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Antiretrovirals, fractures and osteonecrosis in a large international HIV cohort

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Abstract:	Background: Antiretrovirals (ARVs) affect bone density and turnover, but their effect on risk of fractures and osteonecrosis of the femoral head is less understood. We investigated if exposure to ARVs increases the risk of both bone outcomes. Methods: EuroSIDA participants were followed to assess fractures and osteonecrosis. Poisson regression identified clinical, laboratory and demographic predictors of either bone outcome. Ever, current and cumulative exposures to ARVs were assessed. . Results: During 86118 PYFU among 11820 included persons (median age 41y, 75% male, median baseline CD4 440/mm3, 70.4% virologically suppressed), there were 619 fractures (incidence/1000PYFU 7.2; 95%CI 6.6-7.7) and 89 osteonecrosis (1.0;0.8-1.3). Older age, white race, lower BMI, IV drug use, lower baseline CD4, HCV-coinfection, prior osteonecrosis, prior fracture, cardiovascular disease and recent non-AIDS cancer (last 12 months) were associated with fractures. After adjustment, persons who had ever used Tenofovir Disoproxil Fumarate (TDF) (1.40; 1.15-1.70) or who were currently on TDF (1.25; 1.05-1.49) had higher incidence of fractures. There was no association between cumulative exposure to TDF and fractures (1.08/5y exposure; 0.94-1.25). No other ARV was associated with fractures (all p>0.1). Risk of osteonecrosis was associated with white race, lower nadir CD4, prior osteonecrosis, prior fracture and prior AIDS. After mutual adjustment, no ARV was associated with osteonecrosis. Conclusions: In HIV infection, host factors, HIV-specific variables and co-morbidities contribute to risk of fractures and osteonecrosis. Exposure to TDF, but not other ARVs, was an independent risk factor for fractures.

Authors' response: We would like to thank the editor and reviewers for taking the time to read and comment on our paper. Your insightful comments and suggestions have improved our paper and are greatly appreciated.

Please note that the font and color of supplemental figures 1 and 2, as well as Figure 2 and 4, have been formatted to match those of the remaining figures. No change in content has been made.

Reviewer #1:

"Antiretrovirals, fractures, and osteonecrosis in a large international HIV cohort" evaluates the rates and risk factors for fractures and osteonecrosis in the EuroSIDA cohort. The manuscript is well-written and important to the field given the large population studied and solid methods employed. I only have minor comments.

1) In the methods section, the text refers to baseline CD4 and viral load. For me, this would refer to pre-ART values. However, in Supplemental Figure 1, it seems these values refer to baseline at around 1/1/2004 when bone data began to be routinely collected in EuroSIDA rather than pre-ART. This could be clarified in the text.

Authors' response: In the present study, we included persons aged >16 year with baseline data on CD4 counts and viral loads with prospective follow up. Baseline was defined as 1/1/2004, when routine prospective collection of data on fractures and osteonecrosis of the femoral head was initiated in EuroSIDA. This has been clarified in the methods section.

In Table 1, as well as in supplemental table 1, the characteristics listed refer to the last visit for those with no bone event, or at last diagnosis of fracture or osteonecrosis. We believe that providing information at last visit is more clinically meaningful than listing variables measured at baseline, i.e. 1/1/2004.

2) In Table 1, I would list the other race/ethnicities in the cohort.

Authors' response: the n(%) of participants with other race/ethnicities is now listed in Table 1.

3) The ordering and naming of variables in the various tables is not consistent. The ordering and naming of variables should match the ordering and naming of the variables in Table 1 (e.g., age should not be listed almost last in Supplemental Table 1).

Authors' response: the ordering of variables in Supplemental Table 1 is now made consistent with Table 1.

Supplemental Tables 2 and 3 list the variables entered into the multivariate models by predefined criteria. As such, the variables included in the final model for fractures are different from the variables included in the final model for osteonecrosis of the femoral head. We have now listed the variables in common in the same order.

4) It appears that risk group is associated with osteoporotic fracture according to Supplemental Table 1 but this is not listed in the text.

Authors' response: the p-value shown in supplemental table 1 refers to a global comparison across the different transmission risk groups. Despite the statistical significance, the frequencies of risk groups are not very different between participants with non-osteoporotic and osteoporotic fractures. We were unsure as to whether this was clinically relevant but have replaced, on the reviewer's request, the statement "At last visit, participants with osteoporotic fractures were more likely to be older and to come from North Europe compared to participants with other fractures, but had otherwise similar demographic and clinical characteristics" with "At last visit, participants with osteoporotic fractures were more likely to be older, have sexually-acquired HIV and to come from North Europe compared to participants with other fractures, but had otherwise similar demographic and clinical characteristics".

Supplemental Table 1: Participant characteristics at last visit: non-osteoporotic versus osteoporotic fractures

		Non-osteoporotic	Osteoporotic	P-value
Transmission risk	**MSM**	**170 (35.0)**	**52 (39.4)**	**0.030**
	IV drug use	**141 (29.0)**	**27 (20.5)**	
	Heterosexual	**131 (27.0)**	**47 (35.6)**	

5) "Data" is a plural word. Should read "there were not sufficient data" at end of page 8.

Authors' response: "There was not sufficient data" has been replaced with "There were not sufficient data" on page 8.

6) It could be considered surprising that past TDF was associated with fracture in this study as studies in which individuals were switched off of TDF to other ARVs had significant increases in BMD. This finding is especially important now given that many individuals on TDF are now being switched to TAF. It might be good to highlight the results of switch studies off of TDF and how this study's results data suggest that there may still be residual bone abnormalities not fully captured by BMD data after the switch off of TDF.

Authors' response: Our data demonstrated that past and current exposure to TDF, but not cumulative exposure, is associated with fracture risk. We can confirm that participants with recent TDF discontinuations were not like current TDF users. Misclassification, therefore, is not a likely explanation. As shown in the Table below, the median (IQR) time since TDF discontinuation among those that have ever started TDF was shorter among those with bone outcomes when compared to those with no bone event. Only 46 fractures were reported among participants who had stopped TDF within one year before the occurrence of fractures.

The reviewer's point about treatment switch is important because it is known that discontinuation of TDF leads to increase in BMD that takes at least 1 year (switch to TAF and raltegravir data). Unfortunately, there are few participants currently using TAF and raltegravir-based regimens in EuroSIDA. Therefore, as mentioned in the discussion, we are underpowered to investigate this. We are very wary of over-interpreting our data and believe that a discussion on the effects of treatment swift on fracture/osteonecrosis risk would be beyond the scope of our investigation.

Variable	No bone event	Fractures	Osteonecrosis of the femoral head	P-value*
Ever on TDF; n (%)	7635 (64.1)	320 (64.7)	51 (69.9)	0.38
On TDF; n (%)	5492 (46.5)	241 (48.7)	37 (50.7)	0.49
Stopped TDF last 12 months; n(%)	1703 (22.3)	46 (14.4)	8 (15.7)	0.0020
Cumulative exposure TDF; median years (IQR)	2.4 (0.0 – 6.3)	1.4 (0.0 – 4.3)	1.6 (0.0 – 4.6)	<0.0001
Cumulative exposure TDF among those ever started; median years (IQR)	5.2 (2.7 – 7.8)	3.5 (1.6 – 5.3)	3.5 (1.4 – 6.6)	<0.0001
Time since stopping among those that have started; median years (IQR)	3.2 (1.3 – 5.8)	1.6 (0.4 – 3.3)	1.4 (0.5 – 4.0)	<0.0001

Reviewer #2:

Only minor edits would be formatting of Table 1 cells, and changing "homosexual" to MSM in Table.

Authors' response: "Homosexual" has been replaced with "MSM" in Table 1.

However, given sex differences in osteoporosis and fracture risk, particularly at median age 47, Sex-stratified analyses should be conducted as well. If the findings are not

substantially different between men and women, this could be simply stated in the results section, however these additional analyses should be performed.

Authors' response: Approximately 20% of EuroSIDA participants are female. As a result, power for sex-stratified analyses was low, particularly with respect to osteonecrosis of the femoral head. We ran each model by gender (as described in Supplemental tables 2 and 3), and compared the adjusted incidence rate ratios (aIRR) across gender. To reduce the impact of multiple testing, and mindful of the fact that this analysis was not planned a priori, we formally tested the interaction where there was a ≥2-fold difference in aIRRs for a given variable when comparing males and females. P-values for all interactions tested were not significant (i.e. > 0.05). Therefore, findings were not substantially different between men and women. This has been included in the results section.

Title: Antiretrovirals, fractures and osteonecrosis in a large international HIV cohort

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Key words: fractures, osteonecrosis, avascular necrosis, bone, HIV

Running title: Fractures and osteonecrosis during HIV

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Summary: We investigated the association of exposure to antiretroviral drugs with incident fractures and osteonecrosis of the femoral head in the EuroSIDA cohort. We demonstrated that past and current exposure to tenofovir disoproxil fumarate, but no other antiretroviral, was independently associated with higher incidence of fractures. After mutual adjustment, no antiretroviral was associated with osteonecrosis risk.

Abstract

Background: Antiretrovirals (ARVs) affect bone density and turnover, but their effect on risk of fractures and osteonecrosis of the femoral head is less understood. We investigated if exposure to ARVs increases the risk of both bone outcomes.

Methods: EuroSIDA participants were followed to assess fractures and osteonecrosis. Poisson regression identified clinical, laboratory and demographic predictors of either bone outcome. Ever, current and cumulative exposures to ARVs were assessed. .

Results: During 86118 PYFU among 11820 included persons (median age 41y, 75% male, median baseline CD4 440/mm^3, 70.4% virologically suppressed), there were 619 fractures (incidence/1000PYFU 7.2; 95%CI 6.6-7.7) and 89 osteonecrosis (1.0;0.8-1.3). Older age, white race, lower BMI, IV drug use, lower baseline CD4, HCV-coinfection, prior osteonecrosis, prior fracture, cardiovascular disease and recent non-AIDS cancer (last 12 months) were associated with fractures. After adjustment, persons who had ever used Tenofovir Disoproxil Fumarate (TDF) (1.40; 1.15-1.70) or who were currently on TDF (1.25; 1.05-1.49) had higher incidence of fractures. There was no association between cumulative exposure to TDF and fractures (1.08/5y exposure; 0.94-1.25). No other ARV was associated with fractures (all p>0.1). Risk of osteonecrosis was associated with white race, lower nadir CD4, prior osteonecrosis, prior fracture and prior AIDS. After mutual adjustment, no ARV was associated with osteonecrosis.

Conclusions: In HIV infection, host factors, HIV-specific variables and co-morbidities contribute to risk of fractures and osteonecrosis. Exposure to TDF, but not other ARVs, was an independent risk factor for fractures.

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Introduction

Fractures and osteonecrosis of the femoral head have emerged as important manifestations of bone disease during treated HIV infection. According to population-based studies [1-3], HIV positive persons have a 1.5-3.0 greater risk of fractures than the general population. Osteonecrosis of the femoral head, although a rare bone disease, also disproportionally affects HIV positive persons. In the setting of HIV infection, it was estimated that osteonecrosis has a 100-fold excess risk when compared to the general population [4].

Initiation of antiretroviral therapy (ART) is associated with reduction in bone mineral density [5-7] and increases levels of markers of bone turnover [5,8]. The loss of bone mineral density following ART initiation is comparable in magnitude to that occurring during the perimenopausal period [9] and treatment with corticosteroids [10]. Changes in bone mineral density and turnover are observed irrespective of ART regimen, but tenofovir disoproxil fumarate (TDF)-containing regimens causes greater bone loss [6,11] whereas integrase inhibitor-based regimens may cause less bone loss [7]. However, the direct clinical consequences of this are yet to be determined because changes in bone mineral density are not perfect predictors of fracture risk [12] and the effect of ART exposure on the risk of fractures and osteonecrosis remain poorly understood. In a large HIV cohort, we set out to study the association of exposure to antiretroviral drugs with incident fractures and osteonecrosis of the femoral head and to determine factors independently associated with these two bone outcomes.

Methods

The EuroSIDA study, a multinational prospective cohort of 20,854 HIV positive persons from across Europe, Argentina and Israel, has been documented in detail elsewhere [13]. Briefly, detailed information on clinical, virological and immunological parameters has been collected every 6 months with accurate recording of dates of AIDS-defining illnesses and serious non-AIDS-defining events, defined as cardiovascular disease, end-stage hepatic/renal disease, non-AIDS defining malignancies and pancreatitis [14]. Data quality

is assured by yearly monitoring visits to participating clinics and hospitals, when source documentation and dates of reported clinical events are cross-checked. In this study, we included persons aged >16 year with baseline data on CD4 counts and viral loads with prospective follow up. Baseline was defined as 1/1/2004, when routine prospective collection of data on fractures and osteonecrosis of the femoral head was initiated in EuroSIDA. Data on fractures and osteonecrosis was reported in follow up forms by the attending physician. Participating sites reporting no cases of fractures or osteonecrosis or with less than 100 person-years of follow up (PYFU) were excluded due to concerns about underreporting and data quality on bone outcomes.

Poisson regression using generalized estimating equations was used to identify clinical, laboratory and demographic factors associated with fractures and osteonecrosis of the femoral head. More than one outcome per person was included accounting for correlation between and within included persons. Factors adjusted for were chosen on the basis of their epidemiological importance and biological plausibility and included baseline and time-updated variables, as defined in a previous report [14]. To avoid model overfitting, factors with marginal associations ($p<0.1$) in univariate analyses were included in multivariate models. Nadir, baseline, and current CD4 counts, baseline and current viral load, as well as age, were all investigated as categorical and continuous variables with different transformations to identify the best fitting model. Clinical events were modelled as time-updated covariates in different ways to identify the best fitting model, including non-malignant AIDS events, AIDS-defining cancer, non-AIDS-defining malignancies and cardiovascular disease, and each of these as a recent event, i.e. occurring within the last 12 months prior to the diagnosis of fracture or osteonecrosis.

Each antiretroviral was included in the best-fitting multivariate model to assess the effect of its exposure on the subsequent risk of fractures or osteonecrosis of the femoral head. Exposure to each antiretroviral was included as ever (yes/no), current (yes/no) and cumulative exposure. Antiretrovirals tested were zidovudine, didanosine, stavudine, lamivudine, emtricitabine, TDF, abacavir, nevirapine, efavirenz, saquinavir, ritonavir, lopinavir, indinavir, nelfinavir, atazanavir, ritonavir boosted lopinavir, and any other boosted protease inhibitors (grouped into one category). Antiretrovirals which remained independently associated with risk for fractures or osteonecrosis were subsequently

mutually adjusted for. It was not possible to assess other antiretrovirals such as integrase inhibitors owing to their short cumulative exposure among EuroSIDA participants.

We carried out secondary analyses restricted to osteoporotic fractures, grouped as fractures of the spine, arm, wrist and hip. Similar to other cohort studies without data on bone mineral density [15,16], our assumption was that fractures in these locations were more likely to be osteoporotic. Previous investigations reported an increased fracture risk among persons receiving TDF and boosted protease inhibitors concomitantly [16]. Therefore, we also investigated incidence rates of fractures according to three categories of current ART use: 1) current use of TDF but not boosted protease inhibitor; 2) current use of boosted protease inhibitor but not TDF; and 3) concomitant use of TDF and boosted protease inhibitor. Using category 3 as the comparator, we compared the incidence rate ratios of fractures across these 3 categories in models adjusted for demographics, HIV-specific variables and co-morbidities.

Finally, patients presenting with severe *Pneumocystis* pneumonia are likely to be treated with adjuvant corticosteroids. We examined the association between past history of *Pneumocystis* pneumonia, as a proxy of corticosteroid exposure, and subsequent risk for fractures and osteonecrosis.

Results

Of 20,854 EuroSIDA participants, 14,917 had prospective follow-up after baseline and were eligible for inclusion. An additional 1,902 participants were excluded due to missing CD4 counts and/or viral load at baseline. 1,195 persons were excluded from sites with less than 100 PYFU or where no fractures or osteonecrosis were reported. Thus, 11,820 participants were eligible for final inclusion in the analyses (Supplemental Figure 1).

The odds of being excluded because of missing CD4 and viral load were higher in all HIV exposure groups compared to MSM, in all regions of Europe (except North) compared to South, and in those with hepatitis C virus (HCV) infection at baseline. The odds of being excluded due to missing CD4 and viral load were lower in those who had started ART, were older, had a later date of recruitment to EuroSIDA and in those with a higher nadir CD4 count. Participants excluded from small study sites or with no reported events were more likely to be older, female, non-white, non-MSM, and to be on ART and have a later

date of enrolment to EuroSIDA. Table 1 summarizes participant characteristics at the time of diagnosis of fractures or osteonecrosis and at last visit for those without either outcome. There was a higher proportion of fractures and osteonecrosis among IV drug users and HCV coinfected persons. Persons with a bone outcome tended to have a lower current CD4 count than those without an event. Persons with osteonecrosis had significantly higher levels of triglycerides. There were no differences between participants with and without a bone outcome in terms of chronic kidney disease, estimated glomerular filtration rate (eGFR) and vitamin D levels (Table 1).

Among 11,820 included participants during 86,118 PYFU, 496 persons had 619 incident fractures (incidence rate [IR]/1,000 PYFU, 95% confidence interval [CI]: 7.2, 6.6-7.7) and 73 persons developed 89 cases of osteonecrosis of the femoral head (IR: 1.0, 0.8-1.3) (Supplemental Figure 1).

Considering fractures with a known site, the most frequent were fractures of the leg, followed by arm, ribs and foot. 132 fractures (21.3%) were classified as osteoporotic (Supplemental Figure 2). At last visit, participants with osteoporotic fractures were more likely to be older, have sexually-acquired HIV and to come from North Europe compared to participants with other fractures, but had otherwise similar demographic and clinical characteristics (Supplemental Table 1). Information regarding fracture site could not be ascertained for 202 fractures (32.6%). Participants with fractures of unknown sites were more likely to be female, to be ART naïve at study entry, have a prior AIDS diagnosis and have a higher current CD4 count when compared to participants with known fracture sites.

In analyses stratified by time-updated CD4 count, the crude incidence of fractures decreased as CD4 counts increased (P<0.0001). On the other hand, the incidence of osteonecrosis did not vary significantly across different CD4 count strata (P=0.055).

Univariate and multivariate associations between covariates and risk for fractures and osteonecrosis are detailed in supplemental Tables 2 and 3. After adjustment, an increased risk of incident fractures was independently associated with older age, lower BMI, IV drug use, HCV co-infection, prior fracture, prior osteonecrosis of the femoral head, a recent non-AIDS-defining malignancy and recent cardiovascular disease (Figure 1). Another independent predictor of fractures was origin, with participants from Northern and Central Europe at significantly increased risk (Supplemental Table 2). Race other than white and a

higher current CD4 count were associated with lower fracture risk (Figure 1). Findings were not substantially different between men and women (data not shown).

With respect to osteonecrosis of the femoral head, a prior history of osteonecrosis, fractures and AIDS-defining illnesses (both opportunistic infections and AIDS-defining cancers) was independently associated with increased risk (Figure 1). Race other than white and a higher baseline CD4 count were independently associated with a lower risk for osteonecrosis (Figure 1)

In univariate analyses, ever, current and cumulative exposure to TDF was associated with increased fracture risk. Crude incidence rates are depicted in Figure 2. Whether modelled as ever, current or cumulative, no association between exposure to any of the other investigated antiretrovirals and fracture risk was observed (data not shown). After adjustment (Figure 3), persons who had ever used TDF (adjusted incidence rate ratio [aIRR], 95% CI: 1.40, 1.15-1.70 p=0.0008) or who were currently receiving TDF (aIRR 1.25, 1.05-1.49, p=0.012) had a significantly increased incidence of fractures. The association of fracture risk with cumulative exposure to TDF did not retain statistical significance after adjustment (aIRR 1.08, 0.94-1.25, per 5 years of additional exposure, p=0.27). In analyses restricted to osteoporotic fractures, no significant association was seen with ever, current or cumulative exposure to TDF (Figure 3).

In adjusted analyses to investigate incidence rate ratios of fractures for categories of current ART use stratified according to TDF and boosted protease inhibitor, we found no difference in fracture risk in those receiving TDF but not boosted protease inhibitor (aIRR, 95% CI: 0.91, 0.71-1.18 p=0.49) and in those receiving boosted protease inhibitor but not TDF (aIRR, 95% CI: 0.87, 0.65-1.03 p=0.09) when compared to persons simultaneously receiving TDF and boosted protease inhibitor.

After adjustment, persons who had ever used didanosine, indinavir, saquinavir, ritonavir boosted lopinavir, or TDF, had increased incidence rates of osteonecrosis of the femoral head (Figure 4), although the confidence intervals were quite wide reflecting the lower number of outcomes. However, when these antiretrovirals were mutually adjusted for, there was no significant relationship between exposure to any of them and osteonecrosis risk (Figure 4), although all of them were associated with a non-significant increased risk. There was no association between cumulative exposure to any of these antiretrovirals and osteonecrosis. Current use of ritonavir boosted lopinavir or TDF was not associated with

an increased incidence of osteonecrosis of the femoral head. There were not sufficient data to examine current exposure to didanosine, indinavir or saquinavir as these antiretrovirals are no longer commonly used. Whether assessed as ever, current or cumulative, exposure to none of the other investigated antiretrovirals was associated with osteonecrosis of the femoral head.

Pneumocystis pneumonia was associated with both bone outcomes in univariate analyses (IRR, 95% CI: 1.35, 1.02-1.79 p= 0.039, for fractures; and 2.61, 1.41-4.83 p=0.0022, for osteonecrosis); this association, however, was no longer significant after adjustment (adjusted IRR, 95% CI: 0.99, 0.72-1.36 p=0.94, for fractures; and 1.27, 0.63-2.49 p=0.51, for osteonecrosis).

Discussion

In a large international HIV cohort involving participants receiving standard of care, we report that fractures and osteonecrosis of the femoral head are common bone outcomes. The risk of fractures and osteonecrosis seems to be determined by combination of host factors, HIV-specific variables and co-morbidities. We demonstrated that past and current exposure to TDF, but no other antiretroviral, was independently associated with higher incidence of fractures. Persons who had ever used didanosine, indinavir, saquinavir, ritonavir boosted lopinavir, or TDF, had higher risk of osteonecrosis of the femoral head, but this association was no longer significant after mutual adjustment.

Consistent with previous reports, we found that older age [1,16], white race [1,16], lower current CD4 count [17,18], IV drug use [1,19] and HCV co-infection [1,19] were independent predictors of fracture risk. We found that history of prior osteonecrosis, a recent non AIDS-defining cancer and recent cardiovascular disease were also associated with subsequent risk of fracture. The association with cancer and cardiovascular disease may reflect bone loss caused by co-morbidity treatments such as chemotherapy. It is also possible that a shared pathophysiological mechanism may lead to an increased risk of fractures and co-morbidities during HIV infection [20].

Exposure to TDF was linked to an increased risk of fractures in our study. Although smaller studies failed to find this association [15,17], our results are in overall agreement with larger and more recent cohort studies [1,16]. The question as to how TDF directly causes bone loss remains unanswered. Proximal tubular toxicity leading to phosphate

wasting and enhanced bone turnover is a possible mechanism [21]. However, proximal tubular renal dysfunction and enhanced bone turnover do not necessarily coexist [22]. In our study, there was no difference in renal function, as measured by eGFR, between those with and without a bone event. This suggests that TDF-associated bone toxicity and nephrotoxicity may be determined by different mechanisms. However, we did not compare proteinuria, glycosuria and phosphaturia among participants with and without fractures and it is possible that proximal tubular dysfunction among those receiving TDF may have preceded measurable changes in eGFR.

Of note, we found that past and current exposure to TDF, but not cumulative exposure, was associated with a significantly increased risk of fractures. A possible explanation is the fact that decreases in bone mineral density and increases in markers of bone turnover are steepest in the first one or two years following ART initiation [5,6,23]. After a critical initial period, abnormalities in bone metabolism caused by ART may not be progressive [23]. Consistent with this, the risk of fractures was found to be highest during the first two years of ART [15] to then continue to increase less steeply. We found an aIRR for fracture risk of 1.08 per 5 years of additional exposure to TDF. Although this was not statistically significant we cannot exclude the possibility of 8% yearly cumulative risk of fractures with TDF exposure.

The pathophysiology of osteonecrosis of the femoral head is poorly understood [24], but the primary mechanism is vascular occlusion leading to bone hypoxia and necrosis [25]. Increased inflammation and coagulation, as demonstrated by elevated levels of D-dimer and C-reactive protein, were also linked to osteonecrosis risk [26]. In accordance with other studies, we found that history of ADS-defining conditions and lower CD4 counts [27-29] are associated with increased risk of osteonecrosis. White race and history of prior fractures and prior osteonecrosis were factors independently associated with incident osteonecrosis. These same factors predicted fracture risk; which may be potentially explained by a common mechanism underlying the excess risk of osteoporosis and osteonecrosis in the setting of HIV infection [30]. However, whereas osteoporotic fractures are the clinical manifestation of low bone mineral density, osteonecrosis has a different pathogenesis involving impaired circulation [25].

We found past exposure to didanosine, indinavir, saquinavir, ritonavir boosted lopinavir, or TDF to be independently associated with osteonecrosis risk. However, after mutual

adjustment, these associations were attenuated and became no longer significant. A possible association between protease inhibitors and osteonecrosis risk was reported in a meta-analysis of case-control studies [31]. Our study was more methodologically sound because we had detailed time-updated information on exposure to individual antiretrovirals and performed mutual adjustment. However, we cannot exclude the possibility that ART exposure may have a small effect on osteonecrosis risk. If this was the case, the effect of individual antiretrovirals may have been too weak to be identified concomitantly with other bone-active drugs [32].

Our study had important limitations. First, we had no data on bone mineral density and bone events were not centrally adjudicated. Therefore, we could not confirm the osteoporotic nature of fractures. Information on anatomic sites was unavailable for a sizeable proportion of fractures. Magnetic resonance imaging was not systematically performed to diagnose osteonecrosis of the femoral head; the occurrence of bone events was confirmed during monitoring visits only. Second, we had no data on alcohol use and use of corticosteroids, megestrol acetate or testosterone. Exposure to these drugs has been previously reported in relation to risk of fractures and osteonecrosis [29,33,34]. We investigated previous history of *Pneumocystis* pneumonia as a proxy for corticosteroid exposure and found no significant association in adjusted analyses. Third, ART evolves quickly and it is unclear whether our findings are generalizable to tenofovir alafenamide (TAF)- or integrase inhibitor-based ART regimens.

To conclude, fractures and osteonecrosis of the femoral head are manifestations of bone disease among HIV positive persons receiving ARV treatment. Host factors, HIV-specific variables and co-morbidities contribute to the risk of these bone events. Past and current exposure to TDF, but not cumulative exposure, is independently associated with fracture risk. Our data supports cautious use of TDF among HIV positive persons at fracture risk initiating ART as recommended by current guidelines [35] and expert panels [36]. Individual antiretrovirals are not significantly associated with risk of osteonecrosis of the femoral head after mutual adjustment. However, we could not exclude a small effect of ART exposure on osteonecrosis risk. Larger studies are warranted to clarify this.

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Conflicts of Interest

JH's institution has received funding for her participation on Advisory Boards for Gilead Sciences, ViiV Healthcare, Merck and AbbVie. EF and his institution have received research grants from BMS, Gilead, Janssen, MSD and ViiV Healthcare. EF has been investigator of commercial trials sponsored by BMS, Gilead, Janssen, MSD and ViiV Healthcare and has received travel grants from BMS, MSD and Gilead. DS has received lecture fees and travel support from Gilead, Janssen and Abbott. HJS has received honoraria for Advisory Boards and presentations by Gilead Sciences, AbbVie, Janssen-Cilag, Bristol-Myers Squibb, and Merck Sharp & Dohme. VU has received lecture fees and travel support from AbbVie, BMS, Janssen and GlaxoSmithCline. JT has received lecture fees and funding to attend different international meetings from MSD, Janssen, Gilead, GSK, AbbVie, Pfizer and TEVA. PGK has received lecture fees and honoraria from Astellas, ViiV, Janssen, Gilead and MSD. CO has received honoraria, educational grants, travel scholarships and research grants from Gilead Sciences, Merck Sharp & Dohme, Bristol-Myers Squibb, ViiV Healthcare, Janssen, Johnson & Johnson, Boehringer Ingelheim and GlaxoSmithKline. CP has received a research grant from Gilead Sciences. CL has received lecture fees, honoraria and travel support from Abbvie, Bristol Myers Squibb, Gilead and Janssen. CL's institution has received research grants from Abbvie, Bristol Myers Squibb and Gilead. FM has received funding for participation on advisory boards for Gilead Sciences, ViiV Healthcare, Merck, AbbVie, BMS and Janssen. AM has

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AHB, JH, JDL and AM conceived the study. AM performed statistical analyses. AHB drafted the manuscript. All authors contributed to data interpretation, critically revised the manuscript and approved the final version.

References

1. Hansen AB, Gerstoft J, Kronborg G *et al*. Incidence of low and high-energy fractures in persons with and without HIV infection: a Danish population-based cohort study. AIDS, **2012**; 26: 285-293.
2. Güerri-Fernandez R, Vestergaard P, Carbonell C *et al*. HIV infection is strongly associated with hip fracture risk, independently of age, gender, and comorbidities: a population-based cohort study. J Bone Miner Res, **2013**; 28: 1259-1263.
3. Prieto-Alhambra D, Güerri-Fernández R, De Vries F *et al*. HIV infection and its association with an excess risk of clinical fractures: a nationwide case-control study. J Acquir Immune Defic Syndr **2014**; 66: 90-95.
4. Morse CG, Mican JM, Jones EC *et al*. The incidence and natural history of osteonecrosis in HIV-infected adults. Clin Infect Dis, **2007**; 44: 739-748.
5. Stellbrink HJ, Orkin C, Arribas JR *et al*. Comparison of changes in bone density and turnover with abacavir-lamivudine versus tenofovir-emtricitabine in HIV-infected adults: 48-week results from the ASSERT study. Clin Infect Dis, **2010**; 51: 963-972.
6. McComsey GA, Kitch D, Daar ES *et al*. Bone mineral density and fractures in antiretroviral-naive persons randomized to receive abacavir-lamivudine or tenofovir disoproxil fumarate-emtricitabine along with efavirenz or atazanavir-ritonavir: Aids Clinical Trials Group A5224s, a substudy of ACTG A5202. J Infect Dis, **2011**; 203: 1791-801.
7. Brown TT, Moser C, Currier JS *et al*. Changes in Bone Mineral Density After Initiation of Antiretroviral Treatment With Tenofovir Disoproxil Fumarate/Emtricitabine Plus Atazanavir/Ritonavir, Darunavir/Ritonavir, or Raltegravir. J Infect Dis, **2015**; 212: 1241-1249.
8. Bernardino JI, Mocroft A, Mallon PW *et al*. Bone mineral density and inflammatory and bone biomarkers after darunavir-ritonavir combined with either raltegravir or tenofovir-emtricitabine in antiretroviral-naive adults with HIV-1: a substudy of the NEAT001/ANRS143 randomised trial. Lancet HIV, **2015**; 2: 464-473.
9. Finkelstein JS, Brockwell SE, Mehta V *et a*l. Bone mineral density changes during the menopause transition in a multiethnic cohort of women. J Clin Endocrinol Metab, **2008**; 93: 861-868.

10. van Staa TP, Leufkens HG, Cooper C. The epidemiology of corticosteroid-induced osteoporosis: a meta-analysis. Osteoporos Int, **2002**; 13: 777-787.
11. Gallant JE, Staszewski S, Pozniak AL *et al*. Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: a 3-year randomized trial. JAMA, **2004**; 292: 191-201.
12. Rabenda V, Bruyère O, Reginster JY. Relationship between bone mineral density changes and risk of fractures among patients receiving calcium with or without vitamin D supplementation: a meta-regression. Osteoporos Int, **2011**; 22: 893-901.
13. Mocroft A, Ledergerber B, Katlama C *et al*. Decline in the AIDS and death rates in the EuroSIDA study: an observational study. Lancet, **2003**; 362: 22-29.
14. Mocroft A, Reiss P, Gasiorowski J *et al*. Serious fatal and nonfatal non-AIDS-defining illnesses in Europe. J Acquir Immune Defic Syndr, **2010**; 55: 262-270.
15. Yin MT, Kendall MA, Wu X *et al*. Fractures after antiretroviral initiation. AIDS, **2012**; 26: 2175-2184.
16. Bedimo R, Maalouf NM, Zhang S, Drechsler H, Tebas P. Osteoporotic fracture risk associated with cumulative exposure to tenofovir and other antiretroviral agents. AIDS, **2012**; 26: 825-831.
17. Young B, Dao CN, Buchacz K *et al*. Increased rates of bone fracture among HIV-infected persons in the HIV Outpatient Study (HOPS) compared with the US general population, 2000-2006. Clin Infect Dis, **2011**; 52: 1061-1068.
18. Grant PM, Kitch D, McComsey GA *et al*. Low baseline CD4+ count is associated with greater bone mineral density loss after antiretroviral therapy initiation. Clin Infect Dis, **2013**; 57: 1483-1488.
19. Bedimo R, Cutrell J, Zhang S, Drechsler H *et al*. Mechanisms of bone disease in HIV and hepatitis C virus: impact of bone turnover, tenofovir exposure, sex steroids and severity of liver disease. AIDS, **2016**; 30: 601-608.
20. Szulc P. Association between cardiovascular diseases and osteoporosis-reappraisal. Bonekey Rep, **2012**; 1:144.
21. Fux CA, Rauch A, Simcock M *et al*. Tenofovir use is associated with an increase in serum alkaline phosphatase in the Swiss HIV Cohort Study. Antivir Ther **2008**; 13: 1077-1082.
22. Calmy A, Fux CA, Norris R *et al*. Low bone mineral density, renal dysfunction, and fracture risk in HIV infection: a cross-sectional study. J Infect Dis, **2009**; 200: 1746-1754.
23. Grant PM, Kitch D, McComsey GA *et al*. Long-term Bone Mineral Density Changes in Antiretroviral-Treated HIV-Infected Individuals. J Infect Dis, **2016**; 214: 607-611.

24. Yombi JC, Vandercam B, Wilmes D, Dubuc JE, Vincent A, Docquier PL. Osteonecrosis of the femoral head in patients with type 1 human immunodeficiency virus infection: clinical analysis and review. Clin Rheumatol, **2009**; 28: 815-823.
25. Mont MA, Cherian JJ, Sierra RJ, Jones LC, Lieberman JR. Nontraumatic Osteonecrosis of the Femoral Head: Where Do We Stand Today? A Ten-Year Update. J Bone Joint Surg Am, **2015**; 97: 1604-1627.
26. Morse CG, Dodd LE, Nghiem K *et al*. Elevations in D-dimer and C-reactive protein are associated with the development ofosteonecrosis of the hip in HIV-infected adults. AIDS, **2013**; 27: 591-595.
27. Hasse B, Ledergerber B, Egger M *et al*. Antiretroviral treatment and osteonecrosis in patients of the Swiss HIV Cohort Study: a nested case-control study. AIDS Res Hum Retroviruses **2004**; 20: 909-915.
28. Mary-Krause M, Billaud E, Poizot-Martin I *et al*. Risk factors for osteonecrosis in HIV-infected patients: impact of treatment with combination antiretroviral therapy. AIDS, **2006**; 20: 1627-1635.
29. Gutiérrez F, Padilla S, Masiá M *et al*. Osteonecrosis in patients infected with HIV: clinical epidemiology and natural history in a large case series from Spain. J Acquir Immune Defic Syndr, **2006**; 42: 286-292.
30. Fessel WJ, Chau Q, Leong D. Association of osteonecrosis and osteoporosis in HIV-1-infected patients. AIDS, **2011**; 25: 1877-1880.
31. Permpalung N, Ungprasert P, Summachiwakij S, Leeaphorn N, Knight EL. Protease inhibitors and avascular necrosis: a systematic review and meta-analysis. Int J Antimicrob Agents, **2014**; 44: 93-95.
32. Grund B, Peng G, Gibert CL *et al*. Continuous antiretroviral therapy decreases bone mineral density. AIDS, 2009; 23: 1519-1529.
33. Lawson-Ayayi S, Bonnet F, Bernardin E *et al*. Avascular necrosis in HIV-infected patients: a case-control study from the Aquitaine Cohort, 1997-2002, France. Clin Infect Dis, **2005**; 40: 1188-1193.
34. Mazzotta E, Agostinone A, Rosso R *et al*. Osteonecrosis in human immunodeficiency virus (HIV)-infected patients: a multicentric case-control study. J Bone Miner Metab, **2011**; 29: 383-388.
35. European AIDS Clinical Society 2015. European Guidelines for treatment of HIV-infected adults in Europe. Available at: http://www.eacsociety.org/files/guidelines_8.1-english.pdf**.** Accessed 14 December 2016.

36. Brown TT, Hoy J, Borderi M *et al*. Recommendations for evaluation and management of bone disease in HIV. Clin Infect Dis, **2015**; 60: 1242-1251.

Tables

Table 1: Characteristics at last visit for those with no bone event, or at last diagnosis of fracture or osteonecrosis

Supplemental Table 1: Participant characteristics at last visit: non-osteoporotic versus osteoporotic fractures.

Supplemental Table 2: Univariate and multivariate factors associated with any fracture.

Supplemental Table 3: Univariate and multivariate factors associated with osteonecrosis of the femoral head.

Figures

Figure 1: Factors independently associated with fractures and osteonecrosis of the femoral head.

aIRR: adjusted incidence rate ratio. Models adjusted for depicted covariates plus calendar year and region. Only covariates significant ($p<0.1$) in univariate analyses were included in multivariate models to avoid overfitting.

1 not significant for osteonecrosis in univariate analysis and hence not included in multivariate model for osteonecrosis

2 not significant for fractures in univariate analysis and hence not included in multivariate model for fractures

Figure 2: Crude incidence of fractures. TDF use.

CI: confidence interval, PYFU: person-years of follow up, TDF: tenofovir disoproxil fumarate.

Figure 3: Effect of TDF exposure on risk of any fracture and of osteoporotic fractures [a].

a grouped as fractures of the spine, arm, wrist and hip

b adjusted for demographics, HIV-specific variables and co-morbidities.

Figure 4: Relationship between antiretroviral drugs and osteonecrosis.

ARV: antiretroviral, DDI: didanosine, IDV: indinavir, LPV/r: ritonavir boosted lopinavir, SQV: saquinavir, TDF : tenofovir disoproxil fumarate.

Table 1: Characteristics at last visit for those with no bone event, or at last diagnosis of fracture or osteonecrosis				
Variable	**No bone event**	**Fractures**	**Osteonecrosis of the femoral head**	**P-value***
Overall	11266 (95.2)	496 (4.2)	73 (0.6)	
Demographics n(%)/Median (IQR)				
Male gender	8460 (75.4)	357 (72.1)	58 (76.5)	0.22
Age (years)	49 (42,56)	50 (44,59)	47 (43,56)	0.0089
HIV transmission group				
MSM	4800 (42.6)	180 (36.4)	35 (47.9)	0.0032
Heterosexual	3475 (30.9)	150 (30.3)	17 (23.3)	
IDU	2156 (19.1)	130 (26.3)	14 (23.3)	
White ethnicity	9702 (86.1)	445 (89.9)	68 (93.1)	0.013
Asian	207 (1.8)	9 (1.6)	2 (2.7)	
Black	638 (5.7)	21 (4.2)	1 (1.4)	
Other	719 (6.4)	21 (4.2)	2 (2.7)	
Body Mass Index (Kg/m^2)				
≤ 18	926 (9.3)	64 (13.9)	10 (14.9)	0.0006
18-30	4248 (42.8)	214 (46.4)	29 (43.3)	

>30	4760 (47.9)	183 (39.7)	28 (41.8)	
HIV-specific variables n(%)/Median (IQR)				
On ART	10857 (89.1)	486 (97.0)	73 (100)	0.20
CD4 cells/mm^3	540 (340,749)	450 (332,658)	468 (328,621)	0.0004
Nadir CD4 cells/mm^3	176 (72,290)	120 (50,230)	90 (28,168)	<0.0001
HIV RNA <500 cp/ml	10032 (89.1)	441 (89.1)	70 (95.9)	0.17
Co-morbidities n(%)				
HCV positive	2805 (24.9)	157 (31.7)	18 (24.7)	0.012
Current smoking	3248 (28.8)	188 (38.0)	31 (42.5)	<0.0001
Prior osteonecrosis	43 (0.4)	14 (2.8)	20 (27.4)	<0.0001
Prior fracture	202 (1.8)	79 (16.0)	6 (8.2)	<0.0001
Prior AIDS	3526 (31.6)	188 (38.0)	43 (58.9)	< 0.0001
Prior AIDS (not cancer)	3114 (27.6)	176 (35.6)	38 (52.1)	<0.0001
Prior AIDS (cancer)	743 (6.6)	30 (6.1)	14 (19.2)	<0.0001
Prior non-AIDS cancer	1570 (13.9)	80 (16.2)	11 (15.1)	0.37
Prior cardiovascular disease	229 (2.0)	21 (4.2)	3 (4.1)	<0.0001
Recent AIDS event [a]	217 (1.9)	8 (1.6)	0 (0)	0.43
Recent AIDS (not cancer) [a]	172 (1.5)	7 (1.4)	0 (0)	0.58
Recent AIDS cancer [a]	55 (0.5)	1 (0.2)	0 (0)	0.25
Recent non-AIDS cancer [a]	403 (3.6)	23 (4.7)	6 (8.2)	0.052

Recent cardiovascular disease [a]	10 (0.1)	4 (0.8)	0 (0)	<0.0001
Chronic kidney disease [b]	583 (5.3)	26 (5.6)	5 (7.5)	0.71
eGFR [c] mL/min/1.73 m^2 Median (IQR)	95 (80,106)	96 (79,106)	94 (75,106)	0.58
Vitamin D [d] ng/mL Median (IQR)	37 (22,65)	35 (15,83)	31 (9,40)	0.26
Total cholesterol mg/dL Median (IQR)[e]	191 (162 -223)	193 (166 – 223)	196 (170 – 228)	0.43
Triglycerides mg/dL Median (IQR)[f]	136 (92 – 208)	139 (97 – 208)	181 (108 – 328)	0.0025

*global p-values for comparing across the three groups

(a) Occurring in the last 12 months prior to the diagnosis of fracture or osteonecrosis of the femoral head.

(b) chronic kidney disease at any time before last visit or event date; defined as confirmed (> 3 months apart) eGFR < 60 for those with first eGFR > 60, or 25% decline where baseline eGFR< 60. eGFRs were calculated using CKD-EPI formula.

(c) eGFR was available for 11536 (97.5%); 11003 (97.7%) for those with no events, 466 (94.1%) for those with any fracture and 67 (91.8%) for those with osteonecrosis of the femoral head (p<0.0001).

(d) Data available for 3291 (27.8%) overall, 3210 (28.5%) for those with no events, 71 (14.3%) for those with fractures and 10 (13.7%) of those with osteonecrosis of the femoral head (p<0.0001).

(e) Data available for 11687 (98.9%) overall; 11122 (98.7%) for those with no events, 492 (99.4%) for those with fractures and 73 (100%) of those with osteonecrosis of the femoral head (p=0.26)

(f) Data available for 11365 (96.2%) overall; 10813 (96.0%) for those with no events, 482 (97.4%) for those with fractures and 70 (95.9%) of those with osteonecrosis of the femoral head (p=0.26)

Supplemental Table 1: Participant characteristics at last visit: non-osteoporotic versus osteoporotic fractures				
		Non-osteoporotic	**Osteoporotic**	**P-value**
Overall		486 (78.6)	132 (21.4)	
Characteristic **n(%)/Median (IQR)**				
Gender	Male	357 (73.5)	91 (68.9)	0.30
Age (years)		49 (44,59)	52 (46,60)	0.021
HIV transmission group	MSM	170 (35.0)	52 (39.4)	0.030
	IV drug use	141 (29.0)	27 (20.5)	
	Heterosexual	131 (27.0)	47 (35.6)	
Race	White	436 (89.7)	123 (93.2)	0.23
Region	North Europe	211 (43.4)	72 (54.6)	0.0006
	Central and Eastern Europe	47 (9.7)	22 (16.7)	
On ART		468 (96.3)	129 (97.7)	0.42
CD4 cells/mm^3		441 (311,663)	454 (340,651)	0.62
Viral load < 500 copies/mL		427 (87.9)	119 (90.2)	0.47
HBV	Pos	25 (5.1)	5 (3.8)	0.61

HCV	Pos	166 (34.2)	39 (29.6)	0.57
Recent AIDS [a]		7 (1.4)	4 (3.0)	0.22
Chronic kidney disease [b]		28 (5.8)	3 (2.2)	0.10
eGFR [c]		97 (80,106)	95 (77,100)	0.33
Vitamin D [d]		33 (11,72)	34 (23,84)	0.42

(a) in last 12 months prior to diagnosis of fractures

(b) chronic kidney disease at any time before last visit or event date; defined as confirmed (> 3 months apart) eGFR < 60 for those with first eGFR > 60, or 25% decline where baseline eGFR< 60.

(c) eGFR was available for 455 (93.6%) for those with non-osteoporotic fractures and 127 (96.2%) for those with osteoporotic fractures (p=0.26). eGFRs were calculated using CKD-EPI formula.

(d) Data available for 64 (13.2%) for those with non-osteoporotic fractures and 15 (11.4%) for those with osteoporotic fractures (p=0.58).

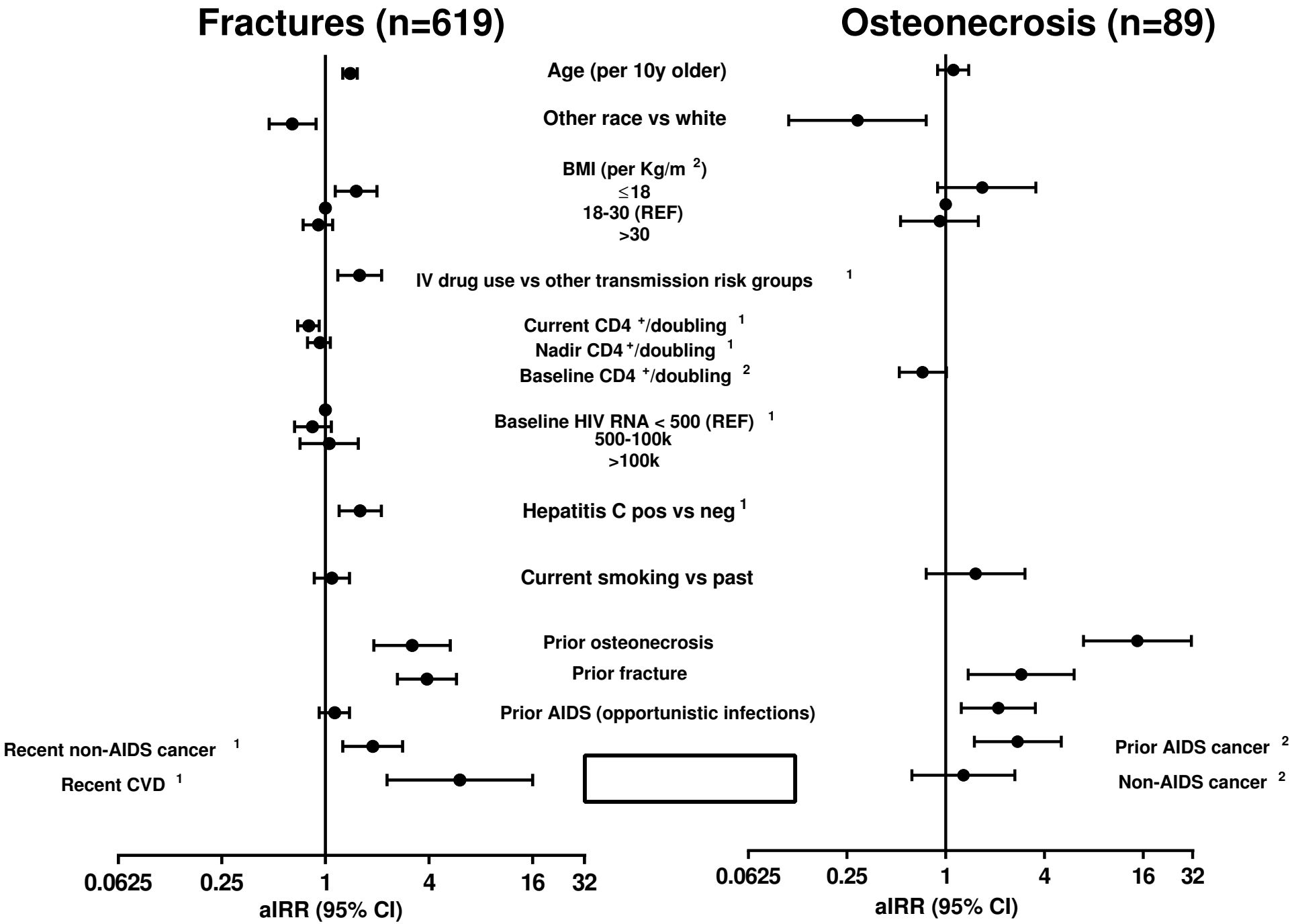
Supplemental Table 2: Univariate and multivariate factors associated with any fracture							
		Univariate			Multivariate		
		IRR	95% CI	P	IRR	95% CI	P
Age	/10 yrs	1.42	1.31 – 1.54	<0.0001	1.39	1.26 – 1.53	<0.0001
HIV transmission group	IDU	1.75	1.39 – 2.14	<0.0001	1.58	1.18 – 2.12	0.0021
	Other	1.00	-	-	1.00	-	-
Race	White	1.00	-	-	1.00	-	-
	Other	0.67	0.50 – 0.90	0.0073	0.64	0.47 – 0.88	0.0056
BMI	≤18	1.69	1.27 – 2.26	0.0004	1.51	1.14 – 1.99	0.0036
	18-30	1.00	-	-	1.00	-	-
	>30	0.90	0.73 – 1.10	0.30	0.91	0.74 – 1.11	0.36
	unknown	0.65	0.44 – 0.98	0.039	0.79	0.52 – 1.18	0.25
CD4*	/doubling	0.73	0.64 – 0.83	<0.0001	0.80	0.69 – 0.92	0.0014
Nadir CD4	/doubling	0.71	0.63 – 0.80	<0.0001	0.92	0.79 – 1.07	0.25
Baseline	<500	1.00	-	-	1.00	-	-
Viral Load	500-100k	0.68	0.53 – 0.87	0.0019	0.84	0.66 – 1.08	0.17
	>100k	1.06	0.70 – 1.60	0.78	1.05	0.71 – 1.55	0.81
Region	S	1.00	-	-	1.00	-	-
	N	1.80	1.32 – 2.47	0.0003	1.83	1.32 – 2.52	0.0003
	C	3.29	2.47 – 4.40	<0.0001	3.63	2.68 – 4.91	<0.0001
	CE	1.58	1.12 – 2.22	0.0092	1.60	1.13 – 2.28	0.0087
Year of follow up*	≤2006	1.00	-	-	1.00	-	-
	07-09	1.58	1.22 – 2.04	0.0005	1.50	1.16 – 1.94	0.0020
	10-12	1.75	1.36 – 2.25	<0.0001	1.56	1.20 – 2.02	0.0007
	>2012	1.28	0.95 – 1.72	0.10	1.09	0.80 – 1.48	0.58
HCV*	Neg	1.00	-	-	1.00	-	-
	Pos	1.70	1.39 – 2.09	<0.0001	1.59	1.20 – 2.11	0.0013
	Unknown	0.68	0.45 – 1.03	0.068	0.81	0.53 – 1.22	0.31

Smoking	Never	1.00	-	-	1.00	-	-
	Current	1.28	1.01 – 1.61	0.040	1.09	0.86 – 1.38	0.47
	Past	0.93	0.70 – 1.29	0.65	0.80	0.58 – 1.11	0.18
	Unknown	0.90	0.70 – 1.15	0.38	0.72	0.57 – 0.92	0.0083
	Arg	0.68	0.35 – 1.41	0.30	0.88	0.42 – 1.82	0.72
Osteonecrosis*	No	1.00	-	-	1.00	-	-
	Yes	4.69	2.95 – 7.46	<0.0001	3.19	1.91 – 5.31	<0.0001
Prior Fracture	No	1.00	-	-	1.00	-	-
	Yes	5.24	3.45 – 7.96	<0.0001	3.89	2.62 – 5.77	<0.0001
AIDS (not cancer)*		1.46	1.20 – 1.77	<0.0001	1.13	0.92 – 1.38	0.25
Recent non-AIDS-defining malignancy*		3.10	2.11 – 4.57	<0.0001	1.88	1.26 – 2.80	0.0018
Recent cardiovascular disease*		9.56	3.68 – 24.82	<0.0001	6.03	2.28 – 15.95	0.0003
All factors fixed at baseline, except *, which were time-updated							

Supplemental Table 3: Univariate and multivariate factors associated with osteonecrosis of the femoral head							
		Univariate			Multivariate		
		IRR	95% CI	P	IRR	95% CI	P
Age	/10 yrs	1.19	0.99 – 1.43	0.068	1.07	0.87 – 1.31	0.54
Race	White	1.00	-	-	1.00	-	-
	Other	0.38	0.15 – 0.95	0.038	0.28	0.11 – 0.76	0.012
Nadir CD4	/doubling	0.52	0.36 – 0.75	0.0005	0.71	0.51 – 1.01	0.054
Prior osteonecrosis	No	1.00	-	-	1.00	-	-
	Yes	20.82	10.32 – 41.99	<0.0001	14.95	6.96 – 32.15	<0.0001
Prior fracture*	No	1.00	-	-	1.00	-	-
	Yes	4.26	2.13 – 8.50	<0.0001	3.12	1.50 – 6.49	0.0023
AIDS (not cancer)*		2.92	1.78 – 4.80	<0.0001	2.13	1.27 – 3.57	0.0043
AIDS-defining cancer*		3.57	1.96 – 6.50	<0.0001	2.69	1.48 – 4.89	0.0012
Non-AIDS-defining malignancies*		2.00	1.03 – 3.91	0.042	1.33	0.65 – 2.69	0.43
All factors fixed at baseline, except *, which were time-updated							

Figure 1

Click here to download Figure (.tif and .eps files only) Figure 1.eps



aIRR: adjusted incidence rate ratio. Models adjusted for depicted covariates plus calendar year and region. Only covariates significant ($p<0.1$) in univariate analyses were included in multivariate models to avoid overfitting.

1 not significant for osteonecrosis in univariate analysis and hence not included in multivariate model for osteonecrosis

2 not significant for fractures in univariate analysis and hence not included in multivariate model for fractures

Figure 2 Click here to download Figure (.tif and .eps files only) Figure 2_final.TIF

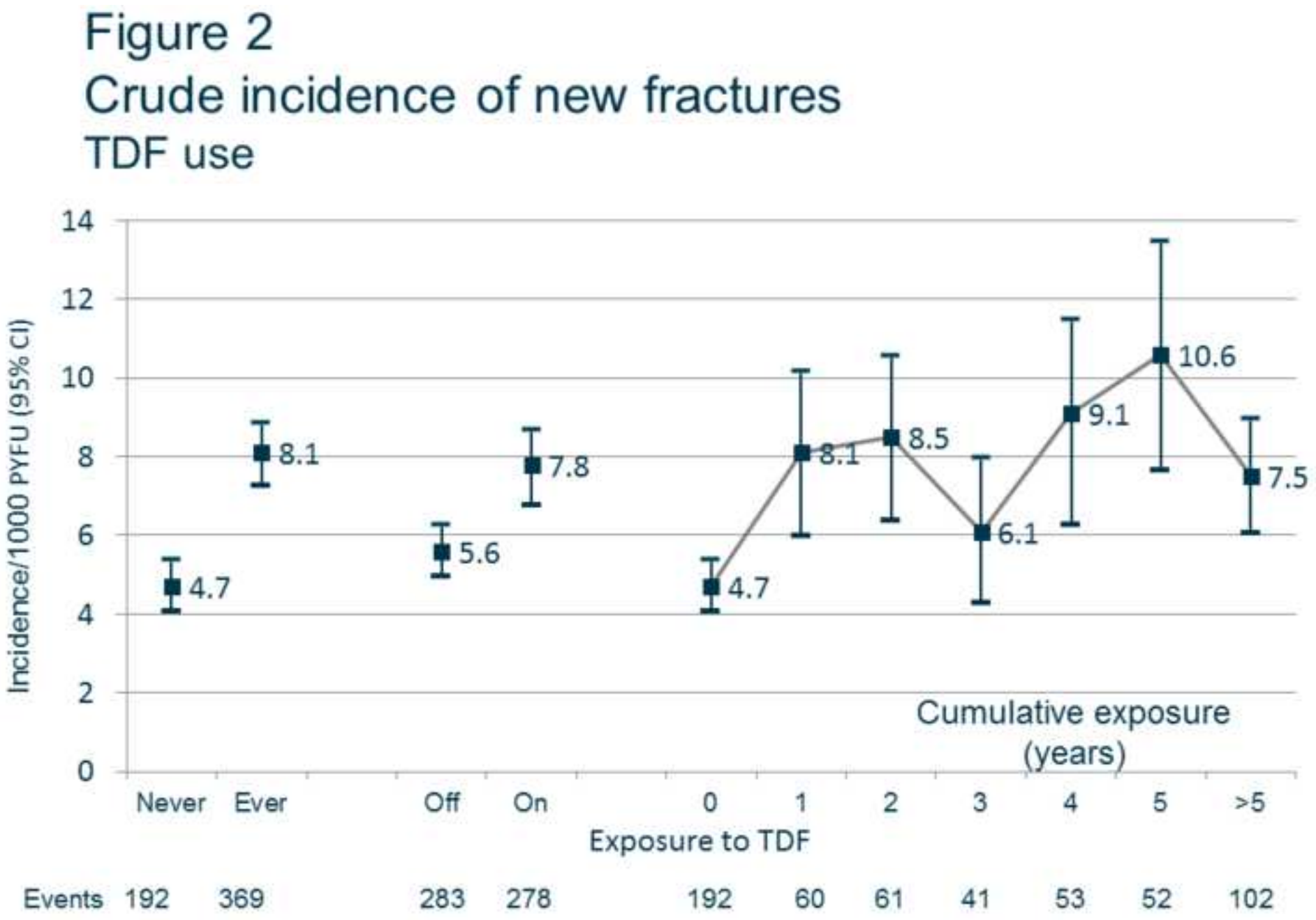
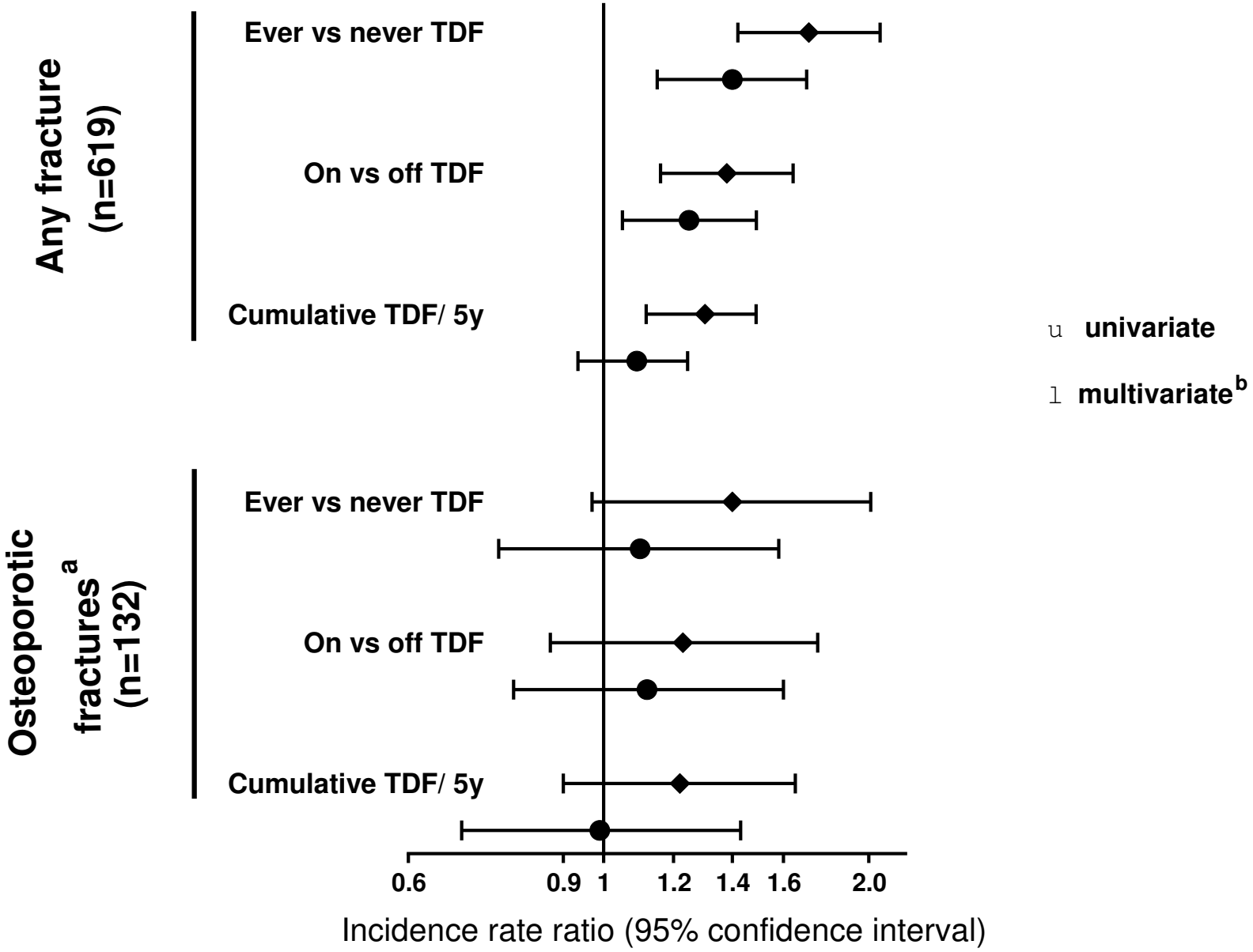


Figure 3

Effect of TDF exposure on risk of any fracture and of osteoporotic fractures[a]

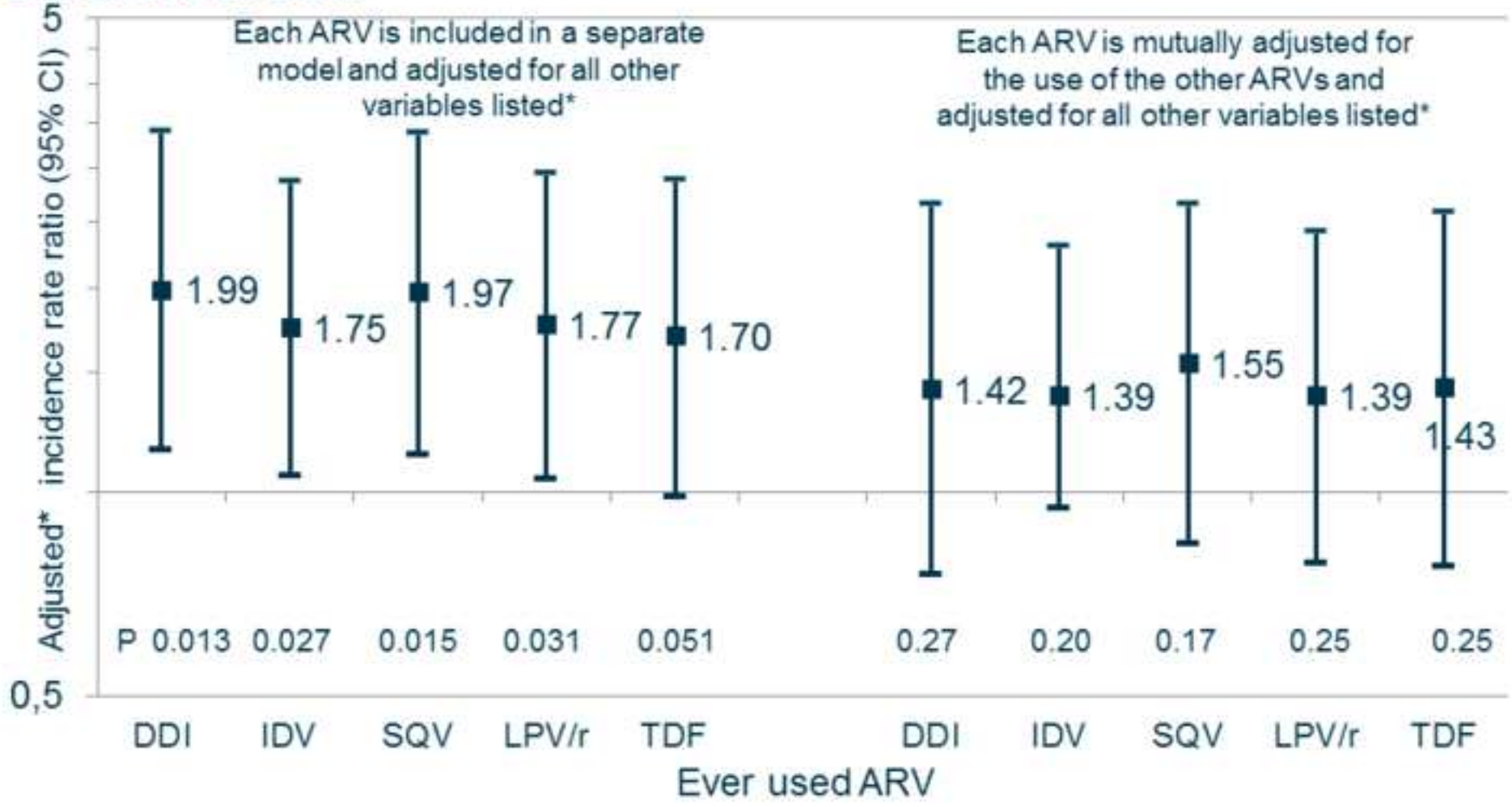


[a] grouped as fractures of the spine, arm, wrist and hip

[b] adjusted for demographics, HIV-specific variables and co-morbidities

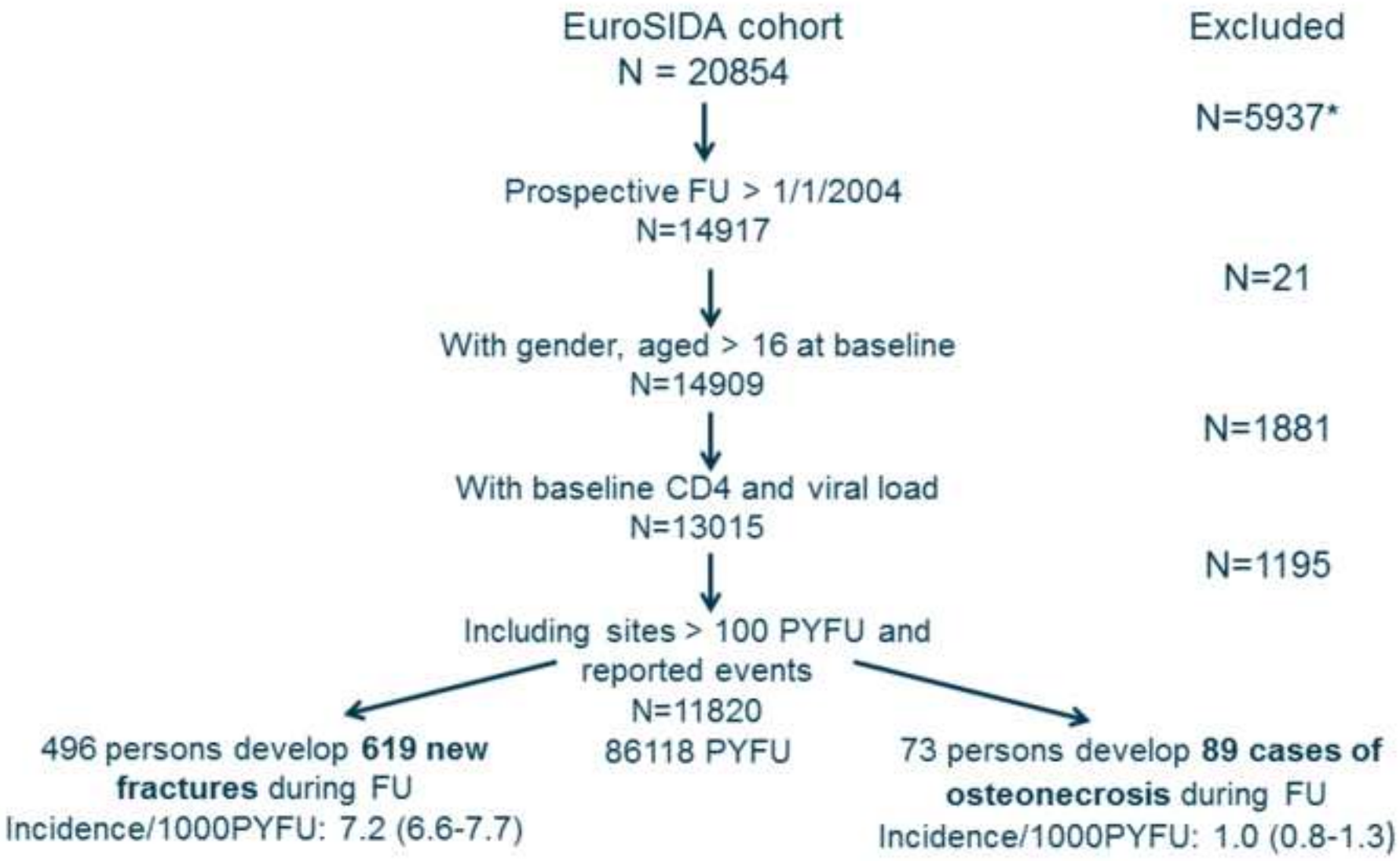
Figure 4 Click here to download Figure (.tif and .eps files only) Figure 4_final.TIF

Figure 4
Relationship between antiretroviral drugs and osteonecrosis



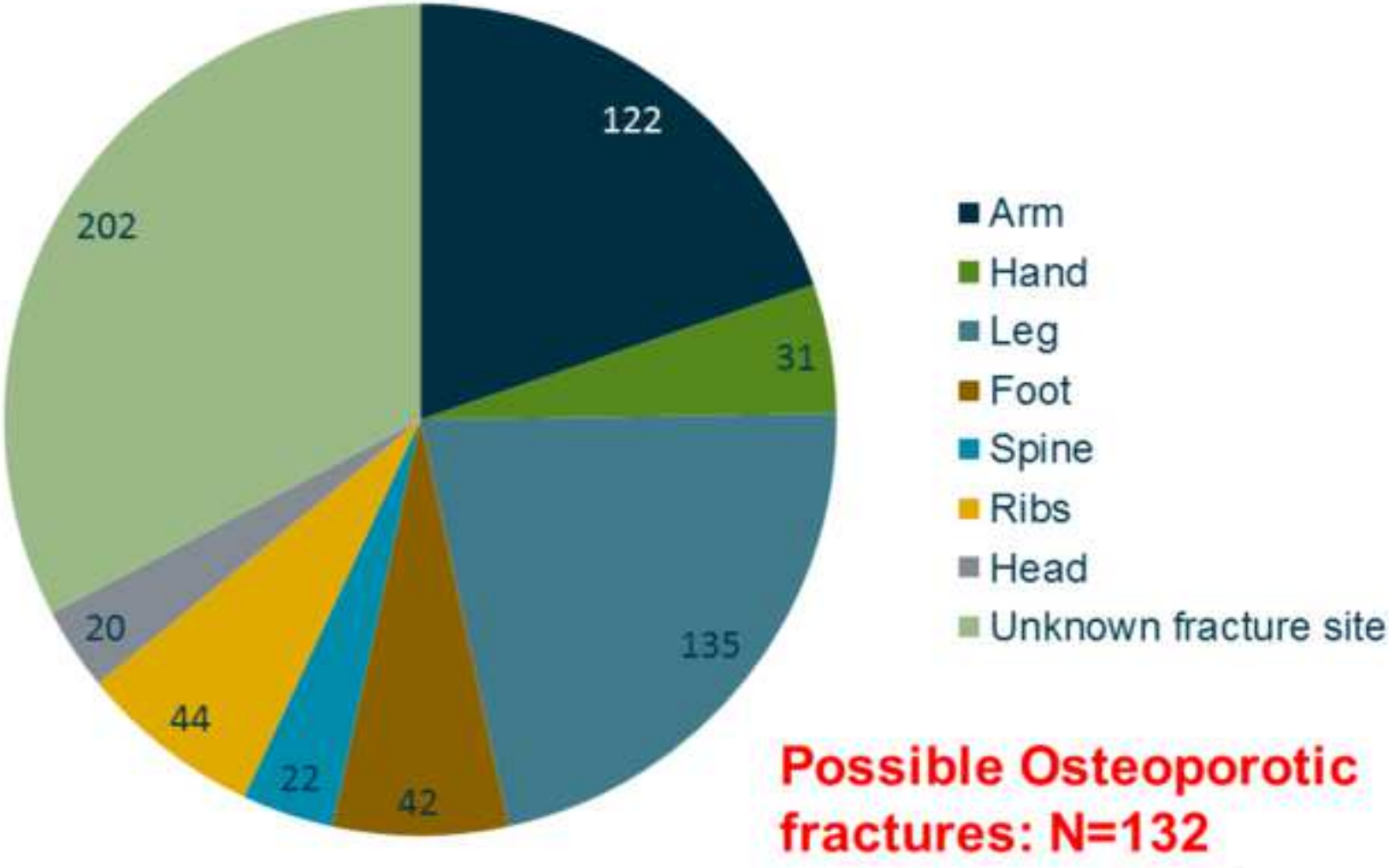
adjusted for race, prior femoral necrosis at baseline, fracture, age, nadir CD4 count, diagnosis of an AIDS defining malignancy*, non-malignant AIDS event* and non-AIDS defining malignancy*
*time-updated variables

Supplemental Figure 1: Inclusion of participants



Baseline: latest of 1 January 2004 and recruitment to EuroSIDA. Baseline CD4/VL defined as last measurement before baseline (and within 6 months) or if none before, first after baseline (and within 6 months).
*Includes persons enrolled into Cohort 10 with only baseline data

Supplemental Figure 2: Fracture sites (N=619)



Title: Antiretrovirals, fractures and osteonecrosis in a large international HIV cohort

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Key words: fractures, osteonecrosis, avascular necrosis, bone, HIV

Running title: Fractures and osteonecrosis during HIV

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Summary: We investigated the association of exposure to antiretroviral drugs with incident fractures and osteonecrosis of the femoral head in the EuroSIDA cohort. We demonstrated that past and current exposure to tenofovir disoproxil fumarate, but no other antiretroviral, was independently associated with higher incidence of fractures. After mutual adjustment, no antiretroviral was associated with osteonecrosis risk.

Abstract

Background: Antiretrovirals (ARVs) affect bone density and turnover, but their effect on risk of fractures and osteonecrosis of the femoral head is less understood. We investigated if exposure to ARVs increases the risk of both bone outcomes.

Methods: EuroSIDA participants were followed to assess fractures and osteonecrosis. Poisson regression identified clinical, laboratory and demographic predictors of either bone outcome. Ever, current and cumulative exposures to ARVs were assessed. .

Results: During 86118 PYFU among 11820 included persons (median age 41y, 75% male, median baseline CD4 440/mm^3, 70.4% virologically suppressed), there were 619 fractures (incidence/1000PYFU 7.2; 95%CI 6.6-7.7) and 89 osteonecrosis (1.0;0.8-1.3). Older age, white race, lower BMI, IV drug use, lower baseline CD4, HCV-coinfection, prior osteonecrosis, prior fracture, cardiovascular disease and recent non-AIDS cancer (last 12 months) were associated with fractures. After adjustment, persons who had ever used Tenofovir Disoproxil Fumarate (TDF) (1.40; 1.15-1.70) or who were currently on TDF (1.25; 1.05-1.49) had higher incidence of fractures. There was no association between cumulative exposure to TDF and fractures (1.08/5y exposure; 0.94-1.25). No other ARV was associated with fractures (all p>0.1). Risk of osteonecrosis was associated with white race, lower nadir CD4, prior osteonecrosis, prior fracture and prior AIDS. After mutual adjustment, no ARV was associated with osteonecrosis.

Conclusions: In HIV infection, host factors, HIV-specific variables and co-morbidities contribute to risk of fractures and osteonecrosis. Exposure to TDF, but not other ARVs, was an independent risk factor for fractures.

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Introduction

Fractures and osteonecrosis of the femoral head have emerged as important manifestations of bone disease during treated HIV infection. According to population-based studies [1-3], HIV positive persons have a 1.5-3.0 greater risk of fractures than the general population. Osteonecrosis of the femoral head, although a rare bone disease, also disproportionally affects HIV positive persons. In the setting of HIV infection, it was estimated that osteonecrosis has a 100-fold excess risk when compared to the general population [4].

Initiation of antiretroviral therapy (ART) is associated with reduction in bone mineral density [5-7] and increases levels of markers of bone turnover [5,8]. The loss of bone mineral density following ART initiation is comparable in magnitude to that occurring during the perimenopausal period [9] and treatment with corticosteroids [10]. Changes in bone mineral density and turnover are observed irrespective of ART regimen, but tenofovir disoproxil fumarate (TDF)-containing regimens causes greater bone loss [6,11] whereas integrase inhibitor-based regimens may cause less bone loss [7]. However, the direct clinical consequences of this are yet to be determined because changes in bone mineral density are not perfect predictors of fracture risk [12] and the effect of ART exposure on the risk of fractures and osteonecrosis remain poorly understood. In a large HIV cohort, we set out to study the association of exposure to antiretroviral drugs with incident fractures and osteonecrosis of the femoral head and to determine factors independently associated with these two bone outcomes.

Methods

The EuroSIDA study, a multinational prospective cohort of 20,854 HIV positive persons from across Europe, Argentina and Israel, has been documented in detail elsewhere [13]. Briefly, detailed information on clinical, virological and immunological parameters has been collected every 6 months with accurate recording of dates of AIDS-defining illnesses and serious non-AIDS-defining events, defined as cardiovascular disease, end-stage hepatic/renal disease, non-AIDS defining malignancies and pancreatitis [14]. Data quality

is assured by yearly monitoring visits to participating clinics and hospitals, when source documentation and dates of reported clinical events are cross-checked. In this study, we included persons aged >16 year with baseline data on CD4 counts and viral loads with prospective follow up. Baseline was defined as 1/1/2004, when routine prospective collection of data on fractures and osteonecrosis of the femoral head was initiated in EuroSIDA. Data on fractures and osteonecrosis was reported in follow up forms by the attending physician. Participating sites reporting no cases of fractures or osteonecrosis or with less than 100 person-years of follow up (PYFU) were excluded due to concerns about underreporting and data quality on bone outcomes.

Poisson regression using generalized estimating equations was used to identify clinical, laboratory and demographic factors associated with fractures and osteonecrosis of the femoral head. More than one outcome per person was included accounting for correlation between and within included persons. Factors adjusted for were chosen on the basis of their epidemiological importance and biological plausibility and included baseline and time-updated variables, as defined in a previous report [14]. To avoid model overfitting, factors with marginal associations ($p<0.1$) in univariate analyses were included in multivariate models. Nadir, baseline, and current CD4 counts, baseline and current viral load, as well as age, were all investigated as categorical and continuous variables with different transformations to identify the best fitting model. Clinical events were modelled as time-updated covariates in different ways to identify the best fitting model, including non-malignant AIDS events, AIDS-defining cancer, non-AIDS-defining malignancies and cardiovascular disease, and each of these as a recent event, i.e. occurring within the last 12 months prior to the diagnosis of fracture or osteonecrosis.

Each antiretroviral was included in the best-fitting multivariate model to assess the effect of its exposure on the subsequent risk of fractures or osteonecrosis of the femoral head. Exposure to each antiretroviral was included as ever (yes/no), current (yes/no) and cumulative exposure. Antiretrovirals tested were zidovudine, didanosine, stavudine, lamivudine, emtricitabine, TDF, abacavir, nevirapine, efavirenz, saquinavir, ritonavir, lopinavir, indinavir, nelfinavir, atazanavir, ritonavir boosted lopinavir, and any other boosted protease inhibitors (grouped into one category). Antiretrovirals which remained independently associated with risk for fractures or osteonecrosis were subsequently

mutually adjusted for. It was not possible to assess other antiretrovirals such as integrase inhibitors owing to their short cumulative exposure among EuroSIDA participants.

We carried out secondary analyses restricted to osteoporotic fractures, grouped as fractures of the spine, arm, wrist and hip. Similar to other cohort studies without data on bone mineral density [15,16], our assumption was that fractures in these locations were more likely to be osteoporotic. Previous investigations reported an increased fracture risk among persons receiving TDF and boosted protease inhibitors concomitantly [16]. Therefore, we also investigated incidence rates of fractures according to three categories of current ART use: 1) current use of TDF but not boosted protease inhibitor; 2) current use of boosted protease inhibitor but not TDF; and 3) concomitant use of TDF and boosted protease inhibitor. Using category 3 as the comparator, we compared the incidence rate ratios of fractures across these 3 categories in models adjusted for demographics, HIV-specific variables and co-morbidities.

Finally, patients presenting with severe *Pneumocystis* pneumonia are likely to be treated with adjuvant corticosteroids. We examined the association between past history of *Pneumocystis* pneumonia, as a proxy of corticosteroid exposure, and subsequent risk for fractures and osteonecrosis.

Results

Of 20,854 EuroSIDA participants, 14,917 had prospective follow-up after baseline and were eligible for inclusion. An additional 1,902 participants were excluded due to missing CD4 counts and/or viral load at baseline. 1,195 persons were excluded from sites with less than 100 PYFU or where no fractures or osteonecrosis were reported. Thus, 11,820 participants were eligible for final inclusion in the analyses (Supplemental Figure 1).

The odds of being excluded because of missing CD4 and viral load were higher in all HIV exposure groups compared to MSM, in all regions of Europe (except North) compared to South, and in those with hepatitis C virus (HCV) infection at baseline. The odds of being excluded due to missing CD4 and viral load were lower in those who had started ART, were older, had a later date of recruitment to EuroSIDA and in those with a higher nadir CD4 count. Participants excluded from small study sites or with no reported events were more likely to be older, female, non-white, non-MSM, and to be on ART and have a later

date of enrolment to EuroSIDA. Table 1 summarizes participant characteristics at the time of diagnosis of fractures or osteonecrosis and at last visit for those without either outcome. There was a higher proportion of fractures and osteonecrosis among IV drug users and HCV coinfected persons. Persons with a bone outcome tended to have a lower current CD4 count than those without an event. Persons with osteonecrosis had significantly higher levels of triglycerides. There were no differences between participants with and without a bone outcome in terms of chronic kidney disease, estimated glomerular filtration rate (eGFR) and vitamin D levels (Table 1).

Among 11,820 included participants during 86,118 PYFU, 496 persons had 619 incident fractures (incidence rate [IR]/1,000 PYFU, 95% confidence interval [CI]: 7.2, 6.6-7.7) and 73 persons developed 89 cases of osteonecrosis of the femoral head (IR: 1.0, 0.8-1.3) (Supplemental Figure 1).

Considering fractures with a known site, the most frequent were fractures of the leg, followed by arm, ribs and foot. 132 fractures (21.3%) were classified as osteoporotic (Supplemental Figure 2). At last visit, participants with osteoporotic fractures were more likely to be older, have sexually-acquired HIV and to come from North Europe compared to participants with other fractures, but had otherwise similar demographic and clinical characteristics (Supplemental Table 1). Information regarding fracture site could not be ascertained for 202 fractures (32.6%). Participants with fractures of unknown sites were more likely to be female, to be ART naïve at study entry, have a prior AIDS diagnosis and have a higher current CD4 count when compared to participants with known fracture sites.

In analyses stratified by time-updated CD4 count, the crude incidence of fractures decreased as CD4 counts increased (P<0.0001). On the other hand, the incidence of osteonecrosis did not vary significantly across different CD4 count strata (P=0.055).

Univariate and multivariate associations between covariates and risk for fractures and osteonecrosis are detailed in supplemental Tables 2 and 3. After adjustment, an increased risk of incident fractures was independently associated with older age, lower BMI, IV drug use, HCV co-infection, prior fracture, prior osteonecrosis of the femoral head, a recent non-AIDS-defining malignancy and recent cardiovascular disease (Figure 1). Another independent predictor of fractures was origin, with participants from Northern and Central Europe at significantly increased risk (Supplemental Table 2). Race other than white and a

higher current CD4 count were associated with lower fracture risk (Figure 1). Findings were not substantially different between men and women (data not shown).

With respect to osteonecrosis of the femoral head, a prior history of osteonecrosis, fractures and AIDS-defining illnesses (both opportunistic infections and AIDS-defining cancers) was independently associated with increased risk (Figure 1). Race other than white and a higher baseline CD4 count were independently associated with a lower risk for osteonecrosis (Figure 1)

In univariate analyses, ever, current and cumulative exposure to TDF was associated with increased fracture risk. Crude incidence rates are depicted in Figure 2. Whether modelled as ever, current or cumulative, no association between exposure to any of the other investigated antiretrovirals and fracture risk was observed (data not shown). After adjustment (Figure 3), persons who had ever used TDF (adjusted incidence rate ratio [aIRR], 95% CI: 1.40, 1.15-1.70 p=0.0008) or who were currently receiving TDF (aIRR 1.25, 1.05-1.49, p=0.012) had a significantly increased incidence of fractures. The association of fracture risk with cumulative exposure to TDF did not retain statistical significance after adjustment (aIRR 1.08, 0.94-1.25, per 5 years of additional exposure, p=0.27). In analyses restricted to osteoporotic fractures, no significant association was seen with ever, current or cumulative exposure to TDF (Figure 3).

In adjusted analyses to investigate incidence rate ratios of fractures for categories of current ART use stratified according to TDF and boosted protease inhibitor, we found no difference in fracture risk in those receiving TDF but not boosted protease inhibitor (aIRR, 95% CI: 0.91, 0.71-1.18 p=0.49) and in those receiving boosted protease inhibitor but not TDF (aIRR, 95% CI: 0.87, 0.65-1.03 p=0.09) when compared to persons simultaneously receiving TDF and boosted protease inhibitor.

After adjustment, persons who had ever used didanosine, indinavir, saquinavir, ritonavir boosted lopinavir, or TDF, had increased incidence rates of osteonecrosis of the femoral head (Figure 4), although the confidence intervals were quite wide reflecting the lower number of outcomes. However, when these antiretrovirals were mutually adjusted for, there was no significant relationship between exposure to any of them and osteonecrosis risk (Figure 4), although all of them were associated with a non-significant increased risk. There was no association between cumulative exposure to any of these antiretrovirals and osteonecrosis. Current use of ritonavir boosted lopinavir or TDF was not associated with

an increased incidence of osteonecrosis of the femoral head. There were not sufficient data to examine current exposure to didanosine, indinavir or saquinavir as these antiretrovirals are no longer commonly used. Whether assessed as ever, current or cumulative, exposure to none of the other investigated antiretrovirals was associated with osteonecrosis of the femoral head.

Pneumocystis pneumonia was associated with both bone outcomes in univariate analyses (IRR, 95% CI: 1.35, 1.02-1.79 p= 0.039, for fractures; and 2.61, 1.41-4.83 p=0.0022, for osteonecrosis); this association, however, was no longer significant after adjustment (adjusted IRR, 95% CI: 0.99, 0.72-1.36 p=0.94, for fractures; and 1.27, 0.63-2.49 p=0.51, for osteonecrosis).

Discussion

In a large international HIV cohort involving participants receiving standard of care, we report that fractures and osteonecrosis of the femoral head are common bone outcomes. The risk of fractures and osteonecrosis seems to be determined by combination of host factors, HIV-specific variables and co-morbidities. We demonstrated that past and current exposure to TDF, but no other antiretroviral, was independently associated with higher incidence of fractures. Persons who had ever used didanosine, indinavir, saquinavir, ritonavir boosted lopinavir, or TDF, had higher risk of osteonecrosis of the femoral head, but this association was no longer significant after mutual adjustment.

Consistent with previous reports, we found that older age [1,16], white race [1,16], lower current CD4 count [17,18], IV drug use [1,19] and HCV co-infection [1,19] were independent predictors of fracture risk. We found that history of prior osteonecrosis, a recent non AIDS-defining cancer and recent cardiovascular disease were also associated with subsequent risk of fracture. The association with cancer and cardiovascular disease may reflect bone loss caused by co-morbidity treatments such as chemotherapy. It is also possible that a shared pathophysiological mechanism may lead to an increased risk of fractures and co-morbidities during HIV infection [20].

Exposure to TDF was linked to an increased risk of fractures in our study. Although smaller studies failed to find this association [15,17], our results are in overall agreement with larger and more recent cohort studies [1,16]. The question as to how TDF directly causes bone loss remains unanswered. Proximal tubular toxicity leading to phosphate

wasting and enhanced bone turnover is a possible mechanism [21]. However, proximal tubular renal dysfunction and enhanced bone turnover do not necessarily coexist [22]. In our study, there was no difference in renal function, as measured by eGFR, between those with and without a bone event. This suggests that TDF-associated bone toxicity and nephrotoxicity may be determined by different mechanisms. However, we did not compare proteinuria, glycosuria and phosphaturia among participants with and without fractures and it is possible that proximal tubular dysfunction among those receiving TDF may have preceded measurable changes in eGFR.

Of note, we found that past and current exposure to TDF, but not cumulative exposure, was associated with a significantly increased risk of fractures. A possible explanation is the fact that decreases in bone mineral density and increases in markers of bone turnover are steepest in the first one or two years following ART initiation [5,6,23]. After a critical initial period, abnormalities in bone metabolism caused by ART may not be progressive [23]. Consistent with this, the risk of fractures was found to be highest during the first two years of ART [15] to then continue to increase less steeply. We found an aIRR for fracture risk of 1.08 per 5 years of additional exposure to TDF. Although this was not statistically significant we cannot exclude the possibility of 8% yearly cumulative risk of fractures with TDF exposure.

The pathophysiology of osteonecrosis of the femoral head is poorly understood [24], but the primary mechanism is vascular occlusion leading to bone hypoxia and necrosis [25]. Increased inflammation and coagulation, as demonstrated by elevated levels of D-dimer and C-reactive protein, were also linked to osteonecrosis risk [26]. In accordance with other studies, we found that history of ADS-defining conditions and lower CD4 counts [27-29] are associated with increased risk of osteonecrosis. White race and history of prior fractures and prior osteonecrosis were factors independently associated with incident osteonecrosis. These same factors predicted fracture risk; which may be potentially explained by a common mechanism underlying the excess risk of osteoporosis and osteonecrosis in the setting of HIV infection [30]. However, whereas osteoporotic fractures are the clinical manifestation of low bone mineral density, osteonecrosis has a different pathogenesis involving impaired circulation [25].

We found past exposure to didanosine, indinavir, saquinavir, ritonavir boosted lopinavir, or TDF to be independently associated with osteonecrosis risk. However, after mutual

adjustment, these associations were attenuated and became no longer significant. A possible association between protease inhibitors and osteonecrosis risk was reported in a meta-analysis of case-control studies [31]. Our study was more methodologically sound because we had detailed time-updated information on exposure to individual antiretrovirals and performed mutual adjustment. However, we cannot exclude the possibility that ART exposure may have a small effect on osteonecrosis risk. If this was the case, the effect of individual antiretrovirals may have been too weak to be identified concomitantly with other bone-active drugs [32].

Our study had important limitations. First, we had no data on bone mineral density and bone events were not centrally adjudicated. Therefore, we could not confirm the osteoporotic nature of fractures. Information on anatomic sites was unavailable for a sizeable proportion of fractures. Magnetic resonance imaging was not systematically performed to diagnose osteonecrosis of the femoral head; the occurrence of bone events was confirmed during monitoring visits only. Second, we had no data on alcohol use and use of corticosteroids, megestrol acetate or testosterone. Exposure to these drugs has been previously reported in relation to risk of fractures and osteonecrosis [29,33,34]. We investigated previous history of *Pneumocystis* pneumonia as a proxy for corticosteroid exposure and found no significant association in adjusted analyses. Third, ART evolves quickly and it is unclear whether our findings are generalizable to tenofovir alafenamide (TAF)- or integrase inhibitor-based ART regimens.

To conclude, fractures and osteonecrosis of the femoral head are manifestations of bone disease among HIV positive persons receiving ARV treatment. Host factors, HIV-specific variables and co-morbidities contribute to the risk of these bone events. Past and current exposure to TDF, but not cumulative exposure, is independently associated with fracture risk. Our data supports cautious use of TDF among HIV positive persons at fracture risk initiating ART as recommended by current guidelines [35] and expert panels [36]. Individual antiretrovirals are not significantly associated with risk of osteonecrosis of the femoral head after mutual adjustment. However, we could not exclude a small effect of ART exposure on osteonecrosis risk. Larger studies are warranted to clarify this.

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Conflicts of Interest

JH's institution has received funding for her participation on Advisory Boards for Gilead Sciences, ViiV Healthcare, Merck and AbbVie. EF and his institution have received research grants from BMS, Gilead, Janssen, MSD and ViiV Healthcare. EF has been investigator of commercial trials sponsored by BMS, Gilead, Janssen, MSD and ViiV Healthcare and has received travel grants from BMS, MSD and Gilead. DS has received lecture fees and travel support from Gilead, Janssen and Abbott. HJS has received honoraria for Advisory Boards and presentations by Gilead Sciences, AbbVie, Janssen-Cilag, Bristol-Myers Squibb, and Merck Sharp & Dohme. VU has received lecture fees and travel support from AbbVie, BMS, Janssen and GlaxoSmithCline. JT has received lecture fees and funding to attend different international meetings from MSD, Janssen, Gilead, GSK, AbbVie, Pfizer and TEVA. PGK has received lecture fees and honoraria from Astellas, ViiV, Janssen, Gilead and MSD. CO has received honoraria, educational grants, travel scholarships and research grants from Gilead Sciences, Merck Sharp & Dohme, Bristol-Myers Squibb, ViiV Healthcare, Janssen, Johnson & Johnson, Boehringer Ingelheim and GlaxoSmithKline. CP has received a research grant from Gilead Sciences. CL has received lecture fees, honoraria and travel support from Abbvie, Bristol Myers Squibb, Gilead and Janssen. CL's institution has received research grants from Abbvie, Bristol Myers Squibb and Gilead. FM has received funding for participation on advisory boards for Gilead Sciences, ViiV Healthcare, Merck, AbbVie, BMS and Janssen. AM has

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References

1. Hansen AB, Gerstoft J, Kronborg G *et al*. Incidence of low and high-energy fractures in persons with and without HIV infection: a Danish population-based cohort study. AIDS, **2012**; 26: 285-293.
2. Güerri-Fernandez R, Vestergaard P, Carbonell C *et al*. HIV infection is strongly associated with hip fracture risk, independently of age, gender, and comorbidities: a population-based cohort study. J Bone Miner Res, **2013**; 28: 1259-1263.
3. Prieto-Alhambra D, Güerri-Fernández R, De Vries F *et al*. HIV infection and its association with an excess risk of clinical fractures: a nationwide case-control study. J Acquir Immune Defic Syndr **2014**; 66: 90-95.
4. Morse CG, Mican JM, Jones EC *et al*. The incidence and natural history of osteonecrosis in HIV-infected adults. Clin Infect Dis, **2007**; 44: 739-748.
5. Stellbrink HJ, Orkin C, Arribas JR *et al*. Comparison of changes in bone density and turnover with abacavir-lamivudine versus tenofovir-emtricitabine in HIV-infected adults: 48-week results from the ASSERT study. Clin Infect Dis, **2010**; 51: 963-972.
6. McComsey GA, Kitch D, Daar ES *et al*. Bone mineral density and fractures in antiretroviral-naive persons randomized to receive abacavir-lamivudine or tenofovir disoproxil fumarate-emtricitabine along with efavirenz or atazanavir-ritonavir: Aids Clinical Trials Group A5224s, a substudy of ACTG A5202. J Infect Dis, **2011**; 203: 1791-801.
7. Brown TT, Moser C, Currier JS *et al*. Changes in Bone Mineral Density After Initiation of Antiretroviral Treatment With Tenofovir Disoproxil Fumarate/Emtricitabine Plus Atazanavir/Ritonavir, Darunavir/Ritonavir, or Raltegravir. J Infect Dis, **2015**; 212: 1241-1249.
8. Bernardino JI, Mocroft A, Mallon PW *et al*. Bone mineral density and inflammatory and bone biomarkers after darunavir-ritonavir combined with either raltegravir or tenofovir-emtricitabine in antiretroviral-naive adults with HIV-1: a substudy of the NEAT001/ANRS143 randomised trial. Lancet HIV, **2015**; 2: 464-473.
9. Finkelstein JS, Brockwell SE, Mehta V *et a*l. Bone mineral density changes during the menopause transition in a multiethnic cohort of women. J Clin Endocrinol Metab, **2008**; 93: 861-868.

10. van Staa TP, Leufkens HG, Cooper C. The epidemiology of corticosteroid-induced osteoporosis: a meta-analysis. Osteoporos Int, **2002**; 13: 777-787.
11. Gallant JE, Staszewski S, Pozniak AL *et al*. Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: a 3-year randomized trial. JAMA, **2004**; 292: 191-201.
12. Rabenda V, Bruyère O, Reginster JY. Relationship between bone mineral density changes and risk of fractures among patients receiving calcium with or without vitamin D supplementation: a meta-regression. Osteoporos Int, **2011**; 22: 893-901.
13. Mocroft A, Ledergerber B, Katlama C *et al*. Decline in the AIDS and death rates in the EuroSIDA study: an observational study. Lancet, **2003**; 362: 22-29.
14. Mocroft A, Reiss P, Gasiorowski J *et al*. Serious fatal and nonfatal non-AIDS-defining illnesses in Europe. J Acquir Immune Defic Syndr, **2010**; 55: 262-270.
15. Yin MT, Kendall MA, Wu X *et al*. Fractures after antiretroviral initiation. AIDS, **2012**; 26: 2175-2184.
16. Bedimo R, Maalouf NM, Zhang S, Drechsler H, Tebas P. Osteoporotic fracture risk associated with cumulative exposure to tenofovir and other antiretroviral agents. AIDS, **2012**; 26: 825-831.
17. Young B, Dao CN, Buchacz K *et al*. Increased rates of bone fracture among HIV-infected persons in the HIV Outpatient Study (HOPS) compared with the US general population, 2000-2006. Clin Infect Dis, **2011**; 52: 1061-1068.
18. Grant PM, Kitch D, McComsey GA *et al*. Low baseline CD4+ count is associated with greater bone mineral density loss after antiretroviral therapy initiation. Clin Infect Dis, **2013**; 57: 1483-1488.
19. Bedimo R, Cutrell J, Zhang S, Drechsler H *et al*. Mechanisms of bone disease in HIV and hepatitis C virus: impact of bone turnover, tenofovir exposure, sex steroids and severity of liver disease. AIDS, **2016**; 30: 601-608.
20. Szulc P. Association between cardiovascular diseases and osteoporosis-reappraisal. Bonekey Rep, **2012**; 1:144.
21. Fux CA, Rauch A, Simcock M *et al*. Tenofovir use is associated with an increase in serum alkaline phosphatase in the Swiss HIV Cohort Study. Antivir Ther **2008**; 13: 1077-1082.
22. Calmy A, Fux CA, Norris R *et al*. Low bone mineral density, renal dysfunction, and fracture risk in HIV infection: a cross-sectional study. J Infect Dis, **2009**; 200: 1746-1754.
23. Grant PM, Kitch D, McComsey GA *et al*. Long-term Bone Mineral Density Changes in Antiretroviral-Treated HIV-Infected Individuals. J Infect Dis, **2016**; 214: 607-611.

24. Yombi JC, Vandercam B, Wilmes D, Dubuc JE, Vincent A, Docquier PL. Osteonecrosis of the femoral head in patients with type 1 human immunodeficiency virus infection: clinical analysis and review. Clin Rheumatol, **2009**; 28: 815-823.
25. Mont MA, Cherian JJ, Sierra RJ, Jones LC, Lieberman JR. Nontraumatic Osteonecrosis of the Femoral Head: Where Do We Stand Today? A Ten-Year Update. J Bone Joint Surg Am, **2015**; 97: 1604-1627.
26. Morse CG, Dodd LE, Nghiem K *et al*. Elevations in D-dimer and C-reactive protein are associated with the development ofosteonecrosis of the hip in HIV-infected adults. AIDS, **2013**; 27: 591-595.
27. Hasse B, Ledergerber B, Egger M *et al*. Antiretroviral treatment and osteonecrosis in patients of the Swiss HIV Cohort Study: a nested case-control study. AIDS Res Hum Retroviruses **2004**; 20: 909-915.
28. Mary-Krause M, Billaud E, Poizot-Martin I *et al*. Risk factors for osteonecrosis in HIV-infected patients: impact of treatment with combination antiretroviral therapy. AIDS, **2006**; 20: 1627-1635.
29. Gutiérrez F, Padilla S, Masiá M *et al*. Osteonecrosis in patients infected with HIV: clinical epidemiology and natural history in a large case series from Spain. J Acquir Immune Defic Syndr, **2006**; 42: 286-292.
30. Fessel WJ, Chau Q, Leong D. Association of osteonecrosis and osteoporosis in HIV-1-infected patients. AIDS, **2011**; 25: 1877-1880.
31. Permpalung N, Ungprasert P, Summachiwakij S, Leeaphorn N, Knight EL. Protease inhibitors and avascular necrosis: a systematic review and meta-analysis. Int J Antimicrob Agents, **2014**; 44: 93-95.
32. Grund B, Peng G, Gibert CL *et al*. Continuous antiretroviral therapy decreases bone mineral density. AIDS, 2009; 23: 1519-1529.
33. Lawson-Ayayi S, Bonnet F, Bernardin E *et al*. Avascular necrosis in HIV-infected patients: a case-control study from the Aquitaine Cohort, 1997-2002, France. Clin Infect Dis, **2005**; 40: 1188-1193.
34. Mazzotta E, Agostinone A, Rosso R *et al*. Osteonecrosis in human immunodeficiency virus (HIV)-infected patients: a multicentric case-control study. J Bone Miner Metab, **2011**; 29: 383-388.
35. European AIDS Clinical Society 2015. European Guidelines for treatment of HIV-infected adults in Europe. Available at: http://www.eacsociety.org/files/guidelines_8.1-english.pdf**.** Accessed 14 December 2016.

36. Brown TT, Hoy J, Borderi M *et al*. Recommendations for evaluation and management of bone disease in HIV. Clin Infect Dis, **2015**; 60: 1242-1251.

Tables

Table 1: Characteristics at last visit for those with no bone event, or at last diagnosis of fracture or osteonecrosis

Supplemental Table 1: Participant characteristics at last visit: non-osteoporotic versus osteoporotic fractures.

Supplemental Table 2: Univariate and multivariate factors associated with any fracture.

Supplemental Table 3: Univariate and multivariate factors associated with osteonecrosis of the femoral head.

Figures

Figure 1: Factors independently associated with fractures and osteonecrosis of the femoral head.

aIRR: adjusted incidence rate ratio. Models adjusted for depicted covariates plus calendar year and region. Only covariates significant ($p<0.1$) in univariate analyses were included in multivariate models to avoid overfitting.

1 not significant for osteonecrosis in univariate analysis and hence not included in multivariate model for osteonecrosis

2 not significant for fractures in univariate analysis and hence not included in multivariate model for fractures

Figure 2: Crude incidence of fractures. TDF use.

CI: confidence interval, PYFU: person-years of follow up, TDF: tenofovir disoproxil fumarate.

Figure 3: Effect of TDF exposure on risk of any fracture and of osteoporotic fractures [a].

a grouped as fractures of the spine, arm, wrist and hip

b adjusted for demographics, HIV-specific variables and co-morbidities.

Figure 4: Relationship between antiretroviral drugs and osteonecrosis.

ARV: antiretroviral, DDI: didanosine, IDV: indinavir, LPV/r: ritonavir boosted lopinavir, SQV: saquinavir, TDF : tenofovir disoproxil fumarate.

Table 1: Characteristics at last visit for those with no bone event, or at last diagnosis of fracture or osteonecrosis				
Variable	**No bone event**	**Fractures**	**Osteonecrosis of the femoral head**	**P-value***
Overall	11266 (95.2)	496 (4.2)	73 (0.6)	
Demographics n(%)/Median (IQR)				
Male gender	8460 (75.4)	357 (72.1)	58 (76.5)	0.22
Age (years)	49 (42,56)	50 (44,59)	47 (43,56)	0.0089
HIV transmission group				
MSM	4800 (42.6)	180 (36.4)	35 (47.9)	0.0032
Heterosexual	3475 (30.9)	150 (30.3)	17 (23.3)	
IDU	2156 (19.1)	130 (26.3)	14 (23.3)	
White ethnicity	9702 (86.1)	445 (89.9)	68 (93.1)	0.013
Asian	207 (1.8)	9 (1.6)	2 (2.7)	
Black	638 (5.7)	21 (4.2)	1 (1.4)	
Other	719 (6.4)	21 (4.2)	2 (2.7)	
Body Mass Index (Kg/m^2)				
≤ 18	926 (9.3)	64 (13.9)	10 (14.9)	0.0006
18-30	4248 (42.8)	214 (46.4)	29 (43.3)	

>30	4760 (47.9)	183 (39.7)	28 (41.8)	
HIV-specific variables n(%)/Median (IQR)				
On ART	10857 (89.1)	486 (97.0)	73 (100)	0.20
CD4 cells/mm^3	540 (340,749)	450 (332,658)	468 (328,621)	0.0004
Nadir CD4 cells/mm^3	176 (72,290)	120 (50,230)	90 (28,168)	<0.0001
HIV RNA <500 cp/ml	10032 (89.1)	441 (89.1)	70 (95.9)	0.17
Co-morbidities n(%)				
HCV positive	2805 (24.9)	157 (31.7)	18 (24.7)	0.012
Current smoking	3248 (28.8)	188 (38.0)	31 (42.5)	<0.0001
Prior osteonecrosis	43 (0.4)	14 (2.8)	20 (27.4)	<0.0001
Prior fracture	202 (1.8)	79 (16.0)	6 (8.2)	<0.0001
Prior AIDS	3526 (31.6)	188 (38.0)	43 (58.9)	< 0.0001
Prior AIDS (not cancer)	3114 (27.6)	176 (35.6)	38 (52.1)	<0.0001
Prior AIDS (cancer)	743 (6.6)	30 (6.1)	14 (19.2)	<0.0001
Prior non-AIDS cancer	1570 (13.9)	80 (16.2)	11 (15.1)	0.37
Prior cardiovascular disease	229 (2.0)	21 (4.2)	3 (4.1)	<0.0001
Recent AIDS event [a]	217 (1.9)	8 (1.6)	0 (0)	0.43
Recent AIDS (not cancer) [a]	172 (1.5)	7 (1.4)	0 (0)	0.58
Recent AIDS cancer [a]	55 (0.5)	1 (0.2)	0 (0)	0.25
Recent non-AIDS cancer [a]	403 (3.6)	23 (4.7)	6 (8.2)	0.052

Recent cardiovascular disease [a]	10 (0.1)	4 (0.8)	0 (0)	<0.0001
Chronic kidney disease [b]	583 (5.3)	26 (5.6)	5 (7.5)	0.71
eGFR [c] mL/min/1.73 m^2 Median (IQR)	95 (80,106)	96 (79,106)	94 (75,106)	0.58
Vitamin D [d] ng/mL Median (IQR)	37 (22,65)	35 (15,83)	31 (9,40)	0.26
Total cholesterol mg/dL Median (IQR)[e]	191 (162 -223)	193 (166 – 223)	196 (170 – 228)	0.43
Triglycerides mg/dL Median (IQR)[f]	136 (92 – 208)	139 (97 – 208)	181 (108 – 328)	0.0025

*global p-values for comparing across the three groups

(a) Occurring in the last 12 months prior to the diagnosis of fracture or osteonecrosis of the femoral head.

(b) chronic kidney disease at any time before last visit or event date; defined as confirmed (> 3 months apart) eGFR < 60 for those with first eGFR > 60, or 25% decline where baseline eGFR< 60. eGFRs were calculated using CKD-EPI formula.

(c) eGFR was available for 11536 (97.5%); 11003 (97.7%) for those with no events, 466 (94.1%) for those with any fracture and 67 (91.8%) for those with osteonecrosis of the femoral head (p<0.0001).

(d) Data available for 3291 (27.8%) overall, 3210 (28.5%) for those with no events, 71 (14.3%) for those with fractures and 10 (13.7%) of those with osteonecrosis of the femoral head (p<0.0001).

(e) Data available for 11687 (98.9%) overall; 11122 (98.7%) for those with no events, 492 (99.4%) for those with fractures and 73 (100%) of those with osteonecrosis of the femoral head (p=0.26)

(f) Data available for 11365 (96.2%) overall; 10813 (96.0%) for those with no events, 482 (97.4%) for those with fractures and 70 (95.9%) of those with osteonecrosis of the femoral head (p=0.26)

Supplemental Table 1: Participant characteristics at last visit: non-osteoporotic versus osteoporotic fractures				
		Non-osteoporotic	**Osteoporotic**	**P-value**
Overall		486 (78.6)	132 (21.4)	
Characteristic **n(%)/Median (IQR)**				
Gender	Male	357 (73.5)	91 (68.9)	0.30
Age (years)		49 (44,59)	52 (46,60)	0.021
HIV transmission group	MSM	170 (35.0)	52 (39.4)	0.030
	IV drug use	141 (29.0)	27 (20.5)	
	Heterosexual	131 (27.0)	47 (35.6)	
Race	White	436 (89.7)	123 (93.2)	0.23
Region	North Europe	211 (43.4)	72 (54.6)	0.0006
	Central and Eastern Europe	47 (9.7)	22 (16.7)	
On ART		468 (96.3)	129 (97.7)	0.42
CD4 cells/mm^3		441 (311,663)	454 (340,651)	0.62
Viral load < 500 copies/mL		427 (87.9)	119 (90.2)	0.47
HBV	Pos	25 (5.1)	5 (3.8)	0.61

HCV	Pos	166 (34.2)	39 (29.6)	0.57
Recent AIDS [a]		7 (1.4)	4 (3.0)	0.22
Chronic kidney disease [b]		28 (5.8)	3 (2.2)	0.10
eGFR [c]		97 (80,106)	95 (77,100)	0.33
Vitamin D [d]		33 (11,72)	34 (23,84)	0.42

(a) in last 12 months prior to diagnosis of fractures

(b) chronic kidney disease at any time before last visit or event date; defined as confirmed (> 3 months apart) eGFR < 60 for those with first eGFR > 60, or 25% decline where baseline eGFR< 60.

(c) eGFR was available for 455 (93.6%) for those with non-osteoporotic fractures and 127 (96.2%) for those with osteoporotic fractures (p=0.26). eGFRs were calculated using CKD-EPI formula.

(d) Data available for 64 (13.2%) for those with non-osteoporotic fractures and 15 (11.4%) for those with osteoporotic fractures (p=0.58).

Supplemental Table 2: Univariate and multivariate factors associated with any fracture							
		Univariate			Multivariate		
		IRR	95% CI	P	IRR	95% CI	P
Age	/10 yrs	1.42	1.31 – 1.54	<0.0001	1.39	1.26 – 1.53	<0.0001
HIV transmission group	IDU	1.75	1.39 – 2.14	<0.0001	1.58	1.18 – 2.12	0.0021
	Other	1.00	-	-	1.00	-	-
Race	White	1.00	-	-	1.00	-	-
	Other	0.67	0.50 – 0.90	0.0073	0.64	0.47 – 0.88	0.0056
BMI	≤18	1.69	1.27 – 2.26	0.0004	1.51	1.14 – 1.99	0.0036
	18-30	1.00	-	-	1.00	-	-
	>30	0.90	0.73 – 1.10	0.30	0.91	0.74 – 1.11	0.36
	unknown	0.65	0.44 – 0.98	0.039	0.79	0.52 – 1.18	0.25
CD4*	/doubling	0.73	0.64 – 0.83	<0.0001	0.80	0.69 – 0.92	0.0014
Nadir CD4	/doubling	0.71	0.63 – 0.80	<0.0001	0.92	0.79 – 1.07	0.25
Baseline	<500	1.00	-	-	1.00	-	-
Viral Load	500-100k	0.68	0.53 – 0.87	0.0019	0.84	0.66 – 1.08	0.17
	>100k	1.06	0.70 – 1.60	0.78	1.05	0.71 – 1.55	0.81
Region	S	1.00	-	-	1.00	-	-
	N	1.80	1.32 – 2.47	0.0003	1.83	1.32 – 2.52	0.0003
	C	3.29	2.47 – 4.40	<0.0001	3.63	2.68 – 4.91	<0.0001
	CE	1.58	1.12 – 2.22	0.0092	1.60	1.13 – 2.28	0.0087
Year of follow up*	≤2006	1.00	-	-	1.00	-	-
	07-09	1.58	1.22 – 2.04	0.0005	1.50	1.16 – 1.94	0.0020
	10-12	1.75	1.36 – 2.25	<0.0001	1.56	1.20 – 2.02	0.0007
	>2012	1.28	0.95 – 1.72	0.10	1.09	0.80 – 1.48	0.58
HCV*	Neg	1.00	-	-	1.00	-	-
	Pos	1.70	1.39 – 2.09	<0.0001	1.59	1.20 – 2.11	0.0013
	Unknown	0.68	0.45 – 1.03	0.068	0.81	0.53 – 1.22	0.31

Smoking	Never	1.00	-	-	1.00	-	-
	Current	1.28	1.01 – 1.61	0.040	1.09	0.86 – 1.38	0.47
	Past	0.93	0.70 – 1.29	0.65	0.80	0.58 – 1.11	0.18
	Unknown	0.90	0.70 – 1.15	0.38	0.72	0.57 – 0.92	0.0083
	Arg	0.68	0.35 – 1.41	0.30	0.88	0.42 – 1.82	0.72
Osteonecrosis*	No	1.00	-	-	1.00	-	-
	Yes	4.69	2.95 – 7.46	<0.0001	3.19	1.91 – 5.31	<0.0001
Prior Fracture	No	1.00	-	-	1.00	-	-
	Yes	5.24	3.45 – 7.96	<0.0001	3.89	2.62 – 5.77	<0.0001
AIDS (not cancer)*		1.46	1.20 – 1.77	<0.0001	1.13	0.92 – 1.38	0.25
Recent non-AIDS-defining malignancy*		3.10	2.11 – 4.57	<0.0001	1.88	1.26 – 2.80	0.0018
Recent cardiovascular disease*		9.56	3.68 – 24.82	<0.0001	6.03	2.28 – 15.95	0.0003
All factors fixed at baseline, except *, which were time-updated							

Supplemental Table 3: Univariate and multivariate factors associated with osteonecrosis of the femoral head							
		Univariate			Multivariate		
		IRR	95% CI	P	IRR	95% CI	P
Age	/10 yrs	1.19	0.99 – 1.43	0.068	1.07	0.87 – 1.31	0.54
Race	White	1.00	-	-	1.00	-	-
	Other	0.38	0.15 – 0.95	0.038	0.28	0.11 – 0.76	0.012
Nadir CD4	/doubling	0.52	0.36 – 0.75	0.0005	0.71	0.51 – 1.01	0.054
Prior osteonecrosis	No	1.00	-	-	1.00	-	-
	Yes	20.82	10.32 – 41.99	<0.0001	14.95	6.96 – 32.15	<0.0001
Prior fracture*	No	1.00	-	-	1.00	-	-
	Yes	4.26	2.13 – 8.50	<0.0001	3.12	1.50 – 6.49	0.0023
AIDS (not cancer)*		2.92	1.78 – 4.80	<0.0001	2.13	1.27 – 3.57	0.0043
AIDS-defining cancer*		3.57	1.96 – 6.50	<0.0001	2.69	1.48 – 4.89	0.0012
Non-AIDS-defining malignancies*		2.00	1.03 – 3.91	0.042	1.33	0.65 – 2.69	0.43
All factors fixed at baseline, except *, which were time-updated							