Bone Density, Microarchitecture, and Tissue Quality After Long-Term Treatment With Tenofovir/Emtricitabine or Abacavir/Lamivudine

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Objectives: HIV infection has been associated with reduced bone mineral density (BMD). Antiretroviral therapy (ART) has a deleterious effect on BMD, but its effect on bone fragility is not clear. The objective of this study is to analyze the BMD, microarchitecture, and tissue quality of bone in patients receiving long-term tenofovir- or abacavir-based ART.

Design: We conducted a cross-sectional study in patients with HIV undergoing tenofovir or abacavir ART for more than 5 years.

Methods: We measured BMD using dual X-ray absorptiometry , bone michroarchitecture using trabecular bone score (TBS), and bone tissue quality using microindentation. TBS is a dual X-ray absorptiometry–based software that is more highly correlated with bone fragility than BMD. Microindentation (BMSi) directly assesses bone quality at the tissue level.

Results: A total of 63 patients were included in this study, with 36 belonging to the TDF-FTC group and 27 to the ABC-3TC group. Patients receiving TDF-FTC treatment showed lower BMD values than those in the ABC-3TC group. We found no differences in TBS or microindentation between the 2 groups. However, after adjusting for sex, age, body mass index, and 25[OH]vitD we found lower BMSi and thus poorer bone properties in the TDF-FTC group than in the ABC-3TC group [beta coefficient −3.594 (confidence interval: 95% −0.12 to −7.61); $P = 0.043$].

Conclusions: Long-term treatment with TDF-FTC leads to impaired bone health, not only in terms of BMD but also in terms of bone quality, another determinant of overall bone strength. To complement BMD-based predictions, these other techniques may also be used to identify patients with excess fracture risk.

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INTRODUCTION

Antiretroviral therapy (ART) and HIV infection have been associated with reduced bone mineral density (BMD) as measured by dual X-ray absorptiometry (DXA). Despite the potentially toxic effects of ART on bones, bone disease in HIV-treated patients is not well characterized. More specifically, the antiretroviral drug tenofovir disoproxil fumarate (TDF) has been associated with reduced BMD and higher levels of bone turnover markers.[1] Several mineral alterations can already be detected soon after ART initiation, and as treatment continues, there is a progressive decrease in BMD and an increase in bone turnover markers. In fact, these changes can even persist for much longer: in longitudinal follow-up studies of up to 96 weeks, ART-treated patients continued to exhibit progressive BMD loss.[2] However, no clear association between fracture risk and antiretroviral drug use has been discovered. Nonetheless, the clinical impact of bone disease among patients undergoing long-term ART is an important issue. In patients with HIV, aside from ART, a combination of several other factors such as harmful lifestyles and altered hormone levels may also have an impact on bone health.

Bone strength is an integration of both bone density and bone quality.[3] As such, measuring DXA only provides limited information about overall bone health. Unfortunately, in many situations, this DXA-measured bone density is not sufficient to thoroughly assess fracture risk.[4–6] A more comprehensive assessment including other key aspects of bone strength, such as bone microarchitecture and mechanical properties (tissue quality), is needed to better evaluate the propensity of bone to fracture. This is especially true in situations where BMD does not fully account for the decrease in bone strength. To assess bone microarchitecture, new DXA-based software has been developed. This enables a trabecular bone score (TBS), an index of bone microarchitecture, to be derived from lumbar spine DXA images.[7] The TBS is a BMD-independent predictor of fracture risk. A recent meta-analysis found that the TBS can significantly

predict fracture risk independently of the FRAX diagnostic tool.[8] With respect to the quality component of bone strength, microindentation techniques can provide direct assessments of the mechanical properties of bone at the tissue level.[5,6,9] Through single-impact microindentation, a direct measurement of the resistance of cortical bone tissue to the appearance of microcracks is obtained. This phenomenon closely mimics the initial crack of a fracture[9,10] and is correlated with the propensity of bone to fracture.[4] Furthermore, as measured by microindentation, patients with HIV show worse mechanical properties than noninfected individuals irrespective of BMD.[11]

The aim of this study was to determine the consequences of long-term tenofovir/emtricitabine (TDF-FTC) or abacavir/lamivudine (ABC-3TC) treatment on bone health. The effects of these 2 common ART regimens were analyzed by measuring BMD, microindentation, TBS-assessed bone microarchitecture, and bone turnover markers.

PATIENTS AND METHODS

Patients

We conducted a cross-sectional study in the outpatient clinic of the Infectious Diseases Department at Hospital del Mar in Barcelona, Spain. The study was approved by the local Clinical Research Ethics Committee, and all participants provided written informed consent (Ethical Committee 2013/5250/I).

For each patient, we obtained information regarding general clinical history, specific HIV history, and past treatments. All patients in the study were HIV infected, older than 50 years, and had been subjected to ART (either TDF-FTC or ABC-3TC treatment) for more than 5 years. All patients displayed good treatment adherence, undetectable viral loads ($<$50 copies/mL), and CD4 T-lymphocyte counts of $>$250/mL. All individuals who had undergone treatment with bone-active drugs or who had a condition that could interfere with bone metabolism were excluded from the study. This included patients with liver disease, severe chronic active alcoholism (defined as $>$40 g/d), malignancy, Cushing syndrome, hypogonadism, hyperthyroidism, hypopituitarism, hyperparathyroidism, chronic kidney disease (defined as glomerular filtration rate $<60\ \text{mL}\cdot\text{min}^{-1}\cdot 1.73\ \text{m}^{-2}$ for at least 3 months), and specifically we excluded patients who presented in any previous control abnormal creatinine levels, and also those cases where therapy was changed because of abnormal kidney function, chronic obstructive pulmonary disease, chronic hepatitis C or B, diabetes mellitus type 1 or type 2, or neuropathic disease. Furthermore, patients who used glucocorticoids or opioids, who were active intravenous drug consumers, or who had a history of switching between TDF-FTC and ABC-3TC treatments were also excluded.

Bone Measurements

For each patient, we recorded risk factors for bone fracture and performed spinal x-rays to detect vertebral fractures. X-rays were assessed by 2 independent observers. We accepted grade I or above (a loss of $>$20% of vertebral height) as a true fracture. We used chemiluminescent immunoassays (CLIAs) to determine several fasting routine bone-specific laboratory values. Each immunoassay had an interassay coefficient of variation of 10%. Specifically, we measured levels of intact parathormone (Siemens); bone alkaline phosphatase (Roche Diagnostics, Switzerland); the amino pro-peptide of type I collagen (PINP), cross-linked collagen type I, C-telopeptide (CTX), and serum 25-hydroxyvitamin D (Roche Diagnostics); bone-specific alkaline phosphatase (Roche Diagnostics) and high-sensitivity C-reactive protein [CLIA (Immulite 2000; Siemens)], erythrocyte sedimentation rate, beta-2 microglobulin (CLIA) (Immulite 2000; Siemens); D-dimer [immunoturbidimetry (ACL TOP300)] and fibrinogen [Clauss method (ACL TOP300)].

BMD was measured at the lumbar spine and the hip by DXA (DXA-Hologic QDR4500SL; S/N 45,329). The coefficient of variation for the DXA measurements was 1% in the spine and 1.7% for the femoral neck. The TBS, a gray-level textural metric that can be extracted from two-dimensional lumbar spine DXA images, is related to bone microarchitecture and provides skeletal information that is not captured in standard BMD values. TBS values are considered to be normal when above 1.35. Values between 1.20 and 1.35 imply a partially impaired microarchitecture, whereas values below 1.20 refer to a degraded microarchitecture. TBS values were evaluated in the same regions that were used for lumbar spine BMD measurements using iNsight v 2.1 (Med.Imaps, Merignac, France). Microindentation tests were performed using a handheld OsteoProbe Reference Point Indenter (Active-Life-Scientific, Santa Barbara, CA) according to a recently published protocol.[12] Briefly, under local anesthesia, software provides the average value of eight 30 N indentations performed on the anterior face of the tibia using a test probe with a conic edge of 4 μm. This average is then normalized to the average of 5 indentations made on a polymethylmethacrylate block. The ratio between the bone and polymethilmethacrylate measurements provides a bone mineral strength index (BMSi) parameter.[12] This technique has previously been validated in humans, specifically within the HIV-infected population.[9,11] Importantly, this procedure takes less than 5 minutes, causes minimal discomfort to the patient, and no complications have been described in published studies. The coefficient of variation for microindentation in our laboratory is 3% (Fig. 1).

Statistical Analysis

Sample size number was calculated based on previous publications.[11] Assuming a type I and II error of 0.05 in a 2-sided test, 27 HIV-infected patients treated with TDF-FTC and 27 treated with ABC-3TC were required to detect a statistically significant difference of $\geq$5 units of BMSi. The common SD was assumed to be 5.

We used 2-sample t tests and χ^2 tests to compared quantitative and categorical variables, respectively, between HIV-infected individuals treated with TDF-FTC and those treated with ABC-3TC. We used multivariable linear

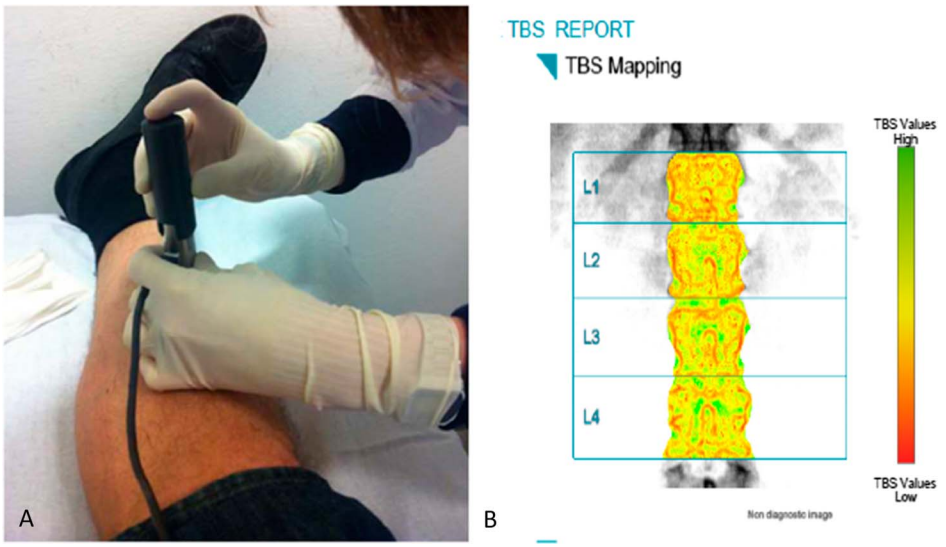

FIGURE 1. A, Microindentation on tibia for the assessment of bone quality. B, TBS for the assessment of bone microarchitecture on spine DXA.

regression modeling with backward stepwise variable selection to identify factors associated with BMSi. The variables included in the model at the first step of variable selection were, after locking age, sex and body mass index (BMI), treatment group, CD4 count, spine BMD, 25[OH]vitD, CTX, spine BMD, and total hip BMD. Any variable with $P > 0.1$ was eliminated from the model, and results with $P < 0.05$ (2-tailed) were considered statistically significant. Analyses were performed using Stata/IC 13.1.

RESULTS

Patients

This study included a total of 63 patients between May 2015 and May 2016, of which 36 belonged to the TDF-FTC group and 27 to the ABC-3TC group. The mean ages of the TDF-FTC and ABC-3TC groups were 56.3 (SD = 6.2) and 63 (SD = 10), respectively ($P = 0.0001$). The median time after initiation of ART was 7.8 years (interquartile range, IQR = 3.2) in the TDF-FTC group versus 8.9 years (IQR = 3.4) in the ABC-3TC group ($P = 0.086$). No current or past differences in renal function were detected among groups. There were no differences in the use of protease inhibitor as a third drug in the TDF versus ABC groups [3 (8%) versus 2(7%); $P = 0.893$], respectively. Most demographic and clinical parameters were well balanced between the 2 groups (Table 1).

The median level of the bone formation marker PINP was higher in patients with HIV-1 receiving TDF-FTC treatment (54.3 ng/mL, IQR = 36.5) than in those receiving ABC-3TC (38.1 ng/mL, IQR = 18.5) ($P = 0.0171$). We observed a similar effect with respect to the median level of bone resorption marker CTX [0.432 ng/mL (IQR = 0.303) and 0.310 (IQR = 0.12) ng/mL, respectively; $P = 0.05$]. However, after adjusting by age, sex, BMI, and vitamin D levels, the differences in PINP, CTX, and bone disappeared between TDF and ABC groups. In contrast, we found no differences in vitamin D levels between groups [27.5 ng/mL (IQR = 21.2) versus 23.1 ng/mL (IQR = 17), respectively; $P = 0.339$].

The median BMD in the lumbar spine of HIV-1–infected patients under TDF-FTC treatment was significantly lower than that of those under ABC-3TC treatment [0.926 g/cm^2 (SD = 0.2) versus 1.015 g/cm^2 (SD = 0.2); $P = 0.04$]. This difference was also reflected in the Z-score of the BMD (table 2). In contrast, there were no significant differences between the groups with respect to the BMD (and Z-scores) in either the femoral neck or the total hip (Table 2).

Furthermore, we found no difference in TBS between the TDF-FTC and ABC-3TC groups, even after adjusting for age and BMI [1.254 (IQR = 0.015) versus 1.221

TABLE 1. Baseline Characteristics of Study Population

	TDF-FTC treated	ABC-3TC treated	*P*
N	36	27	
Age, yrs	56.4 (6.3)*	63 (9.8)	0.0019
Male, n (%)	27 (75)	20 (74.1)	0.93
BMI, kg/m^2	23.8 (2.4)	26.1 (3.4)	0.0031
Smoking, n (%)	14 (38.9)	13 (48.1)	0.46
Alcohol, >10 g/d	5 (13.9)	2 (7.4)	0.42
Ex-IDU, n (%)	5 (13.9)	2 (7.4)	0.42
Recreational drugs, n (%)	1 (2.7)	1 (3.7)	0.84
Previous fracture, n (%)	2 (5.5)	1 (3.7)	0.422
Family history of fracture, n (%)	0	1 (3.7)	0.146
Prevalent spine fractures, n (%)	0	0	
eGFR <60 mL/min any time in follow-up	0	0	
eGFR (CKD-EPI, mL/min)	86 (2.2)	81 (2.3)	0.134
Years since HIV diagnosis	16.8 (6.3)	17.1 (5.2)	0.85
Time undergoing treatment, mo	9 (3)	8 (3)	0.452
Nadir CD4 count (per mL)	217.9 (128.3)	189 (132.4)	0.39
Current CD4 count (per mL)	741.4 (311.3)	712.8 (229.8)	0.69
Ever met AIDS criteria, n (%)	9 (25)	9 (33.3)	0.47
Protease inhibitor, n (%)	3 (8)	2 (7)	0.893

*Results are shown as mean values (SD), unless indicated otherwise.
CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration estimation of Glomerular Filtration Rate; eGFR, estimated glomerular filtration rate; IDU, intravenous drugs users.

TABLE 2. BMD, Markers of Bone Turnover, Calcium Metabolism, and Inflammation and Procoagulation

	TDF-FTC Treated	ABC-3TC Treated	*P*
BMD			
Femoral neck BMD, g/cm^2	0.718 (0.2)	0.740 (0.150)	0.248
Femoral neck T-score	−1.5 (0.85)	−1.28 (0.88)	0.340
Lumbar spine BMD, g/cm^2	0.926 (0.2)	1.015 (0.2)	0.04
Lumbar spine T-score	−1.46 (1.3)	−0.7 (1.36)	0.029
Total hip BMD, g/cm^2	0.886 (0.301)	0.884 (0.150)	0.373
Total hip T-score	−1.05 (1.03)	−0.72 (0.95)	0.207
TBS	1.254 (0.015)	1.221 (0.026)	0.368
Bone turnover			
Amino pro-peptide of type 1 collagen, ng/mL	54.3 (36.5)	38.1 (18.5)	0.017
Bone alkaline phosphatase, µg/mL	20.96 (10.28)	17.46 (16.02)	0.306
C-telopeptide, ng/mL	0.432 (0.303)	0.310 (0.12)	0.051
Calcium metabolism			
Parathormone, pg/mL	60.74 (31.65)	49.04 (25.36)	0.131
25[OH]Vitamin D, ng/mL	29.08 (14.93)	25.6 (12.64)	0.339
Calcium, mg/dL	9.4 (0.5)	9.6 (0.4)	0.062
Phosphorus, mg/dL	3.07 (0.51)	2.95 (0.46)	0.363
Creatinine	0.88 (0.3)	0.96 (0.26)	0.426
Inflammation and coagulation			
High-sensitivity C-reactive protein, mg/dL	0.48 (1.14)	0.62 (0.97)	0.610
LDH	313.11 (47.1)	311.96 (49.04)	0.925
Erythrocyte sedimentation rate, mm/h	5.32 (4.7)	7.48 (5.97)	0.126
D-Dimer, ng/mL	275.29 (236.04)	254.17 (162)	0.710
Fibrinogen, mg/dL	300.48 (64.44)	276.92 (61.73)	0.165
Beta-2 microglobulin, mg/L	1.87 (0.36)	1.9 (0.37)	0.769

Results are shown as median values (IQR), unless indicated otherwise.
LDH, Lactate Dehydrogenase.

(IQR = 0.026); $P = 0.14$]. We also found no differences in BMSi between the TDF-FTC and ABC-3TC groups in the unadjusted analysis [81.02 (IQR = 0.819) versus 82.68 (IQR = 1.3); $P = 0.269$]. The results of the multivariate analysis are shown in Table 3. However, after adjusting for patients' age, sex, BMI, and 25[OH] vitD levels, we found a lower BMSi (ie, poorer bone material properties) in the TDF-FTC group compared with the ABC-3TC group [beta coefficient −3.594 (confidence interval: 95% −-0.12 to −7.61); $P = 0.043$].

In an alternative multivariable analysis, we also locked in the analysis of total hip BMD for an additional adjustment. We used total hip as long as tibial BMD was not available. After this adjustment, differences between groups of treatment remained similar to the previous analysis and statistically different.

For BMSi, we found a strong, significant correlation with 25[OH]vitD levels (r = 0.257; $P = 0.04$), but no correlation with the BMD or TBS. Similarly, we did not observe any correlation for BMD and TBS values with turnover markers, hsCRP, or 25[OH]vitD levels. No fractures were recorded in any of the patients recruited.

DISCUSSION

This study analyzes differences in the bone health of patients with HIV undergoing 2 types of long-term ART. Overall, our results show greater bone impairment in patients treated with TDF-FTC than on those treated with ABC-3TC. In the TDF-FTC group, we observed not only impaired bone density but also poorer mechanical properties of the bone tissue, as measured by microindentation. This deleterious effect is likely worsened by the harm caused to bone by the HIV infection itself.[11] To date, this is the first global assessment of bone health at different levels, namely bone density, bone microarchitecture, and direct tissue testing in HIV-treated patients.

Although ART can reverse many of the immunologic changes induced by HIV, several published articles suggest that it also results in increased bone damage.[13] In our study, patients who had received TDF-FTC treatment for 5 years have a higher rate of bone remodeling than those who received ABC-3TC treatment. However, after adjusting by covariables such as BMI, sex, age, and vitamin D, we observed that the influence of the treatment disappeared.

The initiation of ART induces rapid loss in bone density, especially during the first year. Grant et al[2] reported a greater decline in BMD during the first 96 weeks in patients subjected to ART than in control patients. Thereafter, the rate of decline slowed down, but still remained higher than in HIV-uninfected patients. This was attributed to the persistent inflammatory activation that occurs even after reaching infection control and undetectable viral loads. Moreover, many longitudinal studies have compared the effect of ART on BMD in therapy-naive patients starting treatment to that in individuals who had previously received treatment.[14] These studies uniformly showed that TDF-FTC regimes induced greater bone loss than ABC-3TC-based regimes. Although the mechanism underlying these differences is not clear, Casado et al[15] reported that tubular dysfunction leads to altered phosphate metabolism, and that the subsequent chronic abnormal phosphaturia could at least partly explain the progressive loss of bone during TDF-FTC therapy.

In agreement with Grigsby et al,[15] we report an excess of bone loss in patients undergoing TDF-FTC–based therapy. In these patients, we also observe impaired mechanical properties of bone tissue, but no differences in bone microarchitecture as measured by the TBS. Bone strength is the capacity of bone to absorb energy before fracturing. Although BMD is not an apt parameter to fully assess bone strength, a number of studies have consistently shown that TBS is associated with fracture risk in both cross-sectional and prospective studies.[16,17] Furthermore, TBS is capable of predicting fracture independent of BMD. In our study, both treatment groups had TBS values within the same order of magnitude, indicating that they had very similar bone microarchitecture. This shows that although overall bone density is diminished in the TDF-FTC group, bone microarchitecture remains similar. Although BMD and TBS use DXA as a source for images, they are measuring different aspects of bone strength. While with the former, we measure what could be considered as the "amount of mineral in the tissue" with

TABLE 3. Univariate and Multivariable Analysis

	Univariate Analysis			Multivariable Analysis*		
	Unadjusted—β Coefficient	95% CI	*P*	Adjusted—β Coefficient*	95% CI	*P*
ABC-3TC group	Ref			Ref		
TDF-FTC group	−1.65	1.31 to −4.62	0.268	−3.594	−0.12 to −7.61	0.043
Age	−0.0701	−0.243 to 0.103	0.422	−0.167	−0.35 to 0.025	0.087
Male	Ref			Ref		
Female	−1.32	−4.71 to 2.06	0.437	−2.31	−5.96 to 1.33	0.209
BMI, kg/m^2	0.05	−0.435 to 0.5488	0.819	0.023	−0.57 to 0.62	0.938
25[OH]Vitamin D	0.107	0.003 to 0.212	0.044	0.111	0.0016 to 0.221	0.047
Creatinine, g/dL	4.65	−2.4 to 11.74	0.195			
hsCPR	−0.677	−2.11 to 0.753	0.347			
PTH	−0.013	−0.06 to 0.038	0.613			
Spine BMD, g/cm^2	−0.676	−10.25 to 8.91	0.888			
Total hip BMD, g/cm^2	7.28	−3.01 to 17.57	0.162			
TBS	9.584	−4.01 to 23.18	0.164			
Protease inhibitor	−2.14	−7.60 to 3.31	0.434			

Predictors of BMSi by multiple linear regression—stepwise backward elimination.
*Adjusted for group of treatment, sex, age, BMI, and 25-OH vitamin.
CI, confidence interval; PTH, parathormone hsCRP high sensibility C Reactive Protein.

the last we are measuring how the trabecular bone is disposed at the spine. It is plausible that the group of patients receiving TDF have a greater bone loss through different mechanisms, but probably mainly through phosphorus loss at renal tubule; however, the microarchitecture could remain more stable.

Although we found no differences in TBS between TDF and ABC groups, even after adjusting for potential confounders, the values of TBS in both groups are considered below normal. In fact, values between 1.350 and 1.200 are considered to show a partially deteriorated microarchitecture.[16] Because this is a cross-sectional study, we do not know which is the real role of the treatment on this deterioration. This could, once again, raise the potential role of the HIV infection itself, besides the treatment, in a potential impairment of bone strength.

Bone microindentation can provide extra information about the mechanical properties of bone tissue in ART patients. Although we did not find differences in BMSi in our univariate analysis, after adjusting for well-known fracture risk factors using multivariate linear regression, the TDF-FTC group showed poorer material properties. The small differences observed between the 2 groups, and the need to adjust for other variables to find these differences, lead us to the hypothesis that other HIV-related factors (such as time of HIV infection without treatment) could be important. Even after extensive TDF usage, the incidence of bone fracture does not increase markedly, neither in scientific studies nor in clinical practice. Even in large population-based studies such as the one by Bedimo et al,[18] in patients receiving TDF treatment the relative risk for fracture was 1.06, in contrast to other risk factors such as glucocorticoids or nutritional deficiencies that carried greater increases of risk. The EuroSIDA cohort results showed that the overall incidence of bone fracture among patients exposed to TDF at some point in their lives was nearly 2-fold higher than in patients who had never been treated with this drug.[19] The association between TDF use and fracture risk persisted in a multivariate analysis. However, there was no association between longer durations of TDF treatment and increased risk of fracture.[19] The fact that longer courses of TDF treatment are not associated with an increase in the number of fractures suggests that some individuals are more prone to bone fragility, and therefore more impacted by the drug. Thus, by complementing the information provided by DXA, microindentation could help to identify patients with greater risk of bone fracture.

The main limitations of this study are the small sample size and its cross-sectional design. Moreover, there may be selection bias in patients receiving ABC-3TC treatment, as these patients may have poorer bone conditions when starting ART. This is the first study that uses microindentation to assess the mechanical properties of bone tissue in ART-treated patients. In patients where bone fractures are prevalent despite their relatively preserved BMD values, microindentation could be clinically applicable in the HIV field. However, its ultimate value for monitoring bone health in ART-treated patients remains to be established in larger longitudinal studies.

In conclusion, BMD and BMSi values were lower in TDF-FTC–treated patients. Long-term treatment with TDF-FTC leads to impaired bone health, not only in terms of BMD but also in other key determinants of bone strength such bone quality.

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