Will HIV-Infected Patients Make Old Bones?: Osteoporotic Fractures in the Aging HIV Population

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I. Trends in Morbidity and Mortality: The Changing Face of HIV Disease

a. Increased survival and aging of HIV-infected patients

The use of protease inhibitors (PI) in the context of combination antiretroviral therapy, known as HAART, has had a significant impact on the survival of HIV-infected patients [1, 2]. During the second half of the 1990's decade, mortality of HIV-infected patients in the U.S. and Europe dropped to less than 1/3 of the rate of the previous half-decade [1-3], and continued declines observed in the 2000's [4].
Furthermore the HIV population is significantly aging. It is expected that by 2015, more than one-half of all HIV-infected individuals in the United States will be aged >50 years [5] with expected increase in age-related morbidities such as cardiovascular diseases, cancers and accelerated bone loss [6]. In 2007, the largest number of new HIV/AIDS diagnoses was for persons aged 40–44 years, who accounted for 15% of all HIV/AIDS cases diagnosed during that year [7].

b. Trends in causes of death among persons with HIV/AIDS in the HAART era

The increase in overall survival of HIV-infected patients has been associated with a shift in underlying cause of death among these patients, with lesser representation of "AIDS-related causes" (opportunistic infections and AIDS-defining cancers) and greater representation of "non-AIDS-related" deaths. Between 1999 and 2004, the proportion of deaths due to non–AIDS-related causes increased by 33% in HIV-infected patients in New York [8]. A French cohort observed similar trends between 2000 and 2005. In that study, the proportion of underlying causes of death due to AIDS decreased (36% in 2005 vs. 47% in 2000). The non-AIDS causes of death with increasing in proportion included non-AIDS cancers (17% vs. 11%), chronic liver disease (15% vs. 13%), and cardiovascular disease (8% vs. 7%) [4]. The causes of the persistence of these chronic complications despite optimal antiretroviral therapy are subject of intensive investigation, and some controversy.

We have previously examined the rates of cardiovascular disease and non-AIDS malignancies among HIV-infected patients [9-12]. We have also examined the impact of antiretroviral therapy and co-infection with HCV on these complications. Regarding cardiovascular diseases, we showed that HCV co-infection and advancing age, but not antiretroviral therapy were associated with increased risk of CVD among HIV-infected patients [10, 12]. We have also determined that HIV-infected patients continue to experience significantly higher rates of "non-AIDS" malignancies despite successful antiretroviral therapy resulting in significant immunologic recovery (at least as measured by improved CD4 cell counts) [11].

This greater representation of non-AIDS complications – and the still lower survival of HIV patients compared to the general population – suggest that further reductions in morbidity and mortality of HIV-infected patients will require a greater understanding of the mechanisms and factors associated with these non-AIDS complications and a shift to a more comprehensive health care approach including enhanced screening for malignancy and heart disease as well as preventive measures for substance abuse, liver disease, bone disease, and other metabolic complications.

II. Epidemiology and Risk Factors of Osteoporosis in HIV

a. Epidemiology of Osteoporosis in HIV

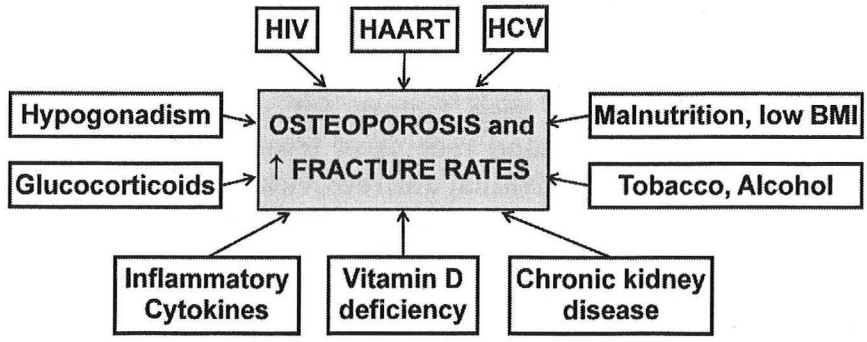
While this was not apparent in early studies [13-15] recent studies generally show higher rates of decreased bone mineral density (BMD) among HIV-infected patients than in the general population [16, 17]. However, the prevalence estimates have varied widely, probably as a consequence of limited sample sizes and selection bias [17, 18]. A meta-analytic review has shown a prevalence of osteoporosis of 15% in HIV-infected individuals, 3.7 times greater than in HIV-uninfected controls [19]. Almost all these studies were cross-sectional and few adjusted for important covariates of decreased BMD such as HIV disease stage, treatment duration, smoking (only reported in two of eleven studies, and controlled for in none), alcohol and drug use. In a large study comparing two HAART regimens – Gilead 903 study [20] – there was a high baseline prevalence of osteopenia of 23% and 28% in the two study groups (mean age of subjects, 36 years), which is significantly higher than the national prevalence among adults in the United States [16]. The underlying mechanisms leading to this increased risk are still unclear but it is thought to be a multifactorial process [17, 19, 21, 22].

On the basis of results of large epidemiologic studies that clearly correlated the risk of decreased BMD (as measured by dual X-ray absorptiometry [DXA]) with an increased incidence of fragility fracture [23], a World Health Organization committee provided definitions of osteoporosis and osteopenia that were based on the difference between a patient's BMD and the mean BMD at the time of peak bone mass among 30-year-old persons, adjusting for race and sex, and expressed as standard deviation (SD) from the mean, or T-score. Osteoporosis is defined as a T score of less than −2.5 SDs. Osteopenia is defined as T score between −2.5 and −1 SDs.

b. Risk Factors and Pathogenic Mechanisms

Bone turnover is related to bone resorption and bone formation. Normally, bone formation and resorption are closely linked and synchronized. Bone remodeling depends on the coupled activity of osteoblasts, which form new bone and osteoclasts, which degrade the bone matrix. The balance between osteoblast and osteoclast activity is a key determinant of bone mass and fracture risk. Several factors regulate osteoclast number and activity, including hormones and inflammatory cytokines via cellular signaling pathways. Osteopenia and osteoporosis can therefore be mediated through uncoupling of the bone formation and resorption, either by decreasing bone formation, increasing bone resorption or both.

Figure 1: Factors likely associated with decreased bone health in HIV



One possible cause of the high prevalence of osteopenia among HIV-infected patients is an enrichment of the

"traditional" risk factors among HIV-infected patients [24]. These include low body mass index, steroid use, IV drug use, smoking, vitamin D deficiency, other nutritional deficiencies, low levels of calcium intake, immobilization, hypogonadism renal insufficiency and derangements of the parathyroid-calcium-vitamin D and the pituitary-gonadal axes (Figure 1) [24, 25]. Opiate use has also been associated with altered bone metabolism and reduced trabecular bone mass, attributable, at least in part, to gonadal deficiency [26, 27].

Beyond these attributable causes, chronic HIV infection itself, HAART, and HCV co-infection could also be associated with an increased risk of low BMD:

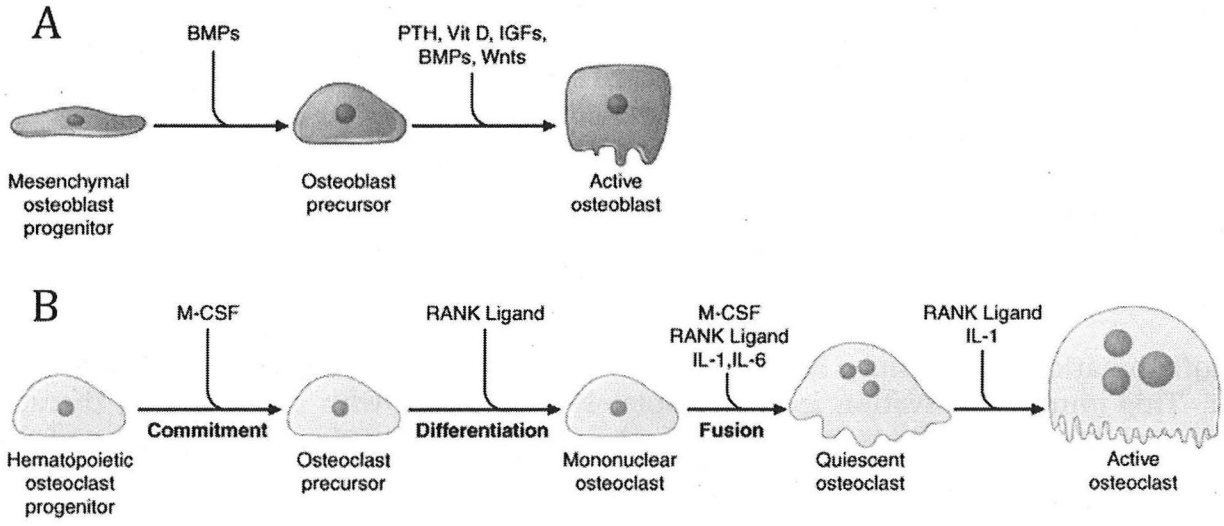
HIV infection and antiretroviral therapy: HIV infection itself may increase the risk of bone loss through enhanced osteoclastic activity due to chronic T cell activation and production of pro-inflammatory cytokines such TNF-alpha and IL-6 [28, 29]. Specific steps in bone formation and/or resorption depicted in figure 2 that have been shown to be impacted by HIV or antiretroviral factors to date include the following:

1. HIV-infected post-menopausal women, particularly those receiving ART, have significantly higher levels of serum TNF-a, N-telopeptide, and C-telopeptide than HIV-negative women [30].
2. HAART leads to increased levels of the N-terminal propeptide of type-I procollagen (PINP) and bone-specific alkaline phosphatase (BALP), reflecting increased bone turnover [31].
3. HIV gp120 upregulates the expression of the receptor of activated NFκB ligand (RANKL) in PBMCs leading to increased osteoclastic activity [28]
4. Increased osteoclastic activity is stimulated by the protease inhibitors ritonavir, nelfinavir, indinavir, and saquinavir but not by lopinavir or amprenavir in a rat model [28, 32].
5. Inhibition of osteoclast differentiation by ritonavir may occur via blockade of RANKL-induced downstream signaling [33].

Increased osteoclast formation by zidovudine in vitro may occur by upregulation of NF-Kβ downstream of RANKL. In mice treated with zidovudine, increased osteoclastogenesis led to decreased BMD [34]. *In vitro* and animal model studies showed that selected (mostly older) protease inhibitors (PI) were associated with increased osteoclast activity [32, 35], and altered vitamin D metabolism leading to a decreased macrophage-based calcitriol activity [36]. There were significant differences among PIs on their effect on bone metabolism. In older studies of untreated HIV subjects with advanced disease, bone resorption markers were elevated and osteocalcin (bone formation marker) levels suppressed, suggesting that normally tightly regulated processes of formation and resorption were "uncoupled" [37]. After ART initiation, markers of bone formation and resorption become correlated [37], although resorption markers are typically elevated [38, 39]. A recent cross-sectional study results was generally consistent with these data [30].

Cross-sectional studies controlling for "traditional" risk factors for osteoporosis do not consistently show an association between low BMD and receipt of treatment with protease inhibitors (PIs) or HAART in general [21, 24, 40]. Individual antiretroviral drugs have actually been found to be protective of bone loss [41, 42]. Longitudinal studies have shown either no changes in BMD associated with cumulative PI, NNRTI or NRTI use [43], or a modest initial change that does not extend beyond the initial period after ART initiation [20]. The clinical impact of these changes in BMD on increased risk of osteoporotic fractures is uncertain.

Furthermore, newer PIs appear to have more favorable metabolic profiles, and *ex vivo* studies suggest that they also might have a more favorable impact on bone health [32]. Longitudinal analysis of the impact of individual antiretrovirals on fracture risk in a large cohort has never been conducted.



Hormones, cytokines, and growth factors that control cell proliferation and differentiation are shown above the arrows. Vit D, vitamin D; IGFs, insulin-like growth factors; M-CSF, macrophage colony-stimulating factor; RANK ligand, receptor activator of NFκB ligand; IL-1, interleukin-1; IL-6, interleukin-6.

Figure 2: Pathways regulating development of (*A*) osteoblasts and (*B*) osteoclasts.

Hypothalamic-pituitary-gonadal (HPG) axis: Low testosterone, a major risk factor for male osteoporosis, is one of the most common endocrine abnormalities observed in men with HIV infection [44]. Conversely, estrone levels are significantly lower in post-menopausal HIV-infected women compared to their non-HIV infected counterparts [30]. At the cellular level, hypogonadism leads to osteoporosis due to upregulation of pro-resorptive cytokines [45] and receptor activator of NFκB ligand (RANKL) [46], and suppression of the bone protective factor osteoprotegerin [47].

Parathyroid-calcium-vitamin D: A large proportion of HIV-infected patients are vitamin D-deficient [39, 43]. Vitamin D deficiency (25-OH-D deficiency less than 30 nmol/l) has been found to be more prevalent among the HIV-infected patients [48], and among HCV-infected patients [49]. Furthermore, HIV+/HCV+ have been found to have higher lower 1,25 (OH)2D levels than HIV+/HCV- patients. Risk factors include HAART therapy [48], liver disease, poor sun exposure and malnutrition. Furthermore, impaired parathyroid secretion and resistance to PTH action have been reported in patients with HIV infection [50, 51].

Chronic kidney disease (CKD): Human immunodeficiency virus-associated nephropathy (HIVAN) and other glomerular lesions (e.g., immunoglobulin A nephropathy and immune complex glomerulonephritis) are frequent complications of HIV infection [52]. The prevalence and epidemiology of these renal lesions remain largely undefined; however, most studies agree that black race is a major risk factor for HIVAN. We have found rates of CKD (glomerular filtration rate [GFR] below 60 cc/min) of greater than 10% in the HAART era, in a previous analysis of the VHA database [53]. Once the GFR falls below 60 cc/min, bone disease is seen in virtually all CKD patients [54]. This condition, previously known as renal osteodystrophy and recently redefined as chronic kidney disease–mineral and bone disorder [55] is multifactorial in etiology.

III. Association of Osteoporosis with other Non-AIDS Morbidities: Role of Inflammation

Chronic inflammation and immune activation is now recognized as a hallmark of chronic HIV infection. This immune activation is not completely normalized with antiretroviral therapy and has been shown to contribute to the increased risk of cardiovascular disease [56], and the association between chronic inflammatory conditions and osteoporosis is well documented. Activated T cells produce the key mediator of osteoclast activity, RANKL. Levels of inflammatory cytokines such as interleukin-1 (IL-1), IL-6 and tumor necrosis factor alpha (TNFα) are increased among HIV-infected patients, and these cytokines stimulate bone resorption [57] and increased cardiovascular risk [58]. Furthermore, inflammatory markers were strongly related to all-cause mortality among HIV-infected patients [59].

Given that some of the potential mechanisms through which HIV disease and HAART might alter bone metabolism could also mediate other metabolic complications [32], a correlation of decreased BMD and other HIV and HAART-associated metabolic complications has been postulated [18, 31]. In the general population, a connection between osteoporosis and a self-reported history of myocardial infection has been found in the cross-sectional analysis of NHANES III [60]. Data from small studies of HIV patients have shown associations between decreased BMD and dyslipidemia and lipodystrophy [61, 62], and lactic acidosis [63]. More recently, an Italian study found a link between coronary artery calcification – a cardiovascular disease marker – and decreased femoral BMD [64], and analysis of a 12-year prospective cohort of antiretroviral-treated patients in France found that osteopenia or osteoporosis were significant predictors of increased cardiovascular risk [65]. The association between osteoporosis or osteoporotic fractures and HAART-associated metabolic complications has never been explored in a large HIV cohort.

Finally, therapies that reduce the inflammatory response to HIV and decrease may warrant investigation as potentially reducing non-AIDS morbidity and mortality. It has recently been suggested in a small observation study that statins reduce all-cause mortality in HIV patients on stable HAART [66]. The impact of statin administration on the morbidity and mortality from non-AIDS complications has also never been explored in a large HIV cohort.

IV. Rates, Determinants and Outcomes of Osteoporotic Fractures among HIV-infected Patients

In non-HIV-infected individuals, the risk of osteoporotic fractures is significantly predicted by low bone mineral density and increased bone turnover [67] (figure 3). The increased prevalence of osteopenia and osteoporosis among HIV-infected patients has been described above. Furthermore the HIV-infected population is aging and the impact of decreased BMD on fracture risk dramatically increases with age [68]. It is therefore expected that HIV-infected patients would have higher rates of osteoporotic fractures and that these rates are expected to further increase significantly in the next few years. Furthermore, the risk of osteoporotic fractures would be expected to be even higher among HIV/HCV co-infected patients than among HIV mono-infected patients.

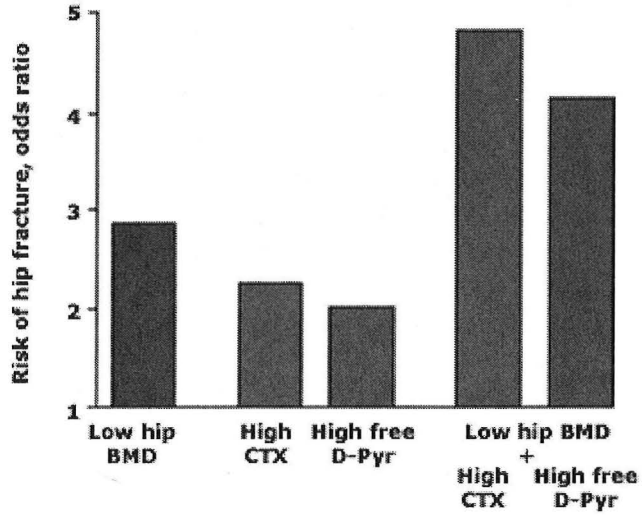


Figure 3: Interaction of low bone mineral density and increased bone turnover in predicting fracture risk.

In women over age 75 years followed prospectively, the odds ratio for hip fracture was increased 2.7-fold in those with a 1 standard deviation reduction in hip bone mineral density (BMD) but normal markers for bone turnover (first column), approximately 2-fold in those with normal BMD but a value for urinary C-terminal collagen crosslink excretion (CTX) or free deoxypyridinoline excretion (D/Pyr) above the premenopausal range (second and third columns), and 4.5-fold when both risk factors were present (last two columns).

The clinical impact of decreased BMD on fracture risk of HIV-infected patients is just beginning to be evaluated. In the ANRS CO8 APROCO-COPILOTE cohort of patients treated with combination antiretroviral therapy since 1997–1999, the incidence density of bone fractures was 3.3 for 1000 patient-years [95% confidence interval (CI) = 2.0–4.6] [69]. A relatively large population-based study compared the rates of fractures among 8525 HIV-infected and 2,208,792 non-HIV-infected who received care from 2002 to 2008 [70]. The authors calculated the period prevalence of fractures from 1996 to 2008 [70]. The overall fracture prevalence was 2.87 vs. 1.77 patients with fractures per 100 persons in HIV-infected, compared with non-HIV-infected patients (P<0.0001).

While this cohort compared rates between HIV-infected and HIV-uninfected patients, these were not comparable populations. Other significant limitations of this analysis include the evaluation of prevalence instead of incidence of fractures, the inability to investigate the role of potential covariates: antiretroviral medication use, smoking, alcohol use, body mass index, socioeconomic status, or medications affecting bone metabolism such as estrogens or steroids. Also, fracture incidence was not calculated. An analysis of Veterans Aging Cohort Study Virtual Cohort (VACS-VC) – 119,318 men, 33% of whom were HIV infected – found that HIV was associated with only a modest increase in risk of osteoporotic fractures after controlling for demographics and other established risk factors. [HR: 1.24 (95% CI: 1.11, 1.39)]. Adjusting for BMI attenuated this association even further [HR: 1.10 (95% CI: 0.97, 1.25)] [71].

Both cohorts evaluated patients only in the HAART era. This prevents an evaluation of a potential pre-HAART to HAART era trends, and impact of changes in antiretroviral drugs and regimens. Also the impact of cumulative exposure to antiretroviral therapy was not explored.

a. VA cohort analysis: Rates of osteoporotic fractures among HIV patients: pre-HAART and HAART eras

Using the Veterans Health Administration (VHA)'s Clinical Case Registry (CCR) spanning a 25-year period including the pre-HAART and HAART eras – from 1984 to 2009 – we examined the rates and determinants of osteoporotic fractures among HIV-infected patients, focusing on the associations of cumulative exposure to different antiretroviral drugs on fracture risk. Osteoporotic fractures were defined as incident vertebral, hip and wrist fractures (selected on the basis of their likelihood of being related to osteoporosis) [72].

We identified 56,660 patients who used VHA services for HIV disease during the study period and were included in CCR, including 22,005 who had clinic/outpatient or inpatient discharge data within the last twelve months of observation (1 January 2009 – 31 December 2009). The total person-years of observation was 305,237 (median: 5.4 person-years per patient). The "traditional" fracture risk factors evaluated included age, gender, race, smoking status, diabetes, CKD and HCV co-infection. Apart from female gender, all risk factors are significantly more prevalent among patients with OF than among patients without OF events. Patients with OF had a higher median age than those without (46 years vs. 44), were more likely to be white (57% vs. 45% of those without OF), smokers (56% vs. 32%), to have diabetes (25% vs. 15%), a BMI below 20 (49% vs. 33%) and have HCV co-infection (51% vs. 31%) ($p < 0.0001$ for all comparisons).

A total of 951 individual patients sustained at least one OF during the period of observation (124 vertebral, 486 wrist and 341 hip). Rates of osteoporotic fractures by age-group per 1000 patient-years are presented in figure 1. Rates for both hip and vertebral fractures increased progressively from the 18-29 years age group (0.02 and 0.00 respectively) to the 70+ years age group (4.49 and 1.65 respectively). The rates of wrist fractures increased progressively from the 18-29 years age group (0.11 per 1000 patient-years) to the 50-59 years age group (2.41), then declined in the later age-groups (1.64 for the 60-69 years and 1.20 for the 70+ years).

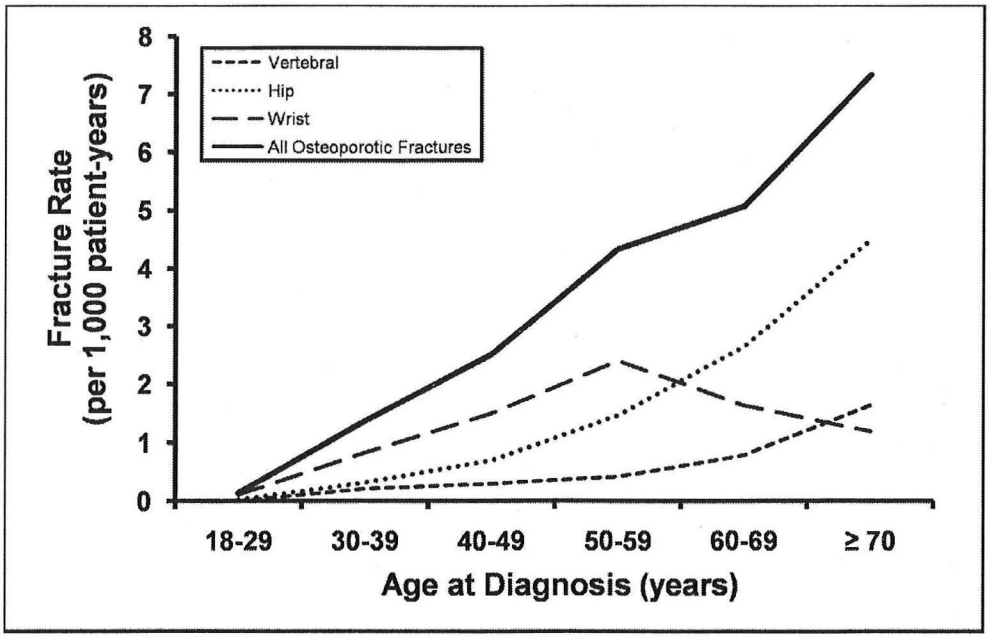


Figure 4: Rates of Osteoporotic Fractures by Age Group

b. Impact of type and duration of antiretroviral therapy on osteoporotic fractures in HIV

While HIV infection itself has adverse skeletal effects, HAART may also contribute to accelerated bone loss [17, 19, 21, 22]. Previous studies have suggested that antiretroviral drugs differ in their impact on bone health, with tenofovir (TDF) use being associated with a greater decline in BMD. TDF has been found to be associated with a greater decline in BMD than stavudine (d4T) [20] or abacavir (ABC) [73]. Prophylactic use of TDF has also been shown to cause a small but significant decline in BMD in HIV-uninfected subjects [74]. Also, earlier studies had suggested that exposure to PIs decreased BMD [17, 24], and it has been recently suggested that atazanavir (ATZ) is associated with increased risk of osteoporosis, compared to efavirenz (EFV) [73]. Antiretroviral initiation has also been shown to be associated with a rapid and significant increase in levels of serologic markers of increased bone turnover (which might signify increased bone fragility) [75-77]. Although these findings have raised concern for increased risk for osteoporotic fractures (OF), there has never been an evaluation of the OF risk associated with cumulative exposure to TDF and other antiretroviral drugs.

In our cohort, we first explored the association of the cumulative exposure to any antiretroviral drug (ARV) use on incident OF. Unadjusted and adjusted hazard ratio (HR) for OF associated with cumulative exposure to antiretroviral therapy and traditional osteoporotic risk factors are presented in table 1. After controlling for other risk factors, ART use was not independently associated with increased risk of OF.

Factors	**Hazard Ratio (95% Confidence Interval; p value)**	
	Univariate Analysis	Multi-variable Analysis
Cumulative ART Use (per year)	1.05 (1.01 – 1.10; p=0.02)	0.99 (0.95 – 1.04; p=0.77)
CKD (eGFR <60)	1.48 (1.04 – 2.09; p=0.03)	1.05 (0.72 – 1.53; p = 0.79)
White Race	1.76 (1.46 – 2.13; p < 0.0001)	1.88 (1.54 – 2.30; p< 0.0001)
Age (per 10 year increase)	1.51 (1.39 – 1.63; p <0.0001)	1.50 (1.37 – 1.64; p< 0.0001)
Tobacco Use	1.25 (1.06 – 1.47; p=0.01)	1.31 (1.09 – 1.56; p=0.003)
Diabetes	1.27 (1.05 – 1.53; p=0.01)	1.10 (0.90 – 1.34; p=0.34)
BMI < 20	1.61 (1.29 – 2.00; p<0.0001)	1.48 (1.18 – 1.87; p=0.007)
HCV Co-infection	1.43 (1.21 – 1.69; p<0.0001)	1.49 (1.25 – 1.77; p< 0.0001)

Table 1: Factors predicting osteoporotic fracture among HIV patients

Patient-days of antiretroviral (ARV) use prior to OF event were then calculated separately for each ARV, and survival analyses done to predict new OF. Categories explored were cumulative exposure to ART containing: tenofovir (TDF), abacavir (ABC), zidovudine or stavudine (AZT/D4T) any ritonavir-boosted protease inhibitors (rPI), and non-nucleoside reverse transcriptase inhibitors (NNRTI). Two multivariable models were constructed to examine the association of these ARV exposures: model 1, Controlling for age, race, tobacco use, diabetes, CKD, HCV and BMI; and model 2, controlling model 1 variables and concomitant exposure to other ARVs. Gender was not included in the model since over 98% of the study population is male. Statistical significance was declared at $p<0.05$.

Two separate analyses were conducted using these survival models: for the entire study population (enrolled from 1988 to 2009), and only for patients entering the cohort in the HAART era (since January 1st, 1996).

Cumulative ARV use and risk of osteoporotic fractures: 1988 – 2009

Table 2 presents person-years of exposure to different antiretroviral drugs and drug classes, as well as HR for OF associated with each one in univariate and multivariate models described above.

Drug or Drug Class	**PY of Exposure**	**Hazard Ratio per Year of Exposure (95% Confidence Interval; p value)**		
		Univariate Analysis	Multi-variable Model 1*	Multi-variable Model 2**
Tenofovir (TDF)	46,062	1.08 (1.02-1.15; <0.001) $	1.06 (0.99-1.12; 0.079)	1.06 (0.99-1.14; 0.106)
Abacavir (ABC)	24,251	0.99 (0.93-1.05; 0.989)	0.96 (0.90-1.03; 0.245)	0.96 (0.90-1.03; 0.224)
Thymidines (AZT or D4T)	94,595	1.02 (0.99-1.05; 0.199)	0.96 (0.95-1.02; 0.311)	0.99 (0.95-1.02; 0.520)
Boosted PI (rPI)	41,336	1.06 (1.01-1.12; 0.015) $	1.04 (0.99-1.10; 0.142)	1.03 (0.97-1.09; 0.349)
NNRTI	59,857	0.99 (0.95-1.03; 0.655)	0.96 (0.92-1.01; 0.094)	0.96 (0.92-1.01; 0.112)

*: Controlling for CKD, age, race, tobacco use, diabetes and BMI;
**: Controlling for Model 1 variables + concomitant exposure to other ARVs.
$: Statistically significant associations (p ,0.05)

Table 2: Antiretroviral Exposure and Risk of Osteoporotic Fractures: 1988-2009

TDF exposure was associated with a yearly HR for OF of 1.08 (95% CI: 1.02 – 1.15; p <0.001) in UV model, 1.06 (0.99 – 1.12; p=0.079) in MV1 and 1.06 (0.99 – 1.14; p=0.106) in MV2. Boosted PI exposure was associated with HR of 1.06 (1.01 – 1.12; p=0.015) in UV model, 1.04 (0.99 – 1.10; p=0.142) in MV1 and 1.03 (0.97 – 1.09; p=0.349) in MV2. Exposure to ABC, AZT/D4T or NNRTI were not significantly associated with increased risk of OF in UV or MV models.

Cumulative ARV use and risk of osteoporotic fractures in the HAART era: 1996 – 2009

As expected, the proportion of patients exposed to antiretroviral therapy was significantly higher among patients who entered the cohort in the HAART era (83.6%, compared to 69.4% in the entire cohort). The rate of osteoporotic fractures was significantly higher in the HAART era (4.09 events/1000 patient-years) compared to the pre-HAART era (1.61 events/1000 patient-years) (figure 5).

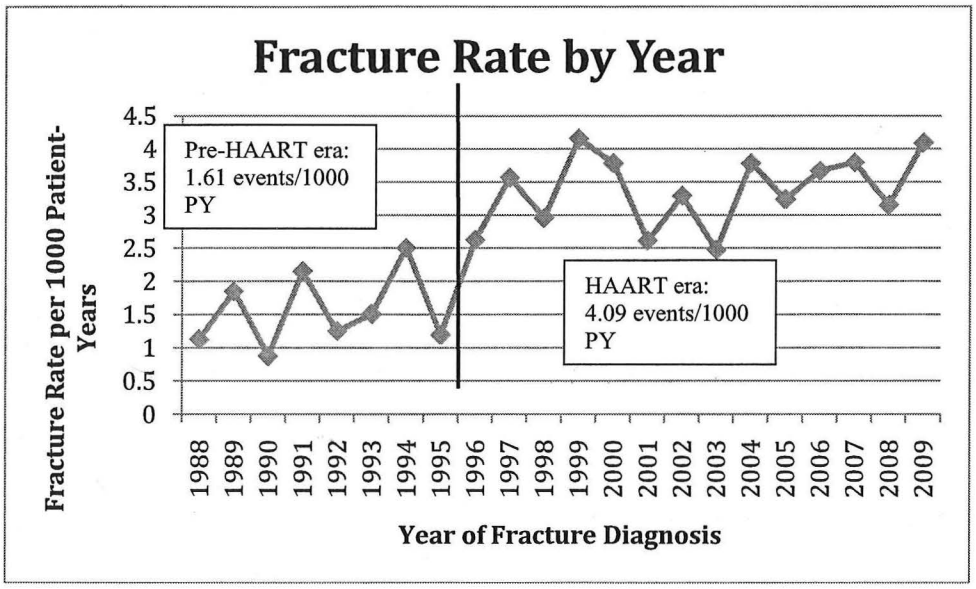


Figure 5: Fracture rates by year of diagnosis: Pre-HAART and HAART eras

In the HAART era, TDF exposure was associated with a yearly HR for OF of 1.16 (95% CI: 1.08 – 1.24; p <0.001) in UV model, 1.13 (1.05 – 1.21; p=0.001) in MV1 and 1.12 (1.03 – 1.21; p=0.011) in MV2. Boosted PI exposure was associated with HR of 1.11 (1.05 – 1.18; p=0.001) in UV model, 1.08 (1.01 – 1.15; p=0.026) in MV1 and 1.05 (0.97 – 1.13; p=0.237) in MV2. Exposure to ABC, AZT/D4T or NNRTI were again not significantly associated with increased risk of OF in UV or MV models.

In the HAART era, we also examined the OF risk associated with specific protease inhibitors with over 10,000 PY of exposure in the database: Nelfinavir (NFV), Indinavir (IDV), Atazanavir (ATV) and Lopinavir/ritonavir (LPV/r) in univariate and multivariate models 1 and 2 as above.

Drug or Drug Class	PY of Exposure	Hazard Ratio per Year of Exposure (95% Confidence Interval; p value)		
		Univariate Analysis	Multi-variable Model 1*	Multi-variable Model 2**
Tenofovir (TDF)	**38,009**	**1.16 (1.08-1.24; <0.0001) $**	**1.13 (1.05-1.21; 0.001) $**	**1.12 (1.03-1.21; 0.011) $**
Abacavir (ABC)	18,885	0.99 (0.92-1.07; 0.842)	0.96 (0.88-1.04; 0.313)	0.95 (0.87 -1.03; 0.194)
Thymidines (AZT or D4T)	68,376	1.02 (0.97-1.06; 0.489)	0.98 (0.93-1.02; 0.289)	0.99 (0.94-1.04; 0.600)
boosted PI (rPI)	32,109	1.11 (1.05-1.18; 0.001) $	1.08 (1.01-1.15; 0.026) $	1.05 (0.97-1.13; 0.237)
NNRTI	48,943	1.01 (0.96-1.06; 0.771)	0.98 (0.93-1.03; 0.409)	0.98 (0.92-1.03; 0.386)

*: Controlling for CKD, age, race, tobacco use, diabetes and BMI;
**: Controlling for Model 1 variables + concomitant exposure to other ARVs.
$: Statistically significant associations (p ,0.05)

Table 3: Antiretroviral Exposure and Risk of Osteoporotic Fractures: HAART era (1996-2009)

Since exposure to TDF and rPI were the only drug or drug classes significantly associated with increased OF risk in univariate analysis, we then evaluated the effect of cumulative exposure to both TDF and rPI, and that of exposure to individual rPIs.

We determined four different exposure categories: 1) exposure to neither TDF nor rPI (referent category); 2) exposure to TDF, but not rPI; 3) exposure to rPI, but not TDF; and 4) concomitant exposure to TDF and rPI. Concomitant exposure to both TDF and rPI associated with a greater OF risk (HR: 1.16; CI: 1.04 – 1.30) than exposure to either TDF without rPI (HR: 1.11; CI: 1.01 – 1.21) or rPI without TDF (HR: 1.10; CI: 1.01 – 1.22).

Regarding exposure to individual PIs, we selected those with the highest PY of exposure in the database: Indinavir (IDV; 12,124 PY), Atazanavir (ATV; 12,685 PY), Nelfinavir (NFV; 14,356 PY) and Lopinavir/ritonavir (LPV/RTV; 15,3190 PY). Only LPV/RTV was associated with significantly increased OF risk in UV model (HR: 1.17; CI: 1.08 – 1.26; p<0.0001). The association remained significant in MV1 (HR: 1.13; CI: 1.04 – 1.22; p=0.005) and barely in MV2 (HR: 1.09; HR: 1.00 – 1.20; p=0.051). Exposure to NFV, IDV and ATV (boosted or unboosted) were not associated with significantly increased OF risk (table 4).

Drug	PY of Exposure	Hazard Ratio per Year of Exposure (95% Confidence Interval; p value)		
		Univariate Analysis	Multi-variable Model 1*	Multi-variable Model 2**
IDV	12,124	1.00 (0.93 - 1.07; 0.947)	0.98 (0.91 - 1.05; 0.579)	0.99 (0.92 - 1.07; 0.755)
ATV	12,685	1.12 (0.98 - 1.27; 0.097)	1.08 (0.95 - 1.24; 0.233)	1.03 (0.89 - 1.18; 0.713)
NFV	14,356	1.00 (0.93 - 1.07; 0.977)	0.98 (0.91 -1.05; 0.509)	0.98 (0.91 - 1.05; 0.512)
LPV/RTV	**15,319**	**1.17 (1.08 - 1.26; <0.0001) $**	**1.13 (1.04 - 1.22; 0.005) $**	**1.09 (1.00 -1.20; 0.051)**
RTV	18,691	1.06 (0.97 - 1.15; 0.2)	1.04 (0.96 - 1.14; 0.349)	1.01 (0.92 - 1.11; 0.79)
ATV/RTV	9546	1.11 (0.95 - 1.31; 0.18)	1.08 (0.91 - 1.27; 0.378)	0.99 (0.84 - 1.18; 0.946)

*: Controlling for CKD, age, race, tobacco use, diabetes and BMI;
**: Controlling for Model 1 variables + concomitant exposure to other ARVs.
$: Statistically significant associations (p ,0.05)

Table 4: Exposure to Specific Protease Inhibitors and Risk of Osteoporotic Fractures: HAART era (1996-2009)

c. Morbidity and Mortality associated with osteoporotic fractures in HIV

Osteoporosis is associated with a significant morbidity and mortality, and healthcare costs [78] in the general population. A 50-year old woman's lifetime risk of dying from a hip fracture is equal to her lifetime risk of dying from breast cancer [79]. Furthermore, at any given age, mortality after osteoporotic fractures is even higher in men than in women [80, 81]. Given that gender difference in mortality associated with fractures, and given that most HIV-infected individuals are men (this is especially the case in the VA HIV population, over 98% of which is male), osteoporotic fractures could be a particularly important problem in the HIV population.

In our cohort, osteoporotic fractures in the HAART era were associated with a hazard ratio of death of 2.47. This increased mortality was no longer significant (HR: 0.89; p=0.155) after controlling for factors osteoporotic risk factors that were independently predictive of high mortality: HCV (HR: 1.23), CKD (HR: 1.95), Age (HR: 1.45 for each 10 year increase), DM (HR: 1.09), BMI<20 (HR: 2.36) and cumulative antiretroviral exposure, which was independently predictive of lower mortality (HR: 0.75).

Ongoing analyses will determine whether there is an association between osteoporotic fractures and cardiovascular outcomes, and other non-AIDS-defining chronic complications among HIV-infected patients.

V. Concluding Remarks and Future Directions

With the advent of HAART, there is increased survival and aging of the HIV population. This has resulted in increasing prevalence of age-related "non-AIDS" complications among HIV patients including non-AIDS malignancies, cardiovascular disease, and chronic liver disease (mostly HCV-related). The morbidity and mortality caused by metabolic bone disease is also beginning to be better understood. Several cohorts have previously reported a high prevalence of osteoporosis among HIV patients, but the clinical implications of this were unclear. Findings from our cohort and other recent data suggest an elevated risk of fracture among HIV patients. This risk might be related to a higher incidence of osteoporotic risk factors as well as independent risk associated with HIV infection (likely due to chronic immune activation) and might be magnified by exposure to specific antiretroviral drugs.

Previous studies have suggested that antiretroviral drugs differ in their impact on bone health, with tenofovir use being associated with a greater decline in BMD. Antiretroviral regimens have also been shown to increase expression of markers of bone turnover. Our findings suggest for the first time that these associations of antiretroviral drugs with either decreased BMD or increased bone turnover markers might be reflected in increased risk of osteoporotic fractures. These findings, if confirmed, might warrant consideration of osteoporotic fracture risk in decisions in ART initiation among HIV-infected patients. Consistent with data from the general population OF were independently associated with advancing age, race other than Black, low BMI, and smoking in HIV-infected individuals.

While we found significant increase in fracture rates in the HAART era, cumulative ART exposure likely does not account for the increased risk. Greater fracture rates, higher (significant) HR for TDF and rPI in the HAART era could be due to longer survival, and exclusion of most patients with no ART, mono, or dual ART. Also, lower BMD was associated with controlled HIV replication in a recent study [82], and controlled viremia is much more likely in the HAART era.

a. Ongoing analyses

An estimated 15 to 30% of HIV-infected patients are co-infected with hepatitis C (HCV). We have shown that among HIV-infected patients, HCV is a significant risk factor for osteoporotic fractures. Likely mechanisms by which HCV infection induces increased bone turnover and decreased BMD include interference with vitamin D metabolism in the liver (25-hydroxylation of Vitamin D3), low hepatic IGF-1 production (inducing impaired osteoblastic proliferation & differentiation), and gonadal hormone deficiency (decreased RANKL/Osteoprotegrin ratio), and finally chronic inflammation (high levels of IL-1 and IL-6). [83, 84]. Furthermore, interestingly, HCV might be associated with a higher fracture risk regardless of its effect on BMD or BTMs [85].

Whether HCV independently predicts higher rate of bone turnover and increased risk of osteoporosis among HIV-infected patients and/or HIV-uninfected patients remains unknown. Among patients receiving care at the VA North Texas, we are currently recruiting cohorts of HIV/HCV co-infected patients (HIV+/HCV+) and cohorts of HIV mono-infected (HIV+/HCV-), HCV mono-infected (HCV+/HIV-), and uninfected (HIV-/HCV-). We will perform analysis of covariance (COANOVA) to compare mean values of BMD, BTMs, calciotropic and gonadal hormones, and inflammatory cytokines, between these groups. Various demographic, anthropometric, medical, and lifestyle variables will be included into the model to control for their confounding impact.

b. Prevention and treatment implications

Interventions used to mitigate osteoporotic fracture risk in the general population (calcium and vitamin D) have been showed to improve BMD of HIV-infected patients [86] and are advocated in HIV guidelines [87]. The expert review makes the point that HIV infection should be considered as a risk factor for bone disease. It recommends screening with DEXA scan patients with fragility fractures, all HIV-infected post-menopausal women, and all HIV-infected men >50 years of age. If the results of the test do not warrant medical treatment, the test should be repeated every 2–5 years, depending on the proximity to thresholds for therapy. DEXA scans in younger HIV-infected persons are probably not indicated, because the risk for fracture is low.

After evaluation and treatment of secondary causes of reduced BMD, the experts recommend pharmacologic treatment of osteoporosis for post-menopausal women and men >50 years with a T-score of the total hip, femoral neck, or lumbar spine less than or equal to −2.5 or in those with a history of fragility fracture, in accordance with the most recent guidelines from the National Osteoporosis Foundation. For those with osteopenia, the 10-year risk for both major osteoporotic fracture (hip, shoulder, wrist, and clinical vertebral combined) and hip fracture alone should be calculated, using the WHO Fracture Risk Assessment Tool (FRAX) *http://www.shef.ac.uk/FRAX/*. If the 10-year risk of all osteoporotic fracture is >20% or risk of hip fracture is >3% (the cost-effective threshold set in the United States), consideration should be given to starting pharmacologic therapy.

Future studies will need to determine whether these and/or changes in antiretroviral therapy will result in decreased risk of osteoporotic fractures. As a cautionary tale, recent data suggest that changes in BMD by such interventions are very poorly predictive of osteoporotic risk in the general population [88].

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