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Vitamin D Level Between Calcium-Phosphorus Homeostasis and Immune System: New Perspective in Osteoporosis

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Abstract Vitamin D is a key molecule in calcium and phosphate homeostasis; however, increasing evidence has recently shown that it also plays a crucial role in the immune system, both innate and adaptive. A deregulation of vitamin D levels, due also to mutations and polymorphisms in the genes of the vitamin D pathway, determines severe alterations in the homeostasis of the organism, resulting in a higher risk of onset of some diseases, including osteoporosis. This review gives an overview of the influence of vitamin D levels on the pathogenesis of osteoporosis, between bone homeostasis and immune system.

Keywords Vitamin D · Osteoporosis · Calcium-phosphorus homeostasis · Immune system · Polymorphisms

Introduction

Osteoporosis is an asymptomatic, chronic, and progressive bone disease characterized by a reduction of bone mass and mineral content, which may lead to an increased risk of bone fractures and consequently cause infirmity with serious complications [1, 2]. It is classified as primary osteoporosis—postmenopausal osteoporosis and age-related or senile osteoporosis—and secondary osteoporosis. The latest is related to various causes such as unbalance in nutritional factors (alcohol, caffeine, fat intake, etc.), lifestyle (smoking, inactivity, low sunlight exposure, obesity, etc.), and numerous medical conditions affecting bone directly, or via other apparatus and tissues thus leading to progressive bone resorption [3].

Osteoporosis is clinically defined as a bone mineral density (BMD) that is 2.5 standard deviation (SD) or more below the mean of healthy individuals (*T* score <-2.5 SD) [1]. Dual energy X-ray absorptiometry (DXA) is the gold standard to measure BMD for osteoporosis diagnostic, in the absence of any other disease [4–6], as highlighted in most international guidelines on osteoporosis [7–12], even though new techniques with different approaches are recently emerging, like bone quantitative ultrasound (QUS) [7–12]. However, the use of BMD alone to make a diagnosis of osteoporosis is not optimal for the detection of individuals at high risk of fracture, due to its high specificity and low sensitivity [1].

Various international population-based cohort studies tried to identify, prospectively, the risk factors (BMD, clinical risk factors: age, sex, weight, fracture familiar history, glucocorticoid intake, lifestyle, etc.) for osteoporotic fractures in order to develop algorithms, such as FRAX®, useful for diagnosis [13]. Other risk factors of potential utility, but less extensively validated, included biochemical markers of bone turnover, which identify the status of bone remodeling [14, 15]. Most international guidelines identified bone alkaline phosphatase (bALP), pro-peptide of pro-collagen I (PINP), and osteocalcin as markers of bone formation, while pyridinoline, deoxypyridinoline, and N- and C-terminal telopeptides of collagen I as markers of bone resorption.

Recently, hypovitaminosis D, even when related to mutations in vitamin D synthetic pathway genes or in its receptor, emerged as a risk factor for osteoporosis and related fractures [7, 8, 16]. Vitamin D, a molecule of fundamental importance in the

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homeostasis of bone metabolism [17, 18], has been proposed as therapy associated with calcium and combined with other bone protective therapies, in elderly people due to their typical insufficiency [19, 20]. Despite recent progresses in understanding the correlation of vitamin D and bone health, some limitations of the existing data—such as control of confounders (baseline vitamin D concentration, skin pigmentation, lifestyle, etc.), vulnerable populations (dark-skinned persons, children, etc.), independent effect from calcium, and lack of established link with functional outcomes—do not allow to draw specific conclusions on the protective role of vitamin D on bone [21]. Many studies have also suggested some degree of correlation between vitamin D levels and different diseases presenting an inflammatory chronic state and an altered bone health homeostasis [22]. Vitamin D was found to have a modulator role in the inflammatory response of cells of the monocyte/macrophage lineage, which in turn were modulated by TGF-β [23]. Interestingly, various literature data indicate that vitamin D and a normally functioning vitamin D receptor (VDR) are important for the proper development of the immune system [24, 25], highlighting the link with inflammation.

Despite the relevance of the topic and the evidence of the association between vitamin D and osteoporosis, only in some countries, the current recommendations suggest the administration of vitamin D and calcium in the population over 50–65 years old, and the measurement of vitamin D level is only considered in cases of overt osteoporosis [7, 8, 10, 16].

In the present review, the effects of altered vitamin D levels on the pathogenesis of osteoporosis have been critically investigated. In particular, our attention was focused on the response of the two major targets affected by low levels of vitamin D, Ca-P homeostasis and the immune system, and the possible involvement of these in osteoporosis development and worsening. The relationship between secondary osteoporosis and its various pre-existing causes (chronic inflammation diseases) has also been considered by taking into account the effects of altered vitamin D levels (Appendix).

Vitamin D

Metabolism

The metabolism of vitamin D has been widely studied in the last 20 years, but only recently, the molecular mechanisms of its synthesis, activation, and degradation have been understood. Vitamin D is a secosteroid, where the typical steroid molecule B ring is replaced by a 5,7-diene conjugated with another double bond to form a *cis*-trienic system, which is important in the interaction with hydroxylases, transporting proteins and vitamin D receptors. There are two forms of vitamin D: D_2—ergocalciferol—produced only in plants by the ultraviolet irradiation of ergosterol and which therefore can be taken exclusively by diet; and D_3—cholecalciferol—of animal origin, that can be taken by diet or synthesized in the Malpighian layer in the skin, by the ultraviolet irradiation of 7-dehydrocholesterol. Both vitamin D forms can be absorbed at the duodenal level, but the major source remains the skin, contributing to more than 90 % of vitamin D serum concentration. Exogenous and endogenous vitamin D are then vehiculated by a specific alpha globulin, vitamin D binding protein (DBP), and may be metabolized or stored in the adipose tissue. Ergocalciferol (D_2) and cholecalciferol (D_3) are biologically inactive and require a series of molecular modifications in order to perform their specific physiological actions (Fig. 1) [26–28]. Both forms are hydroxylated in position 25 becoming calcidiol—25(OH)D, which represents the main metabolite present in plasma (90 % 25(OH)D_3 and 10 % 25(OH)D_2). This hydroxylation is made in the liver by microsomal (vitamin D 25-hydroxylase—*CYP2R1*) and mitochondrial (sterol 26-hydroxylase—*CYP27A1*) cytochrome P450 oxidases.

It has recently been found that several other P450 cytochromes (*CYP2C11*, *CYP3A4*, *CYP2D25*, and *CYP2J3*) exhibit vitamin D 25-hydroxylase activities [29, 30]. 25(OH)D is transported into the plasma by DBP, protecting the metabolite from its inactivation; its half-life, when bonded to DBP, is about 15 days [31]. 25(OH)D levels, the main marker of vitamin D status, are extremely variable (ranging between 25 and 200 nmol/L), depending both on diet and light exposition, with significant differences during the course of the year. 25(OH)D levels below 20 ng/mL (50 nmol/L) are defined as "deficiency"; levels between 20 and 29.9 ng/mL (50–75 nmol/L) are defined "insufficiency," while levels above 30 ng/mL (75 nmol/L) are defined "sufficiency." Serum 25(OH)D levels above 150 ng/mL (about 375 nmol/L) may cause acute vitamin D intoxication with hypercalcemia, hypercalciuria, and extra osseous calcifications [32, 33].

In the kidney, 25(OH)D undergoes a further hydroxylation in position 1 by the product of the *CYP27B1* gene (25-hydroxy vitamin D-1α hydroxylase), producing calcitriol—1,25$(OH)_2$D. This metabolite is more active than its direct precursor, and it is not affected by seasonal variations or sun exposure (between 100 and 200 pmol/L, three orders of magnitude smaller than 25(OH)D). 1,25$(OH)_2$D levels are mainly regulated by parathyroid hormone (PTH), which acts on the kidney inducing posttranslational modifications leading to increased *CYP27B1* activity and synthesis in the short and in the long term, respectively [34]. It has recently been highlighted that the final 1α−hydroxylation to calcitriol does not only occur in the kidneys but also in a variety of extrarenal sites, depending on serum 25(OH)D levels. In addition, 1,25$(OH)_2$D is able to regulate its own synthesis through a negative feedback mechanism, by the inhibition of *CYP27B1* [35, 36]. Calcitriol mediates its effect by binding to the VDR, a nuclear receptor that alters the transcription rate of many genes involved in a wide spectrum of biological responses, such as cell cycle regulation, differentiation, and

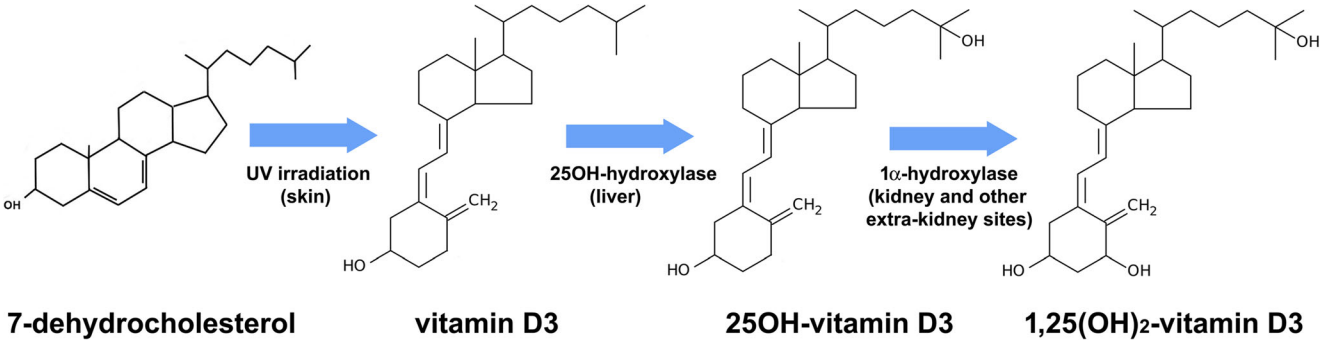

Fig. 1 Biosynthesis of 1α,25(OH)2 vitamin D

apoptosis [37]. The complex VDR-calcitriol dimerizes with the retinoid X receptor (RXR), and the VDR-RXR heterodimer translocates to the nucleus, where it binds vitamin D responsive elements (VDRE) in the promoter regions of vitamin D responsive genes and thus inducing their expression. Aside from this genetic activation pathway, $1,25(OH)_2D$ interacts with a membrane receptor that mediates rapid responses, but whose identity, downstream the signaling pathways and downstream the outcomes, is not well understood. The chaperone protein disulfide isomerase family A member 3 (Pdia3) has been proposed as a candidate membrane receptor for $1,25(OH)_2D_3$ [38].

$1,25(OH)_2D$ is catabolized by $1,25(OH)_2D$ 24-hydroxylase—*CYP24A1*—in position 24 causing its inactivation and further degradation [39–41]. *CYP24A1* expression is upregulated in response to high levels of $1,25(OH)_2D$ and PTH in the kidney, but only to high levels of $1,25(OH)_2D$ in the intestine, suggesting that the intestinal hydroxylase *CYP24A1* is synthesized only in response to VDR transcription factor and not to PTH [36, 42–44]. These pathways of vitamin D activation and inactivation are common to all cell types that are influenced by this hormone.

Biological Role in Calcium and Phosphate Homeostasis

Vitamin D plays a key role in serum homeostasis of Ca and PO_4 ions (Fig. 2), affecting the synthesis of numerous genes that are directly upregulated (e.g., *CYP24A1*, *CaBP-D9k*, *CaBP-D28k*, *BGLAP*, and *TNFSF11*) or downregulated (e.g., *PTH* and *CYP27B1*) by the activation of VDR [45–53]. In Ca homeostasis, $1,25(OH)_2D$ works together with PTH to increase plasma levels of Ca and PO_4. The physiological loop starts when low Ca level is recognized by Ca receptor in the parathyroid gland that in turn secretes PTH. This stimulates the renal enzyme 25OH D-1α hydroxylase, which produces more $1,25(OH)_2D$ from the large circulating pool of 25(OH)D. The resulting increase of cholecalciferol determines an augmentation in the Ca transport to the intestine, the management of bone, and the resorption in the kidney. All these events determine a return to normal plasma Ca levels, stimulating Ca receptors on parathyroid gland cells. The secretion of PTH is turned off by the feedback action of Ca, although the same $1,25(OH)_2D$ level can exert a faster inhibitory effect by activating VDRs that directly suppress PTH synthesis in the parathyroid gland (Fig. 2) [54–56].

The intestinal absorbance of Ca and phosphates is also increased by calcitriol, paramount in the mineralization process of bone, allowing the deposition of crystals onto the collagen fibers of the osteoid matrix; furthermore, $1,25(OH)_2D$ receptors are also present on osteoblasts, where they stimulate their activity [57].

Differently, the primary regulating hormone of PO_4 is fibroblast growth factor 23 (FGF-23), which increases in response to high plasma concentration of PO_4 and $1,25(OH)_2D$. Normally, FGF-23 is produced by the osteocytes and acts on the kidney to increase renal PO_4 excretion via downregulation of the renal type-II transporters, leading to plasma PO_4 decrease. FGF-23 also reduces the plasma level of $1,25(OH)_2D$. This partially counteracts the action of PTH and $1,25(OH)_2D$, which induce

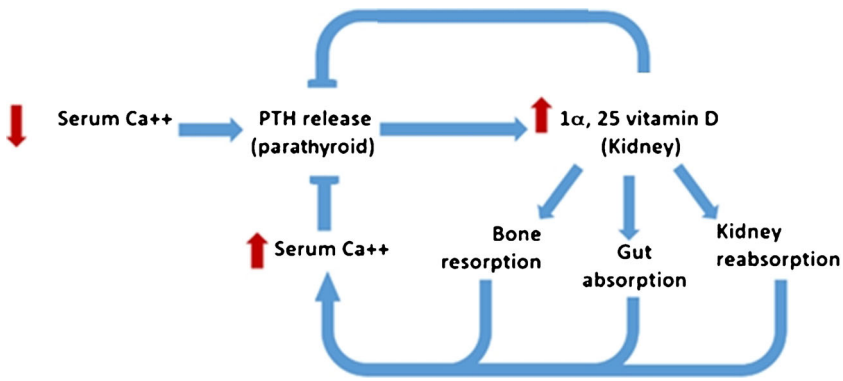

Fig. 2 Representation of the calcium homeostasis system

the release of PO_4 (and Ca) from the skeleton and the upregulation of PO_4 absorption in the intestine [57, 58].

The organism is capable of regulating intestinal Ca intake based on its physiological needs, modulating the synthesis of $1,25(OH)_2D$ through PTH. Unlike its precursor, the half-life of calcitriol is very short, about 10–24 h, and this is justified by the rapid responses of the organism to variations of molecules regulated by it. Other molecules are involved in the regulation of enzyme activity such as Ca, PO_4, FGF-23, and $1,25(OH)_2D$ itself, and this complicates the comprehension of the regulation system [26, 28, 59–61].

Influence on the Immune System

Vitamin D acts as an important regulator of the immune system (Fig. 3); in fact, several studies demonstrated that VDR is expressed on immune cells (B cells, T cells, and antigen-presenting cells) and these cells are also able of synthesizing $1,25(OH)_2D$, which can act in an autocrine and paracrine manner in the local immunologic milieu [62–64]. However, the effects of vitamin D on the immune system have been described only by in vitro or in vivo correlative studies.

Some studies showed that 25(OH)D and its analogs can alter the function of dendritic cells (DC), inducing a more tolerogenic and immature state, characterized by decreased levels of MHC class II and co-stimulatory molecule expression. This results in a reduced antigen presentation, lower IL-12 secretion, and increased synthesis of the anti-inflammatory cytokine, IL-10 [65–67]. It was also reported that 25(OH)D inhibits B cell proliferation, differentiation, and immunoglobulin secretion [68, 69]. For these reasons, high 25(OH)D levels can lead to a shift from a proinflammatory to an anti-inflammatory immune status, with different effects on the immune system. T cell subpopulations are particularly conditioned by 25(OH)D level alteration. In fact, 25(OH)D inhibits proinflammatory T helper cell proliferation and differentiation, negatively modulating the secretion of proinflammatory cytokines such as IL-2, IFN-γ, TNF-α, IL-9, and IL-22 [70–75], while promoting the production of anti-inflammatory cytokines IL-3, IL-4, IL-5, and IL-10 [76].

Recent studies have shown that 25(OH)D is a potent inhibitor of Th17 cell differentiation and $1,25(OH)_2D$ was found to directly suppress IL-17 mRNA synthesis and decrease IL-17, IFN-γ, and IL-21 levels [77, 78]. For these reasons, vitamin D might represent a good candidate for the management of autoimmune diseases, considering that it induces an increased expression of genes typical of regulatory T cells (Tregs) in human primary T cell cultures [79]. In fact, the inhibition of proinflammatory T cell cytokines, such as IL-2 and IL-17, and of toll-like receptors on monocytes [80–83], leads to (a) a significant reduction of the proinflammatory cytokine IL-6, produced by peripheral mononuclear cells [84], and (b) an induction of Tregs, which are crucial for controlling immune responses and avoiding autoreactivity [66]. In addition, Rudensky found that Tregs act to suppress proinflammatory responses by other immune cells and help preventing inappropriate or autoimmune responses [85, 86].

Finally, other works suggest that vitamin D may support the adaptive as well as the innate immune system. Vitamin D enhances the antimicrobial effects of macrophages and monocytes, enhancing chemotaxis, phagocytic capabilities of immune cells, and directly activating the transcription of antimicrobial peptides [82, 87–89]. In fact, monocytes exposed to pathogens show induction of the hydroxylase *CYP27B1* and the VDR by toll-like receptors and other cytokines, leading to the modulation of the expression of its downstream genes. The mechanisms described here seem to be strictly dependent on "sufficient" levels (>75 nmol/L) of 25(OH)D [90–92].

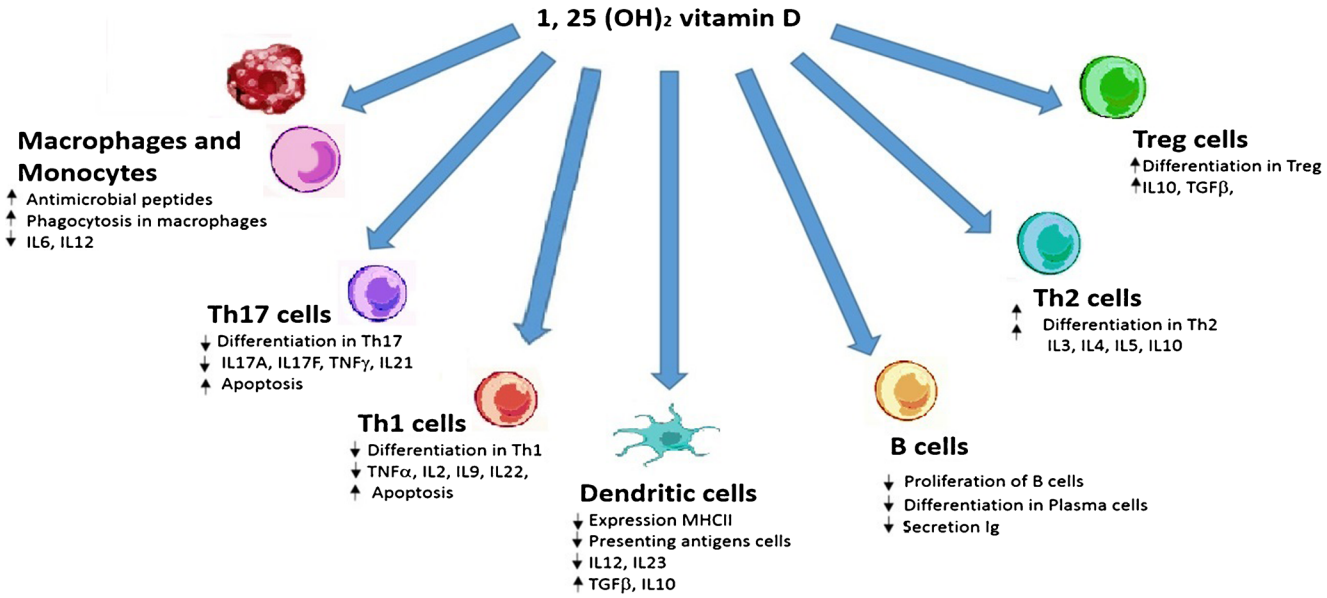

Fig. 3 Effect of 1α,25(OH)2 vitamin D in the cells of the immune system

Level Alterations and Secondary Osteoporosis

25(OH)D insufficiency is often associated with low BMD, an ascertained key risk factor for osteoporotic fracture [32, 93–104]. At the same time, it is interesting to note that patients affected by chronic diseases such as diabetes mellitus (DM), obstructive lung diseases (OLD), rheumatoid arthritis (RA), ankylosing spondylitis (AS), systemic lupus erythematosus (SLE), and multiple sclerosis (MS) present altered vitamin D levels. Indeed, these clinical conditions are correlated with osteoporosis and are characterized by the development of a chronic inflammatory state, also responsible for progressive bone resorption. This is due to the stimulating action of released proinflammatory cytokines, such as IL-1β, IL-6, sIL-6R, TNF-α, etc., stimulating osteoclast differentiation by upregulation of RANKL [22, 92]. Moreover, the chronic use of steroids to contrast systemic inflammation in these diseases promotes the onset of osteoporosis [105], because it alters osteoblastic functions and activity (decreased bone formation) and gonadal (reduction of sexual hormone with an increased resorption of bone) and parathyroid functions (increase PTH release), and finally it alters Ca metabolism by increasing Ca excretion [106]. In vitro and in vivo studies have reported that vitamin D can suppress and prevent inflammatory states in different chronic inflammatory diseases and this is an encouraging finding for promoting further clinical trials, needed to evaluate the potential role of vitamin D in clinical practice [107].

DM is characterized by hyperglycemia and is associated with obesity, aging, and inactivity. Affected subjects develop chronic hyperglycemia that determines glucotoxicity, lipotoxicity, oxidative stress, endoplasmic reticulum stress, and amyloid deposition [108]. It is well known that hypovitaminosis D leads to a deficiency in the secretion of insulin and induces glucose intolerance, while its replacement re-establishes these abnormalities [109]. Various studies highlighted that 25(OH)D levels are significantly lower in DM compared with non-DM patients, and more importantly, vitamin D levels are below the normal range. In particular, it was demonstrated that 25(OH)D deficiency is related to type 2 DM [110, 111]. The condition of hyperglycemia generates advanced glycation end products (AGEs), which accumulate in bone tissue, where they bind their receptors expressed on human bone-derived cells. By doing so, they inhibit the synthesis of type 1 collagen and osteocalcin and enhance osteoclast activity, leading to DM-related bone fragility [112, 113]. Inflammatory molecules can be measured in DM patients' serum, in particular TNF-α, resistin, and free fatty acids. The inflammation is exasperated by the activation of toll-like receptors (TLRs) that usually recognize microbial products, which can bind other molecules, like necrotic cells, oligosaccharides of soluble hyaluronate, and AGEs. In addition to this, high glucose levels increase the expression of TLR-2 and TLR-4, enhancing the inflammatory cascade [114]. Although the correlation between low levels of vitamin D and the production of AGEs is controversial [21, 115], low levels of vitamin D result in an increase of proinflammatory cytokines in DM [116]. The only clinical trial on the administration of vitamin D for DM prevention [117] suggests multiple actions for vitamin D involving the increase of insulin sensitivity, anti-inflammatory actions, and improved lipidic metabolism.

Vitamin D deficiency is a common feature in OLD, as chronic obstructive pulmonary disease (COPD) or bronchial asthma, although its role is not completely understood [118]. There are several studies showing that vitamin D deficiency in OLD is associated with systemic inflammation, decreased lung function, and osteoporosis [119]. In COPD, the majority of patients have low vitamin D levels associated to an inflammatory state [120] and low bone density [121, 122]. In asthma, high vitamin D levels were associated with improved lung function, immunomodulation of inflammation state, better glucocorticoid response, and less airway hyperactivity [123, 124].

In RA, systemic inflammation causes a boosting of bone loss that roughly doubles vertebral and nonvertebral fracture risk. In fact, in RA patients, low 25(OH)D levels were found to be significantly associated with clinical parameters of disease activity, as well as with high serum levels of IL-17 and IL-23 and bone loss. These findings suggest that vitamin D deficiency may play a role in the pathogenesis of osteoporosis in RA patients [125–127].

Osteopenia and osteoporosis of the axial skeleton are also complications of AS, an immune-mediated inflammatory disease, despite that this disease is characterized by excessive bone formation and ankylosis. Several studies suggest that systemic inflammatory mediators may be involved in the pathogenesis of osteoporosis in patients with AS. In particular, IL-6, TNF-α, and IL-17, well-known osteoclast activators, play a pivotal role in the pathogenesis of this bone mineral disorder [128]. Recently, osteopenia and osteoporosis in AS have been associated to low serum level of 25(OH)D and decreased vitamin D absorption and related to chronic inflammatory intestinal lesions or genetic factors (as VDR polymorphisms). Conversely, high levels of serum vitamin D were associated with a decreased risk of AS and showed an inverse relationship with AS activity [129].

In SLE patients, the unbalance in peripheral blood lymphocytic subpopulation, in particular between Th17 and Tregs (and their cytokines), plays an important role in the development of an increased proinflammatory response [76]. In vitro studies have shown that regulatory T cells have the capacity to inhibit osteoclast differentiation and function [130–133]. In particular, IL-17 seems to play a central role in SLE-derived osteoporosis through a direct stimulation of osteoclast

differentiation, as demonstrated in different studies [134–136]. Moreover, in this pathology, patients show very low vitamin D level, which is associated with improved inflammatory state and high risk of osteoporosis [137].

MS, a chronic inflammatory-demyelinating disease of the nervous system, is also characterized by decreased bone formation and osteoporosis. Recent studies have demonstrated an association between decreased vitamin D level and higher rates of MS relapse, as well as higher MS-specific disease activity and disability [138, 139]. Although high doses of intravenous glucocorticoid, used as treatment of this pathology, causes a decrease in bone mass, this effect is usually reversible and osteoporosis in MS patients seems to be independent of the short-term corticosteroid treatment.

Finally, several studies have shown a consistent association between obesity and vitamin D status, thereby an increase in adiposity results in lower serum 25(OH)D levels. The protection of vitamin D related to obesity seems to be due to the stimulation of fatty acid oxidation leading to increased energy expenditure through an upregulation of genes involved in fatty acid oxidation and mitochondrial metabolism [3, 140–144]. Being slightly overweight can have a protective effect on bone, due to the well-established positive effect of mechanical loading conferred by body weight on osteoblast activity and bone formation, thus reducing the risk of fracture and the development of osteoporosis in older adults [145]. Moreover, adipocytes produce estrogens that inhibit osteoclast activity and bone resorption. However, this does not seem to be true when the overweight condition is such as to determine obesity, defined as a state of excess body fat storage resulting from a chronic imbalance between energy intake and energy expenditure [146]. This negative effect has been connected to the conception of obesity as a low-grade chronic inflammation. In fact, in obese subjects, increased levels of inflammatory mediators and acute phase proteins can be observed, together with an increase in macrophages and releasing inflammatory cytokines in adipose tissue. Some of the proinflammatory cytokines produced are also mediator of bone resorption, whose levels raise in obese subjects [147].

Mutations and Polymorphisms of Gene Related to the Vitamin D Pathway

Genetic factors relating vitamin D to primary and secondary osteoporosis involve all genes of vitamin D metabolism enzymes (*CYP27A1*, *CYP27B1*, *CYP2R1*, and *CYP24A1*), transporter DBP (*GC*), and VDR (*VDR*), but the most frequently involved gene in the pathogenesis of osteoporosis seems to be *VDR* and its polymorphisms.

The *VDR* gene is located at the long arm of chromosome 12 and is a member of the nuclear receptor superfamily. Common simple nucleotide polymorphisms (SNP) were identified, namely *BsmI* (rs1544410), *FokI* (rs10735810), *ApaI* (rs7975232), and *TaqI* (rs731236) [148–152]. Most of the polymorphisms associated with the risk of postmenopausal osteoporosis are more frequent in some populations, such as *BsmI* and *ApaI* in Belarusian [153] or *FokI* in Turkish [154, 155]. Studies on *BsmI* polymorphisms reported that (1) women with AA and AG alleles (G is a wild-type allele and A is the allele referred to mutation rs1544410) have an increased and an intermediate risk of low BMD-related disorders, respectively [151, 155]; and (2) the percentages of homozygous dominant (BB) and homozygous recessive (bb) genotypes were, respectively, significantly lower and higher in osteoporotic postmenopausal females in comparison to healthy females [151, 156–158]. A recent preliminary study indicated also that alendronate had a different effect on BMD, depending on the *BsmI* polymorphism, suggesting that b allele carrying patients may be more responsive to treatment compared to patients with the BB genotype [159].

Similarly, for *FokI* polymorphisms, it was reported that postmenopausal women with ff genotype have a significant BMD loss at the femoral neck compared to women with FF and Ff genotypes [156, 158, 160], while for *ApaI* polymorphisms, postmenopausal women with AA genotype had a significant BMD loss at the lumbar spine compared to women with Aa genotype [151, 161].

Mosaad et al. showed that RA and rheumatoid-related osteoporosis are significantly associated to *ApaI*, *BsmI*, and *TaqI* polymorphisms, presenting a high titer of rheumatoid factor [162]. These lines of evidence suggest a pathogenic role for these genotypes in RA-related bone loss, which might be the result of abnormally increased osteoclastic activity. A significant association between lower hip BMD and *BsmI* and *OPG* A163G polymorphisms in RA patients with osteoporosis was also reported [163]. Despite all these interesting results, recent meta-analyses on *VDR* gene polymorphism lead to contrasting conclusions: (1) *BsmI* polymorphism does not seem to be associated with the susceptibility of osteoporosis in overall Caucasian and Asian populations [164]; (2) *BsmI* bb genotype seems to have a protective role against the development of osteoporosis in postmenopausal women [165]; and (3) *ApaI*, *BsmI*, *TaqI*, and *FokI* polymorphisms may be not associated with the risk of fracture in postmenopausal women [166]. Other two *VDR* polymorphisms have been recently investigated: rs2239185 and rs3782905. Physically inactive men with rs2239185 in homozygosis or heterozygosis had a significantly greater risk of lumbar spine and femoral neck osteoporosis than physically active men with the wild-type genotype in homozygosis. Similarly, physically inactive women with rs3782905 genotype in homozygosis or heterozygosis, had a significantly greater risk of femoral neck osteoporosis, than physically active women with the wild-type genotype [167].

Another gene associated to a high risk of osteoporosis is the *GC* gene, encoding for DBP, a highly polymorphic serum

protein, predominantly synthesized in the liver. Three main phenotypic alleles (GC1F, GC1S, and GC2), identified through electrophoretic mobility and that differ in serum level and affinity of 25(OH)D, were reported in the literature [168–170]. Numerous studies attempted to correlate these *GC* isoforms with osteoporosis, with controversial results [169, 171, 172]. Some studies showed a major 25(OH)D affinity in the GC1 isoforms compared to GC2 protein, and others showed that various isoforms had also a different serum concentration of proteins (more in GC2 compared to GC1 isoforms). Specific polymorphisms are associated to osteoporosis. In particular, (TAAA)*n* microsatellite polymorphism in intron 8 was found to lead to changes in the concentrations and activities of transported vitamin D metabolites and to high risk of osteoporosis and vertebral fractures [173, 174]. Several other SNPs within the DBP gene [175–177], the combination of IVS1+827C>T and D432E associated with BMD variations [178] as well as rs4588 and rs7041 polymorphisms associated with the variation in circulating 25(OH)D and total vitamin D intake [179], represent a higher relative risk of osteoporosis.

Other polymorphisms in vitamin D metabolism genes are associated with serum 25(OH)D levels, and these are supported by different studies. For example, the homozygous mutation L99P in exon 2 of *CYP2R1*, causing inactivation of CYP2R1, when associated to those of *GC*, leads to a low level of 25(OH)D [161]. Polymorphisms in *CYP27B1* (rs703842, rs4646536) have been associated to a low level of vitamin D, low BMD, and multiple sclerosis [180–183].

Conclusions

The current review shows that vitamin D is very important for Ca and P homeostasis, acting as a hormone in an autocrine and paracrine manner, and also in the immune system, stimulating immune tolerance in different ways. This last activity has emerged only recently, when it was found that this molecule has to be present at levels of at least 75 nmol/L to be able to carry out its role in the homeostasis of the immune system.

Most of the results of the studies reported in the current review highlight that the relation between the levels of vitamin D and osteoporosis is partially mediated by the immune system, but they lack to provide specific lines of evidence of the pathogenic mechanisms. Levels of insufficiency of vitamin D, in fact, determine alterations in the immune system by inducing the production of a whole series of proinflammatory cytokines, suggesting a key role in inducing secondary osteoporosis in chronic inflammatory diseases. In addition, low values of vitamin D, due to mutations in the genes of the vitamin D pathway or inappropriate lifestyles (i.e., diet), may induce secondary osteoporosis and a state of chronic inflammation, as described in obesity. Nevertheless, the possibility of reverse causations such as in immobilized patients, who get less sunlight, and thus have low level of vitamin D, has not been always considered in reported clinical studies. As for scarce exposition to sunlight as cause of vitamin D level reduction, this is a real complication only for invalidating diseases in an extremely advanced state, although low levels of vitamin D are also observed in early and intermediate state of the abovementioned pathologies [184].

In the authors' current opinion, and by taking into account information derived from this review, future clinical studies on osteoporosis should include investigative steps to understand the implication of a low level vitamin D on the inflammatory state and how its chronic evolution might influence osteoporosis pathogenesis and progression in order to develop new treatment protocols. This review shows how the serum level of vitamin D is involved in developing inflammation in various chronic, degenerative, or autoimmune pathologies, highlighting a possible role of the immune system in mediating the correlation between hypovitaminosis D and secondary osteoporosis in these pathologies. In particular, clinical studies lack in the evaluation of lymphocyte subpopulation balance (pro- and anti-inflammatory cells) in osteoporotic patients with low vitamin D level status. These studies could highlight whether the alteration in vitamin D levels might be a triggering event of osteoporosis development both in primary and in secondary osteoporosis.

Furthermore, there are a few studies that evaluate (a) the presence of polymorphisms in vitamin D pathway genes, (b) the effects of these polymorphisms in the different vitamin D metabolite levels, and (c) the relationship with inflammatory states in osteoporotic patients. The high presence of polymorphisms in osteoporotic patients that determine the observed low level of active vitamin D metabolites can justify the possible lack of efficacy of vitamin D administration and alter the results in some clinical studies. The mutations or polymorphisms that have an effect in vitamin D levels can be considered an additive risk factor in osteoporosis, and can be used to remove the inflammatory perturbations through the actions of active vitamin D metabolites or its analogs.

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Compliance with Ethical Standards

Conflict of Interest All authors have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

Appendix

Search Strategies A literature research of the entire MEDLINE database (PubMed research engine) using the following MeSH database terms was carried out: ("Vitamin D" [Mesh]) AND ("Osteoporosis" [Mesh]). The search was limited to the last 5 years ("2010/01/01" [Date - Entrez]: "2016/06/31" [Date - Entrez]) and abstract availability in English. The references of the retrieved studies and pertinent reviews were then manually assessed by three reviewers. No relevant data were found about the effect of vitamin D in the immune system of osteoporotic patients. We performed a specific research in "PubMed" database including "Vitamin D" [Mesh] AND "Osteoporosis" [Mesh] AND ("2010/01/01" [PDat]: "2016/06/31" [PDat]), "immune system" [Mesh]. The articles found were eight, three of which were discarded because these are not relevant to the subject of the research.

The number of unique papers from the electronic search was 829, which after abstract review was reduced to:

- 4 meta-analysis
- 6 systematic reviews
- 34 reviews
- 5 related to immune system (AND "Immune System" [Mesh])
- 17 related to polymorphisms (AND "Polymorphism" [Mesh])
- 3 related to calcium and phosphorus metabolism (AND "Calcium" [Mesh] AND "Phosphorus" [Mesh])
- 4 guidelines
- 10 other articles

Other 100 (about 54.65 %) papers were included in the final review, including 4 % guidelines, 60 % human, 10 % animal, and 15 % in vitro studies. Most of the human studies were observational (41.7 %, 25 out of 60) and only one controlled study was present (3.3 %, 2 out of 60).

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