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Bone mineral metabolism in inflammatory bowel disease

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Summary

Ulcerous colitis and Crohn's disease constitute the principal components of inflammatory bowel disease (IBD). Osteoporosis is a well-known complication of IBD presenting a multifactorial etiology, although the importance of the inflammatory process in itself seems to be ever greater. The end of this article reviews the existing data on bone mineral metabolism in these patients, both in relation to the prevalence of the loss of bone mass, as in the situation of the markers for bone turnover, the factors involved, as well as the risk factors. In this way, it is intended to shine a light on the importance of osteoporosis in IBD.

Key words: *Ulcerous colitis, Crohn's disease, Inflammatory bowel disease, Bone mineral metabolism.*

Introduction

Inflammatory bowel disease (IBD) is fundamentally comprised of two processes: ulcerous colitis (UC) and Crohn's disease (CD). Although tending to be considered in a combined form, the pathogeny of the disorders is not yet known, each possessing distinct clinical characteristics and histologies. The objective of this work is to carry out a review of the literature on the current knowledge with respect to bone mineral metabolism in patients with IBD.

UC consists of a non-transmural and recurrent inflammatory process which is limited to the colon, and which can manifest itself as proctitis, left-sided colitis, or pancolitis. Typical patients present with bloody diarrhoea (often at night and after meals), accompanied by pus, mucus or both, along with a colic-type abdominal pain, the more infrequent serious symptoms being left-sided colitis and proctitis. The diagnosis is clinical and is confirmed by means of endoscopies and histology.

CD is an inflammatory process, transmural and recurring, of the gastrointestinal mucosa, which can affect any part of the digestive tract, from the mouth to the anus. Typical presentations include segmentary affection of the gastrointestinal tract, with healthy areas of intestine among affected segments, as well as the development of evolving complications among which are included fistulas, abscesses and stenosis. Its diagnosis is based on the combination of clinical data, analysis, X-rays, endoscopies and anatomopathologies¹.

Osteoporosis is a well-known complication of IBD in general. The presence of bone demineralisation and osteoporosis in IBD was reported for the first time by Genant et al in 1976². Transversal studies calculated the prevalence of low bone mass at 30% of patients. In general, the average BMD would be 10% less than in the general population³, but, since they are distinct entities, it seems reasonable to make a differential evaluation.

Bone mineral density in IBD

There are numerous studies which have permitted the documentation of the presence of loss of bone which varies between 18% and 42% (Table 1)³⁻¹². The wide variation in the results obtained in these studies could be influenced by diverse factors, the method and the part of the skeleton where the measurements are carried out, and the selection of the patients, among others. However, these data have clearly shown that those patients with IBD have lower bone mass when compared with a population of healthy controls^{7,13-15}.

The loss of bone seems to be more acute in CD than in UC. A transversal study found a reduction of 7.3% in average BMD in patients with Crohn's disease in relation to those with UC and with healthy subjects¹³. Another found a prevalence of osteoporosis in CD of 59% against 43% in UC¹⁶. However, in the study by Adizzone S et al, despite finding a prevalence of osteopenia of 55% and of osteoporosis of 37% in CD, against 67% of osteopenia and 18% of osteoporosis respectively in UC, the differences were not statistically significant¹⁷. The same happens in other studies^{3,10,18-21}; there is another group that has reported the contrary²¹. On the other hand, although women and men become affected equally, the change can become more serious in males; what's more, although it has not been possible to establish a relationship between the intensity of the loss of bone mass and the duration of the disease, jejunal affection and ileal resection can bring greater risk²².

Markers for bone turnover in IBD

The existing studies in relation to levels of markers for bone turnover and IBD, both in formation and in resorption, have not brought conclusive results on whether or not they found changes with respect to the healthy population. This confusion is due in part to the heterogeneity of the studies, many of which were transversal, with some patients in an active phase and others inactive, not always comparing with healthy controls, and under different treatments, such as glucocorticosteroids (GC) or immunosuppressors, which can have a greater influence over the bone mineral markers than that caused by the disease itself. What's more, there is no uniformity in the markers for formation and measured bone turnover.

The study by Gilman et al, with 47 patients with CD, 25 with UC and their respective healthy controls, found a significant increase in bone alkaline phosphatase (BALP) in the blood, and amino-terminal telopeptide of collagen type 1 (NTX) in the urine, in IBD patients compared with healthy controls, while the levels of osteocalcine (OC) was found to be significantly diminished²³. Another study by Pollack et al, found in 63 patients with CD and 41 with UC, low levels of OC in 7% of those patients, while those levels of NTX were found to have risen in 25% of patients²⁴. Another group found, in a total of 72 patients with IBD, a decrease in levels of OC and an increase in levels

of NTX – the latter negatively correlated with bone mass in the lumbar spinal column and femoral neck²⁵. Arizzone et al found a significant increase in levels of OC and of carboxy-terminal telopeptide of collagen type 1 (CTX) in the 40 patients with UC they evaluated, but not in those 51 with CD¹⁷. On a different tack, in a study only of patients with Crohn's disease, Robinson et al only found elevated levels of urinary deoxypyridoline (DPD) in comparison with 28 healthy controls, but found no differences in levels of OC and CTX²⁶. Neither were differences found in levels of OC in 150 patients with IBD compared with 73 healthy controls, although they did find higher levels of CTX. In addition, in this same study, both patients with CD and with UC who were found to be active showed levels of OC and CTX higher than those were not. In the case of patients with UC, the levels of CTX were found to be higher in those affected by pancolitis as opposed to those who only had left-sided colitis²⁷. On the other hand, Miheller et al, in a study of 23 patients with UC, 26 with CD and 46 healthy controls, found a significant elevation in levels of CTX in both groups with respect to the controls²⁸. In a wider study with 258 patients, only with CD, the levels of urinary DPD and BALP in the blood were found to be in the normal range and there were no difference between those presenting with osteoporosis and those who did not; in terms of the levels of NTX, although these were normal they were significantly higher in those with osteoporosis²⁹.

Finally, although the impact of changes in the markers for bone turnover in IBD on the risk of fracture has not been studied, there is evidence that the increased levels of markers for bone resorption in IBD is associated with the loss of bone mass. Thus, Pollack et al, using an analysis by quartiles, show that the patients with IBD who present with highest urinary concentrations of NTX show a greater loss of bone mass in the lumbar spinal column in comparison with those presenting with lower levels in the urine. The raising of the levels of markers for bone resorption is recognised as a risk factor for fractures, at least in menopausal women^{16,30}.

Risk of fracture in IBD

The consequence of osteoporosis is the development of fractures. However, the increase in the risk of fractures in IBD with respect to the general population is not well established. Klaus et al, in a study developed in Germany, found a high prevalence (22%) of osteoporotic vertebral fractures in 156 patients with CD and Z-score < -1, including in patients younger than 30 years old³¹. In a cohort of 6027 patients in Canada, compared with 60270 controls, Bernstein et al found an increased total risk of fracture of 47%, being higher for vertebral fractures (54%), with no difference between women and men, nor between CD and UC; there was indeed an increased risk of fracture in males with UC in comparison with the women³². On the other hand, Vestergaard et al

Table 1. Prevalence of osteopenia and osteoporosis in IBD

	Population	T-score/ Z-score	Prevalence Osteopenia	Prevalence Osteoporosis
Abitbol et al	CD and UC	Z-score	43%	13,09%
Bjarnasson et al	CD and UC	T-score	78%	29%
Clements et al	CD, others	Z-score	-	30,7%
Compston et al	CD and UC	Z-score	-	30,6%
Gokhale et al	CD and UC	Z-score	-	7%
Martínez et al	CD and UC	-	49,3%	27,4%
Pigot et al	CD and UC	Z-score	-	23%
Pollak et al	CD and UC	T-score	34%	42%
Schoon et al	CD and UC	Z-score	1,5%	26%
Schulte et al	CD and UC	Z-score	-	11%
Siffledeen et al	CD	-	51%	13%
Silvennoinen et al	CD and UC	Z-score	-	5,9%
Sinnot et al	CD	Z-score	-	27%
Staun et al	CD	Z-score	-	20%

found an increased total risk of fracture in women with CD (RR 2.5), but not in males (RR 0.6), nor in patients with UC (RR 1.1), out of a total of 383 patients with Crohn's, 434 with UC and 635 controls in Denmark³³. Although there are discrepancies in this respect. For example, Loftus et al did not detect an increase in the incidence of fractures in a total of 238 patients with CD in the USA, with respect to the control population, with an RR near to 1 in all their findings³⁴.

In general it is accepted that the increased risk of fracture is modest, and comparable between patients with CD and UC. For all types of fracture the relative risk for CD is 1.3 and 1.2 for UC, being somewhat higher in the case of hip fractures (1.5 for CD and 1.4 for UC). Since the majority of studies are based on reports of fractures it is possible that the prevalence of vertebral fractures (and of fractures in general) might be underestimated. In fact the only studies which have used X-ray quantitative morphometry of the spinal column found a very high prevalence of vertebral fractures (14-25%)^{35,36}. Also found were various risk factors for osteoporotic fractures in IBD, such as low BMD, age, use of GC and the activity of the patient. BMD, in turn can be seen as a negative influence on for women at a young age when diagnosed, for the male sex, low body mass index (BMI), duration of the disease, the previous presence of Ileal resection, accumulated dose of GC, reduced physical activity and smoking³⁵. It is important to underline the fact that not all fractures (especially vertebral) are symptomatic and/or vertebral defor-

mities, whose risk is also higher among the population with IBD³¹, and whose presence can be used to identify patients with highest risk of fractures so as to focus prevention.

Pathogenesis of osteoporosis in IBD

The etiology of osteoporosis in IBD is multifactorial (Table 2). The factors which can influence its development can be divided into: a) factors common to those of the rest of the population (low weight, family antecedence, age, female sex, menopause, tobacco,...) and; b) specific factors such as genetic influence, deficiency in vitamins D and K, treatment with GC, hormonal changes and the inflammatory process in itself^{29,33,37,38}.

Genetic factors

There are a number of genes which influence the functioning of the osteoblasts, and it is possible that protein 5 related to the receptor LDL (LRP5) is one of these. Thus, a range of findings have shown that mutations of the gene LRP5, which results in a loss of functionality, gives rise to bone defects similar to those seen in the syndrome osteoporosis-pseudoglioma, supporting the fundamental role of this gene in the integrity of the skeleton. A range of polymorphisms have been described (such as rs491347, rs 1784235, and A1330 V) which are associated with a greater susceptibility to the development of osteoporosis and fractures in humans, supporting therefore the possible role of gene LRP5 in the acquisition of peak bone mass.

Table 2. Principal risk factors for osteoporosis in IBD

<ul style="list-style-type: none"> - Advanced age - Taking corticoids - Malnutrition - Low body mass index - Poor absorption of vitamin D, calcium and vitamin K - Immobilisation - Antecedence of fragility-related fracture - Hypogonadism - Tobacco smoking - Chronic inflammation

On the other hand, the identification of receptors for vitamin D (VDR) in peripheral mononuclear blood cells has boosted an interest in this vitamin as a possible regulator of the immune system. Vitamin D deficit has been related to a range of diseases, among which is osteoporosis mediated by an immune mechanism, as that which appears to occur in IBD. There are various polymorphisms of the gene for VDR associated with the development of osteoporosis which have been studied, above all Bsm I.

Another important candidate for the genetic susceptibility for osteoporosis is the gene coded as TG β -1. Various polymorphisms of this gene have been identified, and a number of works suggest that certain allelic variants of TG β -1 could regulate BMD and susceptibility to osteoporotic fracture.

The list of genes studied is very extensive, such as, for example CYP17 (17-hydroxylase), CYPB1 (cytochrome P450), DBP (binding protein for vitamin D), GH1 (growth hormone 1), GnRH (gonadotropine-releasing hormone), IGF-II (growth factor similar to insulin type II), among many others. However, the relationship of these genes to inflammation, as a possible mechanism for osteoporosis in IBD, is not yet completely clear, but could perform a modulating role in the susceptibility to the development of a metabolic osteopathy in these patients³⁸.

Vitamin D deficiency

A study carried out by Driscoll Jr et al with 82 patients with CD, saw that up to 65% of them presented with low levels of 25-hydroxy-vitamin D (25OHD₃), and 25 of them had a deficiency (<10 ng/ml). The levels were less if there had been a previous resection of the ileum. A bone biopsy was carried out in 9 patients, with 6 of them showing osteomalacia and 3 osteoporosis¹¹. More recently a study with 242 Crohn's patients found that 8% of them showed levels of 25OHD₃ lower than 25 nmol/L, and in 22% levels lower than 40 nmol/L. However, while no differences were detected in relation to BMD in those presenting with normal levels of 25OHD₃, there was indeed biochemical evidence of metabolic bone disease³⁹. Jahnsen et al found levels of 25OHD₃ lower than 30 nmol/L in 27% of 60 patients with CD and in 15% of 60 patients with UC, the patients with Crohn's showing concentrations significantly

lower than those with UC. The levels of 25OHD₃, however, were not related to BMD in any of the findings from skeletal measurements⁴⁰. In the study of Gilman et al, the patients with CD showed levels of 25OHD₃ significantly lower in comparison with the healthy controls, with 19% being lower than 40 nmol/L; in the case of the patients with UC, these also showed levels significantly lower in comparison with the healthy controls, there being levels of 25OHD₃ below 40 nmol/L in 7% of patients²³. Duggan et al and McCarthy et al, also found in patients with CD levels of 25OHD₃ lower than those of healthy controls, with a prevalence of low levels of 7% and 18% respectively^{41,42}. Other authors have also found high levels of 25OHD₃ deficit^{43,44}.

The deficit in 25OHD₃ is due in part to the low level of ingestion of milk products (which are enriched with these vitamins in many countries), but also to their poor absorption. In addition, due to the limitations which this disease brings in its serious state, exposure to the sun for these patients is often lacking (it should be noted that the exposure of the skin to light is the main source of the production of vitamin D). However, many patients with normal levels of 25OHD₃ have osteoporosis, which needs to be explained by other causes.

Vitamin K deficiency

Vitamin K is a necessary cofactor for the carboxylation of the Gla proteins (gamma carboxyglutamate) by the osteoblasts, among which are found osteocalcin and the protein Gla of the matrix, both with a regulatory role in bone mineralization and remodelling. A range of studies have brought evidence of the relationship between a deficit status of vitamin K and bone mineralization. Various works have found vitamin K deficiency status in patients with IBD and a relationship with loss of bone mass. One of the possible causes of this state of deficit could be the taking of antibiotics, which alters the intestinal flora, responsible in good part for the daily requirements of vitamin K^{23,41,44}.

Treatment with glucocorticosteroids

Many patients require GC for the control of their disease. These inhibit the formation of bone, increase its resorption, reduce the absorption of calcium and increase its renal excretion.

The loss of bone mass is more frequent in patients with IBD who have received treatment with GC, above all in the initial months of treatment⁴⁵. A study reported that the incidence of osteopenia was approximately double in patients who had received treatment with GC with respect to those who had not (52% as opposed to 28%)¹⁸. In general, it is accepted that BMD in patients with IBD correlates inversely with the accumulated dose over their lifetimes^{3,13,18,19}. Some studies suggest, what's more, that the loss of bone mass associated with the use of GC is higher in women than in men¹⁹, which means that it is more evident in patients with CD than with UC¹³. Notwithstanding

this, it is difficult to distinguish the degree of the contribution of the use of these drugs to the bone in comparison with the activity of the disease, since a high level of activity and a high degree of inflammation are indications for the use of steroids. Whilst prednisone, methylprednisolon and prednisolon have a systemic action and constitute one of the main factors which contribute to osteoporosis in IBD, budesonide, a corticoid which acts locally with low systemic bioavailability, is coming to be used increasingly in the treatment of IBD, due to its lack of systemic effect, including the loss of bone mass⁴⁶.

Alterations in sexual hormones

Amenorrhea and hypergonadism are frequent in patients with IBD, probably as a consequence of the inhibitory effects of the inflammation and the steroid treatment on the pituitary function⁴⁷.

In men the GCs reduce concentrations of testosterone by at least a third, by inhibiting the secretion of gonadotropines, a known cause of osteoporosis⁴⁸.

Inflammatory activity of the disease

In some patients one sees low bone mass without there being any of the factors indicated. In some of these cases it even is noticed at the moment of diagnosis, without having previously received any type of treatment⁴⁹. In addition, osteoporosis is frequent in patients with IBD who take GC in low doses and who have normal levels of vitamin D¹⁰. Therefore, it is thought that the disease itself could provoke a reduction in bone mass, perhaps measured by an increase in the production of citoquines in the intestine produced by the T lymphocytes and other inflammatory cells such as the macrophages, which could lead to the activation of the osteoclasts, without a compensatory increase in bone formation^{10,18,49,50}. Some of these citoquines implicated could be tumor necrosis factor α (TNF- α), interleukin 6 (IL-6), interleukin 1 (IL-1) and interleukin 2 (IL-2)⁵⁰. Within the mononuclear cells the fundamental transcription factor is nuclear factor kappa- β (NF κ β), which regulates the transcription of IL-1 and IL-6, among others, as well as regulating the expression of other pro-inflammatory genes such as TNF- α and adhesion molecules⁴⁶.

The levels of a range of osteoclast activators with pro-inflammatory activity (including IL-1, IL-6 and TNF- α) are found to be higher in IBD. There is evidence which supports the role of IL-6 in osteoporosis resulting from the loss of male and female steroids. In addition, genetic variants of IL-6 and the antagonist of the receptor of IL-1 have been identified, which are correlated with the clinical course of IBD and the degree of loss of bone mass⁴⁶. On the other hand, it is known that the models of colitis in mice deficient in IL-2 develop colitis and osteopenia⁵¹.

The system constituted for binding the receptor activator of NF κ β (RANKL) and osteoprotegerina (OPG) represents a potential nexus in the

union between inflammation and bone homeostasis, and also an example of osteopenia brought about by inflammation, as occurs in IBD. The equilibrium between RANKL and OPG is of vital importance in osteoclastogenesis, by way of the interaction of RANK, on the surface of the osteoclasts, with its ligand RANKL inducing osteoclastogenesis, whilst OPG proceeding from the osteoblasts blocks this interaction, inhibiting the formation of osteoclasts. The pro-inflammatory citoquines induce the formation of RANKL, and the activated T lymphocytes can activate osteoclastogenesis directly through the RANKL, with the consequent loss of bone mass³⁸. Recent studies suggest that the changes in equilibrium between RANKL and OPG could be responsible for the loss of bone mass in patients with IBD. Thus, plasmatic levels of OPG and RANKL correlate with BMD and the treatment for IBD⁵². In one study, it is seen that the plasmatic levels of OPG are found to be 2.4 times higher in CD and 1.9 times higher in UC. The elevated levels of OPG could represent a continual homeostatic response, an attempt to oppose the osteoclastogenesis induced by RANKL or TNF- α , and thus maintain normal bone mass⁵³.

In relation to the effect of the inflammatory activity of the disease on bone mass. Reffitt et al studied a cohort of 137 patients with IBD and found that their bone mass was higher the greater time they were in remission. In addition, the patients who took azatioprine and were in remission had greater bone mass⁵⁴. In this area there are various studies which have tried to assess the possible effect of anti-TNF drugs (approved for treatment of moderate to serious cases which do not respond to conventional treatment), specifically infliximab, taken to control the inflammatory process, can have on bone metabolism.

Franchimont et al analysed the evolution of bone metabolism in 71 patients with CD treated with infliximab. Baseline markers for formation and resorption were measured and then at 8 weeks on completion of the treatment (a single dose in luminal forms and 3 doses in fistular forms). An increase was seen in the markers for formation (with a median of change of 14-51% according to the marker) and a decrease in that of resorption (median of change 11%). The authors found a clinically significant increase (at least 30%) in the markers for bone formation in 30-61% of the patients (depending on the marker) and a clinically significant decrease (at least 30%) in the marker for resorption in 38% of the patients. No significant association with any of the demographic parameters nor clinical measures (including the clinical or biological response to infliximab), were found. These results, however, were not equal in all patients, in such a way that only 8.5% showed an increase in the markers for formation together with a decrease in those of resorption. The authors conclude that treatment with infliximab produces a rapid improvement in the profile of the markers for bone turnover, independently of the clinical response to it, although the long term effects on

the risk of fracture are to be determined³⁷. In the same vein, another study with 24 patients with active CD treated with a single dose of infliximab found a significant increase in markers for bone formation (BALP and OC) during the 4 months they were followed; whereas the decrease in the marker for bone resorption measured (NTX) was not statistically significant, as neither were the differences found between the responders and non-responders⁵⁵. Abreu et al also found an association between treatment with a dose of infliximab and an increase in the marker for bone absorption (BALP) measured at four weeks, independently from the response to it or the taking of GC; no changes were found with respect to the marker for resorption (NTX)⁵⁶. More recently a study with 103 infant patients with CD, across 54 weeks of treatment with infliximab, found an increase in markers for formation (BALP, N-terminal propeptide of collagen type 1) which was associated with an increase in lineal growth, and which the authors consider would go in favour of blocking the effects of TNF- α on the osteoblasts. Similarly, they also found an increase in markers for bone resorption (CTX, DPD) which the authors justify as reflecting the link between formation and resorption and the increase in lineal growth⁵⁷.

Bernstein et al evaluate the change in bone mass in the femoral neck and lumbar spinal column in 46 patients with CD treated with infliximab as maintenance. There was a gain in bone mineral density at all the points of measurement (2.4% in the lumbar spinal column, 2.8% in the trochanter and 2.6% in the femoral neck), which happened in spite of treatment with GC (28%). Neither was there found any correlation with the taking of calcium and vitamin D supplements, or with the changes in the PCR. Possibly this fact is due to a direct action of the anti-TNF agent on osteoclastogenesis, through the activation of NF κ B, promoting apoptosis by means of caspase⁵⁸. Another retrospective study with 45 patients with Crohn's (15 treated with infliximab and 30 controls), found and improvement in lumbar bone mass in the long term (measured using two DXAs separated by at least a year), independently of their nutritional state or of taking GC⁵⁹.

Finally, Miheller et al, assessed the possible effects of treatment with infliximab in 29 patients with CD on parameters of bone formation and resorption, and their possible relationship to changes in the OPG/RANKL/RANK system. These authors discovered an increase in the parameter of formation measured (OC) and a decrease in OPG (more in responders), at the same time as a decrease in the parameter of resorption measured (CTX) and an increase in RANKL, while the changes in these were not statistically significant. The authors conclude that the high levels of OPG could reflect a counter-regulatory response to factors such as inflammatory citoquines, or could indicate an activation of the T lymphocytes, thus justifying its diminution by the anti-inflammatory action of infliximab⁶⁰.

To date, there are no studies in the literature which have evaluated the affect of adalimumab on the bone metabolism of patients with CD (this drug is not yet approved for CD). However, a study with 50 patients with rheumatoid arthritis treated with adalimumab did not find changes in BMD (neither in the lumbar spinal column not in the femoral neck) over the course of a year, the authors concluding that the blocking of TNF- α could stop the loss of bone mass⁶¹.

Conclusions

Patients with IBD show an increased risk of osteopenia and osteoporosis, and epidemiological studies have shown a high prevalence of low bone mass in these patients. Even though osteoporosis in these patients, which seems to be of high turnover, presents a multifactorial etiology, the inflammatory process which takes place in the intestines has now acquired a preponderant role. A better knowledge of the basic processes which take place at the level of bone, in this context of intestinal inflammation, could provide new therapeutic targets which could control, simultaneously, both sides of the coin (like, for example the anti-TNF drugs), permitting a better control for those patients with IBD, and thus improving their prognosis and quality of life.

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