Osteoprotegerin, s-RANKL, and selected interleukins in the pathology of bone metabolism in patients with Crohn's disease

Iwona Krela-Kaźmierczak[1], Ewa Wysocka[2], Aleksandra Szymczak[1], Piotr Eder[1], Michał Michalak[3], Liliana Łykowska-Szuber[1], Kamila Stawczyk-Eder[1], Katarzyna Klimczak[1], Krzysztof Linke[1], Wanda Horst-Sikorska[4]

[1]Department of Gastroenterology, Human Nutrition and Internal Diseases, Poznan University of Medical Sciences, Poznan, Poland
[2]Department of Clinical Biochemistry and Laboratory Medicine, Poznan University of Medical Sciences, Poznan, Poland
[3]Department of Computer Science and Statistics, Poznan University of Medical Sciences, Poznan, Poland
[4]Department of Family Medicine, Poznan University of Medical Sciences, Poznan, Poland

Prz Gastroenterol 2016; 11 (1): 30–34
DOI: 10.5114/pg.2015.52589

Key words: osteoporosis, Crohn's disease, interleukins, osteoprotegerin, s-RANKL.

Address for correspondence: Iwona Krela-Kaźmierczak MD, PhD, Department of Gastroenterology, Human Nutrition and Internal Diseases, Poznan University of Medical Sciences, 49 Przybyszewskiego St, 60-355 Poznan, Poland, phone: +48 601 256 715, e-mail: krela@op.pl

Abstract

Introduction: Crohn's disease (CD) promotes the development of osteopaenia/osteoporosis, the cytokine background of which is not fully known.

Aim: Evaluation of bone mineral density (BMD), the prevalence of osteopaenia and osteoporosis, and the determination of the levels of selected interleukins (IL), osteoprotegerin (OPG), and s-RANKL proteins in patients with CD in relation to a control group and assessment of the relationship between the tested cytokines, OPG, s-RANKL, and BMD.

Material and methods: Thirty-seven CD patients and 37 healthy volunteers (control group) were enrolled into the study. Densitometry of the lumbar spine (L2–L4) and of the femoral neck using the DXA technique was carried out. Serum levels of: IL-13, IL-4, IL-17, IL-1β, OPG, and s-RANKL were determined using the ELISA method. Progression-of-disease questionnaires were collected.

Results: The prevalence of osteoporosis and osteopaenia in the CD group was: 18.92% and 32.43% in L2–L4; 13.51% and 35.13% in the neck, respectively. The IL-13 and IL-1β concentrations were significantly higher and OPG was significantly lower in CD patients when compared to controls. In the case of all subjects: IL-13 correlated negatively with the BMD of the neck, IL-17 correlated negatively with the *Z*-score of L2–L4, and OPG correlated negatively with the IL-13. In the case of CD patients, IL-4 correlated negatively with the BMD of L2–L4.

Conclusions: The incidence of osteopaenia and osteoporosis in Polish CD patients is high. IL-13, IL-1β, and IL-4 seem to be connected with the pathology of decreased BMD in CD. It can be hypothesised that IL-13 may lower BMD by modulating OPG.

Introduction

Crohn's disease (CD) promotes the development of osteopaenia and osteoporosis, which are among the most common metabolic complications of the disease [1–6]. Due to the gravity of the problem, there are ongoing studies on key mechanisms governing bone metabolic disorders in inflammatory bowel disease (IBD). The importance of a series of cytokines has been raised, especially those of the osteoprotegerin (OPG)-s-RANKL system, which may be modulated by interleukin-13 (IL-13), interleukin-4 (IL-4), interleukin-17 (IL-17), and interleukin-1β (IL-1β) [1-3]. It seems, therefore, that the constant activation of a cascade of inflammatory processes may have a direct impact on the functions of cells regulating bone metabolism. However, studies in this area are still lacking, and the results of some of the previous analyses are inconsistent. In addition, with regard to studies as to the cytokine background of bone mineral density (BMD) disorders in IBD, an important aspect to be considered is that of the characteristics and peculiarities of a given population. It should be emphasised, at this point, that analyses in this area with regards to the inhabitants of Central and Eastern

Europe are few, and there are no data at all concerning the Polish population. It would seem, therefore, that the assessment of the cytokine profile of patients with CD in relation to bone metabolic disorders among Polish patients may be a valuable complement to the knowledge on the importance of osteopaenia and osteoporosis in IBD.

Aim

The aim of the study was to assess BMD, and the incidence of osteopaenia and osteoporosis, as well as to examine the role of selected key cytokines, such as IL-13, IL-4, IL-17, IL-1β, OPG, and s-RANKL, in patients with CD.

Material and methods

The study group consisted of 37 patients with CD aged 31.7 ±8.0 years on average, including 15 women and 22 men, and 37 healthy volunteers aged 29.6 ±8.0 years on average, including 18 women and 19 men, who constituted the control group. Densitometry of the lumbar spine with L2–L4 assessment and densitometry of the proximal epiphysis of the femur with the assessment of the femoral neck was carried out on all patients using the Dual Energy X-ray Absorptiometry (DXA-Lunar DPX-IQ) technique. The analysis took into account the values of BMD as well as the *T*-score and *Z*-score indices. Each patient filled out a specially designed questionnaire concerning the current progress of the disease.

The serum concentrations of selected cytokines: IL-13, IL-4, IL-17, IL-1β, OPG, and s-RANKL, were determined using the ELISA method.

Approval for the conduct of the study was obtained from the Bioethics Committee at the Poznan University of Medical Sciences in Poznan (consent No. 92/09).

Statistical analysis

Statistical analysis was carried out using the Mann-Whitney test. The relationship between the analysed parameters was assessed using the Spearman's rank correlation coefficient, and its significance was assessed by *t*-Student test. The analysis was carried out using Statistica PL10 software (StatSoft). A correlation coefficient level of $p < 0.05$ was regarded as significant in all tests.

Results

The mean BMD (g/cm^2) in the CD group amounted to: 1.109 ±0.193 in L2–L4; and 0.922 ±0.202 in the neck. In the control group, these values amounted to: 1.224 ±0.084 in L2–L4; and 1.0859 ±0.159 in the neck. The prevalence of osteoporosis and osteopaenia in the CD group was as follows: $n = 7$ (18.92%) and $n = 12$ (32.43%) in L2–L4; and based on the assessment of the neck, it amounted to $n = 5$ (13.51%) and $n = 13$ (35.13%). The BMD (neck) *Z*-score and *T*-score in CD were significantly lower when compared with the control group ($p = 0.0007$). The prevalence of osteoporosis and osteopaenia jointly amounted to 53.35% in L2–L4 and 48.6% in the neck in CD patients. Table I presents the characteristics of the control group and patients with CD.

Mean concentrations of investigated cytokines are presented in Table II. The concentration of IL-13, IL-4, and IL-1β was significantly higher, and the concentration of OPG was significantly lower in CD patients, when compared to controls. In the case of the other cytokines, the differences were not statistically significant.

In the case of all subjects: IL-13 correlated negatively with the BMD of the neck ($r = –0.20$, $p = 0.0318$),

Table I. Characteristics of Crohn's disease patients and the control group

Parameter	Crohn's disease		
	n	Mean value	SD
CD patients:			
Age [years]	37	31.75676	8.06365
Weight [kg]	37	62.81081	13.79581
BMI [kg/m^2]	37	20.97953	3.25755
L2–L4 BMD	37	1.10922	0.19362
T-score L2–L4	37	–0.94568	1.56269
Z-score L2–L4	37	–0.42459	1.40937
Neck BMD	37	0.92265	0.20201
T-score neck	37	–0.86324	1.49451
Z-score neck	37	–0.41297	1.28472
Control group:			
Age [years]	37	29.56757	8.00882
Weight [kg]	37	74.56757	14.60696
BMI [kg/m^2]	37	24.73608	3.53956
L2–L4 BMD	37	1.22457	0.08417
T-score L2–L4	37	0.10514	0.72272
Z-score L2–L4	37	0.04378	0.65543
Neck BMD	37	1.08597	0.15916
T-score neck	37	0.49000	1.03907
Z-score neck	37	0.41892	0.98706

Table II. Mean serum concentrations of tested cytokines

Cytokine	Crohn's disease group, mean ± SD	Control group, mean ± SD	Value of *p*
IL-13 [pg/ml]	65.85 ±48.61	32.32 ±11.88	< 0.0001
IL-4 [pg/ml]	0.06 ±0.12	0.03 ±0.03	< 0.0001
IL-17 [pg/ml]	7.71 ±6.77	5.32 ±2.31	NS
IL-1β [pg/ml]	0.73 ±1.18	0.51 ±1.51	0.0008
OPG [pmol/l]	8.76 ±3.22	9.42 ±2.01	< 0.0001
s-RANKL [pmol/l]	284.87 ±213.05	236.84 ±111.63	NS

IL-17 correlated negatively with the *Z*-score of L2–L4 ($r = -0.32$, $p = 0.0005$), and OPG correlated negatively with the IL-13 ($r = -0.51$, $p < 0.0001$) (Figure 1). In the case of CD patients, IL-4 correlated negatively with the BMD of L2–L4 ($r = -0.32$, $p = 0.0491$).

The duration of the disease was 8.05 ±5.29 years in the group of patients with CD and it correlated with the *T*-score and *Z*-score in the neck of the femur. A similar correlation was demonstrated with the number of hospital admissions.

Discussion

The IBD is a common condition that occurs in approximately 1.4 million people in the United States and 2.2 million people in Europe. The prevalence of fractures among patients with IBD is estimated at about 40% higher than in the general population [7, 8]. Osteoporosis and osteopaenia, characterised by low BMD, are common extra-intestinal manifestations of these diseases, and they significantly increase the risk of bone fractures. The overall prevalence of osteopaenia and osteoporosis in patients with IBD varies in the range of 22–77% and 5–41% for osteopaenia and osteoporosis, according to various authors [4–7]. In our study, the prevalence of osteopaenia and osteoporosis was similar, and depending on the tested site, amounted to 32.43–35.13% and 13.5–18.92%, respectively. What is important from the practical point of view, the frequency of BMD disturbances increases in longer-lasting CD and in patients more frequently hospitalised. For a more complete assessment, however, it is necessary to carry out further studies involving a larger number of Polish patients.

The pathogenesis of low BMD in the course of IBD is multifactorial and includes general osteoporosis risk factors such as age and smoking, as well as risk factors specific to IBD, such as glucocorticoid therapy, malnutrition, resection of the small intestine, vitamin D deficiencies, and the effects of pro-inflammatory cytokines [3, 9]. From a molecular point of view, the key processes favouring bone metabolic disorders are considered to be abnormalities in the concentration of individual pro- and anti-inflammatory cytokines, particularly cytokines involved in the regulation of the RANKL/RANK/OPG pathway. RANKL (receptor activator of nuclear factor κB ligand) is a protein that is involved in bone metabolism, activating osteoclasts, belonging to the family of tumour necrosis factors. The OPG, on the other hand, is a protein in the family of tumour necrosis factor receptors (TNFR). The OPG binds with RANKL, acting as its soluble receptor, thereby preventing further binding of RANKL with RANK. In this way, the maturation of osteoclasts is inhibited. The OPG gene expression is increased by individual cytokines (TNF-α, IL-1a, IL-18, TNF-β), bone morphogenetic proteins, 17β-estradiol,

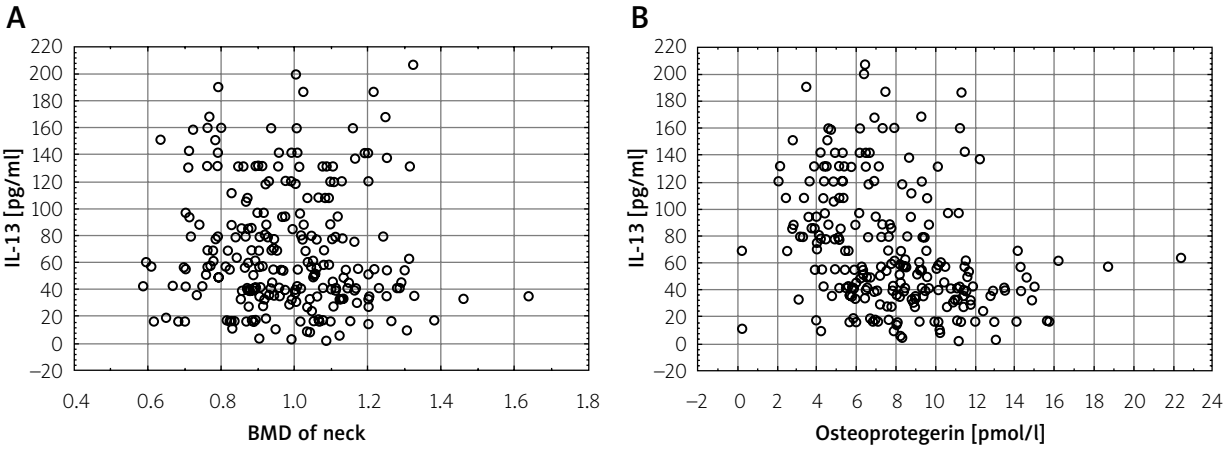

Figure 1. A – Correlation between IL-13 levels and bone mineral density of the neck ($r = -0.2$; $p = 0.3$) in the whole study group. **B** – Correlation between IL-13 and osteoprotegerin levels ($r = -0.5$; $p < 0.0001$) in the whole study group

and bone mechanical stress, and is reduced by drugs (glucocorticoids, immunosuppressants), parathyroid hormone (PTH), prostaglandin PGE2, and fibroblast growth factor (FGF) [3–10].

A network of cytokines with various mechanisms of action plays an important role in the regulation of the function of the RANKL/RANK/OPG system. The IL-13 and IL-4 are anti-inflammatory cytokines, which inhibit the production of pro-inflammatory cytokines. They are produced mainly by stimulated Th2 lymphocytes. The IL-17 and IL-1β, on the other hand, are representatives of key pro-inflammatory cytokines. The IL-17 intensifies the inflammatory process through the induction of the production of IL-1β and TNF [11]. The IL-1 comprises a group of cytokines that includes IL-1β, amongst others. The IL-1β is the secretory form. IL-1, which initiates and intensifies the inflammatory process, stimulates T cells to produce other pro-inflammatory cytokines and intensifies the release of the growth factors [10]. The IL-1β stimulates the osteoclasts, reduces the synthesis of collagen and non-collagenous proteins, and thus plays an important role in the process of osteogenesis and bone resorption.

TNF-α, IL-1, IL-6, IL-7, and IL-17 increase the ratio of RANKL to OPG, thereby promoting bone resorption. IL-4, on the other hand, inhibits the activity of IL-17 and RANKL [10–12]. In an animal model of rheumatoid arthritis, IL-1, IL-7 and IL-17 increased the RANKL/RANK/OPG ratio, which favoured the resorption of bones [11]. Therefore, these cytokines, through their effect on the RANKL/RANK/OPG system, modulate its activity, and thus indirectly participate in the process of bone remodelling [10–14].

The conclusions from the studies assessing the relation between RANKL/RANK/OPG system and BMD in IBD are conflicting. For example, increased levels of OPG in patients with CD were demonstrated in a study carried out by Berstein *et al.* There were no differences in the concentrations of RANKL between patients with CD and the control group as well as the group of patients with ulcerative colitis (UC) [15]. In contrast, Moschen *et al.* showed a statistically significant negative correlation between the concentration of OPG and BMD of the femoral neck; no such correlation was observed with respect to s-RANKL [10]. OPG levels were significantly higher in the CD group, compared with the control group, which was not the case for sRANKL [10].

Similar to Moschen *et al.*, we observed increased levels of OPG in the control group and significantly lower levels in the group of patients with CD, while the levels of RANKL were higher in the group of patients with CD, compared to the control group; however, these differences were not statistically significant. Thus, we showed an increased OPG/RANKL ratio in Polish CD patients. This could promote bone resorption and lead to a decrease in BMD.

The imbalance between pro- and anti-inflammatory cytokines can be the direct cause of OPG/RANKL disturbances. We showed a significant increase in pro-inflammatory IL-13 and IL-1β concentration in CD patients. On the other hand, a crucial anti-inflammatory cytokine, IL-4, correlated negatively with BMD assessed in L2-L4 in the whole study group, indicating that it can also play an important role in BMD regulation in physiological conditions.

Conclusions

The incidence of osteopaenia and osteoporosis in patients with CD is high and increases with the duration of the disease and the number of hospital admissions. This relationship is most likely the result of the progressive nature of the disease in patients with a long-standing history of the disease, and also as a result of its more severe course and the need for more aggressive treatment in patients with multiple hospital admissions. The cytokine background of bone metabolic disorders in CD is complex and includes a number of elements that are directly related to one another. The results of our preliminary studies carried out on patients in Poland suggest that disturbances in the RANKL/OPG/RANK system can play a crucial role in BMD disturbances. This, in turn, can be influenced by an imbalance between pro- and anti-inflammatory cytokines, such as IL-13, IL-1β, or IL-4.

The importance of the RANKL/OPG/RANK pathway in the inflammatory process accompanying bone diseases is confirmed by experiments involving treatment attempts with OPG and other RANK inhibitors [16]. Successful application of a human monoclonal antibody directed against RANKL, denosumab, for the treatment of osteoporosis shows that further studies on the role of cytokine milieu abnormalities in BMD disturbances in different diseases are needed. Our study is in accordance with this trend and provides new data regarding the molecular background of osteoporosis/osteopaenia in Polish patients with CD.

Acknowledgments

This study was financed from the project of the Ministry of Science and Higher Education: NN 402 481 737.

Conflict of interest

The authors declare no conflict of interest.

References

1. Tauseef A, Lam D, Bronze M, et al. Osteoporosis in inflammatory bowel disease. Am J Med 2009; 122: 599-604.

2. Ghosh S, Cowen S, Hannan WJ, et al. Low bone mineral density in Crohn's disease, but not in ulcerative colitis, at diagnosis. Gastroenterology 1994; 107: 1031-9.
3. Targownik LE, Bernstein CN, Leslie WD. Inflammatory bowel disease and the risk of osteoporosis and fracture. Maturitas 2013; 76: 315-9.
4. Tigas S, Tsatsoulis A. Endocrine and metabolic manifestations in inflammatory bowel disease. Ann Gastroenterol 2012; 25: 37-44.
5. Bernstein CN, Leslie WD, Leboff MS. AGA technical review on osteoporosis in gastrointestinal diseases. Gastroenterology 2003; 124: 795-841.
6. Łodyga M, Eder P, Bartnik W, et al. Guidelines for the management of Crohn's disease. Recommendations of the Working Group of the Polish National Consultant in Gastroenterology and the Polish Society of Gastroenterology. Prz Gastroenterol 2012; 7: 317-38.
7. Vestergaard P, Mosekilde L. Fracture risk in patients with celiac disease, Crohn's disease, and ulcerative colitis: a nationwide follow-up study of 16,416 patients in Denmark. Am J Epidemiol 2002; 156: 1-10.
8. Berstein CN, Blanchard JF, Leslie W, et al. The incidence of fracture among patients with inflammatory bowel disease: a population-based cohort study. Ann Intern Med 2000; 133: 795-9.
9. Targownik LE, Bernstein CN, Leslie WD. Risk factors and management of osteoporosis in inflammatory bowel disease. Curr Opin Gastroenterol 2014; 30: 168-74.
10. Moschen AR, Kaser A, Enrich B, et al. The RANKL/OPG system is activated in inflammatory bowel disease and relates to the state of bone loss. Gut 2005; 54: 479-87.
11. Shen F, Ruddy MJ, Plamondon P, et al. Cytokines link osteoblasts and inflammation: microarray analysis of interleukin-17- and TNF-alpha-induced genes in bone cells. J Leukoc Biol 2005; 77: 388-99.
12. Hofbauer LC, Schoppet M. Clinical implications of the osteoprotegerin/RANKL/RANK system for bone and vascular diseases. JAMA 2004; 92: 490-5.
13. Khosla S. Minireview: the OPG/RANKL/RANK system. Endocrinology 2001; 142: 5050-5.
14. Guerrini MM, Takayanagi H. The immune system, bone and RANKL. Arch Biochem Biophys 2014; 561C: 118-23.
15. Berstein CN, Sargent M, Leslie WD. Serum osteoprotegerin is increased in Crohn's disease: a population-based case control study. Inflamm Bowel Dis 2005; 11: 325-30.
16. Cummings SR, San Martin J, McClung MR, et al. Denosumab for prevention of fractures in postmenopausal women with osteoporosis, for the FREEDOM Trial. N Engl J Med 2009; 361: 756-65.

Received: 26.11.2014
Accepted: 11.02.2015