JBM J Bone Metab 2020;27(3):175-186
https://doi.org/10.11005/jbm.2020.27.3.175
pISSN 2287-6375 eISSN 2287-7029

Original Article

Use of Bisphosphonates, Calcium and Vitamin D for Bone Demineralization in Patients with Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome: A Systematic Review and Meta-Analysis of Clinical Trials

Vinícius Magno da Rocha[1,2], Mariana Balardino Bogado Faria[2], Francisco de Assis dos Reis Júnior[3], Carla Ormundo Gonçalves Ximenes Lima[4], Rossano Kepler Alvim Fiorelli[1], Keila Mara Cassiano[5]

[1]Department of General and Specialized Surgery, Medical School, Federal University of the State of Rio de Janeiro, RJ;
[2]Department of Orthopedics and Traumatology, Gaffrée and Guinle University Hospital, RJ;
[3]Dertment of Orthopedics and Traumatology, Central Brazilian Army Hospital, RJ;
[4]Department of Clinical Pathology, National Institute of Traumatology and Orthopedics, RJ;
[5]Department of Statistics, Institute of Mathematics, Federal Fluminense University, RJ, Brazil

Corresponding author
Vinícius Magno da Rocha
Department of General and Specialized Surgery, Medical School, Federal University of the State of Rio de Janeiro, Rua Desembargador Izidro, 18 - Room 912, Tijuca, Rio de Janeiro, RJ 20521-160, Brazil
Tel: +55-21-2209-2194; +55-21-99700-7240
Fax: +55-21-2298-2194
E-mail: viniciusmagnodarocha@gmail.com

Received: April 21, 2020
Revised: July 6, 2020
Accepted: July 8, 2020

Background: The present study performed a systematic review and meta-analysis of clinical trials using bisphosphonates for bone demineralization in human immunodeficiency virus (HIV) patients. **Methods:** A comprehensive literature search was performed from January 2004 to January 2020 considering the bone mineral density (BMD) of the lumbar spine (LS) as the main outcome. Out of 214 titles that met criteria, 9 studies fulfilled the selection criteria. **Results:** A total of 394 patients were identified, and they were allocated into 2 groups: the intervention group (200 patients), to whom a combination of alendronate or zoledronate with calcium and vitamin D was administered; and control group (194 patients), to whom only calcium and vitamin D was administered. Clinical profile and indicators of bone metabolism of the participants were evaluated regarding effect size, homogeneity, and consistency. No substantial heterogeneity between the groups was found for the baseline variables, and there was high consistency to the main outcome. The meta-analysis shows a significant difference in post-treatment BMD, favoring the intervention over the control treatment. The intervention improved LS density up to 0.227 g/cm^2, raising the average to the levels of general population. Adverse effects related to intervention were fever immediately after zoledronate administration and gastrointestinal complaints during alendronate usage. Other adverse effects were barely reported and poorly connected to intervention by studies' authors, despite all of them have been successfully resolved. **Conclusions:** This study provides evidence that BMD post-treatment is better in HIV patients who used bisphosphonates combined with calcium and vitamin D.

Key Words: Antiretroviral therapy, highly active · Bone demineralization, pathologic · Diphosphonates · HIV · Osteoporosis

INTRODUCTION

Advances in the treatment of acquired immune deficiency syndrome (AIDS) impacts directly on the demographic profile of the infection. Highly active antiretro-

viral therapy (HAART) is increasing gradually life expectancy of people living with human immunodeficiency virus (HIV), allowing a better understanding of associations between the virus and the changes inherent to the aging process.[1-4]

Bone mineral density (BMD) reduction is a known finding related to aging that has been discussed for decades as the main pathophysiological drive of osteoporosis – a disease in which bone quality, density, and resistance are reduced, resulting in an increased risk of fractures.[5]

Osteoporosis is defined by a BMD reduction until 2.5 (or more) standard deviation (SD) below the average for the Caucasian population using dual energy X-ray absorptiometry as a measurement tool. Likewise, the disease is also defined by the occurrence of a frailty fracture.[6]

Among HIV patients, osteoporosis is more prevalent than in the general population, despite the age group and the use of HAART, which highlights the virus as an isolated risk factor for bone demineralization.[2,3] Furthermore, additional decreases in BMD were demonstrated after starting HAART, establishing pathways for bone demineralization different of those correlated to virus itself.[7]

As number of people over 50 living with HIV has been growing markedly since HAART introduction, the deleterious effects of HIV and its treatment on bone metabolism is calling the attention of researches and health managers due to the greater risk of frailty fractures in a group of patients with many comorbidities and high rates of post-surgical complications were expected.[8-11]

Driven by this warning, many studies have been conducted in recent decades to clarify the risk of frailty fractures, as well as to identify treatment strategies for bone demineralization in HIV patients.[12-19]

As the use of bisphosphonates, calcium, and vitamin D is a widespread option against bone demineralization in the general population, and we performed a systematic review and meta-analysis about the effects of bisphosphonates, calcium and vitamin D in bone demineralization of patients with HIV/AIDS.

METHODS

A systematic review and meta-analysis of the literature were performed according to the Cochrane Manual for Systematic Reviews of Interventions and Preferred Reporting Items for Systematic Review and Meta-Analysis recommendations.[20,21]

An extensive literature search was conducted in electronic databases Medline, OVID, Cochrane Library, and EMBASE from January 2004 to January 2020, using the following descriptors: "HIV", "AIDS", "acquired immunodeficiency syndrome", "bisphosphonate", "vitamin D", "vitamin D3", "(25-hydroxyvitamin D)", "1,25-hydroxyvitamin D3 (25-hydroxyvitamin D3)", "cholecalciferol", "calcium (calcium)", "1,25-hydroxyvitamin D bone mineral density", "osteopenia", "osteoporosis", "fracture", "frailty, bone disease".

Only trials published in English were selected. An inclusion criterion was the use of bisphosphonates, vitamin D, and calcium on HIV patients against bone demineralization, regardless the dosage used. The use of any other drug to enhance BMD was an exclusion criterion for the trials. The main outcome considered in meta-analysis was the lumbar spine (LS) BMD evaluated by bone densitometry.

Other variables related to BMD were also included in this meta-analysis: patients' age, time of HIV infection, nadir of CD4+ T cells, CD4+ T cells count, body mass index (BMI), tobacco consumption, alcohol consumption, proportion of Caucasians, and LS T-score. Therefore, it is important to show that the intervention and control groups of the global meta-analysis sample were under the same comparison conditions, with no significant differences in baseline variables distribution. So, for BMD outcome and baselines variables, the meta-analysis groups were compared regarding effect size, homogeneity, and consistency. We used Cochrane's Q and I^2 statistics to address heterogeneity and consistency of trials (significant if $P \leq 0.05$ in Q and I^2 greater than 50%). Effect sizes were reported as odds ratios for qualitative factors or as standardized mean difference for continuous variables, and 95% confidence intervals.

Two reviewers, an orthopedist and an infectologist specialized in HIV/AIDS evaluated the search results. Titles and abstracts of trials were submitted for evaluation. Manuscripts were provided in full whenever the information was considered insufficient to meet the eligibility criteria. Data were collected independently by reviewers following a prototype database in an electronic spreadsheet developed by the authors for this purpose. The agreement between the evaluators in the construction of the database was assessed by Cohen's κ coefficient. Divergent choices regarding the selection of a study were solved by consensus. For each tri-

https://doi.org/10.11005/jbm.2020.27.3.175

Table 1. Key features of trials included in the meta-analysis

CT	Reference	Year	Country	Database	Journal	Goal(s)	Methods	Evaluated outcomes	Main results
CT1	Guaraldi et al. [14]	2004	Italy	MEDLINE	HIV Clinical Trials	To evaluate the effects of alendronate combined with vitamin D and calcium on bone metabolism and BMD in HIV-infected men and women using HAART	Prospective, multicentric, randomized (n=41)/Intervention group (n=18): alendronate (70 mg) weekly, calcium (1,000 mg) daily, vitamin D (500 UI) daily/Control group (n=23): calcium (1,000 mg) daily, vitamin D (500 UI) daily/FUT=52 weeks	Changes in bone metabolism assessed by the measurement of collagen type 1 N-tetrapeptide and bone alkaline phosphatase/Femoral neck/BMD Lumbar spine BMD	Alendronate improved lumbar spine BMD and minimized femoral BMD decreasing/Gastrointestinal complaints (about 10% in both groups: intervention and control)
CT2	Mondy et al. [13]	2005	USA	MEDLINE [14], Ovid	Journal of Acquired Immunodeficiency Syndrome	To evaluate the effects of alendronate combined with vitamin D and calcium on bone metabolism and BMD in HIV-infected men and women taking HAART	Prospective, randomized (n=31)/Intervention group (n=15): alendronate (70 mg) weekly, calcium (1,000 mg) daily, vitamin D (500 UI) daily/Control group (n=16): calcium (1,000 mg) daily, vitamin D (500 UI) daily/FUT=52 weeks	Lumbar spine BMD/Femoral neck BMD/Throchanteric BMD/Hip BMD/Whole body BMD	Alendronate improved lumbar spine BMD and minimized femoral BMD decreasing/None adverse effects related by authors
CT3	McComsey et al. [12]	2007	USA	MEDLINE, Ovid	AIDS	To evaluate the effects of alendronate combined with vitamin D and calcium on bone metabolism and BMD of HIV-infected men and women taking HAART with controlled disease	Prospective, multicentric, randomized, double-blinded (n=82)/Intervention group (n=42): alendronate (70 mg) weekly, calcium (1,000 mg) daily, vitamin D (400 UI) daily/Control group (n=40): calcium (1,000 mg) daily, vitamin D (400 UI) daily/FUT=48 weeks	Lumbar spine BMD/Femoral neck BMD/Throchanteric BMD/Hip BMD/Whole body BMD	Alendronate improved lumbar spine BMD and minimized femoral BMD decreasing/Severe adverse effects in 19% in intervention group, but the authors did not discriminate which could not be related to intervention
CT4	Rozenberg et al. [15]	2012	France	MEDLINE, Ovid	AIDS Research and Human Retroviruses	To evaluate the effect of alendronate on the low BMD of HIV-infected patients taking HAART	Prospective, randomized (n=44)/Intervention group (n=20): alendronate (70 mg) weekly, calcium (500 mg) daily, vitamin D (400 UI) daily/Control group (n=24): calcium (500 mg) daily, vitamin D (400 UI) daily/FUT=96 weeks	Lumbar spine BMD/Hip BMD	Alendronate improved the lumbar spine BMD/Severe adverse events in 13 patients (4 in the intervention group and 9 in the control group), but the authors pointed out that none of events was related to the use of biphosphonates
CT5	Natsag et al. [16]	2016	USA	MEDLINE	HIV Medicine	To determine the effect of alendronate on inflammatory markers, OPG, RANKL and bone density in HIV patients taking HAART	Prospective, randomized (n=70)/Intervention group (n=36): alendronate (70 mg) weekly, calcium (1,000 mg) daily, vitamin D (400 UI) daily/Control group (n=34): calcium (1,000 mg) daily, vitamin D (400 UI) daily/FUT=48 weeks	Changes in bone metabolism assessed by measuring OPG, RANKL and other inflammatory markers/Lumbar spine BMD/Hip BMD/Whole body BMD	Alendronate increases TNF-α activity and BMD/The greatest improvement in BMD occurred in patients with lower baseline concentrations of vitamin D/None adverse effects related by authors

(*Continued to the next page*)

https://doi.org/10.11005/jbm.2020.27.3.175

Table 1. Continued

CT	Reference	Year	Country	Database	Journal	Goal(s)	Methods	Evaluated outcomes	Main results
CT6 (phase 1)	Negredo et al. [17]	2015	Spain	MEDLINE	HIV Medicine	To evaluate the efficacy and tolerability of a single dose of zoledronate to treat low BMD in HIV-infected patients taking HAART	Prospective, multicentric, randomized, double-blinded (n=19)/Intervention group (n=9): zoledronate IV (5 mg) 1x, calcium (1,200-1,500 mg) daily, vitamin D (400 UI) daily/Control group (n=10): calcium (1,200-1,500 mg) daily, vitamin D (400 UI) daily/FUT=48 weeks	Lumbar spine BMD/Hip BMD	Zoledronate in a single dose reduced BMD decreasing in HIV-infected patients taking HAART
CT7 (phase 2)	Negredo et al. [17]	2015	Spain	MEDLINE	HIV Medicine	To evaluate the efficacy and tolerability of two doses of zoledronate to treat low BMD in HIV patients taking HAART	Prospective, multicentric, randomized, double-blinded (n=19)/Intervention group (n=12): zoledronate IV (5 mg) 2x, calcium (1,200-1,500 mg) daily, vitamin D (400 UI) daily/Control group (n=10): calcium (1,200-1,500 mg) daily, vitamin D (400 UI) daily/FUT=96 weeks	Lumbar spine BMD/Hip BMD	Zoledronate in a single dose reduced BMD decreasing in HIV-infected patients taking HAART/ The benefits of BMD with a single dose of zoledronate is equivalent to that obtained with two doses of the drug
CT8	Bolland et al. [18]	2007	New Zealand	MEDLINE, Embase	The Journal of Clinical Endocrinology & Metabolism	To evaluate the efficacy and tolerability of zoledronate on the BMD of HIV-infected men taking HAART	Prospective, randomized, double-blinded (n=43)/Intervention group (n=21): zoledronate IV (4 mg) 1x, calcium (400 mg) daily, vitamin D (50,000 UI) monthly/ Control group (n=22): calcium (400 mg) daily, vitamin D (50,000 UI) monthly/ FUT=104 weeks	Changes in bone metabolism assessed by the measurement of collagen type 1 N-tetrapeptide and bone alkaline phosphatase/ Lumbar spine BMD/Hip BMD/Whole body BMD	Zoledronate reduced the decreasing in BMD in HIV-infected patients taking HAART
CT9	Huang et al. [19]	2009	USA	MEDLINE	AIDS	To evaluate the efficacy and tolerability of two doses of zoledronate on the BMD of HIV patients taking HAART	Prospective, randomized, double-blinded (n=30)/Intervention group (n=15): zoledronate IV (4 mg) 1x, calcium (1,000 mg) daily, vitamin D (400 UI) monthly/Control group (n=15): calcium (1,000 mg) daily, vitamin D (400 UI) monthly/FUT=52 weeks	Biomarkers of bone metabolism/Lumbar spine BMD/Hip BMD	Zoledronate reduced the decreasing in BMD in HIV-infected patients taking HAART

CT, clinical trial; BMD, bone mineral density; HIV, human immunodeficiency virus; HAART, highly active antiretroviral therapy; OPG, osteoprotegerin; RANKL, receptor activator of nuclear factor κ-β ligand; FUT, follow-up time; TNF-α, tumor necrosis factor-α.

https://doi.org/10.11005/jbm.2020.27.3.175

Table 2. Characteristics of global sample, intervention and control groups of meta-analysis

Characteristics	Global sample	Intervention group	Control group	*P*-value[a]
Total of patients (k=9)	394 (100.0%)	200 (50.8%)	194 (49.2%)	0.855[b]
Gender				0.815[c]
Female	73 (18.5%)	35 (17.5%)	38 (19.6%)	
Male	321 (81.5%)	165 (82.5%)	156 (80.4%)	
Tobacco consumption (k=6)	198/300 (66.0%)	98/149 (65.8%)	100/151 (66.2%)	0.98
Alcohol consumption (k=5)	107/257 (41.6%)	53/128 (41.4%)	54/129 (41.9%)	0.877
Proportion of Caucasians (k=6)	256/300 (85.3%)	119/149 (79.9%)	137/151 (90.7%)	0.058
Biphosphonate used (k=9)				0.237[c]
Alendronate (5 trials)	268 (68.0%)	131 (65.5%)	137 (70.6%)	
Zoledronate (4 trials)	126 (32.0%)	69 (34.5%)	57 (29.4%)	
Time of HIV infection (yr) (k=6)	10.3±8.0	10.3±6.2	10.2±3.8	0.982[d]
Nadir of CD4+T cells (cells/μL) (k=6)	174.9±302.2	174.3±159.9	175.5±144.2	0.730[d]
CD4+T cells count (células/μL) (k=7)	491.50±434.4	519.3±301.7	462.38±334.7	0.103[d]
Lumbar spine T-score before intervention (k=9)	1.91±0.60	1.97±0.72	1.84±0.67	0.055[d]
Lumbar spine DMO before intervention (g/cm^2) (k=9)	0.96±0.10	0.97±0.10	0.95±0.12	0.098[d]

Quantitative variables are expressed by mean±standard deviation.
[a]*P*-value refers to the comparison of the distributions in intervention and control groups. [b]Binomial test. [c]χ^2 test. [d]Test for differences between means.
k, number of trials that registered the variable; HIV, human immunodeficiency virus; CD4, cluster of differentiation 4.

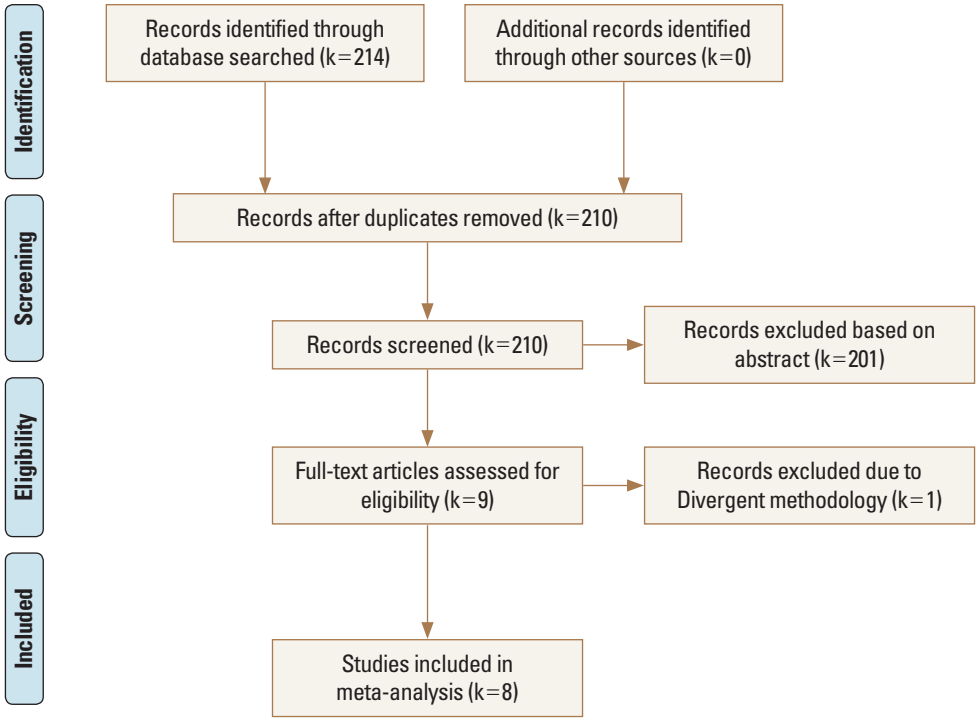


Fig. 1. Flowchart (Preferred Reporting Items for Systematic Reviews and Meta-Analysis [PRISMA flow]) for the identification and selection of trials for systematic review and meta-analysis. k, number of trials that registered the variable.

al selected, the data collected follows in Tables 1 and 2.

Results were presented using an organization chart to studies selection and forest plots for the comprehension of the meta-analysis. We used Cochrane's Q and I^2 statistics to address heterogeneity and consistency of trials (significant if $P \leq 0.05$ in Q and I^2 greater than 50%). The difference between the groups compared (intervention and control) was made at the significance level of 5% ($\alpha = 0.05$).

Table 3. Heterogeneity and inconsistency tests performed in meta-analysis to baseline variables and BMD outcome

Variable	Model	Effect size (CIs of 95%)								Homogeneity			Comment
		n	PE	SE	V	Lower limit	Upper limit	z-value	*P*-value	Q	*P*-value	I^2	
Patients' age	F	9	0.229	0.107	0.011	0.019	0.439	2.139	0.032	-	-	-	Heterogeneity and inconsistency founded
	R[a)]	9	0.071	0.343	0.118	-0.602	0.744	0.208	0.835	78.283	0	89.78	
Time of HIV infection	F[a)]	7	-0.012	0.131	0.017	-0.268	0.244	-0.09	0.928	4.13	0.659	0	
	R	7	-0.012	0.131	0.017	-0.268	0.244	-0.09	0.928	-	-	-	
Nadir of CD4+T Cells	F[a)]	5	0.054	0.158	0.025	-0.255	0.363	0.345	0.73	5.748	0.219	30.4	
	R	5	0.047	0.191	0.036	-0.327	0.421	0.247	0.805	-	-	-	
CD4+T cells count	F[a)]	7	0.186	0.114	0.013	-0.037	0.409	1.632	0.103	8.884	0.18	32.46	
	R	7	0.205	0.143	0.02	-0.075	0.485	1.432	0.152	-	-	-	
Body mass index	F	8	-0.336	0.116	0.013	-0.563	-0.109	-2.899	0.004	-	-	-	Heterogeneity and inconsistency founded
	R[a)]	8	-0.705	0.422	0.178	-1.533	0.123	-1.669	0.095	85.469	0	91.81	
Tobacco consumption	F[a)]	6	0.993	-	-	0.592	1.667	-0.025	0.98	4.828	0.437	0	
	R	6	0.993	-	-	0.592	1.667	-0.025	0.98	-	-	-	
Alcohol consumption	F[a)]	5	1.041	-	-	0.621	1.746	0.154	0.877	6.78	0.148	41.002	
	R	5	1.015	-	-	0.505	2.041	0.043	0.966	-	-	-	
Proportion of Caucasians	F[a)]	6	2.01	-	-	0.975	4.143	1.892	0.058	4.234	0.516	0	
	R	6	2.01	-	-	0.975	4.143	1.892	0.058	-	-	-	
Proportion of Caucasians	F[a)]	9	-0.204	0.102	0.01	-0.405	-0.004	-2.001	0.055	7.313	0.503	0	
	R	9	-0.204	0.102	0.01	-0.405	-0.004	-2.001	0.055	-	-	-	
Proportion of Caucasians	F[a)]	9	-0.169	0.102	0.01	-0.37	0.031	-1.655	0.098	11.631	0.168	31.22	
	R	9	-0.154	0.126	0.016	-0.401	0.094	-1.215	0.224	-	-	-	
Proportion of Caucasians	F[a)]	9	0.227	0.102	0.01	0.026	0.428	2.216	0.027	10.279	0.246	22.17	Significant difference between groups
	R	9	0.241	0.118	0.014	0.009	1.571	2.039	0.041	-	-	-	

[a)]Chosen model.
BMD, bone mineral density; HIV, human immunodeficiency virus; CD4, cluster of differentiation 4; F, fixed model; R, random model; CI, confidence interval; PE, point estimate; SE, standard error; V, variance.

https://doi.org/10.11005/jbm.2020.27.3.175

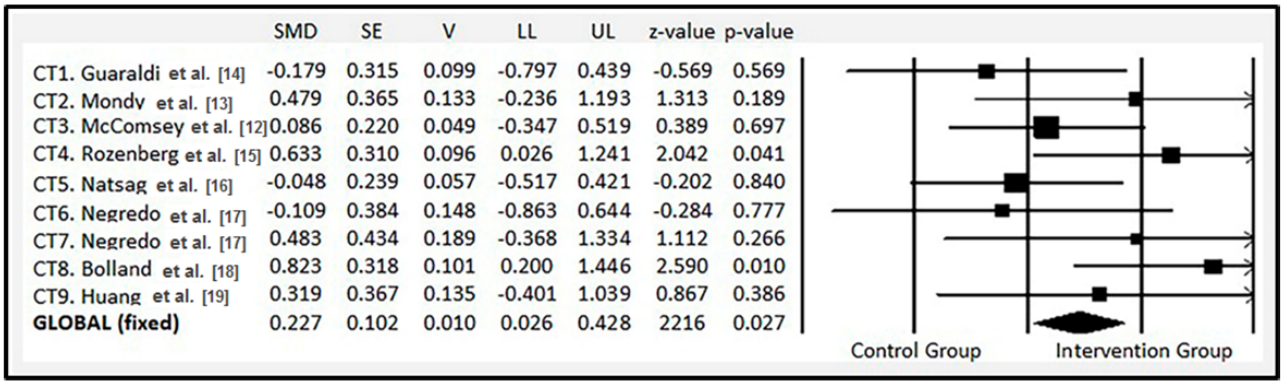


Fig. 2. Forest plot of meta-analysis for lumbar spine bone mineral density after intervention. SMD, standardized mean differences; SE, standard error; V, variance; LL, lower limit; UL, upper limit.

Table 4. Means and standard deviations of lumbar spine BMD after intervention of the CTs

CT	Reference	Year	Intervention group	Control group
CT1	Guaraldi et al. [14]	2004	0.97±0.11	1.00±0.17
CT2	Mondy et al. [13]	2005	0.97±0.08	0.92±0.12
CT3	McComsey et al. [12]	2007	0.94±0.07	0.93±0.07
CT4	Rozenberg et al. [15]	2012	0.91±0.06	0.87±0.08
CT5	Natsag et al. [16]	2016	0.91±0.13	0.91±0.15
CT6 (phase 1)	Negredo et al. [17]	2015	0.98±0.10	0.99±0.14
CT7 (phase 2)	Negredo et al. [17]	2015	1.04±0.04	0.99±0.14
CT8	Bolland et al. [18]	2007	1.25±0.11	1.14±0.16
CT9	Huang et al. [19]	2009	0.88±0.08	0.86±0.08
	Weighted average		0.99±0.09	0.95±0.12

BMD, bone mineral density; CT, clinical trial.

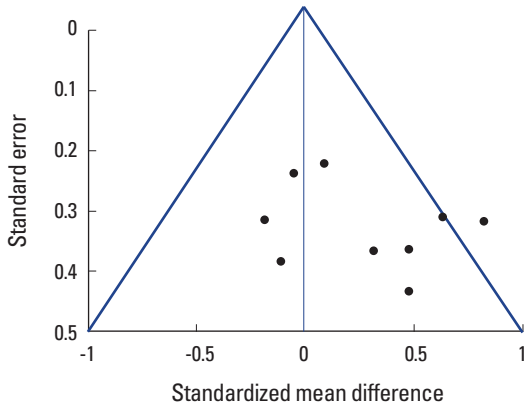


Fig. 3. Funnel plot of publication bias on bone mineral density outcomes.

For bias evaluation, we used the Bias Risk Assessment (BRA) tool recommended by the Cochrane Manual,[20] according to which the reviewers chose and classified the risk of bias to each source of bias and each trial in 4 categories: absent, low, medium, or high. Thus, to each trial and each source of bias was calculated the bias risk as the double of a weighted average of these categories frequencies (risk absent – weight 0; low risk – weight 1; medium risk – weight 2; high risk – weight 3). BRA is a measure that varies from 0 to 100%. The risk of publication bias was assessed using the graphical assessment of the funnel plot on BMD outcome.

Sensitivity analysis testing the influence of each study on the overall results was done by omitting one study at a time to explore potential sources of heterogeneity and to test the stability of regrouped results.

Descriptive analyzes were performed using the IBM SPSS Statistics for Windows (version 20.0; IBM Corp., Armonk, NY, USA), and the software used for the meta-analysis was the Comprehensive Meta-Analysis for Windows (version 3.0; Biostat Software Inc., Englewood, NJ, USA).

RESULTS

Full search results are represented in the flow-chart of Figure 1. Out of the 214 publications identified, 8 trials met the criteria selection and were selected to be critically evaluated in relation to authenticity, methodological quality, and the importance of the information (κ=0.92 of concordance between the 2 reviewers). All of them were controlled and compared the effects of biphosphonates (alendronate or zoledronate) combined with vitamin D and calcium on

Table 5. Bias risk assessment for each trial according to source

	Source of bias						Frequency of bias risk classification				
	Selection	Allocation	Blinding	FUT[a]	Result presentation		Absent	Low	Moderate	High	BRA
					Mean[b]	SD[c]					
CT											
CT1[d]	Absent	Absent	Moderate	High	Absent	Absent	83.3%	0.0%	16.7%	0.0%	5.57%
CT2[e]	Absent	Absent	Moderate	High	Low	Moderate	50.0%	16.7%	33.3%	0.0%	13.88%
CT3[f]	Absent	Absent	Absent	High	High	High	66.7%	0.0%	0.0%	33.3%	16.65%
CT4[g]	Absent	Absent	Absent	Moderate	Moderate	Moderate	50.0%	0.0%	50.0%	0.0%	16.67%
CT5[h]	Absent	Absent	Absent	High	Low	High	66.7%	16.7%	0.0%	16.7%	11.13%
CT6[i]	Absent	Absent	Moderate	Moderate	Absent	Absent	66.7%	0.0%	33.3%	0.0%	11.1%
CT7[j]	Absent	Absent	Moderate	Moderate	Absent	Absent	66.7%	0.0%	33.3%	0.0%	11.1%
CT8[k]	Absent	Absent	Absent	Moderate	Low	Moderate	50.0%	16.7%	16.7%	0.0%	8.35%
CT9[l]	Absent	Absent	Absent	High	Low	Moderate	83.3%	0.0%	16.7%	0.0%	5.57%
Global sample							64.82%	5.57%	22.22%	5.56%	11.11%
Absent	100.0%	100.0%	55.55%	55.55%	33.33%	33.33%					
Low	0.0%	0.0%	0.0%	0.0%	44.44%	0.0%					
Moderate	0.0%	0.0%	44.44%	44.44%	11.11%	44.44%					
High	0.0%	0.0%	0.0%	0.0%	11.11%	22.22%					
Global sample BRA	0.0%	0.0%	14.81%	14.81%	16.67%	25.92%					

The BRA tool used was the one recommended by the Cochrane Manual [20].
CT, clinical trial; BRA, bias risk assessment; FUT, follow-up time; SD, standard deviation.
[a]FUTs differed significantly in CTs (48 to 104 weeks) and it was considered a possible source of bias. Applying BRA tool, we considered of 52 weeks of FUT to be without risk of bias, and the 96 weeks (or more) of FUT to be at risk of bias. [b]Some CTs presented results using median instead of means, which was considered a potential source of bias during BRA. [c]Some CTs presented interquartile distances and range values instead of SD, which was considered a potential source of bias during BRA. [d]CT1: Guaraldi et al. [14], 2004. [e]CT2: Mondy et al. [13], 2005. [f]CT3: McComsey et al. [12], 2007. [g]CT4: Rozenberg et al. [15], 2012. [h]CT5: Natsag et al. [16], 2016. [i]CT6: Negredo et al. [17], 2015. [j]CT7: Negredo et al. [17], 2015. [k]CT8: Bolland et al. [18], 2007. [l]CT9: Huang et al. [19], 2009.

HIV patients using HAART and presenting osteopenia or osteoporosis. Vitamin D and calcium was used in all trials by the control groups as a comparison parameter. Table 1 gathers the characteristics of the trials selected, as the bisphosphonates used in each of them, its dosage, and adverse effects related to use.

The trial by Negredo et al. [17] made an experiment with 2 different treatments over 2 years. In this review, we considered each treatment as an isolated trial to perform the meta-analysis (CT6 and CT7). The trial conducted by Pepe et al. [22] had a methodological approach different from the other trials, separating the treatment and control groups into other subgroups according to the patients' gonadal status. As none of the other trials had an approach like that used by Pepe et al. [22], this study was removed from the meta-analysis.

Random allocation of patients in groups was performed in all trials. The effects of intervention in bone metabolism were inferred by variables such as collagen n-tetra-peptide type 1, bone-specific alkaline phosphatase, osteoprotegerin, receptor activator of nuclear factor κ-β ligand (RANKL), femoral neck BMD, LS BMD, femoral head BMD, whole-body BMD. As the measurement of LS BMD (g/cm^2) using bone densitometry was the unique variable assessed in all clinical trials, this was chosen as the main outcome of meta-analysis. The follow-up time (FUT) was quite discrepant among trials, ranging from 48 to 104 weeks (Table 1).

After gathering the participants of all trials, the global sample for meta-analysis had 394 patients, of whom 200 (50.8%) received bisphosphonate, calcium, and vitamin D (intervention group); the other 194 patients (49.2%) received only calcium and vitamin D (control group). For these patients, the average of FUT was 62.9 weeks. The proportion of men was greater than women, 82.5 and 17.5%, respectively; however, this disparity occurred in both study groups without statistical difference between the joint distribution of men and women in intervention and control groups. Characteristics of global sample, intervention and

https://doi.org/10.11005/jbm.2020.27.3.175

control groups of a meta-analysis are described in Table 2.

Table 3 summarizes the tests for heterogeneity and inconsistency performed with the model used in the analysis of SMD or, considering fixed or random effects for each variable. Meta-analysis for baselines variables showed no significant difference between groups before intervention (forest plots for baselines variables available as Supplementary Appendix 1); on the contrary, for the LS BMD after intervention (main outcome), a significant difference was found ($P=0.027$) and the overall diamond does not cross value 0 (Fig. 2).

Most trials of meta-analysis have demonstrated some efficiency of bisphosphonates in reducing bone resorption and BMD increasing, however, some of them could not found statistical significance between the interventions and control groups. Table 4 shows the averages and SDs for LS BMDs after intervention.

The reviewers used and judged the possible sources of bias in the selected clinical trials: no random selection, no blinding, atypical FUT, absence of media, and SD of the variables in the papers, which were estimated by other biased statistics. Regarding the occurrence of bias, Table 5 shows the BRA for each trial and a global sample of meta-analysis. The Global BRA is 11.1%, considering, in the scale from 0% to 100%, refer to low bias. The possible impact of publication bias on BMD outcomes was explored by the funnel plot (Fig. 3). The studies were not evenly distributed across both sides of the funnel plot, reveling soft asymmetry. So, the visual inspection of funnel plots suggests that studies publishing negative effects may be missing. However, the Egger and Begg tests results suggest the absence of this source of bias ($P=0.23$ for the Egger test and $P=0.33$ for the Begg test). Sensitivity analysis demonstrated that results remained statistically significant despite all deletions.

DISCUSSION

In the present meta-analysis, we found that the use of alendronate or zoledronate with calcium and vitamin D could significantly improve the BMD in patients with HIV/AIDS taking HAART (up to 0.227 g/cm^2). Considering the normal values of LS BMD (1.237 g/cm^2), the gain obtained with the intervention is sufficient to bring the average of LS BMD of patients with HIV/AIDS (1.176 g/cm^2) to the levels of not infected population, suggesting the existence of clinical benefits.[23]

The decrease in the risk of fractures due to bone frailty is certainly the main clinical benefit expected; however, establishing this correlation is a complex task that has been tried by other authors with minor success.[10,24-29] The hardest part of this correlation stems from the number of accumulated variables associated with the risk of fractures in HIV patients.[26,29] Obviously, since the reduction of BMD is an isolated risk factor for fractures in the non-infected population, we infer that the same should occur among HIV patients, although with a reduction in relative risk different from that observed in the general population.[30] Believing in this theoretical reduction in the risk of fractures when an increase in BMD is obtained, trials with HIV patients have been conducted investigating the role of vitamin D, calcium, and bisphosphonates for this purpose. As noted in previous reviews, the selected clinical trials did not allow us to establish a correlation between improved BMD and decreased risk of fracture.[27,31-34] The short FUT of patients was an important obstacle in this regard. As frailty fractures can occur at any time after the onset of osteoporosis, short-term trials do not allow us to establish this relationship, despite patients are using bisphosphonates or not.

Equally important regarding the use of bisphosphonates is the duration of its effects when its use is stopped. Studies with bisphosphonates in postmenopausal women suggest that patients with a t-score below -2.5 in the femoral neck still maintain a high risk of vertebral fractures even after 3 to 5 years of treatment, suggesting that prolonged use may be beneficial. Up to the time of writing this manuscript, the only study evaluating the prolonged use of a bisphosphonate (zoledronate) in patients with HIV/AIDS demonstrated that the effects of 2 annual doses lasted for five years after the second medication.[18] However, this study was relatively small and did not have enough strength to detect significant differences in fracture risk; the authors also recruited relatively young male patients with a t-score below -0.5 and with well-controlled disease, which makes it impossible to generalize their results to populations of patients with HIV/AIDS, low BMD and infection uncontrolled. In this meta-analysis, none of trials allowed us to assess adequately the effects of treatment interruption, nor the minimal time of use for an ideal outcome, either in terms of improving BMD or in the duration of the results obtained.

In this meta-analysis the biphosphonates used were alendronate orally, and zoledronate intravenously. Both of them were, in general, well-tolerated. No effect related to its withdrawal was reported. The most common adverse effects related to alendronate use were gastrointestinal complaints, with similar frequencies in their occurrence in trials. Possibly, due to the longer use of alendronate in protocols for the treatment of osteoporosis, care in administering the drug is already widespread (avoiding the decubitus position after taking it and ingesting it with a large amount of liquid), reducing the occurrence of adverse effects. It was related to taking the medication. Considerations regarding the prolonged use of bisphosphonates, such as osteonecrosis of the jaw and atypical femoral fractures due to suppression of bone turnover, were not reported by the authors of the analyzed trials, possibly due to the rarity of these adverse events and the short duration of the trials analyzed. There is a greater hypothetical risk of adverse gastrointestinal events with the use of alendronate in relation to the use of zoledronate, especially with regard to the direct action of alendronate on the gastroesophageal mucosa, however, this risk cannot be assessed in the meta-analysis as it has not been properly presented for studies. Zoledronate, on the other hand, is used intravenously, being less frequently related to adverse gastrointestinal events, however, the occurrence of events related to the venous infusion is expected and was presented by trials that tested this drug. Regarding the route of administration, alendronate has the advantage of being able to be taken by patients themselves at home, while zoledronate needs hospitalization and a health team for its administration. In contrast, zoledronate has the dosage convenience when administered every six months, while alendronate is used weekly, which could be advantageous in patients taking several drugs, such as those used in HAART. Respecting the annual cost of treatment, zoledronate (2 annual doses) is more expensive than alendronate, hampering to use it on a large scale both for public health programs and for studies unlinked to the pharmaceutical industry that aim to measure its effectiveness and adverse effects.

The use of calcium and vitamin D was not homogeneous between studies, with calcium doses ranging from 400 to 1,500 mg/day, and vitamin D doses ranging from 400 to 800 IU/day. Bolland et al. [18] used a monthly dose of 50,000 IU of vitamin D (1.25 mg of cholecalciferol). An adequate intake of vitamin D calcium is important for preventing bone demineralization and reducing the risk of fractures. The physiological needs of these nutrients vary throughout life, increasing with aging.[32,35,36] As vitamin D deficiency is prevalent in HIV patients, supplementation of this vitamin was used in association with calcium in both the treatment and control groups in all trials.[32] With regard to the doses of vitamin D used, previous studies carried out in patients who do not have HIV suggest a dose-dependent effect when demonstrating that supplementation with 400 IU/day has less effect on the risk of fracture than its use in doses equal to or greater than 600 IU/day. [37-39] Among HIV patients there is still no consensus in the literature regarding the dose required for supplementation and ideal serum levels of 1,25-hydroxy-vitamin D3 (25[OH]D3) for improving BMD and preventing frailty fractures.[40] Some authors consider that when the food intake of these elements is insufficient in HIV patients, Screening for hypovitaminosis should be done before starting the replacement of vitamin D and calcium.[40] In our review, only trials testing the effects of zoledronate considered serum levels of 1,25(OH)D3 as baseline characteristics among the study groups.[17-19,41]

Some risk factors for bone demineralization analyzed by the trials were included in the meta-analysis, such as age, white race, smoking, drinking, HIV infection time, indicators of the immune profile (nadir and CD4+ T cells), BMI and the t-score of the LS before treatment. For these factors, there was no significant difference between the treatment and control groups in the meta-analysis sample. However, other risk factors for bone demineralization, such as physical activity, daily calcium intake, drugs used in HAART and serum calcium and vitamin D levels before interventions were not adequately presented by the authors and did not could be included in the meta-analysis, figuring as a significant limitation of this review. Other important limitations were the incomplete assessment of risk factors for bone loss among trials, the small number of trials, the short FUTs of the participants, and the methodological differences of study designs.

As some trials could not find by itself statistical differences between groups tested, despite all limitations above mentioned, the meta-analysis performed was relevant insofar as revealed no substantial heterogeneity, with high consistency and statistical significance to the main out-

https://doi.org/10.11005/jbm.2020.27.3.175

come considered. Consequently, as well as in the general population, the combination of Alendronate (orally) or zoledronate (intravenously), with calcium and vitamin D (orally) is effective to increase LS BMD in HIV patients. Further randomized clinical trials controlling both the variables related to bone demineralization and adverse effects can be useful to improve therapeutic protocols against bone loss in HIV.

CONCLUSIONS

This study provides enough evidence that BMD post-treatment is higher in HIV patients who used bisphosphonates combined with calcium and vitamin D.

DECLARATIONS

Ethics approval and consent to participate

Not applicable.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

ORCID

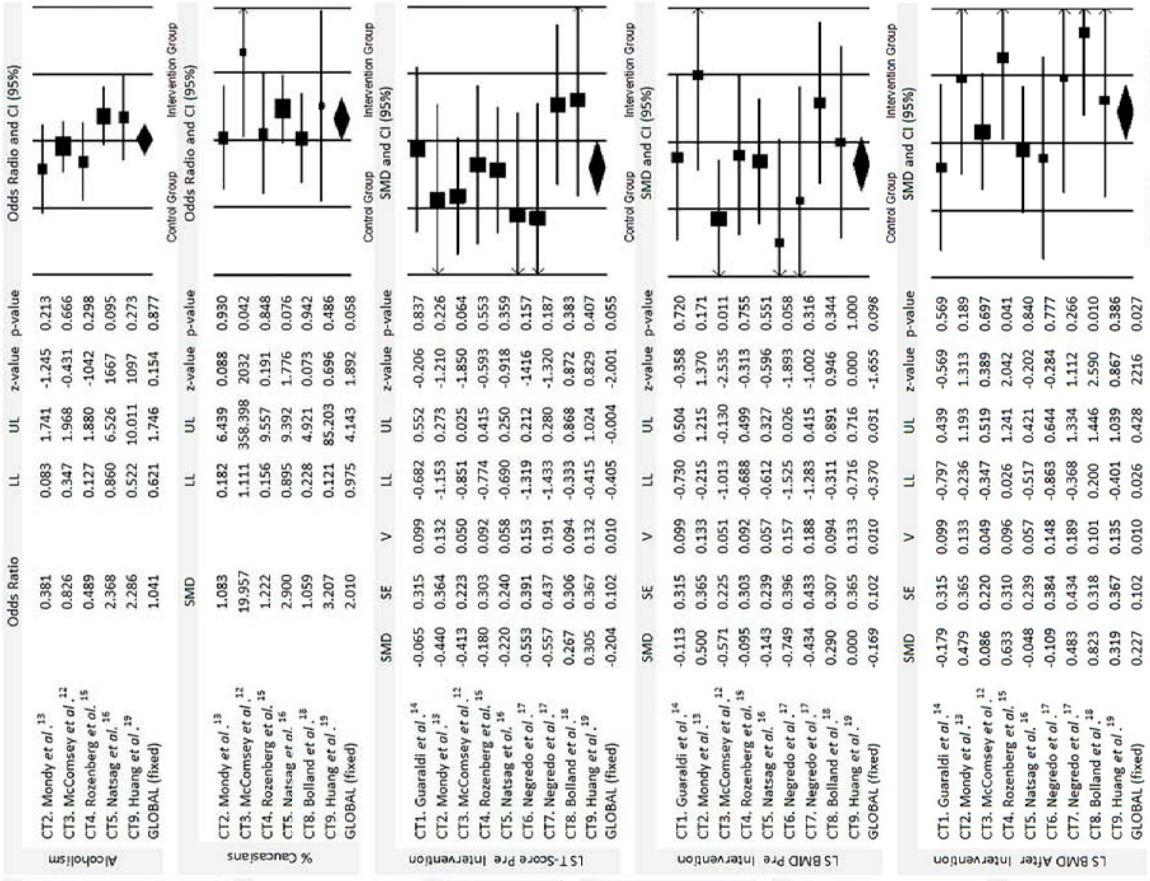
Vinícius Magno da Rocha
https://orcid.org/0000-0001-5441-0679
Mariana Balardino Bogado Faria
https://orcid.org/0000-0002-9237-5664
Francisco de Assis dos Reis Júnior
https://orcid.org/0000-0003-0452-4525
Carla Ormundo Gonçalves Ximenes Lima
https://orcid.org/0000-0003-4649-5101
Rossano Kepler Alvim Fiorelli
https://orcid.org/0000-0001-5236-0903
Keila Mara Cassiano
https://orcid.org/0000-0002-5675-6953

REFERENCES

1. Hoy JF, Grund B, Roediger M, et al. Immediate initiation of antiretroviral therapy for HIV infection accelerates bone loss relative to deferring therapy: findings from the START bone mineral density substudy, a randomized trial. J Bone Miner Res 2017;32:1945-55.
2. Amorosa V, Tebas P. Bone disease and HIV infection. Clin Infect Dis 2006;42:108-14.
3. Saccomanno MF, Ammassari A. Bone disease in HIV infection. Clin Cases Miner Bone Metab 2011;8:33-6.
4. Althoff KN, Smit M, Reiss P, et al. HIV and ageing: improving quantity and quality of life. Curr Opin HIV AIDS 2016;11:527-36.
5. Peck WA, Burckhardt P, Christiansen C, et al. Consensus development conference: diagnosis, prophylaxis, and treatment of osteoporosis. Am J Med 1993;94:646-50.
6. World Health Organization. WHO scientific group on the assessment of osteoporosis at primary health care level. Brussels, BE: World Health Organization; 2004.
7. Ofotokun I, Weitzmann MN. HIV-1 infection and antiretroviral therapies: risk factors for osteoporosis and bone fracture. Curr Opin Endocrinol Diabetes Obes 2010;17:523-9.
8. Dalla Grana E, Rigo F, Lanzafame M, et al. Relationship between vertebral fractures, bone mineral density, and osteometabolic profile in HIV and hepatitis B and C-infected patients treated with ART. Front Endocrinol (Lausanne) 2019;10:302.
9. Premaor MO, Compston JE. The hidden burden of fractures in people living with HIV. JBMR Plus 2018;2:247-56.
10. Sharma A, Shi Q, Hoover DR, et al. Frailty predicts fractures among women with and at-risk for HIV. AIDS 2019;33:455-63.
11. Guaraldi G, Zona S, Menozzi M, et al. Cost of noninfectious comorbidities in patients with HIV. Clinicoecon Outcomes Res 2013;5:481-8.
12. McComsey GA, Kendall MA, Tebas P, et al. Alendronate with calcium and vitamin D supplementation is safe and effective for the treatment of decreased bone mineral density in HIV. AIDS 2007;21:2473-82.
13. Mondy K, Powderly WG, Claxton SA, et al. Alendronate, vitamin D, and calcium for the treatment of osteopenia/osteoporosis associated with HIV infection. J Acquir Immune Defic Syndr 2005;38:426-31.
14. Guaraldi G, Orlando G, Madeddu G, et al. Alendronate reduces bone resorption in HIV-associated osteopenia/osteoporosis. HIV Clin Trials 2004;5:269-77.
15. Rozenberg S, Lanoy E, Bentata M, et al. Effect of alendronate on HIV-associated osteoporosis: a randomized, double-blind, placebo-controlled, 96-week trial (ANRS 120). AIDS Res Hum Retroviruses 2012;28:972-80.
16. Natsag J, Kendall MA, Sellmeyer DE, et al. Vitamin D, os-

teoprotegerin/receptor activator of nuclear factor-kappaB ligand (OPG/RANKL) and inflammation with alendronate treatment in HIV-infected patients with reduced bone mineral density. HIV Med 2016;17:196-205.
17. Negredo E, Bonjoch A, Pérez-Álvarez N, et al. Comparison of two different strategies of treatment with zoledronate in HIV-infected patients with low bone mineral density: single dose versus two doses in 2 years. HIV Med 2015;16:441-8.
18. Bolland MJ, Grey AB, Horne AM, et al. Annual zoledronate increases bone density in highly active antiretroviral therapy-treated human immunodeficiency virus-infected men: a randomized controlled trial. J Clin Endocrinol Metab 2007;92:1283-8.
19. Huang J, Meixner L, Fernandez S, et al. A double-blinded, randomized controlled trial of zoledronate therapy for HIV-associated osteopenia and osteoporosis. AIDS 2009;23:51-7.
20. Higgins JPT, Green S, editors. Cochrane handbook for systematic reviews of interventions version 5.1.0. London, UK: The Cochrane Collaboration; 2011.
21. Moher D, Liberati A, Tetzlaff J, et al. Items de referencia para publicar Revisiones Sistemáticas y Metaanálisis: La Declaración PRISMA. Rev Esp Nutr Hum Diet 2014;18:172-81.
22. Pepe J, Isidori AM, Falciano M, et al. Effect of risedronate in osteoporotic HIV males, according to gonadal status: a pilot study. Endocrine 2014;47:456-62.
23. Cotter AG, Sabin CA, Simelane S, et al. Relative contribution of HIV infection, demographics and body mass index to bone mineral density. AIDS 2014;28:2051-60.
24. 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;52:1061-8.
25. Torti C, Mazziotti G, Soldini PA, et al. High prevalence of radiological vertebral fractures in HIV-infected males. Endocrine 2012;41:512-7.
26. 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;28:1259-63.
27. Shiau S, Broun EC, Arpadi SM, et al. Incident fractures in HIV-infected individuals: a systematic review and meta-analysis. AIDS 2013;27:1949-57.
28. Borderi M, Calza L, Colangeli V, et al. Prevalence of sub-clinical vertebral fractures in HIV-infected patients. New Microbiol 2014;37:25-32.
29. Yin MT, Shiau S, Rimland D, et al. Fracture prediction with modified-FRAX in older HIV-infected and uninfected men. J Acquir Immune Defic Syndr 2016;72:513-20.
30. Kanis JA, McCloskey E, Johansson H, et al. FRAX(®) with and without bone mineral density. Calcif Tissue Int 2012;90:1-13.
31. Brown TT. Challenges in the management of osteoporosis and vitamin D deficiency in HIV infection. Top Antivir Med 2013;21:115-8.
32. Castronuovo D, Cacopardo B, Pinzone MR, et al. Bone disease in the setting of HIV infection: update and review of the literature. Eur Rev Med Pharmacol Sci 2013;17:2413-9.
33. Reid IR, Bolland MJ, Grey A. Effects of vitamin D supplements on bone mineral density: a systematic review and meta-analysis. Lancet 2014;383:146-55.
34. Hileman CO, Eckard AR, McComsey GA. Bone loss in HIV: a contemporary review. Curr Opin Endocrinol Diabetes Obes 2015;22:446-51.
35. Gallagher JC. Vitamin D and aging. Endocrinol Metab Clin North Am 2013;42:319-32.
36. Curtis EM, Moon RJ, Dennison EM, et al. Prenatal calcium and vitamin D intake, and bone mass in later life. Curr Osteoporos Rep 2014;12:194-204.
37. Lips P, Graafmans WC, Ooms ME, et al. Vitamin D supplementation and fracture incidence in elderly persons. A randomized, placebo-controlled clinical trial. Ann Intern Med 1996;124:400-6.
38. Meyer HE, Smedshaug GB, Kvaavik E, et al. Can vitamin D supplementation reduce the risk of fracture in the elderly? A randomized controlled trial. J Bone Miner Res 2002;17:709-15.
39. Vieth R. Why the optimal requirement for Vitamin D3 is probably much higher than what is officially recommended for adults. J Steroid Biochem Mol Biol 2004;89-90:575-9.
40. Ryom L, Boesecke C, Gisler V, et al. Essentials from the 2015 European AIDS Clinical Society (EACS) guidelines for the treatment of adult HIV-positive persons. HIV Med 2016;17:83-8.
41. Ofotokun I, Titanji K, Lahiri CD, et al. A single-dose zoledronic acid infusion prevents antiretroviral therapy-induced bone loss in treatment-naive HIV-infected patients: A phase IIb trial. Clin Infect Dis 2016;63:663-71.

https://doi.org/10.11005/jbm.2020.27.3.175



Supplementary Appendix 1. Forest plots of baseline variables meta-analysis. SMD, standardized mean differences; SE, standard error; V, variance; LL, lower limit; UL, upper limit; CI, confidence interval.