lower BMI, higher HIV-1 RNA level, and PI

and TDF assignment were all independently associated with loss of BMD at 96 weeks in multivariable analysis.

We found a significant interaction between lower baseline CD4+ count and higher HIV-1 RNA levels and BMD loss (p=0.043). In subjects with higher HIV-1 RNA levels, the negative effect of lower CD4+ count was greater than in those subjects with lower HIV-1 RNA levels.

Discussion

In this pooled analysis of nearly 800 individuals initiating ART in randomized clinical trials, we found a strong and independent association between low baseline CD4+ count and total BMD loss in the first two years of treatment. Additionally, we found independent associations between older age, female sex, lower BMI, higher HIV-1 RNA level, PI use, and TDF use and BMD loss. We did not find evidence that the extent of immune reconstitution, as measured by CD4+ count increase at 16 weeks, was associated with BMD change after controlling for baseline CD4+ count.

There are conflicting reports in the literature on the relationship between baseline CD4+ count and the risk for BMD loss on ART [17, 18]. Our study benefits from the large number of included subjects and the comprehensive data that was gathered on subjects in the setting of clinical trials. We found that even after controlling for multiple potential confounders such as BMI that there was a robust relationship between low baseline CD4+ count and greater bone loss after ART initiation. The underlying reason for the relationship between low baseline CD4+ count and bone loss with ART initiation is not known but suggests a potential role for the

immune system in skeletal maintenance. In animal models, T cells are key mediators of bone loss [19, 20]. T-cell knockout mice do not lose bone with parathyroid hormone administration until after reimplantation of T cells [21]. Potentially, an Immune Reconstitution Inflammatory Syndrome (IRIS)-like phenomenon could underlie ART-associated bone loss. However, our study did not find a relationship between the magnitude of the CD4+ count increase with ART and bone loss, a relationship which would have supported an IRIS-like phenomenon underlying ART-associated bone loss.

In addition to HIV disease stage, we found traditional osteoporosis risk factors and specific antiretroviral medications placed individuals at increased risk for bone loss after ART initiation. We found older individuals, women, and those with lower BMI to be at greater risk for bone loss with ART initiation. Older HIV-infected individuals and those with lower BMI have been shown to be at increased risk for osteoporosis in prior cross-sectional studies [22]. However, our finding that these groups and also women are at increased risk for BMD loss after ART initiation is concerning given their already lower pre-ART BMD and the potential for falls and subsequent fragility fractures in these groups [23] .

TDF use has been associated with BMD loss in multiple studies [5, 24] and more recently has also been associated with fracture risk [25]. Studies evaluating the effect of PI use on bone health have been conflicting, partially dependent on the site being evaluated (e.g., spine vs. hip vs. total BMD) [5, 16]. After pooling studies, we found a strong and independent association between randomization to a PI and BMD loss. Previous studies have also shown an association between

the use of certain PIs and incident fractures, reinforcing that PIs can have negative effects on bone metabolism [25].

There are several limitations to our study that deserve highlighting. We assessed change in total BMD rather than site-specific BMD. While there is good correlation between the two measures when measured cross-sectionally, the correlation between the change in the two measures has not been evaluated to our knowledge in an HIV-infected population. In the general population, BMD changes in total body underestimate losses in lumbar spine, total hip, and distal forearm in the early post-menopausal period [26]. However, similar to site-specific BMD, total BMD is also a valid surrogate for fracture risk [15]. The BMD changes found at two years in our study were relatively modest and of unclear clinical significance. It has been noted that fracture incidence is highest in the first two years after ART initiation compared to in subsequent years [27]. Additionally, the significance of the loss could be compounded in persons with pre-existing low BMD or if the loss continues throughout the course of ART. Another potential limitation is that we pooled the bone loss data for all PIs used in our study (nelfinavir, lopinavir/ritonavir, and atazanavir/ritonavir), and it is possible that there are differences in bone effects among the PIs. Some of the regimens in these studies are no longer in common use, but when controlling for parent study in multivariate analysis, study was not a significant factor. We did not have complete data on covariates such as tobacco or alcohol use, testosterone or vitamin D levels, or use of relevant concomitant medications (e.g., calcium, vitamin D, bisphosphonates, etc.) that could affect BMD. However, given the randomized nature of the three studies, it is unlikely that such unmeasured covariates could have biased our estimates of measured covariates. Additionally, since this was a post-hoc analysis with multiple comparisons, marginally

significant associations should be interpreted cautiously. Finally, due to inadequate power, we were unable to determine the precise baseline CD4+ count threshold where bone loss after ART initiation was greater than those in the highest CD4+ count stratum.

In conclusion, we found that low pre-treatment CD4+ count, but not early CD4+ count change with ART, was a strong and independent risk factor for bone loss after ART initiation, providing further evidence for the benefits of the early initiation of ART. Initiation of ART at a higher CD4+ count may reduce the burden of osteoporosis and fragility fractures among HIV-infected patients.

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Conflict of Interest Statement

G.M. has served as a scientific advisor or speaker for Bristol Myers Squibb, GlaxoSmithKline, Janssen, Merck, and Gilead Sciences, has received research grants from Bristol Myers Squibb, GlaxoSmithKline, and Gilead Sciences, and is currently serving as the Data and Safety Monitoring Board (DSMB) Chair for a Pfizer-sponsored study. M.D. has received grant funding from Merck and Gilead Sciences. R.H. reports having received honoraria or consultant fees from

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Table 1: Baseline Characteristics of Included Subjects by Parent Study

Characteristics	All Subjects (n=796)	A5005s (n=93)	A5142 (n=503)	A5224s (n=200)
Median Age (IQR)	39 (32, 44)	37 (32, 45)	38 (32, 44)	40 (33, 44)
Sex				
Male	83%	88%	82%	85%
Female	17%	12%	18%	15%
Race/Ethnicity				
Black non-Hispanic	36%	30%	39%	30%
White non-Hispanic	45%	57%	39%	52%
Hispanic	18%	13%	20%	16%
Other	1%	0%	2%	2%
Median BMI - kg/m^2 (IQR)	25 (22, 28)	24 (22, 27)	25 (22, 28)	25 (22, 28)
HCV antibody positive	9%	N.A.*	9%	7%
Median baseline CD4+ count - cells/μL (IQR)	208 (68, 328)	265 (96, 423)	184 (49, 305)	255 (131, 336)
Median baseline viral load - $\log_{10}$ c/mL (IQR)	4.8 (4.4, 5.2)	5.1 (4.6, 5.6)	4.8 (4.5, 5.3)	4.6 (4.3, 4.9)
Randomized to PI-containing regimen	62%	69%	67%	46%
Initial regimen TDF-containing	27%	0%	23%	49%

* HCV testing was not performed in A5005s; PI=protease inhibitor; TDF=tenofovir disoproxil fumarate

Table 2: Associations of Total Bone Mineral Density Change (%, Week 0-96)

Covariate/Level	Reference	Univariate Analyses		Multivariable Analysis	
		Estimated mean change (95% CI)	**p-value**	**Estimated mean change (95% CI)**	**p-value**
Age	Continuous (per 1 year older)	-0.05 (-0.07, -0.02)	**<0.001**	-0.05 (-0.07, -0.02)	**<0.001**
Male	Female	0.45 (-0.18, 1.08)	0.16	0.84 (0.24, 1.45)	**0.007**
Race/ethnicity			0.50		
White Non-Hispanic	Hispanic	0.35 (-0.31, 1.00)			
Black Non-Hispanic	Hispanic	0.10 (-0.57, 0.78)			
Baseline Body Mass Index	Continuous (per 1 kg/m^2 higher)	0.12 (0.07, 0.17)	**<0.001**	0.10 (0.06, 0.15)	**<0.001**
HCV antibody positive †	Negative	0.22 (-0.66, 1.10)	0.62		
Parent Study			**0.013**		
A5005s	A5224s	0.21 (-0.61, 1.04)			
A5142	A5224s	-0.65 (-1.20, -0.10)			
Baseline CD4+ count			**<0.001**		**<0.001**
<50	≥500	-3.27 (-4.28, -2.26)		-2.27 (-3.30, -1.25)	
50-<200	≥500	-1.58 (-2.57, -0.60)		-0.74 (-1.72, 0.24)	
200-<350	≥500	-0.99 (-1.96, -0.02)		-0.62 (-1.57, 0.33)	
350-<500	≥500	-0.76 (-1.82, 0.30)		-0.60 (-1.63, 0.42)	
Baseline HIV-1 RNA	Continuous (per 1 $\log_{10}$ copy/mL higher)	-0.95 (-1.28, -0.61)	**<0.001**	-0.56 (-0.92, -0.20)	**0.002**
Randomized to Protease Inhibitor	Not	-0.59 (-1.07, -0.10)	**0.017**	-0.80 (-1.27, -0.33)	**0.001**
Initial Regimen TDF[1]-containing	Not	-1.13 (-1.66, -0.60)	**<0.001**	-1.38 (-1.90, -0.87)	**<0.001**
Week 16 Absolute CD4+ Change	Continuous (per 100 cells/μL higher)	0.06 (-0.16, 0.28)	0.62		
Week 16 Relative CD4+ Change ‡	Continuous (per 10 fold higher)	-2.46 (-3.13, -1.80)	**<0.001**		

† -Tested only in A5142 and A5224s; 1 TDF= tenofovir disoproxil fumarate; ‡ - Relative CD4+ Change= $\log_{10}$[CD4 week16/CD4 week0];

Figure 1: Week 96 Bone Mineral Density Change by Baseline CD4+ Count Category (—- = group mean)