

# Vitamin D deficiency in HIV infection: an underestimated and undertreated epidemic

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**Abstract.** – Hypovitaminosis D is a very common disorder, regarding both Western and developing countries. A growing amount of data over the last years have shown vitamin D deficiency to be high prevalent among HIV-positive subjects. In addition to "classic" risk factors, such as female sex, low dietary intake, dark skin pigmentation and low sun exposure, HIV-related factors, including immune activation and anti-retroviral adverse effects, may affect vitamin D status. Even if both protease inhibitors and non-nucleoside reverse transcriptase inhibitors have been associated with low vitamin D levels, available evidences have failed to univocally associate hypovitaminosis D with specific antiretroviral class effects.

Low vitamin D is known to have a negative impact not only on bone health, but also on neurocognitive, metabolic, cardiovascular and immune functions. Similarly to the general population, several studies conducted on HIV-infected subjects have associated hypovitaminosis D with a greater risk of developing osteopenia/osteoporosis and fragility fractures. Analogously, vitamin D deficiency has been described as an independent risk factor for cardiovascular disease and metabolic disorders, such as insulin resistance and type 2 diabetes mellitus.

Last EACS guidelines suggest to screen for hypovitaminosis D every HIV-positive subject having a history of bone disease, chronic kidney disease or other known risk factors for vitamin D deficiency. Vitamin D repletion is recommended when 25-hydroxyvitamin D levels are below 10 ng/ml. Furthermore, it may be indicated in presence of 25OHD values between 10 and 30 ng/ml, if associated with osteoporosis, osteomalacia or

increased parathyroid hormone levels. The optimal repletion and maintenance dosing regimens remain to be established, as well as the impact of vitamin D supplementation in preventing comorbidities.

*Key Words:*

HIV, Vitamin D, HAART, Hypovitaminosis D, Bone disease.

## Introduction

The natural history of Human Immunodeficiency Virus (HIV) infection has been profoundly changed by the introduction of highly active antiretroviral therapy (HAART)<sup>1</sup>. Current treatment has dramatically reduced AIDS-related morbidity and mortality, since in most patients it guarantees undetectable levels of plasma HIV RNA and it leads to immune restoration; however, HAART cannot eradicate HIV<sup>2-14</sup>. Furthermore, increased life expectancy and drugs-associated adverse events expose HIV-infected subjects to age-related morbidities, including metabolic and cardiovascular disease (CVD), cancer, neurocognitive disorders, renal and bone disease<sup>15-32</sup>. Many of these conditions appear to occur earlier in patients with HIV if compared with the general population. There is a large body of evidences that identifies chronic inflammation and immune activation as key-factors to explain

premature age-associated non-AIDS-related events in patients receiving HAART<sup>33</sup>; indeed, it has been shown that plasma levels of several inflammatory and coagulopathic biomarkers, such as interleukin-6 (IL-6), D-Dimer and highly sensitive C-reactive protein (hs-CRP), are higher in the setting of HIV and correlate with the outcome of HIV infection<sup>34</sup>.

Bone disease represents one of the most common long-term complications of HIV infection<sup>35-37</sup>. The prevalence of reduced bone mineral density (BMD) and the risk of fragility fractures are higher among HIV-infected people<sup>38-41</sup>. Vitamin D deficiency is a well-established risk factor for bone disease in the general population; considering the high prevalence of hypovitaminosis D among HIV-infected subjects, it is not surprising to find an association between vitamin D deficiency and osteopenia/osteoporosis in several HIV-positive cohorts<sup>42-48</sup>. In addition, recent data suggest that vitamin D is not only involved in calcium homeostasis, but it also has non-skeletal functions, including cardiovascular and immune regulation, cancer prevention and brain health<sup>49-53</sup>.

In this review, we first briefly describe vitamin D metabolism and biological functions; then, we focus on the most recent experimental and epidemiological data dealing with the relationship between vitamin D deficiency and HIV infection, evaluating the extent of the problem, the pathogenic mechanisms, the clinical implications and potential benefits of vitamin D supplementation among HIV-infected subjects.

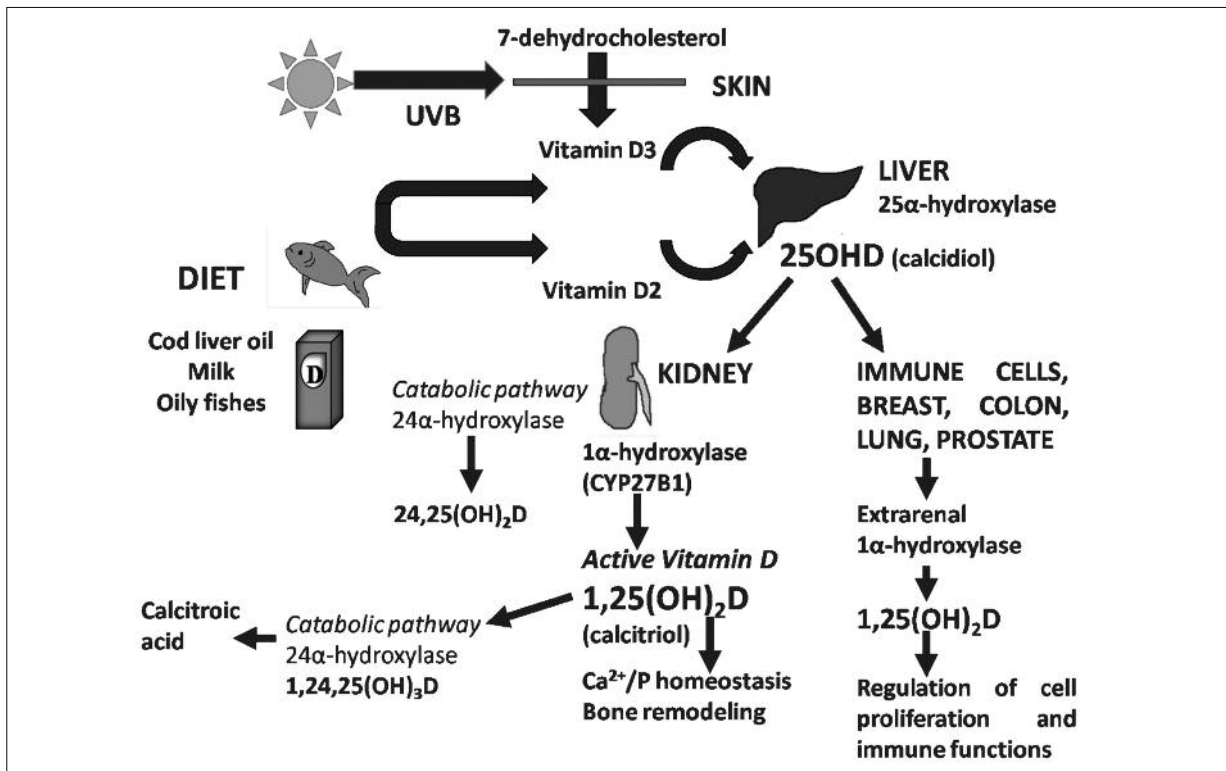
### ***Vitamin D Metabolism and Optimal Circulating Levels: an Overview***

Vitamin D was first identified as a key regulator of calcium homeostasis, since its deficiency was associated with rickets and osteomalacia<sup>54</sup>. Humans can introduce vitamin D with the diet, even if few foods, like oily fishes, cod liver oil and dairy products, naturally contain it<sup>55</sup>; the major production of vitamin D occurs in the skin, where the photochemical action of UVB light is able to transform 7-dehydrocholesterol to previtamin D, which is converted in turn to vitamin D through a non-enzymatic thermal isomerization. Vitamin D is then metabolized in the liver to 25-hydroxyvitamin D (25OHD) by 25 $\alpha$ -hydroxylase; 25OHD is subsequently converted to the biologically active compound 1,25-dihydroxyvitamin D (1,25(OH)<sub>2</sub>D) by 1 $\alpha$ -hydroxylase (or CYP27B1)<sup>55,56</sup>. While 1 $\alpha$ -hydroxylase is pre-

dominantly expressed in the kidney, it is now well known that several extrarenal tissues and cells, like monocyte-macrophages, are also able to convert 25OHD to 1,25(OH)<sub>2</sub>D<sup>50,57</sup>. A catabolic pathway involving 24 $\alpha$ -hydroxylase (CYP24A1) is responsible for 25OHD and 1,25(OH)<sub>2</sub>D hydroxylation to inactive metabolites, named 24,25(OH)<sub>2</sub>D and 1,24,25(OH)<sub>3</sub>D, respectively<sup>58</sup> (Figure 1).

In the kidney, 1 $\alpha$ -hydroxylase activity is strictly controlled by calcium homeostatic signals, especially by parathyroid hormone (PTH), whose release by parathyroid glands is elicited by hypocalcemia. 1,25(OH)<sub>2</sub>D response to low serum calcium levels drives osteoclasts to release calcium from the bone, stimulates intestinal calcium absorption and reduces renal calcium excretion<sup>55</sup>. To exert its functions, vitamin D interacts with vitamin D receptor (VDR), which is expressed by a large number of organs, including brain, muscles, adipose tissue, pancreas, colon, breast and immune cells. Activation of these VDRs is responsible for so-called nonclassic effects of vitamin D<sup>49-51</sup>. Several studies have linked vitamin D status with autoimmune diseases, like type 1 diabetes mellitus<sup>59</sup>, cardiovascular disease<sup>60</sup>, cancer<sup>61,62</sup> and infections<sup>53,63-66</sup> (Figure 2). In this context, 1,25(OH)<sub>2</sub>D has been recognized as an important mediator of both innate and adaptive immune responses. As previously described, antigen presenting cells (APCs), like macrophages and dendritic cells (DCs), are able to locally produce 1,25(OH)<sub>2</sub>D<sup>67</sup>; VDR is expressed not only on APCs, but also on T and B cells<sup>68</sup>. Vitamin D shows strong antimicrobial functions: activation of Toll-like receptor (TLR) pathways by pathogen-associated membrane patterns (PAMPs), shed by microbial agents, like *Mycobacterium tuberculosis*, is able to stimulate 1,25(OH)<sub>2</sub>D synthesis in monocyte-macrophages, leading to the production of antimicrobial peptides, like defensins and cathelicidin<sup>63,64,67</sup>. Furthermore, 1,25(OH)<sub>2</sub>D<sub>3</sub> was shown to trigger autophagy in human macrophages, thus resulting in inhibition of HIV replication<sup>69</sup>.

Vitamin D status is usually assessed by measuring 25OHD circulating levels; optimal 25OHD plasma levels are still matter of debate: currently, 25OHD values > 30 ng/ml (75 nmol/l) are believed to be an adequate cut off for vitamin D sufficiency; 25OHD levels < 20 ng/ml (50 nmol/l) define vitamin D deficiency, whereas 25OHD levels ranging between 20 and 30 ng/ml indicate vitamin D insufficiency<sup>55,70</sup>.

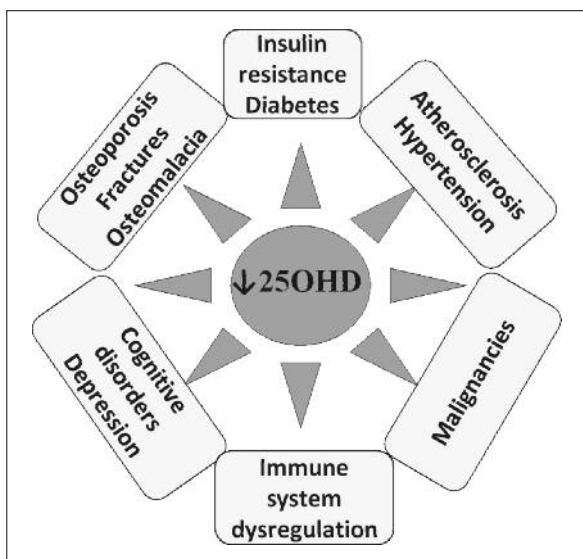


**Figure 1.** Vitamin D metabolism. 25OHD: 25-hydroxyvitamin D; 1,25(OH)<sub>2</sub>D: 1,25-dihydroxyvitamin D; 24,25(OH)<sub>2</sub>D: 24,25-dihydroxyvitamin D; Ca<sup>2+</sup>: calcium; P: phosphate.

**Prevalence of Hypovitaminosis D in HIV-Infected Subjects**

Hypovitaminosis D is a worldwide disorder, with a high prevalence in the general population of both developing and Western countries<sup>71</sup>. Ac-

ording to the results coming from the National Health and Nutrition Examination Survey (NHANES), it is estimated that only 20-25% of American population has a serum 25OHD level of at least 30 ng/ml, whereas 25-30% is thought to be vitamin D deficient<sup>72,73</sup>. From this perspective, it is not surprising to find high rates of hypovitaminosis D even among HIV-infected subjects. A large US prospective cohort study (SUN study) assessed the prevalence of hypovitaminosis D in 672 HIV-positive subjects, demonstrating that 70.3% of them had 25OHD levels below 30 ng/ml, compared with 79.1% of HIV-negative US adults<sup>74</sup>. In a cross-sectional study evaluating vitamin D status in HIV-infected postmenopausal women living in New York, Stein *et al*<sup>44</sup> found that 74% out of 89 HIV-positive women had 25OHD levels < 30 ng/ml; the prevalence rate was similar, however, in HIV-negative controls. They also found no differences in 1,25(OH)<sub>2</sub>D levels, which were normal in both groups. As already described in other reports, 25OHD levels were significantly lower among African-American women, in comparison with Hispanic women. Hypovitaminosis D was recently reported to be highly prevalent also among



**Figure 2.** Association between hypovitaminosis D and some morbidities.

HIV-positive premenopausal women. In a study enrolling 100 HIV-positive and 68 HIV-negative women, Yin et al<sup>45</sup> observed that only 9% in both groups had 25OHD levels > 32 ng/ml; again, no difference by HIV status was found.

Data coming from EuroSIDA study, a prospective, observational work on a large cohort of HIV-positive subjects across 31 European countries, Israel and Argentina, confirmed hypovitaminosis D to be very common among HIV-positive individuals<sup>75</sup>: 23.7% out of 1985 patients had indeed 25OHD below 10 ng/ml, 65.3% between 10 and 30 ng/ml and only 11% above 30 ng/ml. As expected, seasonality affected 25OHD levels, since the number of patients with hypovitaminosis D was higher among winter sampled individuals, in comparison with summer sampled ones. Similarly to the general population, older people were at higher risk of hypovitaminosis D; analogously, black ethnic origin was associated with a four-time odds to have low 25OHD. Furthermore, hypovitaminosis D was independently associated with a higher risk of AIDS events and all-cause mortality. Kaplan-Meier curves of progression to these endpoints over a median 5-year follow up showed that the incidence of AIDS-defining events and death was significantly higher among vitamin D-deficient subjects, thus suggesting the possibility to use vitamin D as a new, independent, prognostic marker in HIV infection. In a French cohort of 2994 HIV-positive patients, Allavena et al<sup>77</sup> have recently observed low 25OHD levels in 86.7% of subjects, including 55.6% with vitamin D insufficiency and 31.1% with vitamin D deficiency. In UK, Welz et al<sup>76</sup> have demonstrated that 91% out of 1077 HIV-positive individuals had 25OHD below 75 nmol/l, with more than one-third showing severe vitamin D deficiency (25OHD < 25 nmol/l). In a recent Italian cross-sectional study, carried out by Vescini et al<sup>78</sup> with a large cohort of HIV-infected subjects (n=810), 47% and 6% of subjects were found to be vitamin D insufficient (30-75 nmol/l) and vitamin D deficient (< 30 nmol/l), respectively. Of interest, the Authors found a correlation between vitamin D insufficiency and the risk of cardiovascular events, diabetes mellitus and renal disease over a median 6.5-year follow up (relative hazard (RH) = 1.60,  $p = 0.05$ ); furthermore, 25OHD levels below 30 nmol/l seemed to predict faster HIV progression (RH = 2.11,  $p = 0.08$ ).

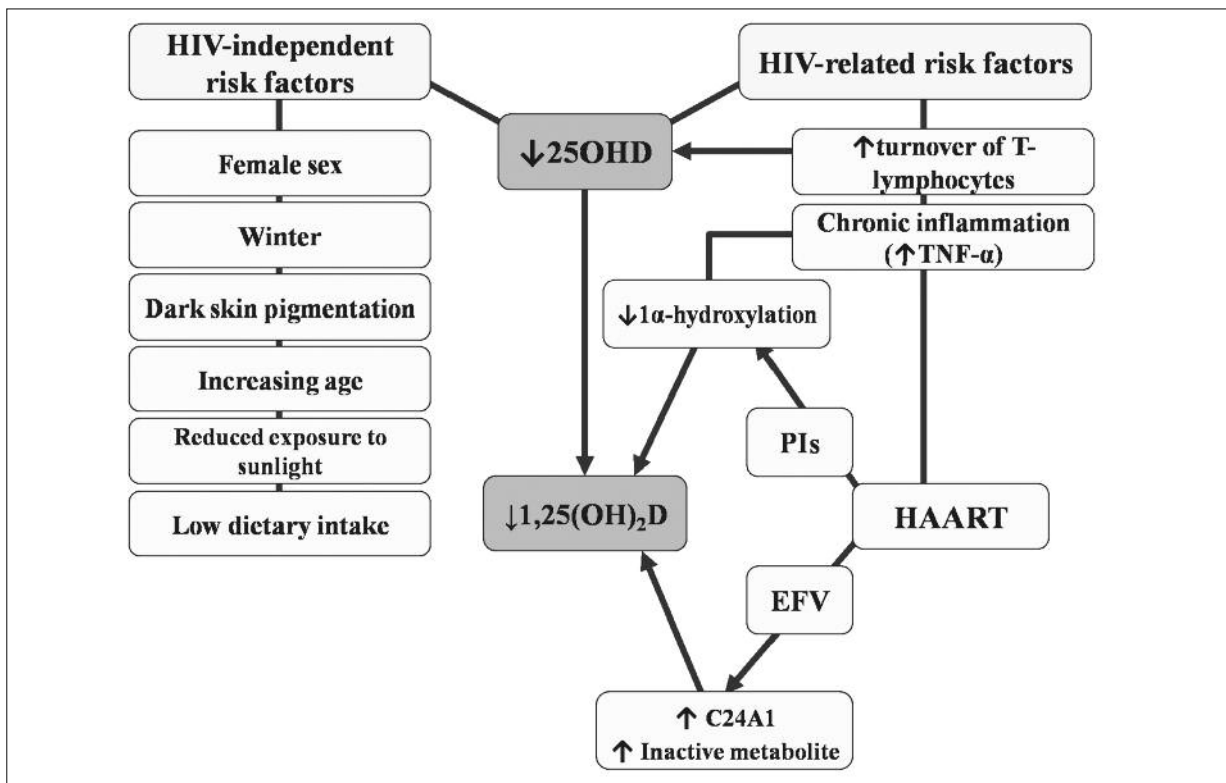
These data are in keeping with a number of previous investigations<sup>46,47,79,80</sup> conducted on small HIV cohorts over the past few years, which had al-

ready described high prevalence rates of hypovitaminosis D in HIV-positive cohorts. In a group of 57 ambulatory HIV-positive patients, 25OHD levels below 32 ng/ml were reported in 74.4% of individuals by Rodriguez et al<sup>81</sup>. In Denmark, in a report that included 115 HIV-infected males, Bang et al<sup>82</sup> observed that only 13% of subjects had 25OHD levels > 75 nmol/l. In 2010, another report revealed that 64% out of 200 HIV-subjects living in the South-Central United States had 25OHD < 20 ng/ml and 20.5% had 25OHD < 10 ng/ml. In multivariate analysis a significant correlation between low 25OHD levels and African-American race was found<sup>83</sup>. A similar prevalence rate for 25OHD deficiency was described by Mueller et al<sup>84</sup> in the setting of the Swiss HIV cohort study. In a cohort of 211 HIV-positive individuals, 42% of patients in spring and 14% in fall had 25OHD levels below 30 nmol/l. It is important to note that 1-hydroxylation rate was significantly higher in patients with lower 25OHD, thus, suggesting a compensatory PTH-mediated response. Moreover, considering that normal 1,25(OH)<sub>2</sub>D levels may hide vitamin D deficiency and/or hyperparathyroidism, 1,25(OH)<sub>2</sub>D measurement does not represent a reliable option to evaluate vitamin D status. In addition, the Authors measured 1,25(OH)<sub>2</sub>D levels in a subgroup of 74 individuals and observed a significant association between a history of AIDS-defining events and lower 1,25(OH)<sub>2</sub>D, but not lower 25OHD values. A number of investigations had previously reported 1,25(OH)<sub>2</sub>D to be lower in patients with advanced HIV infection<sup>48,85</sup>. It has been suggested that immunological hyperactivity and in particular tumor necrosis factor (TNF)- $\alpha$  overproduction may be responsible for the impairment of renal 1 $\alpha$ -hydroxylase, with the subsequent decrease in 1,25(OH)<sub>2</sub>D levels<sup>85</sup>.

### ***Risk Factors for Vitamin D Deficiency in HIV Infection***

The evaluation of risk factors for hypovitaminosis D in the setting of HIV infection includes the assessment of HIV-specific and HIV-independent risk factors (Figure 3); it is often challenging, however, to differentiate the direct impact of HIV infection from the effect of traditional risk factors which may be over-expressed in HIV-positive cohorts.

Some risk factors, such as female sex<sup>79</sup>, winter season<sup>75,76,78</sup>, increasing age<sup>45,75,78</sup>, low vitamin D dietary intake<sup>80,81,83</sup> and dark skin pigmentation, are similar to those reported in HIV-negative cohorts<sup>71</sup>.



**Figure 3.** Risk factors for hypovitaminosis D in HIV infection. HIV: human immunodeficiency virus; 25OHD: 25-hydroxyvitamin D; 1,25(OH)<sub>2</sub>D: 1,25-dihydroxyvitamin D; HAART: highly active antiretroviral therapy; Pis: protease inhibitors; EFV: efavirenz; TNF- $\alpha$ : tumor necrosis factor- $\alpha$ .

Most reports have described a positive correlation between African American ethnicity and low 25OHD<sup>44,45,74-76,83,84</sup>. This observation is not unexpected, considering that in black people vitamin D cutaneous synthesis is markedly reduced<sup>86</sup> and a greater exposure to UVB is necessary to produce the same amount of 25OHD as white individuals<sup>87</sup>. Conflicting data are available about the impact of body mass index (BMI) on 25OHD levels. As in the general population, some Authors<sup>74,83,84</sup> have found a negative correlation between 25OHD serum levels and BMI, possibly because of vitamin D storage in adipose tissue<sup>88</sup>. By contrast, others have associated hypovitaminosis D with low BMI<sup>89</sup> or have related vitamin D insufficiency with a higher risk of wasting, defined as BMI<sup>90</sup> less than 18 kg/m<sup>2</sup>.

The relationship between 25OHD levels and CD4+ T-cell count is not clear cut. Some studies<sup>44,48,85</sup> have described a positive correlation, some others<sup>74,79,82,83,90-92</sup> have failed to demonstrate a significant association.

### Vitamin D Status and HAART

Several *in vitro* and *in vivo* researches have evaluated the impact of antiretroviral drugs on vi-

tamin D metabolism. Both protease inhibitors (Pis) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) have been associated with the impairment of vitamin D metabolic pathways.

Pis have been shown to inhibit vitamin D 1 $\alpha$ - and 25 $\alpha$ -hydroxylation in hepatocyte and monocyte cultures<sup>93</sup>: reduced conversion of 25OHD to its active metabolites may potentially explain the increased 25OHD levels found by some Authors in subjects with low 1,25(OH)<sub>2</sub>D<sup>74,79</sup>. As refers to NNRTIs, there is an increasing amount of data associating efavirenz (EFV) with compromised vitamin D homeostasis. EFV has been described indeed to increase 25OHD catabolism, through the induction of CYP24<sup>94,95</sup> and reduced transcription of CYP2R1, a 25-hydroxylase<sup>96</sup>. A number of *in vivo* studies over the past years have supported this observation, describing an association between EFV assumption and low 25OHD levels. Welz et al<sup>76</sup> found current EFV, but not nevirapine (NVP) use, to be associated with severe vitamin D deficiency (odds ratio (OR) 2 (C.I. 1.5-2.7),  $p < 0.001$ ). In a recent report<sup>97</sup>, NNRTIs current use correlated with lower 25OHD levels: Pasquet et al found indeed an as-

sociation between hypovitaminosis D and exposure to NNRTIs ( $p = 0.05$ ), but not to EFV and NVP, probably because of a lack of statistical power of their analysis. However, considering the crude and adjusted coefficients for EFV and NVP in their regression models, the Authors suggested a NNRTI class effect, rather than a specific EFV impact, on vitamin D levels. Other studies<sup>74,76,98-100</sup> have reported opposing results, describing EFV, but not NVP influence on vitamin D homeostasis. Fux et al<sup>99</sup> evaluated 25OHD levels in a cohort of 209 HIV-positive patients, before and one year after starting HAART. A significant reduction in 25OHD median values was observed among EFV-treated subjects ( $p < 0.001$ ), but not among patients receiving a NVP-based or PI-based treatment. Consistent with these findings, the Authors also reported the results of a cross-sectional study performed on 262 individuals, showing 25OHD levels to be higher in patients exposed to NVP ( $p = 0.04$ ) and PIs ( $p = 0.048$ ), in comparison with those exposed to EFV. Nevertheless, after adjusting for confounding factors, 25OHD levels remained significantly lower in the EFV group, in comparison with the PI group ( $p = 0.006$ ), but not the NVP group ( $p = 0.2$ ). Of interest, a recent article of Boven et al<sup>101</sup> showed that vitamin D levels did not significantly change in comparison with baseline values among patients receiving the new NNRTI rilpivirine over 48 weeks, whereas a significant decrease was observed among those starting a EFV-based regimen. Moreover, of the patients with baseline 25OHD insufficiency/deficiency, a smaller proportion developed severe 25OHD deficiency with rilpivirine than EFV. Analogously, Allavena et al<sup>77</sup> found vitamin D deficiency to be associated with the administration of EFV [adjusted OR 1.89 (1.45-2.47)]; Brown et al<sup>95</sup> showed a significant decline in 25OHD serum levels after the initiation of a EFV-based regimen, in comparison with a non-EFV-based regimen ( $p < 0.001$ ). In addition, subjects receiving EFV had a 1.8 times increased Odds to develop vitamin D deficiency, if compared with those starting PIs. Similar results have been shown by other groups<sup>84,89</sup>: Conesa-Botella et al<sup>89</sup>, for instance, described a 3-fold increased risk to have 25OHD levels below 20 ng/ml among subjects receiving NNRTIs ( $p = 0.02$ ) after 12 months of HAART. In the MONET study<sup>102</sup>, in which virologically controlled patients were switched to a darunavir-based regimen, lower baseline vitamin D levels were associated with efavirenz ( $p =$

0.0062) and zidovudine ( $p = 0.015$ ) use and a 27% increase in 25OHD values was observed in subjects discontinuing EFV ( $p = 0.007$ ). Van Den Bout-Van Den Beukel et al<sup>79</sup> reported higher 25OHD levels in white subjects receiving PIs in comparison with those receiving NNRTIs ( $p = 0.007$ ) or treatment-naïve ( $p = 0.049$ ). Similarly, in the EuroSIDA cohort<sup>75</sup>, patients receiving a PI-based antiretroviral regimen were at low risk of hypovitaminosis D, whereas no significant association with EFV or TDF use was found.

One drawback of most studies is their cross-sectional design, so that causal relationships cannot be inferred. Taken together, these data suggest the need for large prospective studies, properly designed to evaluate the specific effects and clinical impact of antiretroviral drugs on vitamin D status.

#### ***HIV Infection, Bone Disease and Vitamin D***

Bone disease is one of the most common long-term complications in HIV-infected individuals<sup>35-37</sup>. Many studies have shown low BMD values, including osteopenia and osteoporosis, among subjects with HIV, with an increased risk of fragility fractures<sup>38-41,103</sup>.

The pathophysiological mechanisms of bone disease in HIV infection are complex and partially unknown. In addition to the classical risk factors, shared with uninfected subjects (low BMI, corticosteroid use, increasing age, hypogonadism, including post-menopausal status in women, prolonged immobility and smoking), other factors are probably involved, such as the direct effects of HIV on osteoblast and osteoclast functions, chronic inflammation and antiretroviral interference with bone turnover<sup>35</sup>. In particular, TDF assumption has been associated with low BMD, because of its capability to induce proximal renal tubular dysfunction, which may cause in turn renal phosphate wasting and finally BMD loss<sup>104</sup>. Patients starting a TDF-based regimen have a greater BMD loss in comparison with those treated with other antiretrovirals<sup>105,106</sup>. In addition, higher PTH levels have been described in subjects receiving TDF with suboptimal 25OHD levels<sup>107-109</sup>. Childs et al<sup>109</sup> reported an independent association between PTH levels and TDF assumption ( $p = 0.017$ ), with the highest PTH levels among TDF-treated subjects with 25OHD below 30 ng/ml ( $p = 0.045$ ). A similar association between PTH concentration, TDF use and 25OHD levels has been described by Pocaterra et al<sup>110</sup>. The role of PIs on bone home-

ostasis appears controversial, since some studies have described an association between BMD loss and PI-based therapy<sup>38,41,111-114</sup>, but others have not<sup>37,47</sup>. When comparing HAART-naïve and HAART-experienced subjects, most cross-sectional studies reported similar BMD values<sup>36,37,46,111,113</sup>. On the contrary, a meta-analysis of pooled data coming from ten cross-sectional studies conducted over a 5-year period, between 2000 and 2005, none showing significant differences individually, reported a significant BMD reduction in HAART-treated patients (OR 2.5 (95% CI 1.8-3.7))<sup>39</sup>. Longitudinal studies evaluating the changes in BMD in subjects starting HAART have shown that the initiation of antiretroviral therapy is associated with a 2%-6% decrease in BMD within the first year<sup>106,115</sup>. The negative impact of HAART on bone health has been confirmed in the SMART study, where subjects receiving continuous HAART were described to have a greater BMD loss in comparison with those on intermittent HAART<sup>116</sup>.

Hypovitaminosis D is considered a traditional risk factor for bone disease in the general population<sup>35</sup>; similarly, in the setting of vitamin D deficient HIV cohorts, low 25OHD values have been associated with BMD loss. In premenopausal HIV-positive women, Yin et al<sup>45</sup> found that vitamin D levels below 20 ng/ml, but not antiretroviral therapy, were predictive of FN (femoral neck) bone loss; Stein et al<sup>44</sup> described a weak association between serum 25OHD and LS (lower spine) BMD in Hispanic ( $r = 0.33$ ,  $p = 0.01$ ), but not African-American postmenopausal HIV-infected women.

Some prospective randomized trials<sup>117-119</sup> have evaluated the effects of alendronate on BMD in HIV-positive subjects with osteopenia and osteoporosis, showing that the combination of weekly alendronate with daily calcium and vitamin D supplementation was effective and safe in increasing LS BMD at one year; of interest, even though in the placebo group receiving vitamin D and calcium supplements alone BMD variations were not statistically significant, a trend towards BMD increase was seen. These data highlight the need for further prospective studies, specifically designed to evaluate the effect of vitamin D replacement on BMD in vitamin D deficient HIV-infected subjects; in addition, considering the low vitamin D dose administered in the trials mentioned above, further investigations looking at the effects of higher dose on BMD are warranted.

### **Association Between HIV, Hypovitaminosis D and Cardiovascular Disease (CVD)**

Several studies have described the association between HIV and increased risk of CVD<sup>120-123</sup>. HIV infection itself is considered an independent risk factor for atherosclerosis: in fact, the prevalence of atherosclerosis is higher among HIV-positive subjects and it occurs earlier as compared with uninfected individuals<sup>124,125</sup>. In addition, antiretroviral drugs-related dyslipidemia, chronic inflammation and immune activation may also affect atherogenesis in the setting of HIV<sup>33,126</sup>.

In the general population, vitamin D deficiency has been linked to CVD<sup>60</sup>. Considering the high prevalence of both hypovitaminosis D and CVD in patients with HIV, the observation of a relationship between low 25OHD and atherosclerosis in HIV-positive subjects is not surprising. In a cross-sectional study of 139 patients, Choi et al<sup>127</sup> have shown an independent association between vitamin D insufficiency and cIMT (carotid intima-media thickness), a common marker for atherosclerotic vascular disease. Furthermore, they found that mean cIMT was 0.13 mm greater in vitamin D insufficient subjects, as compared to individuals with normal 25OHD levels. Similarly, Ross et al<sup>128</sup> observed a 10.62 higher odds to have cIMT above the median value among subjects with 25OHD values below 30 ng/ml ( $p = 0.01$ ). A recent report by Shikuma et al<sup>129</sup> has described a significant correlation between 25OHD and brachial artery flow-mediated dilation (FMD), a early marker of endothelial dysfunction ( $r = 0.3$ ;  $p = 0.01$ ), but not with cIMT, possibly because of the small sample size. Lai et al<sup>130</sup> found that 25OHD deficiency was independently associated with a greater than two-fold increase in the risk of significant coronary stenosis in a cohort of cardiovascularly asymptomatic HIV-positive African Americans.

Other traditional risk factors for CVD, such as insulin resistance and diabetes mellitus, are frequently seen in HIV-positive individuals<sup>131,132</sup> and, as in the general population<sup>133</sup>, an association between vitamin D status and type 2 diabetes has been described<sup>134</sup>. In a recent cross-sectional study, Szep et al<sup>134</sup> have reported lower 25OHD levels among subjects with type 2 diabetes, in comparison with those without diabetes ( $p < 0.001$ ), although vitamin D deficiency was highly prevalent in both groups. Furthermore, vitamin D deficiency was independently associated with dia-

betes (OR 1.85 (CI 1.03-3.32),  $p = 0.038$ ), but not with metabolic syndrome. One explanation may be vitamin D involvement in the regulation of insulin secretion and insulin-mediated glucose transport<sup>135-137</sup>. In addition, in diabetic HIV-negative subjects vitamin D supplementation was able to reduce insulin-resistance<sup>138</sup>. In the setting of HIV, the effects of vitamin D supplementation on insulin sensitivity need to be evaluated with large, prospective studies since nowadays only few data are available. A small prospective study, conducted by Van den Bout et al<sup>139</sup>, has surprisingly shown that cholecalciferol supplementation (2000 IU/day for 14 weeks, 1000 IU/day until 48 weeks) led to increased insulin resistance, evaluated with HOMA index and fasting glucose levels, after 24 weeks, whereas no differences were seen after 48 weeks. An explanation may be the inhibition of the expression of PPAR- $\gamma$  receptor by 1,25(OH)<sub>2</sub>D<sup>140-142</sup>, considering that the activation of PPAR- $\gamma$  pathway leads to increased insulin sensitivity<sup>143</sup>. It remains to be clarified if the effect of cholecalciferol on insulin sensitivity seen by Van den Bout et al<sup>139</sup> is dose- or time-dependent, but this report further suggests the importance of clinical trials extensively evaluating the pros and cons of supplementing HIV-infected individuals with vitamin D.

Chronic inflammation may contribute to the increased cardiovascular risk of HIV-positive subjects. In fact, hs-CRP and IL-6 have been shown to predict both cardiovascular and HIV disease progression and mortality<sup>34</sup>. Recent studies have described an association between hypovitaminosis D and increased inflammation markers in the setting of HIV infection. Anseman et al<sup>144</sup> found severe vitamin D deficiency to correlate with increased hs-CRP ( $p = 0.04$ ) and IL-6 ( $p = 0.001$ ) levels in a cohort of 263 HIV-infected patients; analogously, Poudel-Tandukar et al<sup>145</sup> reported that the risk of having high inflammation (CRP > 3 mg/l) was greater among HIV-positive people with a 25(OH)D serum level of < 20 ng/ml. However, given the cross-sectional design and the small size of these researches, there is a need for large, prospective studies, evaluating the causal relationship between inflammation and hypovitaminosis D and the safety and efficacy of vitamin D supplementation.

#### **Management of Hypovitaminosis D in HIV-Positive Individuals**

Last European AIDS Clinical Society (EACS) guidelines suggest to screen for vitamin D status

every HIV-positive subject having a history of low bone mineral density and/or fracture, high risk for fracture or chronic kidney disease. It is also suggested to test all HIV-infected individuals having other risk factors for hypovitaminosis D. If 25OHD levels are below 10 ng/ml, replacement is recommended, whereas in vitamin D insufficient individuals vitamin D supplementation is suggested in presence of osteoporosis, osteomalacia or increased PTH<sup>146</sup>.

In the general population, current recommended dietary allowances (RDA) of vitamin D are 700-800 IU cholecalciferol/day<sup>55</sup>. Serum 25OHD levels generally increase by approximately 1 ng/ml for every 100 IU of vitamin D intake<sup>147</sup>. Few data coming from small cohorts are available about the efficacy of vitamin D repletion in HIV-infected subjects. Some Authors have described vitamin D supplementation as a safe and well tolerated option to successfully increase 25OHD levels: Van den Bout et al<sup>139</sup>, for instance, treated 20 vitamin D deficient subjects with 2000 IU cholecalciferol/day for 14 weeks, after which the dosage was lowered to 1000 IU/day until 48 weeks. The evaluation of 25OHD<sub>3</sub> and 1,25(OH)<sub>2</sub>D<sub>3</sub> levels at 24 weeks showed normalized values, but after 48 weeks only serum 25OHD<sub>3</sub> was significantly different from baseline, whereas 1,25(OH)<sub>2</sub>D<sub>3</sub> returned to baseline levels, probably because of the reduction in cholecalciferol dose. Arpadi et al<sup>91</sup> reported the benefits of orally administered bimonthly doses of 100,000 IU cholecalciferol and 1g/day calcium in a cohort of HIV-infected children and adolescents during a 12-month period: 25OHD levels were found to be significantly higher among supplemented subjects, in comparison with individuals receiving placebo; 44.4% of subjects in the group receiving cholecalciferol and calcium had 25OHD > 30 ng/ml after 1 year vs 11.1% observed in the placebo group. Havens et al<sup>148</sup> demonstrated a rapid increase in 25OHD serum concentration in a cohort of 207 adolescents and young adults with HIV when orally administering vitamin D<sub>3</sub> (50000 IU in three doses at monthly intervals). By contrast, other studies reported less encouraging results. In a small study, only 40% of patients receiving oral vitamin D supplements had 25OHD levels > 30 ng/ml after a median 16 week follow up<sup>149</sup>; similarly, just a 46% reduction in hypovitaminosis D was found in a cohort of HIV-positive young adults receiving 50000 IU vitamin D<sub>3</sub>/week for 12 weeks<sup>150</sup>. Lastly, a recent randomized clinical



trial reported a significant increase in 25OHD after 12 weeks of daily supplementation with 4000 IU vitamin D<sub>3</sub> only in subjects receiving a non-EFV-based regimen ( $p = 0.011$ )<sup>151,152</sup>.

## Conclusions

Vitamin D deficiency is emerging as a matter of great concern among HIV-infected subjects. Considering that hypovitaminosis D represents a risk factor for skeletal and extra-skeletal health, clinicians should consider screening all HIV patients at risk for vitamin D deficiency. Prospective studies are necessary to determine the optimal dosage of vitamin D supplementation and to assess the impact of vitamin D supplementation on prevention of comorbidities among HIV-infected people. Furthermore, the exact association between exposure to antiretroviral drugs and hypovitaminosis D needs to be clarified.

## Conflict of Interest

None declared.

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