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Bone disease in the setting of HIV infection: update and review of the literature

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Abstract. – The advent of highly active antiretroviral therapy (HAART) in the mid-1990s has transformed Human Immunodeficiency Virus (HIV) infection into a chronic disease. HIV-infected patients are living longer and are facing several non-AIDS-associated morbidities related with aging, including diabetes mellitus, cardiovascular disease, osteoporosis, osteopenia and fragility fractures.

The prevalence of bone disease is higher among HIV-infected subjects. In addition to traditional risk factors, HAART, chronic inflammation and the virus itself have been suggested to contribute to bone loss in the setting of HIV infection.

In the present review, we summarize the current knowledge about risk factors for low bone mineral density in HIV-positive patients as well as current recommendations for fracture screening and treatment in this specific population.

Key Words:
HIV, HAART, Osteoporosis, Fracture, Bone, Vitamin D.

Introduction

The advent of highly active antiretroviral therapy (HAART) has transformed Human Immunodeficiency Virus (HIV) infection into a chronic disease, with life expectancy close to that of the general population for patients with good access to high quality medical care[1]. HIV-infected patients are living longer and are facing several non-AIDS-associated morbidities related with aging, including diabetes mellitus, malignancies, cardiovascular disease, osteoporosis, osteopenia and fragility fractures[2-37]. However, HAART cannot eradicate HIV infection[38-51].

Osteoporosis is defined as a systemic skeletal disorder characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and fracture[1]. Bone mineral density (BMD) can be measured by imaging modalities, such as dual X-ray absorptiometry (DXA), which helps identifying patients at high risk of fractures[1].

The World Health Organization has grouped reduced BMD into two categories. Osteoporosis is defined as a bone density less than 2.5 standard deviations of the mean BMD of a sex-matched, young healthy population, i.e. a T-score less than < 2.5. Osteopenia is an intermediate category of bone loss defined as a T-score between 1 and 2.5. Although these categories were created to classify postmenopausal women, they are often applied to other adult populations[1].

Osteoporosis is likely to become an important cause of morbidity and mortality as the HIV-infected population ages. It has been related not only to "traditional" risk factors, such as smoking, alcohol use, opiate use, physical inactivity, low body weight, hypogonadism and vitamin D deficiency, but also to chronic immune activation and antiretroviral side effects[52,53].

In the present review, we summarize the current knowledge about risk factors for low BMD in HIV-positive patients as well as current recommendations for fracture screening and treatment in the setting of HIV infection.

Etiology of Low BMD in HIV Infection

Among HIV-infected patients the etiology of osteoporosis is multifactorial.

Some risk factors are shared with the general population and may be more prevalent in HIV-infected populations, i.e. low body mass, sedentary lifestyle, smoking, alcohol abuse, glucocorticoid therapy, low consumption of calcium and vitamin D[54-56]. In addition, immune dysregulation and chronic inflammation, as well as antiretroviral drugs, have been shown to negatively impact

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bone health[54]. Cytokines and other soluble immune factors are involved in the modulation of osteoblast maturation and osteoclastic bone resorption[54-57]. Moreover, bone is richly innervated by both autonomic and sensory neurons. In healthy individuals, several factors control bone metabolism, including the neuroimmune network and the neuroendocrine-immune regulatory system (e.g., adrenocorticotropin hormone; parathyroid hormone (PTH) and calcitonin)[54-57].

The PTH pathway is especially important, as it regulates the production of the proinflammatory cytokine interleukin (IL)-6 and the Receptor Activator of Nuclear Factor kappa-B Ligand (RANKL)[58-59].

In untreated HIV infection, bone resorption and bone formation are uncoupled because of both direct viral effects and proinflammatory mechanisms. *In vitro* studies demonstrated that HIV viral proteins, like vpr and gp120, may promote osteoclast activity, whereas p55-gag is able to suppress osteoblast activity and increase osteoblast apoptosis[58-60]. In addition, proinflammatory cytokines, like tumor necrosis factor (TNF)-α and IL-6, stimulate osteoclastogenesis and bone resorption[61-62]. High HIV RNA viral load and T-cell activation have been associated with elevated levels of RANKL, which may lead to osteoclast formation and increased bone turnover[63-64]. RANKL plasma concentrations have been positively correlated to HIV RNA viral load[62]. Interferon-γ is a physiological inhibitor of RANKL signaling, whose levels are remarkably downregulated in advanced HIV infection[65]. Therefore, a limited capacity to suppress RANKL during HIV infection may lead to increased osteoclast activation and bone resorption. Osteopontin (OPN) is produced by osteoblasts and several immune cells, including macrophages, neutrophils, dendritic cells and T and B cells. In immune metabolism, OPN is endowed with chemotactic properties that promote cell recruitment to inflammatory sites as well as adhesion properties to several integrin receptors which promote T-cell activation, cytokine production and regulation of apoptosis. OPN carries complex molecular and epigenetic regulatory roles in osteogenesis and immune regulation[66]. OPN regulates osteoclast activity and the development of TH1 and TH17 cells. Vikulina et al[67] showed that bone loss in HIV transgenic rats was associated with an increase in RANKL and a parallel decline in OPG levels, thus leading to increased osteoclastic bone resorption.

Role of HAART in Bone Loss

In addition to the direct and immune-mediated effects of HIV on the skeleton, several antiretroviral regimens have been associated with bone loss, although the mechanisms and degree of BMD loss vary according to the antiretroviral class[54].

Initiation of HAART has been associated with a marked and clinically significant loss of BMD (2%-6%), regardless of the antiretroviral regimen, followed by stabilization and increase in BMD within 1-2 years after HAART initiation. In a meta-analysis carried out in 2006 to compare HAART-naive and HAART-treated patients, there was a 2.5 fold increase in the prevalence of low BMD in the treated population, after adjusting for other risk factors for osteoporosis[52]. BMD decrease seems to be higher when initiating tenofovir (TDF) and/or protease inhibitor (PI)-based regimens, in comparison with non-nucleoside reverse transcriptase inhibitors (NNRTI)[68,69].

TDF has been associated with bone demineralization[70,71]. TDF has been shown to cause nephrotoxicity, with epithelial damage in the proximal tubule and hypophosphatemia, which may be responsible in turn for increased PTH levels and bone resorption. In addition, impaired renal function may cause decreased 1-alphahydroxylation of the vitamin D precursor 25-hydroxyvitamin D (25OHD), thus reducing the levels of the active metabolite 1,25-dihydroxyvitamin D ($1,25OH_2D$)[68]. A retrospective study conducted in Los Angeles showed that foot fractures were more frequent in HIV-infected patients treated with TDF than non-TDF-containing HAART. Median time from TDF initiation until fracture was 2.57 years[44]. The TDF group had higher median plasma concentrations of alkaline phosphatase, PTH, 25OHD and a lower white blood cell count[72].

The ASSERT study examined 385 HAART-naïve patients who were randomized to receive either abacavir-lamivudine (ABC/3TC) or tenofovir-emtricitabine (TDF/FTC) with efavirenz (EFV)[73]. Bone turnover markers (osteocalcin, bone specific alkaline phosphatase, procollagen 1 N-terminal propeptide and serum type 1 collagen cross-linked C telopeptide (CTx)) were assessed. Bone turnover markers increased in both groups over the first 6 months and then stabilized, with greater increase in the group receiving TDF/FTC and EFV at 24 weeks[73]. Analogously, McComsey et al confirmed that TDF/FTC-treated subjects had significantly greater decrease in spine and hip BMD than those receiving ABC/3TC at week

96; in addition, they found greater BMD loss at the spine at 96 weeks among patients receiving ATV/ritonavir (r) compared with EFV[74].

In another study, HAART-naive patients were treated with zidovudine(ZDV)/3TC/lopinavir (LPV)/r or nevirapine(NVP)/LPV/r; in both groups, a rapid decrease in femoral neck and lumbar spine BMD after initiation of HAART was found[75]. Lumbar spine bone loss stabilized in the second year of treatment, whereas progressive bone loss in the femoral neck was observed in the same period only in the ZDV/3TC/LPV/r group[75]. Moreover, markers of bone formation and resorption significantly increased after HAART initiation in all patients, indicating an increase in bone turnover. Both ZDV and 3TC have been shown to enhance osteoclastogenesis, potentially leading to bone loss[75,76].

The effects on BMD of new antiretroviral classes, including integrase and entry inhibitors, remain to be established.

Several questions are still unanswered. In fact, the mechanisms leading to an acute decrease in BMD after HAART initiation are unclear; in addition, although a BMD recovery is observed after the first 1-2 years on HAART, the long-term clinical impact of such a BMD loss has not been clarified yet.

Screening for Bone Disease in HIV

Current EACS guidelines recommend to screen and treat secondary causes of low BMD[60]. Laboratory work up includes complete blood count, calcium, phosphate, albumin, creatinine, 25-hydroxyvitamin D as well as PTH, thyroid-stimulating hormone and 24-hour urine collection. For patients on TDF, urinary phosphorus levels should also be evaluated. The expert panel also suggests checking testosterone levels in men and estradiol, prolactin, follicle-stimulating hormone and luteinizing hormone in premenopausal women with amenorrhea[77].

The National Osteoporosis Foundation recommends osteoporosis screening with DXA for all women aged >65 years and men aged >70 years, regardless of clinical risk factors, and for adults aged >50 with additional risk factors for osteoporosis[52].

FRAX algorithm (http://www.shef.ac.uk/FRAX/) is used to calculate 10-year fracture risk by integrating information coming from patients risk factors for osteoporosis and BMD. However, FRAX algorithm has not been formally validated for HIV-positive patients, because it may underestimate the fracture risk and may not discriminate between patients who have osteopenia and those who have not[78,79]. TDF should be used with prudency in patients with low trauma or atraumatic fractures or very low BMD, due to the association with proximal tubule dysfunction[77].

Treatment

Expert consensus panels suggest screening and treatment of secondary causes of low BMD[77]. Pharmacologic treatment of osteoporosis should be undertaken for postmenopausal women and men aged >50 years with fragility fractures or a T-score of the hip, femoral neck or lumbar spine ≤2.5; pharmacological treatment should also be considered if the 10-year probability of hip fracture is ≥3% or the 10-year risk for major osteoporosis-related fractures is ≥20% using the FRAX score[54].

Prior to treatment, calcium and vitamin D intake should be estimated. Guidelines suggest to increase intake from dietary sources and administer 1000-1500 mg of calcium and 800-1000 IU of vitamin D daily[80]. Thirty minutes of weight-bearing (i.e. jogging and walking) and muscle-strengthening exercise at least 3 days a week are also recommended, as such exercise may increase bone density[77]. Smoking cessation and limitation of alcohol intake are also advised[77]. The optimal vitamin D replacement regimen is unknown: many studies suggest to reach a target 25OHD range of 30-50 ng/mL[77]. Vitamin D can be replaced by vitamin D2 (ergocalciferol) or the more bioavailable vitamin D3 (cholecalciferol)[64]. Recently, the US Endocrine Society recommended the administration of 1000-2000 IU of vitamin D daily. Larger doses [i.e. 50,000 IU (orally weekly for 8 weeks or 300,000 IU by intramuscular injection every 3 months] may be required for patients with more severe vitamin D deficiency[80].

Alendronate is a bisphosphonate which inhibits osteoclast-mediated bone resorption and has been approved for the treatment of osteoporosis in men and women[81-83].

The ANRS 120 Fosivir trial examined the effect of alendronate on BMD in HIV-infected patients with a T-score <2.5 at the lumbar spine and/or total hip[83]. Patients were randomized to receive either extended-release alendronate 70 mg weekly or placebo for 96 weeks; all the patients also received daily calcium carbonate (500 mg) and vitamin D (400 IU). Alendronate 70 mg weekly for 96 weeks was shown to improve BMD in HIV-infected patients on HAART[83]. In another double-blind, ran-

domized, placebo-controlled trial, the authors evaluated the effects of two annual 4-mg doses of intravenous zoledronate in a cohort of 43 HIV-infected men with BMD T score <0.5. The authors found that the antiresorptive effects of zolendronate persisted for at least 5 years after the second dose[84]. Oral bisphosphonates have been associated with esophageal irritation and dyspepsia; even if rare, osteonecrosis of the jaw has been reported in patients receiving bisphosphonates[54]. In addition, chronic suppression of bone turnover with bisphosphonates has been suggested to predispose to fracture in some patients as it may prevent the repair of microdamage to the bone architecture[54]. Considering the increased life expectancy of HIV-infected people, there is a need for clinical trials evaluating the long-term safety and the optimal duration of treatment with bisphosphonates.

Second-line osteoporosis therapies, including estrogen-replacement therapy, the selective estrogen receptor modulator raloxifene for postmenopausal women, as well as the PTH analogue teriparatide, have not been specifically evaluated in the setting of HIV infection.

Conclusions

The prevalence of low BMD is higher among HIV-infected patients in comparison with the general population. The pathogenesis of bone disease is multifactorial and includes traditional risk factors, such as hypogonadism, smoking and low body weight, and HIV-related risk factors, such as chronic immune activation and antiretroviral toxicities. Considering that HAART has transformed HIV infection into a chronic disease, systematic screening for bone disease is crucial to reduce fracture risk and improve the quality of life of HIV-infected subjects.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Harris VW, Brown TT. Bone loss in the HIV-infected patient: evidence, clinical implications, and treatment strategies. J Infect Dis 2012; 205: S391-398.
2) Nunnari G, Coco C, Pinzone MR, Pavone P, Berretta M, Di Rosa M, Schnell M, Calabrese G, Cacopardo B. The role of micronutrients in the diet of HIV-1-infected individuals. Front Biosci (Elite Ed) 2012; 4: 2442-2456.
3) Zanet E, Berretta M, Di Benedetto F, Talamini R, Ballarin R, Nunnari G, Berretta S, Ridolfo A, Lleshi A, Zanghì A, Cappellani A, Tirelli U. Pancreatic cancer in HIV-positive patients: a clinical case-control study. Pancreas 2012; 41: 1331-1335.
4) Berretta M, Garlassi E, Cacopardo B, Cappellani A, Guaraldi G, Cocchi S, De Paoli P, Lleshi A, Izzi I, Torresin A, Di Gangi P, Pietrangelo A, Ferrari M, Bearz A, Berretta S, Nasti G, Di Benedetto F, Balestreri L, Tirelli U, Ventura P. Hepatocellular carcinoma in HIV-infected patients: check early, treat hard. Oncologist 2011; 16: 1258-1269.
5) Berretta M, Lleshi A, Cappellani A, Bearz A, Spina M, Talamini R, Cacopardo B, Nunnari G, Montesarchio V, Izzi I, Lanzafame M, Nasti G, Basile F, Berretta S, Fisichella R, Schiantarelli C, Garlassi E, Ridolfo A, Guella L, Tirelli U. Oxaliplatin based chemotherapy and concomitant highly active antiretroviral therapy in the treatment of 24 patients with colorectal cancer and HIV infection. Curr HIV Res 2010; 8: 218-222.
6) Berretta M, Cappellani A, Di Benedetto F, Lleshi A, Talamini R, Canzonieri V, Zanet E, Bearz A, Nasti G, Lacchin T, Berretta S, Fisichella R, Balestreri L, Torresin A, Izzi I, Ortolani P, Tirelli U. Clinical presentation and outcome of colorectal cancer in HIV-positive patients: a clinical case-control study. Onkologie 2009; 32: 319-324.
7) Berretta M, Zanet E, Basile F, Ridolfo AL, Di Benedetto F, Bearz A, Berretta S, Nasti G, Tirelli U. HIV-positive patients with liver metastases from colorectal cancer deserve the same therapeutic approach as the general population. Onkologie 2010; 33: 203-204.
8) Zanet E, Berretta M, Martellotta F, Cacopardo B, Fisichella R, Tavio M, Berretta S, Tirelli U. Anal cancer: Focus on HIV-positive patients in the HAART era. Curr HIV Res 2011; 9: 70-81.
9) Nunnari G, Xu Y, Acheampong Ea, Fang J, Daniel R, Zhang C, Zhang H, Mukhtar M, Pomerantz RJ. Exogenous IL-7 induces Fas-mediated human neuronal apoptosis: potential effects during human immunodeficiency virus type 1 infection. J Neurovirol 2005; 11: 319-328.
10) 10 Di Benedetto F, Tarantino G, Ercolani G, Baccarani U, Montalti R, De Ruvo N, Berretta M, Adani GL, Zanello M, Tavio M, Cautero N, Tirelli U, Pinna AD, Gerunda GE, Guaraldi G. Multicenter Italian Experience in liver transplantation for hepatocellular carcinoma in HIV-infected patients. Oncologist 2013; 18: 592-599.
11) Tavio M, Grossi P, Baccarani U, Scudeller L, Pea F, Berretta M, Adani G, Vivarelli M, Riva A, Tirelli U, Bresadola V, Viale P, Risaliti A. HIV infected patients and liver transplantation: who, when and why. Curr HIV Res 2011; 9: 120-127.
12) Spina M, Carbone A, Gloghini A, Serraino D, Berretta M, Tirelli U. Hodgkin's disease in patients with HIV infection. Adv Hematol 2011; 2011.
13) Spina M, Chimienti E, Martellotta F, Vaccher E, Berretta M, Zanet E, Lleshi A, Canzonieri V, Bulian P, Tirelli U. Phase 2 study of intrathecal, long-act-

ing liposomal cytarabine in the prophylaxis of lymphomatous meningitis in human immunodeficiency virus-related non-Hodgkin lymphoma. Cancer 2010; 116: 1495-1501.

14) Martellotta F, Berretta M, Vaccher E, Schioppa O, Zanet E, Tirelli U. AIDS-related Kaposi's sarcoma: state of the art and therapeutic strategies. Curr HIV Res 2009; 7: 634-638.

15) Simonelli C, Tedeschi R, Gloghini A, Talamini R, Bortolin MT, Berretta M, Spina M, Morassut S, Vaccher E, De Paoli P, Carbone A, Tirelli U. Plasma HHV-8 viral load in HHV-8 related lymphoproliferative disorders associated with HIV infection. J Med Virol 2009; 81: 888-896.

16) Berretta M, Zanet E, Di Benedetto F, Simonelli C, Bearz A, Morra A, Bonanno S. Berretta S, Tirelli U. Unusual presentation of metastatic hepatocellular carcinoma in an HIV/HCV coinfected patient: case report and review of the literature. Tumori 2008; 94: 589-591.

17) Di Benedetto F, Di Sandro S, De Ruvo N, Berretta M, Montalti R, Guerrini GP, Ballarin R, DE Blasiis MG, Spaggiari M, Smerieri N, Iemmolo RM, Guaraldi G, Gerunda GE. Human Immunodeficiency virus and liver tranplantation: our point of view. Transplant Proc 2008; 40: 1965-1971.

18) Di Benedetto f, Di Sandro S, De Ruvo N, Berretta M, Masetti M, Montalti R, Ballarin R, Cocchi S, Potenza L, Luppi M, Gerunda GE. Kaposi's sarcoma after liver transplantation. J Cancer Res Clin Oncol. 2008; 134: 653-658.

19) Di Benedetto F, De Ruvo N, Berretta M, Masetti M, Montalti R, Di Sandro S, Ballarin r, Codeluppi M., Guaraldi G, Gerunda GE. Hepatocellular carcinoma in HIV patients treated by liver transplantation. Eur J Surg Oncol 2008; 34: 442-447.

20) Berretta M, Martellotta F, Simonelli C, Di Benedetto F, De Ruvo N, Drigo A, Bearz A, Spina M, Zanet E, Berretta s, Tirelli U. Cetuximab/targeted chemotherapy in an HIV-positive patient with metastatic colorectal cancer in the HAART era: a case report. J Chemother 2007; 19: 343-346.

21) Di Benedetto F, De Ruvo n, Berretta M, Masetti M, Montalti R, Di Sandro S, QuintiNI C, Codeluppi M, Tirelli U, Gerunda GE. Dont'deny liver transplantation to HIV patients with hepatocellular carcinoma in the highly active antiretroviral therapy era. J Clin Oncol 2006; 24: e26-27.

22) Berretta M, Tirelli U. Colorectal cancer screening in HIV-infected patients 50 years of age and older: missed opportunities for prevention. Am J Gastroenterol 2006; 101: 907.

23) Berretta M, Di Benedetto F, Simonelli C, Bearz A, Berretta S, Maugeri D, Tirelli U. Multidisciplinary approach in a HIV/HCV positive patient with liver metastases by colorectal cancer in the HAART era. Ann Oncol 2006; 17: 1333-1334.

24) Nasti G. Martellotta F, Berretta M, Mena M, Fasan M, Di Perri G, Talamini R, Pagano G, Montroni M, Cinelli R, Vaccher E, D'Arminio Monforte A, Tirelli U; GICAT; ICONA. Impact of Highly active antiretroviral therapy on the presentino features and outcome of patients with acquired immunodeficiency syndrome-related Kaposi sarcoma. Cancer 2003; 98: 2440-2446.

25) Berretta M, Cinelli R, Martellotta F, Spina M, Vaccher E, Tirelli U. Therapeutic approaches to AIDS-related malignancies. Oncogene. 2003 Sep 29; 22: 6646-6659.

26) Spina M, Berretta M, Tirelli U. Hodgkin's disease in Hiv. Hematol Oncol Clin North Am. 2003 Jun; 17: 843-858.

27) Nunnari G, Pomerantz RJ. IL-7 as a potential therapy for HIV-1-infected individuals. Expert Opin Biol Ther 2005; 5: 1421-1426.

28) Nunnari G, Berretta M, Pinzone MR, Di Rosa M, Cappellani A, Berretta S, Tirelli U, Malaguarnera M, Schnell JM, Cacopardo B. Hepatocellular carcinoma in HIV positive patients. Eur Rev Med Pharmacol Sci 2012; 16: 1257-1270.

29) Martellotta F, Berretta M, Cacopardo B, Fisichella R, Schioppa O, Zanghì A, Spartà D, Cappellani A, Talamini R, Izzi I, Ridolfo A, Torresin A, Fiorica F, Tirelli U. Clinical presentation and outcome of squamous cell carcinoma of the anus in HIV-infected patients in the HAART-era: a GICAT experience. Eur Rev Med Pharmacol Sci 2012; 16: 1283-1291.

30) Berretta M, Di Benedetto F, Dal Maso L, Cacopardo B, Nasti G, Facchini G, Bearz A, Spina M, Garlassi E, De Re V, Fiorica F, Lleshi A, Tirelli U. Sorafenib for the treatment of unresectable hepatocellular carcinoma in HIV-positive patients. Anticancer Drugs 2013; 24: 212-218.

31) Di Rosa M, Malaguarnera G, De Gregorio C, Palumbo M, Nunnari G, Malaguarnera L. Immunomodulatory effects of vitamin D3 in human monocyte and macrophages. Cell Immunol 2012; 280: 36-43.

32) Di Rosa M, Malaguarnera L, Nicolosi A, Sanfilippo C, Mazzarino C, Pavone P, Berretta M, Cosentino S, Cacopardo B, Pinzone MR, Nunnari G. Vitamin D3: an ever green molecule. Front Biosci (Schol Ed) 2013; 5: 247-260.

33) Pinzone MR, Fiorica F, Di Rosa M, Malaguarnera G, Malaguarnera L, Cacopardo B, Zanghì G, Nunnari G. Non-AIDS-defining cancers among HIV-infected people. Eur Rev Med Pharmacol Sci 2012; 16: 1377-1388.

34) Bearz A, Vaccher E, Talamini R, Berretta M, Tirelli U. Comment on 'Lung cancer in the Swiss HIV Cohort Study: role of smoking, immunodeficiency and pulmonary infection'. Br J Cancer 2012; 106: 1899-1900.

35) Nunnari G, Smith JA, Daniel R. HIV-1 Tat and AIDS-associated cancer: targeting the cellular anti-cancer barrier. J Exp Clin Cancer Res 2008; 27: 3.

36) Pinzone MR, Celesia BM, Di Rosa M, Cacopardo B, Nunnari G. Microbial translocation in chronic liver diseases. Int J Microbiol 2012; 2012: 694629.

37) Pinzone MR, Di Rosa M, Malaguarnera M, Madeddu G, Focà E, Ceccarelli G, D'ettorre G, Vullo V,

Fisichella R, Cacopardo B, Nunnari G. Vitamin D deficiency in HIV infection: an underestimated and undertreated epidemic. Eur Rev Med Pharmacol Sci 2013; 17: 1218-1232.

38) Pinzone MR, Di Rosa M, Cacopardo B, Nunnari G. HIV RNA suppression and immune restoration: can we do better? Clin Develop Immunol 2012; 2012: 515962.

39) Nunnari G, Otero M, Dornadula G, Vanella M, Zhang H, Frank I, Pomerantz RJ. Residual HIV-1 disease in seminal cells of HIV-1-infected men on suppressive HAART: latency without on-going cellular infections. AIDS 2002; 16: 39-45.

40) Nunnari G, Leto D, Sullivan J, Xu Y, Mehlman Ke, Kulkosky J, Pomerantz RJ. Seminal reservoirs during an HIV type 1 eradication trial. AIDS Res Hum Retroviruses 2005; 21: 768-775.

41) Nunnari G, Sullivan J, Xu Y, Nyirjesy P, Kulkosky J, Cavert W, Frank I, Pomerantz RJ. HIV type 1 cervicovaginal reservoirs in the era of HAART. AIDS Res Hum Retroviruses 2005; 21: 714-718.

42) Nunnari G, Gussio M, Pinzone MR, Martellotta F, Cosentino S, Cacopardo B, Celesia BM. Cryptococcal meningitis in an HIV-1-infected person: relapses or IRIS? Case report and review of the literature. Eur Rev Med Pharmacol Sci 2013; 17: 1555-1559

43) Pomerantz RJ, Nunnari G. HIV and GB virus C--can two viruses be better than one? N Engl J Med 2004; 350: 963-965.

44) Dornadula G, Nunnari G, Vanella M, Roman J, Babinchak T, De Simone J, Stern J, Braffman M, Zhang H, Pomerantz RJ. Human immunodeficiency virus type 1-infected persons with residual disease and virus reservoirs on suppressive highly active antiretroviral therapy can be stratified into relevant virologic and immunologic subgroups. J Infect Dis 2001; 183: 1682-1687.

45) Otero M, Nunnari G, Leto D, Sullivan J, Wang FX, Frank I, Xu Y, Patel C, Dornadula G, Kulkosky J, Pomerantz RJ. Peripheral blood Dendritic cells are not a major reservoir for HIV type 1 in infected individuals on virally suppressive HAART. AIDS Res Hum Retroviruses 2003; 19: 1097-1103.

46) Nunnari G, Argyris E, Fang J, Mehlman KE, Pomerantz RJ, Daniel R. Inhibition of HIV-1 replication by caffeine and caffeine-related methylxanthines. Virology 2005; 335: 177-184.

47) Smith JA, Nunnari G, Preuss M, Pomerantz RJ, Daniel R. Pentoxifylline suppresses transduction by HIV-1-based vectors. Intervirology 2007; 50: 377-386.

48) Pinzone MR, Cacopardo B, Condorelli F, Di Rosa M, Nunnari G. Sirtuin-1 and HIV-1: An Overview. Curr Drug Targets 2013; 14: 648-652.

49) Wang FX, Xu Y, Sullivan J, Souder E, Argyris EG, Acheampong EA, Fisher J, Sierra M, Thomson MM, Najera R, Frank I, Kulkosky J, Pomerantz RJ, Nunnari G. IL-7 is a potent and proviral strain-specific inducer of latent HIV-1 cellular reservoirs of infected individuals on virally suppressive HAART. J Clin Invest 2005; 115: 128-137.

50) Pinzone MR, Di Rosa M, Celesia BM, Condorelli F, Malaguarnera M, Madeddu G, Martellotta F, Castronuovo D, Gussio M, Coco C, Palermo F, Cosentino S, Cacopardo B, Nunnari G. LPS and HIV gp120 modulate monocyte/macrophage CYP27B1 and CYP24A1 expression leading to vitamin D consumption and hypovitaminosis D in HIV-infected individuals. Eur Rev Med Pharmacol Sci 2013; 17: 1938-1950.

51) Celesia BM, Castronuovo D, Pinzone MR, Bellissimo F, Mughini MT, Lupo G, Scarpino MR, Gussio M, Palermo F, Cosentino S, Cacopardo B, Nunnari G. Late presentation of HIV infection: predictors of delayed diagnosis and survival in eastern Sicily. Eur Rev Med Pharmacol Sci 2013; 17: 2218-2224.

52) Brown TT, Qaqish RB. Antiretroviral therapy and the prevalence of osteopenia and osteoporosis: a meta-analytic review. AIDS 2006; 20: 2165-2174.

53) Rothman MS, Bessesen MT. HIV Infection and osteoporosis: pathophysiology, diagnosis, and treatment options. Curr Osteoporos Rep 2012; 10: 270-277.

54) McComsey GA, Tebas P, Shane E, Yin MT, Overton ET, Huang JS, Aldrovandi GM, Cardoso SW, Santana JL, Brown TT. Bone disease in HIV infection: a practical review and recommendations for HIV care providers. Clin Infect Dis 2010; 51: 937-946.

55) Tebas P, Powderly WG, Claxton S, Marin D, Tantisiriwat W, Teitelbaum SL, Yarasheski KE. Accelerated bone mineral loss in HIV-infected patients receiving potent antiretroviral therapy. AIDS 2000; 14: F63-F67.

56) Brown TT. HIV: An under-recognized secondary cause of osteoporosis? J Bone Miner Res 2013; 28: 1256-1258.

57) Panayiotopoulos A, Bhat N, Bhangoo A. Bone and vitamin D metabolism in HIV. Rev Endocr Metab Disord 2013 [Epub ahead of print]

58) Yin M. Vitamin D, bone, and HIV infection. Top Antivir Med 2012; 20: 168-172.

59) Huang JC, Sakata T, Pfleger LL, Bencsik M, Halloran BP, Bikle DD, Nissenson RA. PTH differentially regulates expression of RANKL and OPG. J Bone Miner Res 2004; 19: 235-244.

60) Fakruddin JM, Laurence J. HIV envelope gp120-mediated regulation of osteoclastogenesis via receptor activator of nuclear factor kappa B ligand (RANKL) secretion and its modulation by certain HIV protease inhibitors through interferon-gamma/RANKL cross-talk. J Biol Chem 2003; 278: 48251-48258.

61) Fakruddin JM, Laurence J. Interactions among human immunodeficiency virus (HIV)-1, interferon-gamma and receptor of activated NF-kappa B ligand (RANKL): implications for HIV pathogenesis. Clin Exp Immunol 2004; 137: 538-545.

62) Gibellini D, Borderi M, De Crignis E, Cicola R, Vescini F, Caudarella R, Chiodo F, Re C. RANKL/OPG/TRAIL plasma levels and bone mass loss evaluation in antiretroviral naïve HIV-1-positive men. J Med Virol 2007; 79: 1446-1454.

63) Gazzola L, Bellistri GM, Tincati C, Ierardi V, Savoldi A, del Dole A, Tagliabue L, d'Arminio Monforte A, Marchetti G. Association between peripheral T-Lymphocyte activation and impaired bone mineral density in HIV-infected patients. J Transl Med 2013; 11: 51.

64) Josien R, Wong BR, Li HL, Steinman RM, Choi Y. TRANCE, a TNF family member, is differentially expressed on T cell subsets and induces cytokine production in dendritic cells. J Immunol 1999; 162: 2562-2568.

65) Barkhordarian A, Ajaj R, Ramchandani MH, Demerjian G, Cayabyab R, Danaie S, Ghodousi N, Iyer N, Mahanian N, Phi L, Giroux A, Manfrini E, Neagos N, Siddiqui M, Cajulis OS, Brant XM, Shapshak P, Chiappelli F. Osteoimmunopathology in HIV/AIDS: a translational evidence-based perspective. Patholog Res Int 2011; 2011: 359242.

66) Malizia AP, Cotter E, Chew N, Powderly WG, Doran PP. HIV protease inhibitors selectively induce gene expression alterations associated with reduced calcium deposition in primary human osteoblasts. AIDS Res Hum Retroviruses 2007; 23: 243-250.

67) Vikulina T, Fan X, Yamaguchi M, Roser-Page S, Zayzafoon M, Guidot DM, Ofotokun I, Weitzmann MN. Alterations in the immuno-skeletal interface drive bone destruction in HIV-1 transgenic rats. Proc Natl Acad Sci USA 2010; 107: 13848-13853.

68) Bedimo R, Maalouf NM, Zhang S, Drechsler H, Tebas P. Osteoporotic fracture risk associated with cumulative exposure to tenofovir and other antiretroviral agents. AIDS 2012; 26: 825-831.

69) Madeddu G, Spanu A, Solinas P, Calia GM, Lovigu C, Chessa F, Mannazzu M, Falchi A, Mura MS, Madeddu G. Bone mass loss and vitamin D metabolism impairment in HIV patients receiving highly active antiretroviral therapy. Q J Nucl Med Mol Imaging 2004; 48: 39-48.

70) Heath KV, Montaner JS, Bondy G, Singer J, O'Shaughnessy MV, Hogg RS. Emerging drug toxicities of highly active antiretroviral therapy for human immunodeficiency virus (HIV) infection. Curr Drug Targets 2003; 4: 13-22.

71) Schafer JJ, Manlangit K, Squires KE. Bone health and human immunodeficiency virus infection. Pharmacotherapy 2013; 33: 665-682.

72) Horizon AA, Joseph RJ, Liao Q, Ross ST, Pakes GE. Characteristics of foot fractures in HIV-infected patients previously treated with tenofovir versus non-tenofovir-containing highly active antiretroviral therapy. HIV AIDS (Auckl) 2011; 3: 53-59.

73) Stellbrink HJ, Orkin C, Arribas JR, Compston J, Gerstoft J, Van Wijngaerden E, Lazzarin A, Rizzardini G, Sprenger HG, Lambert J, Sture G, Leather D, Hughes S, Zucchi P, Pearce H; ASSERT Study Group. Comparison of changes in bone density and turnover with abacavir-lamivudine versus tenofovir-emtricitabine in HIV-infected adults: 48-week results from the ASSERT study. Clin Infect Dis 2010; 51: 963-972.

74) McComsey GA, Kitch D, Daar ES, Tierney C, Jahed NC, Tebas P, Myers L, Melbourne K, Ha B, Sax PE. Bone mineral density and fractures in antiretroviral-naive persons randomized to receive abacavir-lamivudine or tenofovir disoproxil fumarate-emtricitabine along with efavirenz or atazanavir-ritonavir: Aids Clinical Trials Group A5224s, a substudy of ACTG A5202. J Infect Dis 2011; 203: 1791-1801.

75) van Vonderen MG, Lips P, van Agtmael MA, Hassink EA, Brinkman K, Geerlings SE, Sutinen J, Ristola M, Danner SA, Reiss P. First line zidovudine/lamivudine/lopinavir/ritonavir leads to greater bone loss compared to nevirapine/lopinavir/ritonavir. AIDS 2009; 23: 1367-1376.

76) Hansen AB, Obel N, Nielsen H, Pedersen C, Gerstoft J. Bone mineral density changes in protease inhibitor-sparing vs. nucleoside reverse transcriptase inhibitor-sparing highly active antiretroviral therapy: data from a randomized trial. HIV Med 2011; 12: 157-165.

77) European Aids Clinical Society. Guidelines: prevention and management of non-infectious comorbidities in HIV. Available at http: //www.europeanaidsclinicalsociety. org/images/stories/EACSPdf/Eacs-Guidelines-v6.12edition.pdf. Accessed May 2013.

78) FRAX WHO Fracture Risk Assessment Tool. University of Sheffield; UK: http://www.shef.ac.uk /FRAX/

79) Lundgren JD, Battegay M, Behrens G, De Wit S, Guaraldi G, Katlama C, Martinez E, Nair D, Powderly WG, Reiss P, Sutinen J, Vigano A; EACS Executive Committee. European AIDS Clinical Society (EACS) guidelines on the prevention and management of metabolic diseases in HIV. HIV Med 2008; 9: 72-81.

80) Watts NB, Adler RA, Bilezikian JP, Drake MT, Eastell R, Orwoll ES, Finkelstein JS; Endocrine Society. Osteoporosis in men: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2012; 97: 1802-1822.

81) McComsey GA, Kendall MA, Tebas P, Swindells S, Hogg E, Alston-Smith B, Suckow C, Gopalakrishnan G, Benson C, Wohl DA. Alendronate with calcium and vitamin D supplementation is safe and effective for the treatment of decreased bone mineral density in HIV. AIDS 2007; 21: 2473-2482.

82) Mondy K, Powderly WG, Claxton SA, Yarasheski KH, Royal M, Stoneman JS, Hoffmann ME, Tebas P. Alendronate, vitamin d, and calcium for the treatment of osteopenia/osteoporosis associated with HIV infection. J Acquir Immune Defic Syndr 2005; 38: 426-431.

83) Rozenberg S, Lanoy E, Bentata M, Viard JP, Valantin MA, Missy P, Darasteanu I, Roux C, Kolta S, Costagliola D; ANRS 120 Fosivir Study Group. Effect of alendronate on HIV-associated osteoporosis: a randomized, double-blind, placebo-controlled, 96-week trial (ANRS 120). AIDS Res Hum Retroviruses 2012; 28: 972-980.

84) Bolland MJ, Grey A, Horne AM, Briggs SE, Thomas MG, Ellis-Pegler RB, Gamble GD, Reid IR. Effects of intravenous zoledronate on bone turnover and bone density persist for at least five years in HIV-infected men. J Clin Endocrinol Metab 2012; 97: 1922-1928.