Università degli Studi di Salerno
Facoltà di Medicina e Chirurgia

Corso di Dottorato di Ricerca in Medicina Traslazionale dello Sviluppo e dell'Invecchiamento Attivo
Dipartimento di Medicina, Chirurgia e Odontoiatria
"Scuola Medica Salernitana"

Tesi di Dottorato

ROLE OF VITAMIN D IN THE MODULATION OF CHRONIC BOWEL INFLAMMATION AND IN THE GENESIS OF BONE MASS LOSS IN PATIENTS WITH INFLAMMATORY BOWEL DISEASES.

Relatore
Chiar.ma Prof.ssa Paola Iovino

Candidato
Antonella Santonicola
Matr. 8800900018

ANNO ACCADEMICO 2018/2019

CONTENTS

ABBREVIATIONS AND ACRONYMS..…. 3

ABSTRACT ...…….4

1. INTRODUCTION ...…….....…..6

- 1.1 Inflammatory bowel disease and Bone Mass……..… .6
- 1.2 Vitamin D and Bone……..9
- 1.3 Vitamin D, Immunity and Inflammation……...11
- 1.4 New tools for assessment of bone metabolism……..14

2. AIM…….......17

3. METHODS.....18

3.1 Study Setting and Population.....18

3.2 Clinical, Socio-Demographic and Laboratory Variables.....18

3.3 Statistic Analysis 19

4. RESULTS 20

5. DISCUSSION 24

6. CONCLUSIONS 27

7. REFERENCES 28

ABBREVIATIONS AND ACRONYMS

APCs= Antigen Presenting Cells

BMD=Bone Mineral Density

BMI=Body Mass Index

CD=Crohn's Disease

CoTh=Cortical Thickness

CRP=C-reactive protein.

CSA=Cross-Sectional Area

DBP= vitamin D binding protein

DXA= dual-energy X-ray absorptiometry

EIM= ExtraintestinalManifestation

GI= Gastro-intestinal

IBD = InflammatoryBowelDisease

NF-kB=nuclear factor kB

pQCT= peripheral Quantitative Computed Tomography

PTH= Parathyroid Hormone

TLR=Toll Like Receptors

UC= Ulcerative Colitis

UVB= ultraviolet B irradiation

VDR=vitamin D receptor

ABSTRACT

Background: Inflammatory bowel disease (IBD) is a chronic immune-mediated inflammatory disorder of the gastrointestinal tract consisting two principal categories, ulcerative colitis (UC) and Crohn's disease (CD). Bone alterations in IBD population are frequently described and appear to have a multifactorial etiology: inflammation, changes in diet, malabsorption of calcium and vitamin D, decreases in physical exercise, and the use of osteotoxic medications. Bone alterations increased the risk of osteoporosis and fractures in IBD patients. Vitamin D is an immunoregulatory factor that seems to play a significant role in the pathogenesis of IBD by affecting both the gut microbiome and the inflammatory response.

Aim: This study aimed to evaluate the levels of the active form of vitamin D the 1,25(OH2)VitD in IBD patients and the possible correlation with the risk of osteoporosis. Secondly, we aimed to study the bone status in patients affected by IBD using the peripheral Quantitative Computed Tomography (pQCT), a new tool for the assessment of Bone Mineral Density (BMD).

Methods: Patients with IBD (CD and UC) were consecutively enrolled from November to May. For each patient calcium, phosphate, creatinine, serum protein/albumin, 25(OH)VitD, 1,25(OH2)VitD, and PTH levels were measured. C-reactive protein (CRP) and fecal calprotectin were used to assess the presence of inflammation. All patients underwent pQCT both at forearm and lower leg. Tibia and radius BMD, T and Z score were calculated.

Results: Forty-five IBD patients (25 CD and 20 UC) were enrolled. There were no statistically significant differences between the CD and UC patients in terms of age, gender and disease duration. There was a high prevalence (71%) of vitamin D insufficiency in IBD patients (76% CD and 65% UC patients). The prevalence if vitamin D insufficiency was

significantly higher in patients with elevated fecal calprotectin (≥100 mg/kg) (83.3 vs 37.5%, p=0.035) but it was not significantly different in patients with elevated CRP (31.6 vs 20%, p=0.5). Mean BMD, T score and Z score were significantly lower CD compared to UC patients ($p<0.05$ in all cases). The prevalence of osteoporosis was higher in CD than UC patients, although the difference did not reach the statistical significance (44 vs 20%, p=0.09). A significant inverse correlation was found between radius and tibia BMD values and disease duration (r=-0.35, p=0.05 and r=-0.33, p=0.06, respectively) and between radius and tibia BMD and treatment with biologics (r=-0.45, p=0.002 and r=-0.44, p=0.003, respectively).no correlations were found between PTH, 25(OH)VitD, and 1,25(OH)2VitD levels and radius and tibia BMD values ($p>0.05$).

Conclusions: Vitamin D insufficiency was highly prevalent in IBD population. Thus, it would be beneficial for all IBD patients, especially those with active bowel inflammation, to be checked regularly for Vitamin D status. Low BMD was common in IBD patients, and it does not correlate to laboratory indices. pQCT could be a useful tool to assess the bone status of IBD patient using very low radiation dosage.

INTRODUCTION

- **Inflammatory bowel disease and Bone Mass**

Inflammatory Bowel Disease (IBD) is a chronic immune-mediated inflammatory disorder of the gastrointestinal (GI) tract with relapsing and remitting periods. IBD includes two forms, Crohn's Disease (CD) and Ulcerative Colitis (UC). The etiology of IBD is not completely understood; however, it is believed that genetic factors such as a mutation of the nucleotide-binding oligomerization domain-containing protein 2 (NOD2) gene, gut microbiome, immune and environmental factors have important roles in its pathogenesis[1]. The typical clinical manifestations of IBD patients include: diarrhea, abdominal pain, weight loss, anemia, and hematochezia. Furthermore, 10%-40% of IBD patients may suffer from at least one extra-intestinal manifestation (EIM)[2], such as ankylosing spondylitis, erythema nodosum, pyoderma gangraenosum, iritis, episcleritis, scleritis, anterior uveitis, primary sclerosing cholangitis[3]. They may occur before the onset of the intestinal disease[4] and may not correlate with disease activity (primary sclerosing cholangitis and ankylosing spondylitis) but generally EIMs tend to follow the clinical course of IBD and may have a high impact on quality of life, morbidity and even mortality in these patients[3].

EIMs affecting the musculoskeletal system are frequent, and in particular those involving the bone tissue, such as osteoporosis[5].

Osteoporosis is a systemic disease characterized by increased risk of fractures and reduction of bone strength due to a decrease in bone mineral density (BMD) and a deterioration in bone quality[6]. According to the World Health Organization the diagnosis of osteoporosis is based on BMD values equal or lower than 2.5 standard deviations (SD) from the average values for young healthy women (T-score < -2.5 SD) in post-menopausal women and men aged ≥ 50 years, while, osteopenia is defined by BMD values between -1 to -2.5 SD (T-score -1< and > -

2.5)[7].BMD is usually measured by dual-energy X-ray absorptiometry (DXA); T-score is a parameter comparing the BMD of the patient with the average bone density of young healthy adults of the same sex, while, Z-score compares each BMD with the average BMD of a person with the same age and sex[8].

The prevalence of low BMD varies across the studies, ranging from 22% to 77% [9], probably due to the variability in patient's selection, different methods used to evaluate bone density, and different body sites studied at DXA (i.e., radius vs lumbar spine or hip). A Swiss cohort study on 877 IBD patients showed a prevalence of low BMD in 20% of patients and identified, by multivariate logistic regression analysis, corticosteroid usage, long disease duration and perianal disease as independent risk factors[5].A recent meta-analysis demonstrated a significant association between IBD and osteoporosis, with a pooled OR of 1.32 (95% CI, 1.2 - 1.4)[10]. American guidelines recommend the BMD testing in presence of hypogonadism, prior fractures, low Body Mass Index (BMI), and family history of osteoporosis, but specifically recommended testing for IBD patients with age greater than 60 years and/or cumulative steroid exposure greater than 3 months[11].

Although CD and UC are both classified as IBDs, they show considerable differences in the anatomic location and distribution of the intestinal lesions as well as in the underlying pathogenic mechanisms. This might have an influence on the incidence of bone alterations in each condition. Some authors did not find any differences in T scores for spine or hip of patients with CD and UC[12, 13]. However, other authors showed that BMD was significantly lower at all measured sites in CD patients compared to UC[14].

With the increased risk of developing osteoporosis, patients are at a greater risk of suffering osteoporotic fractures. IBD patients can have an increased risk of fractures by 40% as compared to the general population[15].

Bone alterations in IBD population appear to have a multifactorial etiology: cumulative steroid dose, hypogonadism induced by IBD (absence of menstrual period in women),

malabsorption of calcium and vitamin D, low body mass index and disease activity/elevated inflammatory cytokines. Other risk factors are previous fragility fracture, a positive family history, immobilization and life style risk factors (smoking, excessive alcohol intake, physical inactivity)[3, 16]. In IBD, the immune response, mediated by T lymphocytes and other inflammatory cells like macrophages, leads to production of various proinflammatory cytokines such as interleukin (IL)-2 and tumor necrosis factor (TNF), that stimulate the production and maturation of osteoclasts, the cells responsible for bone resorption[9]. Specifically, osteoclasts develop from their precursor cells under the influence of nuclear factor kappa B (NF-KB),which is produced when the receptor activator of NF-kB (RANK) is stimulated. In the setting of chronic intestinal inflammation, T-cells increase the production of soluble RANK ligand (RANK-L), which binds to RANK, generating the NF-KB which in turn promotes excessive osteoclast activity. Conversely, osteoblasts produce a RANK inhibitor called osteoprotegerin (OPG),which may rise in response to increasing levels of soluble RANK-L. Rising levels of OPG counteracts osteoclast activity, though not enough to completely prevent bone loss[17]. Several factors such as parathyroid hormone (PTH), 1,25-(OH)2vitamin D, prostaglandin E2 stimulate RANKL expression and inhibit OPG production, thus increasing osteoclastogenesis, whereas 17β-estradiol increases OPG and decreases RANKL, reducing osteoclastogenesis[18].

However, recent studies demonstrated that probably vitamin D not only plays a role in bone metabolism in IBD patients but also, as an immunoregulatory factor, could contribute to the composition of the gut microbiome and the inflammatory response in IBD subjects[19]

Furthermore, it has been reported that 38.1% of CD patients and 31.6% of UC patients suffer from vitamin D deficiency[20], that could influence the inflammatory pathways in IBD patients.

- **Vitamin D and Bone**

Vitamin D sources are the diet and the endogenous synthesis in the skin triggered by ultraviolet B irradiation (UVB). Vitamin D is naturally present in some fatty fish (i.e. Salmon, Swordfish, Sardines) and egg yolks, and in lower amounts in some other foods. Daily sun exposure for about a quarter of an hour produces up to 10,000 IU vitamin D[21]. Thus, the sunlight-stimulated vitamin D represents the main constituent of the body pool for the majority of the population[22, 23].

Once in the general circulation, vitamin D is bound to its serum carriers: the vitamin D binding protein (DBP) and the albumin. Then, it undergoes sequential hydroxylations to generate the metabolically active metabolite. The first hydroxylation occurs in the liver, where Vitamin D is hydroxylated to form 25-hydroxyvitamin D (25(OH)VitD), the major intermediate, inactive vitamin D metabolite. The 25(OH)VitD re-enters the circulation and it is carried by DBP or albumin to the tubular epithelial cells of the kidney. Subsequent hydroxylation of 25(OH)VitD occurs in the kidneys and forms 1,25-dihydroxyvitamin D (1,25(OH)2VitD), which is the most metabolically active form of the vitamin. Other tissues, such as the placenta, may also produce 1,25(OH)2VitD for local autocrine/paracrine effects[24].

The renal hydroxylation is strictly controlled by PTH and phosphate (stimulation and inhibition, respectively)[25].

The 25(OH)D3-1alfa-hydroxylase (CYP27B1) is the rate limiting enzyme for biologically active vitamin D production. Cyp27B1 is expressed in kidney but also in the intestine, to drive local production of 1,25(OH)2VitD. Interestingly, colonic Cyp27B1 expression is influenced by toll-like receptor activation and colonic inflammation[26, 27].

The 1,25(OH)2VitD promotes intestinal absorption of calcium and phosphate in the gut. Low intake or absorption of calcium and/or low vitamin D status leads to the increased production of PTH, that both stimulates the renal hydroxylation of 25(OH)VitD to 1,25(OH)2VitD and increases bone resorption, mobilizing calcium from the bones to maintain serum calcium concentration in the physiological range[23].Some recent studies examined the relationship between 25(OH)VitD and PTH, but results have been conflicting in terms of the strength of the correlations and the putative value at which PTH starts to increase[28].

25(OH)VitD has a 2-week half-life, a plasma concentration of approximately 30-100 ng/ml, and is considered a nutritional marker but has low biological activity. Vice versa, 1,25(OH)2VitD has a 4-hour half-life, a very low plasma concentrations (pg/mL, approximately 1000-time lower than 25(OH)VitD) and is considered the biologically active form[29]. Globally, there is a general consensus that blood 25(OH)VitD levels below 25 nmol/l (or 10 ng/ml) qualify as 'deficient', but beyond this there is currently no standard definition or agreement as to 'optimal' 25(OH)VitD level. However, the consensus generated the clinical consequence of the need for replacement of vitD below 25 nmol/l[30].

Some authors expressed concerns about most of the existing data that the 25(OH)VitD have a direct effect on bone[31]. It has been clearly shown, in fact, that a reduced calcium absorption seen in severe vitamin D deficiency only occurs when serum 25(OH)VitD is so low that there is not enough substrate to maintain 1,25(OH2)VitD even with maximal stimulation by PTH[32]. If this is true, there is no evidence supporting the 25(OH)VitD supplementation following the assessment of low serum levels. However, there is no clear-cut evidence that 25(OH)VitD has no biological effects as it is before transformation in the active form.

- **Vitamin D, Immunity and Inflammation**

Apart from regulating the homeostasis of minerals and parathyroid hormone, 1,25(OH)2VitD has been shown to be involved in the regulation of the innate and the adaptive immune systems[16]. The healthy intestine is certainly pivotal for vitamin D and calcium absorption and metabolism and a target site for immunity. The immunologic effects of 1,25(OH)2VitD can be summarized as affecting the toll like receptors (TLR), decreasing Th1/Th17 CD4+ T cells and cytokines, increasing regulatory T cells, downregulating T cell-driven IgG production, and inhibiting antigen presenting cells (APCs) differentiation. The tolerogenic capacities of APCs rely on their ability to express the CYP27B1 enzyme. By use of this enzyme, these cells can achieve high concentration of activated vitamin D which is important for developing their immunomodulatory effects[33]. The production of both B and T lymphocytes and T cell maturation is also regulated by 1,25(OH)2VitD [34]. Calcitriol can inhibit differentiation, proliferation and memory cell generation as well as inducing apoptosis of B cell lymphocytes. The sum of these effects on B cells is crucial for modulating auto reactive antibody production during development of an autoimmune process. Vitamin D also hassome direct effects on T cells. Calcitriol can suppress T helper lymphocytes production, and negatively regulate the production of proinflammatory cytokines, which include IL-2, IL-9 and TNF-α[35]. Along with suppression of these cytokines, other anti-inflammatory cytokines such as IL10 are increased. Also regulatory T cells, that are important in maintaining an anti-inflammatory state, are activated either directly by calcitriol or indirectly by 25(OH)D[35].

A key player for all is the vitamin D receptor (VDR), a nuclear receptor mediating 1,25(OH2) vitamin D functions. Polymorphisms in the VDR gene have been associated with increased risk of multiple autoimmune diseases, including diabetes and celiac disease, although the role

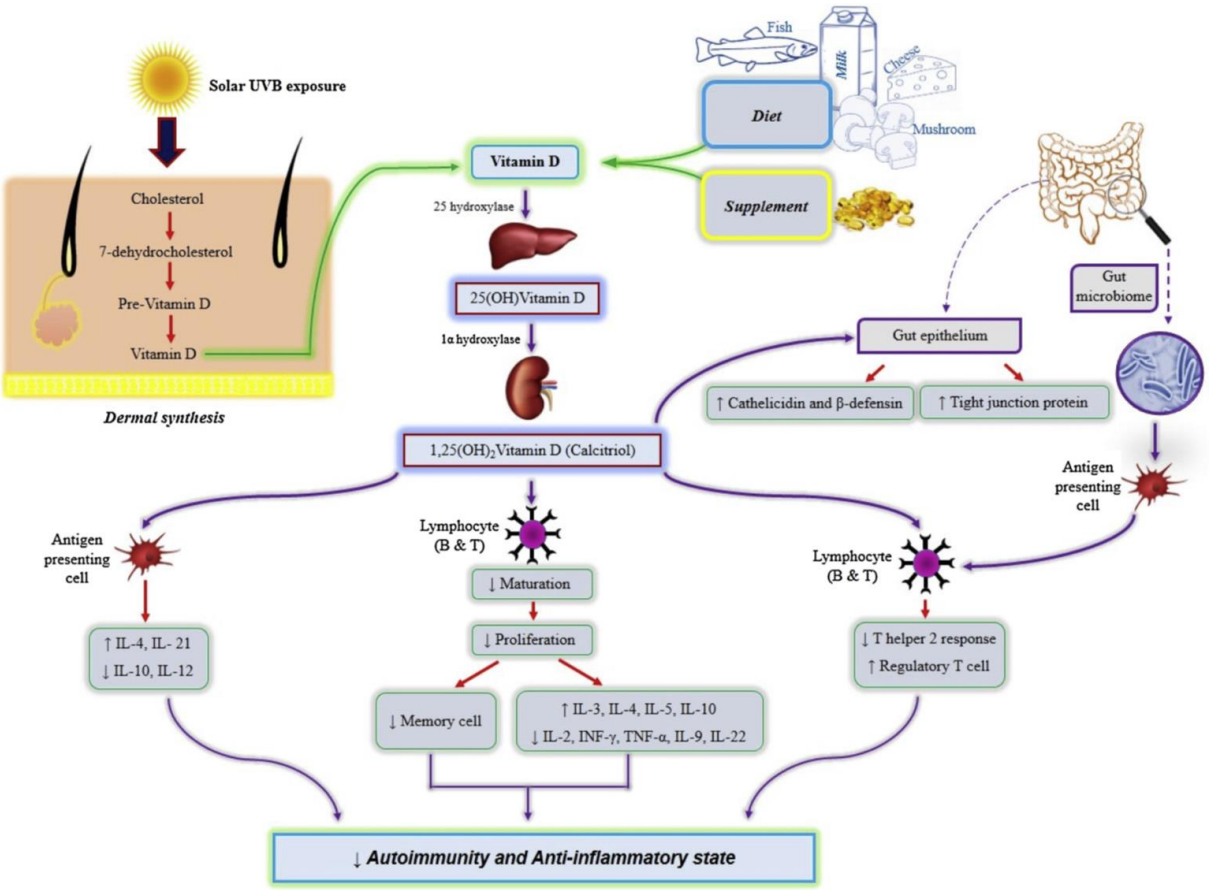
of this alteration in these diseases is far to be understood[36]. VDR mediates all the genomic effects of 1,25(OH)2VitD that take several hours to days to be fully apparent. However, 1,25(OH)2VitD action generates also rapid responses within 1–2 min, known as non-genomic effects. Some of the non-genomic effects of 1,25(OH)2VitD are the rapid hormonal stimulation of calcium absorption, direct insulin stimulation in the pancreas, ion channels opening in bone, migration of endothelial cells in culture, stimulation of monocytes and a direct stimulation of several second messenger phosphoproteins[36].

The nuclear factor kB (NF-kB) is a major regulator of gene transcription involved in immune, inflammatory and stress responses. Recent data indicates that the VDR receptor signaling inhibits experimental colitis in rat by blocking NF-κB activation and thus reducing intestinal cells apoptosis[37].

VDR also acts as an important regulator of homeostasis in intestinal tissue; in fact, studies in selective intestinal VDR knock-out mice demonstrated that decreased production of lysozyme which further results in defective autophagy and stimulate the development of colitis[38].

1,25(OH)2D3 affects the expression of different genes such as B2 defensines (DEFB2/HBD2) and cathelicidin antimicrobial peptides (CAMP), that have antimicrobial effects[39].

Figure 1: The role of vitamin D in regulating immune system and its possible effects on the pathogenesis of IBD[39].



- **New tools for assessment of bone metabolism**

Recently, new tools are available to assess body calcium homeostasis: a reliable test for serum 1,25(OH2)vitD and the assessment of BMD by the peripheral Quantitative Computed Tomography (pQCT),a device that through peripheral scans at very low radiation dosage provides separate estimates of trabecular and cortical bone[40, 41]. The effective dose of radiation for this study has been estimated as 0.0035 millisieverts (mSv), which is within the dose constraints for children and adults and represents a negligible risk[42].

It is most commonly applied to the forearm or to the lower leg.

The patient's forearm is placed pronated in the pQCT gantry with the elbow resting on a block and the hand gripping the hand fixture. Initially the forearm length is measured as the distance between the tip of the ulnar styloid and the olecranon. Typically, scan locations with single-slice CT scanners are distal sites (4 % of radius length), containing mainly trabecular bone, and a shaft location (15–65 % of radius length), consisting predominantly of cortical bone.

After the participant placed their leg, shoeless, through the gantry of the pQCT scanner and the research assistant secured their foot to the pQCT footrest. A scout scan identified the distal epiphysial plate and allowed placement of a reference line at its proximal edge. The researcher then identified the 4% (ankle) and66% (shin) sites of the tibia using this reference line in relation to the limb length[43].

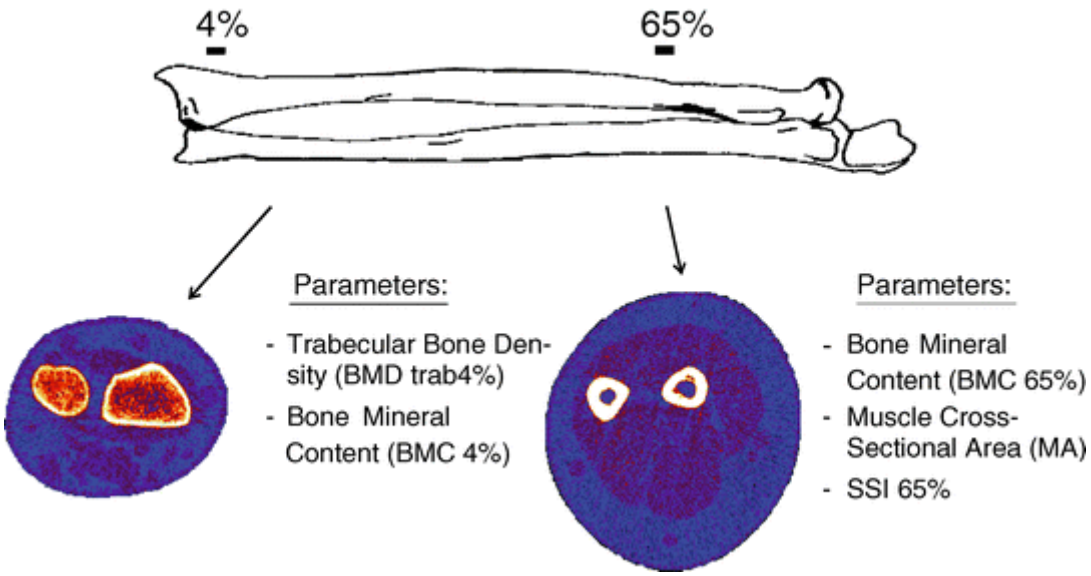
Multi-slice scanners at a scan speed of 20 mm/s, slice thickness of 2.4 mm and voxel size of 0.4 mm were obtained.

Figure 2: Peripheral quantitative computed tomograph (pQCT) for the measurement at femur, radius or tibia

A coronal scout scan is performed and a reference line is placed to bisect the medial border of the end of the distal radius in adults[44]. pQCT provides an automatical scan analysis of trabecular and cortical bone compartments, calculating not only their bone mineral density (BMD), but also bone geometrical parameters, such as marrow and cortical Cross-Sectional Area (CSA), Cortical Thickness (CoTh), both periosteal and endosteal circumference, as well as biomechanical parameters like Cross-Sectional Moment of Inertia (CSMI), a measure of bending, polar moment of inertia, indicating bone strength in torsion, and Strength Strain Index (SSI). Also CSA of muscle and fat can be extracted. Muscles, which are thought to stimulate bones to adapt their geometry and mineral content, are determinant to preserve or

increase bone strength; thus, pQCT provides an evaluation of the functional 'muscle-bone unit', defined as BMC/muscle CSA ratio.

Figure 3: Bone mineral density (BMD) and other bone geometrical parameters calculated by pQCT of forearm.



This functional approach to bone densitometry can establish if bone strength is normally adapted to the muscle force, and if muscle force is adequate for body size, providing more detailed insights to targeted strategies for the prevention and treatment of bone fragility[45].

The International Society for Clinical Densitometry (ISCD) stated that pQCT is in a rapid development as an accurate method for fracture risk assessment, for diagnosis, treatment initiation, and monitoring BMD for the clinical assessment of osteoporosis[46]. The pQCT has the advantage to provide an accurate measure of bone mass. In fact, the larger surface

trabecular bone reacts faster to changes in bone metabolism than cortical bone. Bone loss or the success of a therapy can be diagnosed earlier and more significantly with pQCT than with methods that cannot differentiate between cortical and trabecular bone. In fact, in contrast to traditional DXA, pQCT measures actual density in true volumetric value (g/cm^3) and not the area-projected mass (g/cm^2) as for DXA that misses the information about the size of bone. Another advantage is that pQCT allows the muscle cross sectional area measurement and the comparison of muscle and bone parameter. Bone strength is adapted to the maximum muscle force. With a comparison of muscle and bone cross sectional area it can be recognized if bone is adapted to muscle and an osteopenia caused by a sarcopenia can be differentiated from a primary osteoporosis. In the former case the reduced bone strength is a result of a physiological adaptation process to the reduced muscle force, therefore bone is healthy and the muscle should be treated. In the latter case bone is not adapted to muscle and the bone must be treated.

AIM

The aims of this study were:

- to evaluate the levels of the active form of vitamin D the 1,25(OH2)VitD in IBD patients and the possible correlation with the risk of osteoporosis.
- to study the bone status in patients affected by IBD using pQCT

METHODS

Study Setting and Population

Patients with IBD (CD and UC)were consecutively enrolled from the outpatient clinic devoted to diagnosis and follow-up of IBD of the University of Salerno. All the participants gave their informed consent.

Inclusion criteria: all adult patients (≥18 and ≤65 years) with a verified diagnosis of IBD based on endoscopic, biochemical and histological findings and the ability to read and to give their consent to participate into the study. The participants were enrolled in the months from November to May to minimize the effect of sunlight in vitamin D skin production (which can be relevant in Southern Italy, with a variation from 4 to 17% according to the season).

Exclusion criteria: Withdrawn of informed consent; age below 18 or over 65 years; severe kidney or liver disease; mental illness; alcohol abuse; pregnancy and/or breast feeding; use of drugs interfering with calcium metabolism; patients who were on Vitamin D supplementation.

Clinical, Socio-Demographic and Laboratory Variables

At enrollment, the following data were collected: anthropometry, smoking habits, GI symptoms, therapy, and routine laboratory tests.

After the enrolment visit, serum samples were collected and quickly stored at –80° for subsequent assessment of

- 25(OH)VitD and 1,25(OH)2VitD levels; they were analyzed by one central accredited laboratory by in vitro chemiluminescent immunoassay (LIAISON® XL, Dia Sorin Saluggia, Vicenza, Italy).

Vitamin D deficiency was defined as 25(OH)VitD concentration < 10 ng/mL, vitamin D insufficiency was defined as a 25(OH)VitD concentration of 10 - 30 ng/mL; a 25(OH)VitD concentration of 30 - 100 ng/mL was considered sufficient, whereas >100 ng/mL was considered toxic. Values of 1,25(OH)2VitD of 16-55 pg/mL were considered "normal".

- PTH; it was measured using chemiluminescent immunoassay CLIA (LIAISON® 1-84 PTH Dia Sorin Saluggia, Vicenza, Italy); normal valued were considered 10-60 pg/mL.

- Calcium, phosphate, creatinine and serum protein/albumin.

C-reactive protein (CRP) and fecal calprotectin were used to assess the presence of inflammation. A fecal calprotectin level of≥100 mg/kg[47]and/or a CRP level ≥5 mg/L[48] indicated active inflammation.

All participants underwent evaluation on Bone density by pQCT. The pQCT scanner is a Stratec XCT 2000 device (Stratec Medizintechnik GmbH, Pforzeim, Germany, software version 5.50d).According to the World Health Organization, osteopenia was defined if T-score -1< and > -2.5; osteoporosis was defined if T-score < 2.5[7].

Statistical Analysis

The data are expressed in frequencies and percentages for qualitative variables, as Mean ± SD for quantitative ones, unless otherwise indicated. Significance was expressed at $p< 0.05$ level. When appropriate, a $\chi 2$ test for categorical data and analysis of variance (ANOVA) for continuous data were used. The relationship between variables was assessed by bivariate and partial correlation using Pearson's coefficient for parametric and Spearman's coefficient for nonparametric variables as appropriate.The SPSS for Windows version 15.0 statistical package (SPSS Inc, Chicago, IL., USA) was used for statistical analysis.

RESULTS

Forty-five IBD patients were enrolled. Among them 25 were affected by CD and 20 by UC. There were no statistically significant differences between the UC and CD patients in terms of age, gender and disease duration (Table 1). Smoking was more frequent in CD compared to UC (p=0.04).Furthermore, more CD patients were receiving treatment with biologics than UC patients (48% vs. 20%, p=0.05).

Table 1. Demographic characteristics and anthropometric data in CD and UC patients. Data are expressed as percentage (%) or as mean±SD

	CD ***n = 25***	**UC** ***n = 20***	***p***
Gender (M/F)	15/10	8/12	0.18
Age (Years)	40.2 ± 15.4	38.5 ± 13.5	0.69
BMI (Kg/m^2)	23.8 ± 4.2	23.8 ± 4.5	0.98
Smoking	39%	8%	0.04
Disease duration(years)	9.9±7.2	8.7±10.1	0.7
CRP≥5 mg/L	26.3%	30%	0.8
Fecal calprotectin≥100 mg/kg	63.6%	66.7%	0.9
Current use of medication, n (%)			
• **Biologics**	48%	20%	0.05
• **5-ASA**	52%	45%	0.64
• **Prednisolone**	16%	10%	0.55
• **AZA/MTX**	12%	20%	0.46

Vitamin D deficiency, defined as 25(OH)VitD concentration <10 ng/mL, was found in 2 patients (1 CD and 1 UC). Moreover, 76% of CD patients and 65% of UC patients showed vitamin D insufficiency defined as a 25(OH)VitD concentration of 10-30 ng/mL.

The prevalence of vitamin D insufficiency was significantly higher in patients with elevated fecal calprotectin (≥100 mg/kg) compared to those with normal fecal calprotectin values (83.3 vs 37.5%, p=0.035). On the contrary, vitamin D insufficiency rate was not significantly different in patients with elevated CRP (31.6 vs 20%, p=0.5) and in IBD patients receiving treatment with biologics (p=0.34) and was not correlated to the disease duration (r=0.23; p=0.19).

Table 2 showed the studied biochemical parameters both in CD and UC patients.

Table 2. Biochemical parameters in CD and UC patients. Data are expressed as mean±SD.

	Normal range	**CD** ***n = 25***	**UC** ***n = 20***	***p***
Calcium	8,5 - 10,5 mg/dL	9.9 ± 0.7	10 ± 0.6	0.7
Phosphate	2,5 - 5,5 mg/dL	3.1 ± 0.5	4.2 ± 2.9	0.08
Albumin	3.5- 5.5 g/dl	4.2 ± 0.5	4.3 ± 0.4	0.38
PTH	10 - 60 pg/mL	52.8 ± 43.2	33.8 ± 22.3	0.08
25(OH)vitD	>30 ng/mL	22.3 ± 10.9	23.2 ± 10.1	0.80
1,25(OH)2vitD	16 -55 pg/mL	52.3 ± 16.6	51.1 ± 14.6	0.79

Mean values of calcium, phosphate, albumin, PTH, 25(OH)VitD, and 1,25(OH)2vitD were similar in male and female subjects (p>0.05 in all cases).

Seventy-two% of CD and 55% of UC patients showed normal values of 1,25(OH)2VitD (16-55 pg/mL). PTH levels were higher than normal range in 7/25 (28%) of CD and 2/20 (10%) of UC patients (p=0.11). Moreover, a significant direct correlation was found between PTH values and age ($r = 0.49$, $p = 0.001$), whereas there were not any significant correlations between PTH and 25(OH)VitD values (r=-0.21, p=0.16), PTH values and BMI (r=0.17, p=0.25), and PTH values and gender (r=-0.18, p=0.23).

All patients underwent pQCT both at forearm and lower leg. Table 3 reported tibia and radius BMD, T and Z score both in CD and UC patients.

Table 3. Tibia and radius BMD, T and Z score both in CD and UC patients. Data are expressed as mean±SD.

	CD ***n = 25***	**UC** ***n = 20***	***p***
Radius BMD	274.5 ± 61.3	319.0 ± 78.1	0.04
Tibia BMD	245.0 ± 66.2	274.6 ± 51.6	0.1
T score	- 2.3 ± 1.1	-1.4 ± 1.2	0.019
Z score	-1.94 ± 1.0	-1.2 ± 1.1	0.03

Mean BMD, T score and Z score were significantly lower CD compared to UC patients (p<0.05 in all cases). The prevalence of osteoporosis defined as a T-score < -2.5 SD was higher in CD than UC patients, although the difference did not reach the statistical significance (44 vs 20%, p=0.09).The prevalence of osteopenia defined as a T-score -1< and > -2.5 was similar in the two groups (52 vs 55%, p=0.84).

A significant inverse correlation was found between radius and tibia BMD values and disease duration (r=-0.35, p=0.05 and r=-0.33, p=0.06, respectively) and between radius and tibia BMD and treatment with biologics(r=-0.45, p=0.002 and r=-0.44, p=0.003, respectively).On the contrary, there were not any significant correlations between radius and tibia BMD values and age, gender and BMI (p>0.05 in all cases). Moreover, no correlations were found between PTH, 25(OH)VitD, and 1,25(OH)2VitD levels and radius and tibia BMD values (p>0.05).

DISCUSSION

This study demonstrated a high prevalence (71%) of vitamin D insufficiency in IBD patients (76% CD and 65% UC patients); this value was slightly higher than those described by other authors, who reported a prevalence ranging from 49.8% and 62%[49, 50]. The observed percentage value was particularly high if we consider that this study was conducted on a cohort of patients of Southern Italy for whom geographical latitude is not considered a risk factor in the onset of vitamin D deficiency, as in other countries. However, some factors could explain these controversial findings: firstly, the different cut-off points considered for the vitamin D status. In the study by Ulitsky et. al, in fact, vitamin D deficiency was defined as <20 ng/dL. Secondly, vitamin D status assessment strategy plays an important role. In fact, we choose to enroll our cohort of IBD patients in the months from November to May to minimize the effect of sunlight in vitamin D skin production. Burrelli Scotti et al. separately evaluated vitamin D levels during summertime (June–November) and wintertime spring(December–May). The reported prevalence of vitamin D insufficiency in their cohort of patients during wintertime was about 75%, comparable to that observed in our IBD cohort.

The relation between inflammation and vitamin D status has been also investigated in several studies. We demonstrated that the prevalence of vitamin D insufficiency was significantly higher in IBD patients with elevated fecal calprotectin, whereas no difference was found in patients with elevated CRP. Also Garg et al.[16] previously demonstrated an inverse correlation between 25(OH)VitD and calprotectin in IBD patients (r=-0.37, p=0.003), but not with systemic markers such as CRP. On the contrary, other authors demonstrated that high levels of CRP were associated with an increased risk of Vitamin D deficiency[50].They hypothesized that the inadequate sun exposure or dietary restrictions may lead to the reduced intake of calcium and vitamin D in IBD patients with active disease.

Furthermore, we revealed that disease duration was not an adjunctive risk factor for Vitamin D deficiency, and it is consistent with other studies[50, 51].

We did not find any significant correlation between PTH and 25(OH)VitD values. Several recent studies examined the relationship between PTH and 25(OH)D levels but results have been conflicting[28, 52]. Ferrone et al.[28] demonstrated an inverse association between PTH and 25(OH)VitD ($r=-0.178$, $p < 0.001$) and between PTH and age ($r=0.322$, $p < 0.001$). The first correlation was probably related to the expression by parathyroid cells of megalin (a vitamin D binding protein receptor) that promotes the internalization of 25(OH)VitD, that is then converted to active 1,25(OH)2VitD by CYP27B[28, 52]. We confirmed the direct significant correlation between PTH values and age. The increase in PTH with advancing has been attributed to many mechanisms: reduced intestinal calcium absorption, decreased 1,25(OH)2VitD production[53], increased resistance to target organs[28].

In this study osteoporosis was found in 33.3% of IBD patients (40% CD and 20% UC patients) and osteopenia in 53.3%of IBD patients (52% CD and 55% UC patients).The prevalence of osteopenia and osteoporosis in IBD patients varies significantly depending on the study populations, location, and design, but ranges from 22%-77% and 17%-41%, respectively[9].We described that the prevalence of osteoporosis was higher in CD than UC patients, although the difference did not reach the statistical significance (44 vs 20%, $p=0.09$).This is a controversial issue. Some authors[14, 54] in fact demonstrated a higher frequency of low BMD in CD patients than UC patients. Other authors did not report this difference[12, 55, 56]. Bjarnason et al.[12]demonstrated, in fact, that disease type, location and severity of IBD were not related to BMD reduction. Also Ardizzone et al.[56] showed that the prevalence of osteopenia and osteoporosis were similar in CD and UC patients.

A significant inverse correlation was found between radius and tibia BMD values and disease duration ($r=-0.35$, $p=0.05$ and $r=-0.33$, $p=0.06$, respectively) and between radius and tibia BMD and treatment with biologics ($r=-0.45$, $p=0.002$ and $r=-0.44$, $p=0.003$, respectively).

Most of biologics used in IBD patients are anti-TNFα agents; their effects on BMD are not completely known. In fact, it is not clear whether the effects of anti-TNFα agents on bone are the consequence of a direct interference with the process of bone modeling or if these effects are simply due to a decreased disease activity and subsequent improvement of mineral absorption[57].TNF-α not only plays a central role in the pathogenesis of IBD but is also involved in bone metabolism, promoting bone resorption through regulation of osteoclast activity. However, data on the effect of TNF-α on bone metabolism are still conflicting. Bernstein demonstrated in 46 CD patients a reduced BMD in 43% of patients at the lumbar spine and 46% at the left femur. At 1 year after anti-TNFα agents (infliximab), mean BMD significantly increased at the lumbar spine (p= 0.002), at the femoral trochanter (p= 0.03), and at the femoral neck (p= 0.001).Recently, Maldonado-Pérez et al.[58] did not show in a longitudinal cohort study on 71 IBD patients at 7 years of follow-up, any differences in the incidence of vertebral fracture and value of bone mass between the group of patients treated with anti-TNF-α and the control group which did not receive biological treatment.

In our study cohort we reported a significant inverse correlation between radius and tibia BMD values and treatment with biologics (r=-0.45, p=0.002 and r=-0.44, p=0.003, respectively).Probably in our clinical setting, patients on biologics had a history of severe disease activity or corticosteroid use, and this may negatively impact on bone status and explain the reduced BMD values compared to patients without biologics.

CONCLUSION

This study demonstrated a high prevalence (71%) of vitamin D insufficiency in IBD patients who live in the South of Italy, a country in which inadequate sunlight exposure is not considered a risk factor. Vitamin D insufficiency correlated with the markers of intestinal inflammation. Thus, it would be beneficial for all IBD patients, especially those with active bowel inflammation, to be checked regularly for Vitamin D status.

Also low BMD was common in IBD patients, and it does not correlate to laboratory indices. Treatment with anti-TNF-α does not seem to impact on bone status. The pathogenesis of bone alterations in IBD patients is complex and several factors are involved and other perspective study on large cohort of patients are needed. pQCT could be a useful tool to assess the bone status of IBD patient using very low radiation dosage and could positively impact on clinical management of these patients but larger samples of patients are needed.

REFERENCES

1 Cosnes J, Gower-Rousseau C, Seksik P, Cortot A. Epidemiology and natural history of inflammatory bowel diseases. *Gastroenterology* 2011; **140**(6): 1785-1794 [PMID: 21530745 DOI: 10.1053/j.gastro.2011.01.055]

2 Sheth T, Pitchumoni CS, Das KM. Musculoskeletal manifestations in inflammatory bowel disease: a revisit in search of immunopathophysiological mechanisms. *Journal of clinical gastroenterology* 2014; **48**(4): 308-317 [PMID: 24492406 DOI: 10.1097/mcg.0000000000000067]

3 Rothfuss KS, Stange EF, Herrlinger KR. Extraintestinal manifestations and complications in inflammatory bowel diseases. *World journal of gastroenterology* 2006; **12**(30): 4819-4831 [PMID: 16937463 PMCID: PMC4087615 DOI: 10.3748/wjg.v12.i30.4819]

4 Vavricka SR, Rogler G, Gantenbein C, Spoerri M, Prinz Vavricka M, Navarini AA, French LE, Safroneeva E, Fournier N, Straumann A, Froehlich F, Fried M, Michetti P, Seibold F, Lakatos PL, Peyrin-Biroulet L, Schoepfer AM. Chronological Order of Appearance of Extraintestinal Manifestations Relative to the Time of IBD Diagnosis in the Swiss Inflammatory Bowel Disease Cohort. *Inflammatory bowel diseases* 2015; **21**(8): 1794-1800 [PMID: 26020601 DOI: 10.1097/mib.0000000000000429]

5 Schule S, Rossel JB, Frey D, Biedermann L, Scharl M, Zeitz J, Freitas-Queiroz N, Kuntzen T, Greuter T, Vavricka SR, Rogler G, Misselwitz B. Widely differing screening and treatment practice for osteoporosis in patients with inflammatory bowel diseases in the Swiss IBD cohort study. *Medicine* 2017; **96**(22): e6788 [PMID: 28562531 PMCID: PMC5459696 DOI: 10.1097/md.0000000000006788]

6 Consensus development conference: diagnosis, prophylaxis, and treatment of osteoporosis. *The American journal of medicine* 1993; **94**(6): 646-650 [PMID: 8506892 DOI: 10.1016/0002-9343(93)90218-e]

7 Organization WH. WHO Criteria for Diagnosis of Osteoporosis *http://www4bonehealthorg/education/world-health-organization-criteria-diagnosis-osteoporosis/*

8 Baim S, Binkley N, Bilezikian JP, Kendler DL, Hans DB, Lewiecki EM, Silverman S. Official Positions of the International Society for Clinical Densitometry and executive summary of the 2007 ISCD Position Development Conference. *Journal of clinical densitometry : the official journal of the International Society for Clinical Densitometry* 2008; **11**(1): 75-91 [PMID: 18442754 DOI: 10.1016/j.jocd.2007.12.007]

9 Ali T, Lam D, Bronze MS, Humphrey MB. Osteoporosis in inflammatory bowel disease. *The American journal of medicine* 2009; **122**(7): 599-604 [PMID: 19559158 PMCID: PMC2894700 DOI: 10.1016/j.amjmed.2009.01.022]

10 Hidalgo DF, Boonpheng B, Phemister J, Hidalgo J, Young M. Inflammatory Bowel Disease and Risk of Osteoporotic Fractures: A Meta-Analysis. *Cureus* 2019; **11**(9): e5810 [PMID: 31720198 PMCID: PMC6823062 DOI: 10.7759/cureus.5810]

11 American Gastroenterological Association medical position statement: guidelines on osteoporosis in gastrointestinal diseases. *Gastroenterology* 2003; **124**(3): 791-794 [PMID: 12612916 DOI: 10.1053/gast.2003.50107]

12 Bjarnason I, Macpherson A, Mackintosh C, Buxton-Thomas M, Forgacs I, Moniz C. Reduced bone density in patients with inflammatory bowel disease. *Gut* 1997; **40**(2): 228-233 [PMID: 9071937 PMCID: PMC1027054 DOI: 10.1136/gut.40.2.228]

13 Vazquez MA, Lopez E, Montoya MJ, Giner M, Perez-Temprano R, Perez-Cano R. Vertebral fractures in patients with inflammatory bowel disease compared with a healthy

population: a prospective case-control study. *BMC gastroenterology* 2012; **12**: 47 [PMID: 22584049 PMCID: PMC3438096 DOI: 10.1186/1471-230x-12-47]

14 Jahnsen J, Falch JA, Aadland E, Mowinckel P. Bone mineral density is reduced in patients with Crohn's disease but not in patients with ulcerative colitis: a population based study. *Gut* 1997; **40**(3): 313-319 [PMID: 9135518 PMCID: PMC1027079 DOI: 10.1136/gut.40.3.313]

15 Bernstein CN, Blanchard JF, Leslie W, Wajda A, Yu BN. The incidence of fracture among patients with inflammatory bowel disease. A population-based cohort study. *Annals of internal medicine* 2000; **133**(10): 795-799 [PMID: 11085842 DOI: 10.7326/0003-4819-133-10-200011210-00012]

16 Garg M, Lubel JS, Sparrow MP, Holt SG, Gibson PR. Review article: vitamin D and inflammatory bowel disease--established concepts and future directions. *Alimentary pharmacology & therapeutics* 2012; **36**(4): 324-344 [PMID: 22686333 DOI: 10.1111/j.1365-2036.2012.05181.x]

17 Luo G, Li F, Li X, Wang ZG, Zhang B. TNFalpha and RANKL promote osteoclastogenesis by upregulating RANK via the NFkappaB pathway. *Molecular medicine reports* 2018; **17**(5): 6605-6611 [PMID: 29512766 PMCID: PMC5928634 DOI: 10.3892/mmr.2018.8698]

18 Huang JC, Sakata T, Pfleger LL, Bencsik M, Halloran BP, Bikle DD, Nissenson RA. PTH differentially regulates expression of RANKL and OPG. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research* 2004; **19**(2): 235-244 [PMID: 14969393 DOI: 10.1359/jbmr.0301226]

19 Tabatabaeizadeh SA, Tafazoli N, Ferns GA, Avan A, Ghayour-Mobarhan M. Vitamin D, the gut microbiome and inflammatory bowel disease. *Journal of research in medical sciences : the official journal of Isfahan University of Medical Sciences* 2018; **23**: 75 [PMID: 30181757 PMCID: PMC6116667 DOI: 10.4103/jrms.JRMS_606_17]

20 Del Pinto R, Pietropaoli D, Chandar AK, Ferri C, Cominelli F. Association Between Inflammatory Bowel Disease and Vitamin D Deficiency: A Systematic Review and Meta-analysis. *Inflammatory bowel diseases* 2015; **21**(11): 2708-2717 [PMID: 26348447 PMCID: PMC4615394 DOI: 10.1097/mib.0000000000000546]

21 Stamp TC, Haddad JG, Twigg CA. Comparison of oral 25-hydroxycholecalciferol, vitamin D, and ultraviolet light as determinants of circulating 25-hydroxyvitamin D. *Lancet (London, England)* 1977; **1**(8026): 1341-1343 [PMID: 69059 DOI: 10.1016/s0140-6736(77)92553-3]

22 Calvo MS, Whiting SJ, Barton CN. Vitamin D intake: a global perspective of current status. *The Journal of nutrition* 2005; **135**(2): 310-316 [PMID: 15671233 DOI: 10.1093/jn/135.2.310]

23 Roth DE, Abrams SA, Aloia J, Bergeron G, Bourassa MW, Brown KH, Calvo MS, Cashman KD, Combs G, De-Regil LM, Jefferds ME, Jones KS, Kapner H, Martineau AR, Neufeld LM, Schleicher RL, Thacher TD, Whiting SJ. Global prevalence and disease burden of vitamin D deficiency: a roadmap for action in low- and middle-income countries. *Annals of the New York Academy of Sciences* 2018; **1430**(1): 44-79 [PMID: 30225965 DOI: 10.1111/nyas.13968]

24 Liu NQ, Hewison M. Vitamin D, the placenta and pregnancy. *Archives of biochemistry and biophysics* 2012; **523**(1): 37-47 [PMID: 22155151 DOI: 10.1016/j.abb.2011.11.018]

25 Omdahl JL, Morris HA, May BK. Hydroxylase enzymes of the vitamin D pathway: expression, function, and regulation. *Annual review of nutrition* 2002; **22**: 139-166 [PMID: 12055341 DOI: 10.1146/annurev.nutr.22.120501.150216]

26 Liu N, Nguyen L, Chun RF, Lagishetty V, Ren S, Wu S, Hollis B, DeLuca HF, Adams JS, Hewison M. Altered endocrine and autocrine metabolism of vitamin D in a mouse model

of gastrointestinal inflammation. *Endocrinology* 2008; **149**(10): 4799-4808 [PMID: 18535110 PMCID: PMC2582909 DOI: 10.1210/en.2008-0060]

27 Mukherji A, Kobiita A, Ye T, Chambon P. Homeostasis in intestinal epithelium is orchestrated by the circadian clock and microbiota cues transduced by TLRs. *Cell* 2013; **153**(4): 812-827 [PMID: 23663780 DOI: 10.1016/j.cell.2013.04.020]

28 Ferrone F, Pepe J, Danese VC, Fassino V, Cecchetti V, De Lucia F, Biamonte F, Colangelo L, Ferrazza G, Panzini E, Scillitani A, Nieddu L, Blocki F, Rao SD, Minisola S, Cipriani C. The relative influence of serum ionized calcium and 25-hydroxyvitamin D in regulating PTH secretion in healthy subjects. *Bone* 2019; **125**: 200-206 [PMID: 31129357 DOI: 10.1016/j.bone.2019.05.029]

29 Zerwekh JE. Blood biomarkers of vitamin D status. *The American journal of clinical nutrition* 2008; **87**(4): 1087S-1091S [PMID: 18400739 DOI: 10.1093/ajcn/87.4.1087S]

30 Spiro A, Buttriss JL. Vitamin D: An overview of vitamin D status and intake in Europe. *Nutrition bulletin* 2014; **39**(4): 322-350 [PMID: 25635171 PMCID: PMC4288313 DOI: 10.1111/nbu.12108]

31 Need AG, Nordin BE. Misconceptions - vitamin D insufficiency causes malabsorption of calcium. *Bone* 2008; **42**(6): 1021-1024 [PMID: 18343737 DOI: 10.1016/j.bone.2008.01.012]

32 Lips P. Which circulating level of 25-hydroxyvitamin D is appropriate? *The Journal of steroid biochemistry and molecular biology* 2004; **89-90**(1-5): 611-614 [PMID: 15225848 DOI: 10.1016/j.jsbmb.2004.03.040]

33 Baeke F, Takiishi T, Korf H, Gysemans C, Mathieu C. Vitamin D: modulator of the immune system. *Current opinion in pharmacology* 2010; **10**(4): 482-496 [PMID: 20427238 DOI: 10.1016/j.coph.2010.04.001]

34 van Etten E, Mathieu C. Immunoregulation by 1,25-dihydroxyvitamin D3: basic concepts. *The Journal of steroid biochemistry and molecular biology* 2005; **97**(1-2): 93-101 [PMID: 16046118 DOI: 10.1016/j.jsbmb.2005.06.002]

35 Prietl B, Treiber G, Pieber TR, Amrein K. Vitamin D and immune function. *Nutrients* 2013; **5**(7): 2502-2521 [PMID: 23857223 PMCID: PMC3738984 DOI: 10.3390/nu5072502]

36 Haussler MR, Jurutka PW, Mizwicki M, Norman AW. Vitamin D receptor (VDR)-mediated actions of 1alpha,25(OH)(2)vitamin D(3): genomic and non-genomic mechanisms. *Best practice & research Clinical endocrinology & metabolism* 2011; **25**(4): 543-559 [PMID: 21872797 DOI: 10.1016/j.beem.2011.05.010]

37 Liu W, Chen Y, Golan MA, Annunziata ML, Du J, Dougherty U, Kong J, Musch M, Huang Y, Pekow J, Zheng C, Bissonnette M, Hanauer SB, Li YC. Intestinal epithelial vitamin D receptor signaling inhibits experimental colitis. *The Journal of clinical investigation* 2013; **123**(9): 3983-3996 [PMID: 23945234 PMCID: PMC3754241 DOI: 10.1172/jci65842]

38 Wu S, Zhang YG, Lu R, Xia Y, Zhou D, Petrof EO, Claud EC, Chen D, Chang EB, Carmeliet G, Sun J. Intestinal epithelial vitamin D receptor deletion leads to defective autophagy in colitis. *Gut* 2015; **64**(7): 1082-1094 [PMID: 25080448 PMCID: PMC4312277 DOI: 10.1136/gutjnl-2014-307436]

39 Parizadeh SM, Jafarzadeh-Esfehani R, Hassanian SM, Mottaghi-Moghaddam A, Ghazaghi A, Ghandehari M, Alizade-Noghani M, Khazaei M, Ghayour-Mobarhan M, Ferns GA, Parizadeh SMR, Avan A. Vitamin D in inflammatory bowel disease: From biology to clinical implications. *Complementary therapies in medicine* 2019; **47**: 102189 [PMID: 31779998 DOI: 10.1016/j.ctim.2019.08.023]

40 Jiang H, Robinson DL, Yates CJ, Lee PVS, Wark JD. Peripheral quantitative computed tomography (pQCT)-based finite element analysis provides enhanced diagnostic performance in identifying non-vertebral fracture patients compared with dual-energy X-ray absorptiometry. *Osteoporosis international : a journal established as result of cooperation*

between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 2020; **31**(1): 141-151 [PMID: 31720708 DOI: 10.1007/s00198-019-05213-1]

41 Kontulainen SA, Johnston JD, Liu D, Leung C, Oxland TR, McKay HA. Strength indices from pQCT imaging predict up to 85% of variance in bone failure properties at tibial epiphysis and diaphysis. *Journal of musculoskeletal & neuronal interactions* 2008; **8**(4): 401-409 [PMID: 19147978]

42 Damilakis J, Adams JE, Guglielmi G, Link TM. Radiation exposure in X-ray-based imaging techniques used in osteoporosis. *European radiology* 2010; **20**(11): 2707-2714 [PMID: 20559834 PMCID: PMC2948153 DOI: 10.1007/s00330-010-1845-0]

43 Vlok J, Simm PJ, Lycett K, Clifford SA, Grobler AC, Lange K, Ismail N, Osborn W, Wake M. pQCT bone geometry and strength: population epidemiology and concordance in Australian children aged 11-12 years and their parents. *BMJ open* 2019; **9**(Suppl 3): 63-74 [PMID: 31273017 PMCID: PMC6624036 DOI: 10.1136/bmjopen-2018-022400]

44 Adams JE. Quantitative computed tomography. *European journal of radiology* 2009; **71**(3): 415-424 [PMID: 19682815 DOI: 10.1016/j.ejrad.2009.04.074]

45 Stagi S, Cavalli L, Cavalli T, de Martino M, Brandi ML. Peripheral quantitative computed tomography (pQCT) for the assessment of bone strength in most of bone affecting conditions in developmental age: a review. *Italian journal of pediatrics* 2016; **42**(1): 88 [PMID: 27670687 PMCID: PMC5037897 DOI: 10.1186/s13052-016-0297-9]

46 Engelke K, Adams JE, Armbrecht G, Augat P, Bogado CE, Bouxsein ML, Felsenberg D, Ito M, Prevrhal S, Hans DB, Lewiecki EM. Clinical use of quantitative computed tomography and peripheral quantitative computed tomography in the management of osteoporosis in adults: the 2007 ISCD Official Positions. *Journal of clinical densitometry : the official journal of the International Society for Clinical Densitometry* 2008; **11**(1): 123-162 [PMID: 18442757 DOI: 10.1016/j.jocd.2007.12.010]

47 Kristensen V, Klepp P, Cvancarova M, Roseth A, Skar V, Moum B. Prediction of endoscopic disease activity in ulcerative colitis by two different assays for fecal calprotectin. *Journal of Crohn's & colitis* 2015; **9**(2): 164-169 [PMID: 25518057 DOI: 10.1093/ecco-jcc/jju015]

48 D'Haens G, Sandborn WJ, Feagan BG, Geboes K, Hanauer SB, Irvine EJ, Lemann M, Marteau P, Rutgeerts P, Scholmerich J, Sutherland LR. A review of activity indices and efficacy end points for clinical trials of medical therapy in adults with ulcerative colitis. *Gastroenterology* 2007; **132**(2): 763-786 [PMID: 17258735 DOI: 10.1053/j.gastro.2006.12.038]

49 Ulitsky A, Ananthakrishnan AN, Naik A, Skaros S, Zadvornova Y, Binion DG, Issa M. Vitamin D deficiency in patients with inflammatory bowel disease: association with disease activity and quality of life. *JPEN Journal of parenteral and enteral nutrition* 2011; **35**(3): 308-316 [PMID: 21527593 DOI: 10.1177/0148607110381267]

50 Burrelli Scotti G, Afferri MT, De Carolis A, Vaiarello V, Fassino V, Ferrone F, Minisola S, Nieddu L, Vernia P. Factors affecting vitamin D deficiency in active inflammatory bowel diseases. *Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver* 2019; **51**(5): 657-662 [PMID: 30587439 DOI: 10.1016/j.dld.2018.11.036]

51 Kabbani TA, Koutroubakis IE, Schoen RE, Ramos-Rivers C, Shah N, Swoger J, Regueiro M, Barrie A, Schwartz M, Hashash JG, Baidoo L, Dunn MA, Binion DG. Association of Vitamin D Level With Clinical Status in Inflammatory Bowel Disease: A 5-Year Longitudinal Study. *The American journal of gastroenterology* 2016; **111**(5): 712-719 [PMID: 26952579 DOI: 10.1038/ajg.2016.53]

52 Vieth R, El-Hajj Fuleihan G. There is no lower threshold level for parathyroid hormone as 25-hydroxyvitamin D concentrations increase. *Journal of endocrinological investigation* 2005; **28**(2): 183-186 [PMID: 15887868 DOI: 10.1007/bf03345365]

53 Nordin BE, Need AG, Morris HA, O'Loughlin PD, Horowitz M. Effect of age on calcium absorption in postmenopausal women. *The American journal of clinical nutrition* 2004; **80**(4): 998-1002 [PMID: 15447911 DOI: 10.1093/ajcn/80.4.998]

54 Ezzat Y, Hamdy K. The frequency of low bone mineral density and its associated risk factors in patients with inflammatory bowel diseases. *International journal of rheumatic diseases* 2010; **13**(3): 259-265 [PMID: 20704624 DOI: 10.1111/j.1756-185X.2010.01542.x]

55 Lima CA, Lyra AC, Mendes CMC, Lopes MB, Coqueiro FG, Rocha R, Santana GO. Bone mineral density and inflammatory bowel disease severity. *Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas* 2017; **50**(12): e6374 [PMID: 29069227 PMCID: PMC5649869 DOI: 10.1590/1414-431x20176374]

56 Ardizzone S, Bollani S, Bettica P, Bevilacqua M, Molteni P, Bianchi Porro G. Altered bone metabolism in inflammatory bowel disease: there is a difference between Crohn's disease and ulcerative colitis. *Journal of internal medicine* 2000; **247**(1): 63-70 [PMID: 10672132 DOI: 10.1046/j.1365-2796.2000.00582.x]

57 Sgambato D, Gimigliano F, De Musis C, Moretti A, Toro G, Ferrante E, Miranda A, De Mauro D, Romano L, Iolascon G, Romano M. Bone alterations in inflammatory bowel diseases. *World journal of clinical cases* 2019; **7**(15): 1908-1925 [PMID: 31423424 PMCID: PMC6695530 DOI: 10.12998/wjcc.v7.i15.1908]

58 Maldonado-Perez MB, Castro-Laria L, Caunedo-Alvarez A, Montoya-Garcia MJ, Giner-Garcia M, Arguelles-Arias F, Romero-Gomez M, Vazquez-Gamez MA. Does the Antitumor Necrosis Factor-alpha Therapy Decrease the Vertebral Fractures Occurrence in Inflammatory Bowel Disease? *Journal of clinical densitometry : the official journal of the International Society for Clinical Densitometry* 2019; **22**(2): 195-202 [PMID: 30205986 DOI: 10.1016/j.jocd.2018.07.010].