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ORIGINAL RESEARCH

Inverse Correlation Between Vascular Calcification and Bone Mineral Density in Human Immunodeficiency Virus-Infected Patients

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Abstract HIV-infected individuals suffer from accelerated aging, which manifests as premature cardiovascular and bone disease. However, little is known of the association of these two disorders in the HIV population. Our objective was to investigate the association between a marker of atherosclerosis (coronary artery calcium [CAC]) and low bone mineral density (BMD) in a cross-sectional cohort of HIV-infected patients. The study was conducted at the University of Modena and Reggio Emilia, Italy. A total of 636 consecutive middle-aged, HIV-infected subjects were recruited between January 2006 and December 2010. All patients underwent CAC and BMD assessment. Patients were categorized according to a CAC score <100 or >100 units based on previous literature that identified this cut-point as a marker of increased risk. Low femoral and lumbar spine BMD was defined as <25th percentile value for the study cohort. Logistic regression and bootstrap analysis were used to assess the independent association between CAC and BMD. The main outcome measure was a CAC score >100. Patients with CAC > 100 were older and more likely to be men, diabetic, and overweight. Patients with CAC < 100 had better renal function and a lower cardiovascular risk profile. After adjusting for age, sex, traditional and HIV-specific risk factors, vitamin D level, and PTH level, there was a significant association between CAC > 100 and low BMD for the femur (OR = 2.33, 95 % CI 1.09–4.99; $p = 0.02$) but not for the spine. Bootstrap analyses confirmed these findings. In summary, CAC was independently associated with low femoral BMD in HIV-infected patients. Future studies should test whether therapies that attenuate cardiovascular risk in HIV favorably impact bone health.

Keywords Coronary artery calcification · Bone mineral density · HIV

Introduction

Patients with HIV infection suffer events related to accelerated aging such as premature coronary artery disease and

The authors have stated that they have no conflict of interest.

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osteoporotic fractures [1]. Traditional and HIV-specific risk factors such as chronic inflammation, chronic immune activation, immunosenescence, and antiretroviral therapy toxicity may be responsible for the increased cardiovascular (CV) and bone disease frequency among HIV-infected individuals [1, 2].

Coronary artery calcium (CAC) is a marker of subclinical atherosclerosis and has been clearly linked with an unfavorable outcome in the general population [3]. Additionally, there appears to be an association between vascular and bone disease in the general population [4]. Arterial wall smooth muscle cells can turn into osteoblast-like cells under appropriate triggering [5]; and factors such as fetuin-A, osteocalcin, pyrophosphate, and matrix Gla protein may regulate both CAC accumulation and bone mineralization [4]. To date, there have been no reports of the association of bone and vascular disorders in HIV patients, an association that has been reported both in the general population and in chronic kidney disease patients. In this study we assessed the existence of an association between CAC as a marker of vascular disease and low bone mineral density (BMD) in a cohort of HIV-infected subjects.

Materials and Methods

Consecutive ($n = 636$) HIV-infected patients were recruited between January 2006 and December 2010 at the University of Modena and Reggio Emilia, Italy. Patients were adults with serologically documented HIV-1 infection. All patients signed an informed consent to participate and were excluded from the study if they had a prior history of myocardial infarction, stroke, coronary artery bypass surgery, angioplasty, and peripheral arterial disease. Demographic and clinical data were extracted from chart review. Body mass index (BMI) was calculated as weight in kilograms divided by height in square meters. The glomerular filtration rate (eGFR) was estimated via the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula (http://mdrd.com/). The 10-year CV risk was estimated via the Framingham risk score for women and for men. Study approval was obtained from our institutional ethical review board, and we adhered to the principles of the Declaration of Helsinki for medical research involving human subjects.

Laboratory and Imaging Procedures

We measured biochemical and immunological biomarkers (complete blood cell count, $CD4^+$ lymphocytes count, serum creatinine, 25-hydroxyvitamin D [25(OH)D], intact parathyroid hormone [PTH]) at enrollment in a fasting state. 25(OH)D levels were measured by the DiaSorin (Stillwater, MN) 25(OH)D chemiluminescence immunoassay. The reported reference range is 10–100 ng/mL. Values <10 ng/mL, 10–30 ng/mL, and >100 ng/mL were used to define 25(OH)D deficiency, sufficiency, and toxicity, respectively.

Intact PTH levels were measured with the Access Intact PTH chemiluminescent immunoassay (Beckman Coulter, Fullerton, CA, USA). The reported reference range is 15–90 pg/mL.

BMD of the lumbar spine and femoral neck was assessed by dual-energy X-ray absorptiometry (DXA) with a Hologic (Waltham, MA, USA) scan (Delphi-QDR4500 Discovery-Bone Densitometer) at study inception. Calibration of the densitometer was performed every day via a standard phantom.

CAC was assessed by cardiac computed tomography using a volume CT 64-slice scanner (GE Medical Systems, Milwaukee, WI, USA) during a single breath-hold (settings: 320 mAs, 140 kV, prospective image acquisition at 80 % of the R-to-R interval, slice 2.5 mm, field of view 20 cm^2, pixel size 0.39 mm^2, and voxel size 0.4 mm^3). Images were transferred to an offline workstation for scoring according to the Agatston method, as previously described. The published median interscan CAC score variability is 8–10 % for the Agatston score [6, 7]. Independent skilled operators blinded to other patients' clinical characteristics measured BMD and CAC.

Statistical Analyses

We examined the cross-sectional association between CAC and low BMD in the lumbar spine and the femoral neck. Because the CAC scores were not normally distributed, neither quadratic nor logarithmic transformation normalized their distribution, and a CAC score >100 has been associated with an increased risk of events in the general population [3], subjects were divided in two groups according to a baseline CAC score ≥100 or <100. Because we were interested in the relationship between arterial and bone disease in our patient group, we defined low BMD as <25th percentile for the population under analysis (0.7 g/cm^2 and 0.9 g/cm^2 for femoral neck and lumbar spine, respectively). Logistic regression was used to assess the independent association between CAC and BMD. Odds ratios (ORs) were initially calculated after adjustment for age and sex (model 1); further adjustments were made for diabetes mellitus, BMI, 10-year Framingham risk and hypertension (model 2), $CD4^+$ nadir, exposure to protease inhibitor and tenofovir in association with boosted atazanavir (model 3); eGFR, 25-(OH)D deficiency, and intact PTH levels (model 4). All variables entered into the models were selected a priori because they are known to be associated with either CAC or BMD based on the

Table 1 Patients' characteristics according to level of coronary artery calcium (CAC)

	CAC < 100 ($n = 568$)	CAC ≥ 100 ($n = 68$)	p
Age (years) (SD)	46.6 (7.4)	54.6 (7.8)	<0.001
Men (%)	69.6	88.0	0.001
eGFR (mL/min/1.73 m^2) (SD)	100.7 (10.7)	97 (11)	0.003
Diabetes mellitus (%)	7.4	26.6	<0.001
Hypertension (%)	18.0	36.0	<0.001
Systolic blood pressure (mmHg) (SD)	118 (14)	126 (18)	0.001
Diastolic blood pressure (mmHg) (SD)	77 (12)	79 (10)	0.23
10-year CV Framingham risk score (interquartile range)	5 (1–9)	11 (8–16)	<0.001
Smoking			0.81
Nonsmokers (%)	60.3	58.8	
Smokes <10 cigarettes/day (%)	14.9	13.7	
Smokes ≥10 cigarettes/day (%)	24.6	27.4	
Body mass index (SD)	23.7 (3.7)	25.2 (4.3)	0.004
Current $CD4^+$ lymphocyte count (interquartile range)	157 (64–258)	176 (58–275)	0.21
Protease inhibitor exposure (months) (interquartile range)	38 (12–73)	48 (16–71)	0.36
Tenofovir exposure (months) (interquartile range)	17 (0–33)	22 (0–39)	0.21
Tenofovir associated with atazanavir exposure (months) (interquartile range)	0 (0–13)	0 (0–22)	0.38
Intact PTH (pg/mL) (interquartile range)	57 (44–78)	60 (45–77)	0.47
Vitamin D deficiency (%)	24.0	29.0	0.34
Low femoral BMD (%)	14.6	22.6	0.10
Low lumbar spine BMD (%)	15.0	16.0	0.95

eGFR estimated glomerular filtration rate, estimated via CKD-EPI formula, *BMD* bone mineral density, *PTH* parathyroid hormone, *Vitamin D* 25(OH) vitamin D, *CV* cardiovascular

available medical literature. Because we implemented several assumptions (CAC > 100 AU and BMD < 25th percentile of the study population), the models' predictive performance was internally validated through a nonparametric bootstrapping process [8]. Case deletion was used to handle the missing values. $p < 0.05$ was considered statistically significant. All analyses were completed using R version 2.9.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

The clinical characteristics of the 636 enrolled patients are shown in Table 1; several CV risk factors were more prevalent in patients with CAC > 100, while there was no difference between groups as far as HIV-specific characteristics. The prevalence of low BMD among patients with CAC > 100 was greater but not statistically significant in the femur (23.5 vs. 13.9 %, $p = 0.10$) and in the lumbar spine (16.2 vs. 14.4 %, $p = 0.95$). All multivariable models confirmed a statistically significant association between CAC > 100 and low BMD of the femur but not for the spine (models 1–4 described above). Patients with a low femoral BMD had a significantly increased risk of CAC score ≥100 (OR = 2.33, 95 % confidence interval [CI] 1.09–4.99; $p = 0.02$). Bootstrap analyses confirmed the association of low BMD with CAC score ≥100 at the femoral level only (OR = 2.73, 95 % CI 1.77–5.23) (Table 2). 25-(OH)D deficiency, antiretroviral agents, hypertension, and sex were excluded in the majority of bootstrap models, suggesting that these variables are not significantly associated with CAC. Similarly, bootstrap analyses confirmed the lack of an association between lumbar spine BMD and CAC ≥ 100. Of note, the strength of these associations was not modified by gender (i.e., p value for interaction test not significant).

Discussion

In a cohort of 636 HIV-infected patients we showed an inverse correlation between CAC and low BMD of the femur, while such an inverse correlation was absent for the lumbar spine. HIV patients have an increased CV risk compared to age- and sex-matched individuals and demonstrate characteristics of accelerated aging [9, 10] such as osteopenia and increased fracture rates [11].

The existence of a link between bone and CV health has been reported in the general population as well as specific populations such as diabetic patients, chronic kidney disease, or osteoporotic patients [4] but never in HIV patients.

A complicated interaction of traditional and HIV-specific osteoporotic risk factors may contribute to the osteopenia of

Table 2 Predictors of extensive coronary artery calcium (score >100 Agatston units) using a nested model and bootstrap analytical method: only variables included in >50 % of the bootstrap models are shown

Models testing the independent association between CAC and femoral BMD			
	OR	95 % CI	*p*
Model 1	1.73	0.88–3.38	0.10
Model 2	2.01	0.97–4.19	0.06
Model 3	2.33	1.11–4.86	0.02
Model 4	2.33	1.09–4.99	0.02
Bootstrap analyses			
Variable	OR	95 % CI	Inclusion frequency
Age (1-year increase)	1.12	1.06–1.18	0.99
eGFR (1 mL/min/1.73 m^2 increase)	1.05	1.02–1.09	0.86
Diabetes mellitus (yes vs. no)	2.77	1.77–5.18	0.78
Low femoral BMD (yes vs. no)	2.72	1.77–5.23	0.74
Models testing the independent association between CAC and lumbar spine BMD			
	OR	95 % CI	*p*
Model 1	0.94	0.46–1.92	0.87
Model 2	1.16	0.55–2.42	0.68
Model 3	1.14	0.54–2.39	0.72
Model 4	1.17	0.55–2.48	0.67
Bootstrap analyses			
Variable	OR	95 % CI	Inclusion frequency
Age (1-year increase)	1.12	1.05–1.19	0.99
Diabetes mellitus (yes vs. no)	2.70	1.77–4.91	0.78
eGFR (1 mL/min/1.73 m^2 increase)	1.05	1.02–1.09	0.76
Low lumbar spine BMD (yes vs. no)	1.33	0.30–3.04	0.21[a]

Model 1: adjusted for age and sex

Model 2: adjusted for model 1 + traditional cardiovascular risk factors (diabetes mellitus, body mass index, 10-year risk of hard cardiovascular events according to the Framingham risk score, hypertension)

Model 3: adjusted for model 2 + HIV-specific factors ($CD4^+$ lymphocyte count, current exposure to tenofovir, protease inhibitor and tenofovir associated with atazanavir)

Model 4: adjusted for model 3 + estimated glomerular filtration rate, 25(OH) vitamin D deficiency, and intact parathyroid hormone level

CAC coronary artery calcium, *BMD* body mass index, *eGFR* estimated glomerular filtration rate, estimated via CKD-EPI formula

[a] Low lumbar spine BMD is not significantly associated with extensive CAC and selected only in 20 % of the boostrap models as a significant predictor of extensive CAC

these patients [11]. However, our results suggest that there may be a link between bone demineralization and subclinical atherosclerosis (i.e., CAC) independent of traditional and HIV-specific risk factors; thus, these two disease states could be aspects of the same syndrome. A few clinical studies have shown that interventions that impact one system might exert an effect on the other. A substudy of a randomized trial [12] and a subsequent meta-analysis [13] suggested a lack of efficacy of calcium supplements to reduce osteoporotic fractures in postmenopausal women and a simultaneous increased rate of hard CV events. Sympathetic tone deregulation appears to be associated with osteopenia and the use of β-blockade as antihypertensive agents has been linked with a significant 20–30 % reduction in the risk of fracture in large study cohorts [14]. A few isolated studies suggested that serotonin reuptake inhibitors might increase the risk of osteoporotic fractures [15] as well as CV events [16]. Biphosphonates, non-calcium-containing phosphate binders, and calcimimetic agents have been associated with attenuation of CAC progression in chronic hemodialysis patients [17]. Although earlier studies suggested that bone loss might be attributable to therapy with protease inhibitors, others have failed to confirm such an association. The nucleoside reverse-transcriptase inhibitor tenofovir has been also associated with an acute decrease in BMD [18]. The mechanisms involved in bone loss with various antiretroviral regimens

are not well understood. Tenofovir may have an indirect effect on bone mineralization through proximal tubule toxicity, resulting in phosphate wasting and increased bone turnover [19], whereas protease inhibitors may affect vitamin D metabolism [2]. Of note, the current literature suggests that vitamin D deficiency is linked with an increased risk of arterial calcification; hence, long-term exposure to antiretroviral therapy may contribute to the vascular aging process of HIV-infected subjects via inhibition of vitamin D metabolism [20].

The limitations of this study were its cross-sectional nature, which did not allow us to draw cause-and-effect conclusions or to understand whether low BMD is due to active bone loss or failure to build adequate bone. Furthermore, the relatively small number of patients with CAC $\geq$ 100 did not permit us to take into account several other factors that might be associated with high CAC or low BMD in HIV-infected patients (i.e., unmeasured confounders). The lack of an association between CAC and low lumbar BMD is unexplainable with these data. However, as previously reported, the presence of calcification of the abdominal aorta renders DXA less reliable than CT-based BMD measurements [21]. Since we did not image the abdominal aorta, we cannot exclude that patients with higher CAC scores also had a larger amount of calcium in the aorta, which would have reduced the accuracy of lumbar BMD measured by DXA. Finally, the lack of information about menopausal status and testosterone levels is, in the authors' opinion, another limitation of the present analyses. Consequently, we adopted a priori a highly conservative statistical approach (multivariable adjustment and bootstrap analyses) to compensate for some of the study limitations (i.e., cross-sectional design, lack of standardization of clinical end points).

In summary, we showed a link between arterial disease and low femoral BMD in HIV patients. The association was independent of traditional CV disease-and HIV-specific risk factors, suggesting an active crosstalk between the two systems. The identification of potential mechanisms subtending such a link is beyond the scope of the current analyses, but future studies should further investigate the clinical implications of the interaction of vascular and bone disorders in HIV-infected patients.

References

1. Aberg JA (2012) Aging, inflammation, and HIV infection. Top Antivir Med 20:101–105
2. Cozzolino M, Vidal M, Arcidiacono MV, Tebas P, Yarasheski KE, Dusso AS (2003) HIV-protease inhibitors impair vitamin D bioactivation to 1,25-dihydroxyvitamin D. AIDS 17:513–520
3. Detrano R, Guerci A, Carr J, Bild D, Burke G, Folsom A, Liu K, Shea S, Szklo M, Bluemke D, O'Leary D, Tracy R, Watson K, Wong N, Kronmal R (2008) Coronary calcium as a predictor of coronary events in four racial or ethnic groups. N Engl J Med 358:1336–1345
4. Raggi P, Giachelli C, Bellasi A (2007) Interaction of vascular and bone disease in patients with normal renal function and patients undergoing dialysis. Nat Clin Pract Cardiovasc Med 4:26–33
5. Jono S, McKee MD, Murry CE, Shioi A, Nishizawa Y, Mori K, Morii H, Giachelli CM (2000) Phosphate regulation of vascular smooth muscle cell calcification. Circ Res 87:E10–E17
6. Achenbach S, Ropers D, Mohlenkamp S, Schmermund A, Muschiol G, Groth J, Kusus M, Regenfus M, Daniel WG, Erbel R, Moshage W (2001) Variability of repeated coronary artery calcium measurements by electron beam tomography. Am J Cardiol 87(210–213):A218
7. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr, Detrano R (1990) Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol 15: 827–832
8. Sauerbrei W, Schumacher M (1992) A bootstrap resampling procedure for model building: application to the Cox regression model. Stat Med 11:2093–2109
9. Guaraldi G, Zona S, Alexopoulos N, Orlando G, Carli F, Ligabue G, Fiocchi F, Lattanzi A, Rossi R, Modena MG, Esposito R, Palella F, Raggi P (2009) Coronary aging in HIV-infected patients. Clin Infect Dis 49:1756–1762
10. Guaraldi G, Zona S, Orlando G, Carli F, Ligabue G, Fiocchi F, Rossi R, Modena MG, Raggi P (2012) Progression of coronary artery calcium in men affected by human immunodeficiency virus infection. Int J Cardiovasc Imaging 28:1857–1860
11. Brown TT, Qaqish RB (2006) Antiretroviral therapy and the prevalence of osteopenia and osteoporosis: a meta-analytic review. AIDS 20:2165–2174
12. Bolland MJ, Barber PA, Doughty RN, Mason B, Horne A, Ames R, Gamble GD, Grey A, Reid IR (2008) Vascular events in healthy older women receiving calcium supplementation: randomised controlled trial. BMJ 336:262–266
13. Bolland MJ, Avenell A, Baron JA, Grey A, MacLennan GS, Gamble GD, Reid IR (2010) Effect of calcium supplements on risk of myocardial infarction and cardiovascular events: meta-analysis. BMJ 341:c3691
14. Reid IR, Gamble GD, Grey AB, Black DM, Ensrud KE, Browner WS, Bauer DC (2005) β-Blocker use, BMD, and fractures in the study of osteoporotic fractures. J Bone Miner Res 20:613–618
15. Richards JB, Papaioannou A, Adachi JD, Joseph L, Whitson HE, Prior JC, Goltzman D, Group CMOSR (2007) Effect of selective serotonin reuptake inhibitors on the risk of fracture. Arch Intern Med 167:188–194
16. Von Ruden AE, Adson DE, Kotlyar M (2008) Effect of selective serotonin reuptake inhibitors on cardiovascular morbidity and mortality. J Cardiovasc Pharmacol Ther 13:32–40
17. Raggi P, Chertow GM, Torres PU, Csiky B, Naso A, Nossuli K, Moustafa M, Goodman WG, Lopez N, Downey G, Dehmel B, Floege J, Group AS (2011) The ADVANCE study: a randomized study to evaluate the effects of cinacalcet plus low-dose vitamin D on vascular calcification in patients on hemodialysis. Nephrol Dial Transplant 26:1327–1339
18. Mccomsey GA, Tebas P, Shane E, Yin MT, Overton ET, Huang JS, Aldrovandi GM, Cardoso SW, Santana JL, Brown TT (2010) Bone disease in HIV infection: a practical review and recommendations for HIV care providers. Clin Infect Dis 51:937–946
19. Fux CA, Rauch A, Simcock M, Bucher HC, Hirschel B, Opravil M, Vernazza P, Cavassini M, Bernasconi E, Elzi L, Furrer H, Study SHC (2008) Tenofovir use is associated with an increase in serum alkaline phosphatase in the Swiss HIV Cohort Study. Antivir Ther 13:1077–1082

20. Shroff R, Egerton M, Bridel M, Shah V, Donald AE, Cole TJ, Hiorns MP, Deanfield JE, Rees L (2008) A bimodal association of vitamin D levels and vascular disease in children on dialysis. J Am Soc Nephrol 19:1239–1246

21. Raggi P, Bellasi A, Ferramosca E, Block GA, Muntner P (2007) Pulse wave velocity is inversely related to vertebral bone density in hemodialysis patients. Hypertension 49:1278–1284