

In patients with rheumatoid arthritis, Dickkopf-1 serum levels are correlated with parathyroid hormone, bone erosions and bone mineral density

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Abstract

Objective

The objective of this study is to compare the serum levels of Dickkopf-1 (DKK1), a natural inhibitor of Wnt signalling, with parathyroid hormone (PTH) and bone involvement in patients with rheumatoid arthritis (RA).

Methods

This cross-sectional study includes 154 postmenopausal women with RA and 125 healthy controls. DKK1, 25OH vitamin D (25OHD), bone turnover markers, and PTH serum levels were measured by ELISA; lumbar spine and hip bone mineral density (BMD) and the erosion score were obtained.

Results

The RA patients and healthy controls were not significantly different in terms of age, body mass index, and 25OHD serum levels. The mean level of DKK1 and PTH were significantly higher in patients with RA than in healthy controls (172 ± 68 [SD] vs. 96 ± 55 pmol/L, and 30 ± 15 vs 22 ± 11 , respectively; $p < 0.0001$). DKK1 serum levels were positively correlated with age ($p < 0.05$) only in the healthy controls, while they were correlated with PTH serum levels only in the RA patients ($p < 0.0001$). Among the RA patients, DKK1 levels adjusted for age, PTH and disease duration were significantly higher in patients with bone erosions (176 vs. 167 pmol/L, respectively; $p < 0.05$). DKK1 levels adjusted for age and PTH were negatively correlated with total hip BMD ($p < 0.05$). In the RA patients not on treatment with bisphosphonates, DKK1 serum levels positively correlated with C-terminal telopeptides of type I collagen serum levels ($p < 0.05$).

Conclusion

In patients with RA, serum levels of DKK1 are significantly increased, correlate with PTH and are associated with increased risk of bone erosions and osteoporosis. However, this finding deserves confirmation in a larger and more selected population.

Key words

Dickkopf-1, parathyroid hormone, rheumatoid arthritis, bone erosion, bone mineral density

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Introduction

Recent animal and clinical studies have suggested that Dickkopf-1 (DKK1), a natural inhibitor of canonical Wnt/ β catenin signalling (1), appears to be a master regulator of joint remodelling and that it may play an important role in mediating the juxta-articular bony changes of rheumatoid arthritis (RA) (2). Wnt pathway not only boosts bone formation by fostering osteoblast activity, but it can also inhibit osteoclastic bone resorption (3, 4). A hallmark of the joint pathology in the animal models of inflammatory arthritis is not only increased focal bone erosion, but also decreased bone formation at sites adjacent to focal osteoclast-mediated bone resorption (5). Diarra *et al.* implicated DKK1 in both the enhanced bone resorption and the impaired bone formation characterising an animal model of the disease (2). DKK1 was found increased in the inflamed synovium and up-regulated by TNF- α in cultured synovial fibroblasts and its levels were found elevated in sera from individuals with RA (2, 6), as well as in their inflamed synovium (2). DKK1 serum levels correlate with bone erosions and inflammation in RA (7, 9) and a polymorphism in DKK1 regulating genes is associated with higher serum levels of functional DKK1 and more progressive joint destruction over time (10). It has been reported that blockage of DKK1 abolished bone erosion in an inflammatory mouse model (2) and the neutralisation of DKK1 protects from systemic bone loss during inflammation (11).

We have recently observed that the presence of bone erosions in RA correlates with high serum parathyroid hormone (PTH) levels (12). It is known that the long-term treatment with PTH increases serum DKK1 (13, 14) and that serum PTH positively correlate with DKK1 both in healthy women and in patients with primary hyperparathyroidism (15). It is therefore conceivable that high PTH levels are associated with more aggressive disease activity by promoting the expression of DKK1. The objective of this study is to compare the serum levels of PTH and DKK-1 in patients with RA and healthy control and to explore how they correlate with bone involvement.

Patients and methods

The cross-sectional study population includes 154 consecutive postmenopausal women with RA recruited from our rheumatology department, and 125 healthy sex- and age-matched controls participating in a screening visit for osteoporosis. All patients fulfilled the 1987 American College of Rheumatology (ACR) revised criteria for RA (16). Exclusion criteria were patients unable to walk without assistance or affected by diabetes or hepatic or renal impairment.

Clinical evaluation

All patients were interviewed and went through a full physical examination for information on disease and treatment history. Disease-related findings included disease duration and counts of 28 tender and swollen joints. Radiographs of hands and feet were analysed locally and subjects were categorised by the presence or absence of radiographic erosions. Clinical measurements of RA disease activity included the Italian version of the Health Assessment Questionnaire Disability Index (HAQ) (17) and the 3-variable Disease Activity Score 28-joint count (DAS28), calculated using C-reactive protein (CRP) by the Nijmegen algorithm (available online: <http://www.reuma-nijmegen.nl/www.das-score.nl/index.html>) (18). Information was collected on RA-specific treatments, including disease-modifying anti-rheumatic drugs (DMARD: methotrexate, cyclosporine, sulfasalazine, anti-malarials, and azathioprine), glucocorticoids and TNF- α blockers. Patients were classified as glucocorticoid or TNF- α blocker non-users when they had never been treated or when treatment had been discontinued for more than 6 months. Patients were interviewed on past and current use of drugs affecting bone metabolism including bisphosphonates, calcium, and vitamin D supplements. Daily intake of calcium was assessed by a simplified validated questionnaire (19). In all subjects, body weight and height (Harpender stadiometer) were assessed and body mass index (BMI; kg/m²) calculated. Bone mineral density (BMD) was measured in RA patients by dual-

Competing interests: none declared.

energy x-ray absorptiometry (DXA) at the spine and total hip by making use of Hologic 4500 (Hologic Inc. Waltham, Ma, USA).

Laboratory assessment

CRP, erythrocyte sedimentation rate (ESR), rheumatoid factor (RF), cyclic citrullinated peptide antibody (anti-CCP) and routine biochemistry were measured. Serum samples were collected, aliquoted and stored at -50°C . Bone specific alkaline phosphatase (BAP) and serum C-terminal telopeptides of type I collagen (CTX) were measured by ELISA (IDS Ltd, Boldon, UK). Intra-assay coefficients of variation in our laboratory were 2% for CTX and 4% for BAP. Inter-assay coefficients of variation were 8% for CTX and 6% for BAP. Serum DKK1 was measured using an enzyme immunoassay (Biomedica Medizinprodukte GmbH & Co KG, Wien, Austria) with a sensitivities of 0.38 pmol/L and an intra-assay coefficients of variation of 7.3%. Serum 25OH Vitamin D (25OHD) and serum PTH (1-84) were determined by ELISA (IDS Ltd, Boldon, UK), with intra-assay CVs of 6% and 3%, respectively and a inter-assay CVs of 7% and 6%, respectively. The study was approved by the local institutional review board and an informed consent was obtained from all patients and controls.

Statistical analysis

All continuous variables are reported as mean \pm standard deviations (SD). The clinical characteristics of patients with and without erosions or controls were compared by Student *t*-test for continuous variables. Analysis of variance (ANOVA) and then a two sided Student's *t*-test were used to estimate the absolute differences between two groups (patients and controls) and for adjusted values by ANCOVA. The relationships between variables of interest were defined using the Pearson correlation. All analyses were performed with SPSS, version 13.0 (SPSS Inc., Chicago, IL, USA).

Results

Clinically relevant characteristics of the RA patients and healthy controls are

Table I. Characteristics of the 154 postmenopausal women with RA and 125 healthy controls (mean \pm SD) ($^{\circ}p<0.001$ between groups; $p>0.1$ between groups for all other variables).

	RA patients (154)	Controls (125)
Age (years)	65 \pm 7	64 \pm 6
BMI (kg/m ²)	24.7 \pm 4.1	24.5 \pm 3.7
Duration of RA (months)	145 \pm 105	-
HAQ	1.12 \pm 0.86	-
CRP (mg/dl)	1.1 \pm 2.1	-
DAS28	3.83 \pm 0.94	-
ESR (mm ³ 1h)	32.9 \pm 20.2	-
Calcium Intake mg/day	791 \pm 321	812 \pm 398
Serum Calcium (mg/dl)	9.0 \pm 1.1	8.9 \pm 1.2
Serum Phosphate (mg/dl)	3.3 \pm 0.7	3.4 \pm 0.9
Serum 25OHD (ng/ml)	27 \pm 17	27 \pm 22
Serum PTH (pg/ml)	30 \pm 15	22 \pm 11 $^{\circ}$
Serum CTX (ug/L)*	0.415 \pm 0.263	-
Serum BAP (ng/ml)*	12 \pm 6	-
Serum DKK1 (pmol/L)	172 \pm 68	96 \pm 55 $^{\circ}$
Lumbar spine BMD T score	-1.85 \pm 1.23	-1.79 \pm 1.34
Total hip BMD T score	-1.69 \pm 1.14	-1.56 \pm 1.27

*in patients not in treatment for osteoporosis.

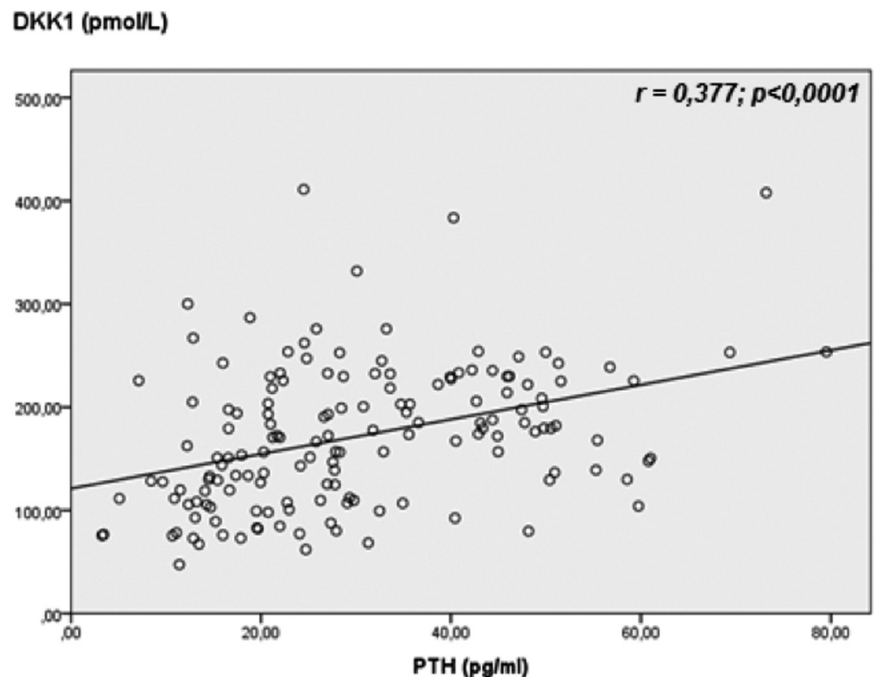


Fig. 1. Correlation between PTH serum level and DKK1 in RA patients.

listed in Table I. RA patients and controls were not significantly different for age, BMI, daily intake of calcium, calcium, phosphate, 25OHD serum levels and lumbar spine or total hip BMD.

90% of patients were taking DMARDs. 53 and 61% were RF or anti-CCP positive patients, respectively. 93% were on glucocorticoid treatment (mean daily dose 4.5 \pm 4.3 mg prednisone equivalents) with a cumulative prednisone equivalent intake of 11.2 \pm 14.0 g. Forty-

six patients (30%) were being treated with anti-TNF- α , generally in association with methotrexate. Sixty-seven patients (44%) were on treatment with bisphosphonates for the prevention or treatment of osteoporosis. No significant differences were observed in DKK1 and PTH serum levels between glucocorticoid, anti-TNF- α or bisphosphonate users or non-users.

The mean serum level of DKK1 and PTH were significantly higher in pa-

tients with RA than in healthy controls (172 ± 68 vs. 96 ± 55 pmol/L, and 30 ± 15 vs. 22 ± 11 , respectively; $p=0.0001$).

DKK1 serum levels were significantly correlated with age ($p<0.05$) only in the healthy controls, while they were correlated with PTH serum levels only in the RA patients ($r = 0.377$, $p<0.0001$; Fig. 1). In the RA patients the serum DKK1 levels were significantly correlated with disease duration, HAQ and DAS28, but the latter two correlations lost significance for data adjusted for age.

In Table II characteristics of the patients with erosive and non-erosive disease are shown. Significant differences between the two groups were observed for HAQ, DAS28, disease duration ($p<0.001$). After adjustment for age, PTH and disease duration, patients with erosions (61%) had serum levels of DKK1 higher than those without erosions (176 ± 10 vs. 167 ± 7 SE pmol/L, respectively; $p<0.05$; Fig. 2). Mean serum phosphate was significantly lower in patients with erosions (3.2 ± 0.8 vs. 3.4 ± 0.6 mg/dl).

Total hip BMD T-score, but not lumbar spine BMD T-score, was significantly lower in RA patients with bone erosions in comparison with patients without bone erosions (-1.9 ± 1.1 vs. -1.4 ± 1.1 , respectively) (Table II). After adjustment for age, PTH and disease duration, a significant negative correlation between DKK1 and total hip BMD T-score was observed ($r = -0.234$, $p<0.05$) (Fig. 3).

In the 87 patients not on treatment with bisphosphonates DKK1 serum levels positively correlated with serum CTX levels ($r = 0.213$; $p<0.05$).

Discussion

We observed for the first time that in patients with RA serum PTH and DKK1, other than significantly higher than in control subjects, are correlated each other and significantly associated with the presence of overt bone erosions and low BMD. We think that the very slight difference in DKK1 between patients with erosive disease *versus* those without might be explained by the limitations of this study: its cross-sectional nature and the binary erosion score used. The role of over-production

Table II. Characteristics of the patients with or without bone erosions (mean \pm SD) ($^{\circ}p<0.001$, $^{*}p<0.05$ between groups).

	RA patients with erosions (94)	RA patients without erosions (60)
Age (years)	66 \pm 7	64 \pm 7
BMI (kg/m ²)	24.7 \pm 3.9	24.7 \pm 4.4
Duration of RA (months)	170 \pm 108 [°]	98 \pm 82
HAQ	1.28 \pm 0.92 [*]	0.87 \pm 0.69
CRP (mg/dl)	1.2 \pm 1.8	0.8 \pm 2.6
DAS28	4.06 \pm 0.94 [°]	3.44 \pm 0.79
ESR (mm ³ 1h)	35.0 \pm 20.6	29.7 \pm 19.2
Calcium Intake mg/day	787 \pm 391	802 \pm 387
Serum Calcium (mg/dl)	8.9 \pm 1.1	9.1 \pm 1.1
Serum Phosphate (mg/dl)	3.2 \pm 0.8 [*]	3.4 \pm 0.6
Serum 25OHD (ng/ml)	26 \pm 14	27 \pm 19
Serum PTH (pg/ml)	31 \pm 16	28 \pm 14 [°]
Serum CTX (ug/L) [*]	0.419 \pm 0.235	0.407 \pm 0.315
Serum BAP (ng/ml) [*]	13 \pm 6	12 \pm 6
Lumbar spine BMD T score	-1.83 \pm 1.24	-1.88 \pm 1.23
Total hip BMD T score	-1.88 \pm 1.13 [*]	-1.39 \pm 1.08

^{*}in patients not in treatment for osteoporosis.

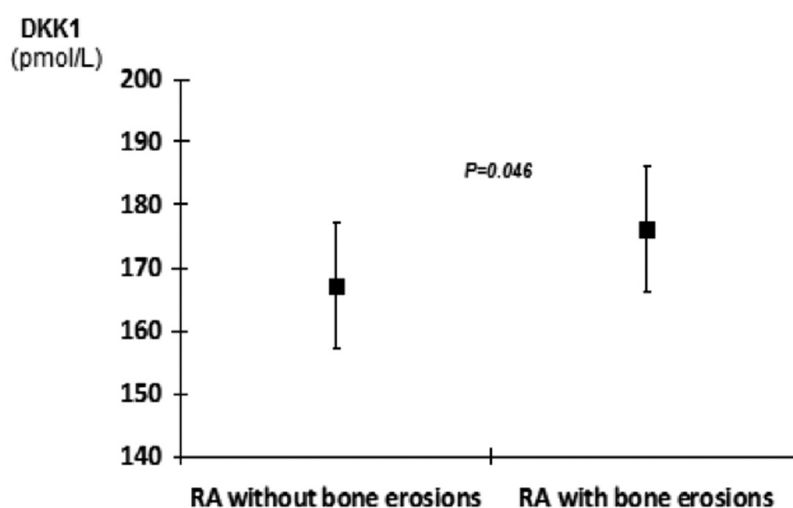


Fig. 2. DKK1 serum levels (mean \pm SE), adjusted for age, PTH and disease duration, in RA patients with or without erosions.

of DKK1 in the pathogenesis of bone lesions in RA is supported by a number of experimental observations. Diarra *et al.* reported an over-production of DKK1 by synovial tissues from patients with RA particularly when exposed to TNF- α . (2). Walsh *et al.* showed at sites of joint erosions in an animal model of RA that osteoblast function is impaired (5), but it can be resumed by blocking inflammation (20). A decrease in DKK1 levels has been reported during RA treatment (21). The lack of correlation between DKK1 and DAS28 in our study might be explained by the large percentages of patients were taking DMARDs (90%), glucocorticoids (93%) or anti-TNF- α (30%).

It was also reported that the exposure to neutralising anti DKK1 antibodies did improve bone repair but inhibited also local bone resorption, an effect observed also in another experimental model (2) attributed to increased number of functionally active osteoblasts with upregulation of the production of osteoprotegerin, the potent inhibitor of RANKL (22). From this point of view it is of interest the positive correlation we found in this study between circulating DKK1 and serum CTX, a marker of bone resorption, suggesting that over-production of DKK1 contributes both to locally increased bone resorption and impaired bone repair. It is conceivable that DKK1 is involved in the expres-

sion of osteoclast differentiation factors. Fujita *et al.*, found that DKK1 facilitates osteoclastogenesis by enhancing RANKL/RANK interaction (23). In other studies DKK1 was found to play an important role in the promotion of synovial angiogenesis (24) which is crucially important in the development of membrane synovial pannus in RA.

We have recently reported that the presence of bone erosions in RA correlates with high serum PTH and low BMD (12), and generalised bone loss is a predictor of radiographic damage (25).

There are a number of direct and indirect evidences (26, 27) that persistent PTH signalling results in increased RANKL expression and then of osteoclast systemic bone resorption, recognised mechanism of bone loss also in inflammatory disorders (28). This effect may be amplified at the site of bone erosions. In this study we do not observed in patients with bone erosions significantly higher mean serum levels of PTH, probably for the small number of cases and the variability of the assay, but the lower mean serum levels of phosphate that we found in these patients might be consequence of this metabolic alteration.

The significant positive correlation observed in this study between DKK1 and PTH is consistent with our recent similar observation both in healthy subjects and in patients with primary hyperparathyroidism (15) and with the observation that long-term treatment of postmenopausal osteoporotic women with the PTH analogue, teriparatide, is associated with significant increases in serum levels of DKK1 (13, 14). Altogether these results suggest that PTH, together with inflammation and autoimmunity (29-31), might be a determinant of local DKK1 over-expression. Thus, high serum PTH levels may directly enhance local bone resorption and impair bone repair by promoting DKK1 expression.

So, PTH and DKK1 together might be involved in the common mechanisms for bone erosions and osteoporosis: in Figure 4 we describe a hypothesised relationship between DKK1, PTH and bone involvement in RA.

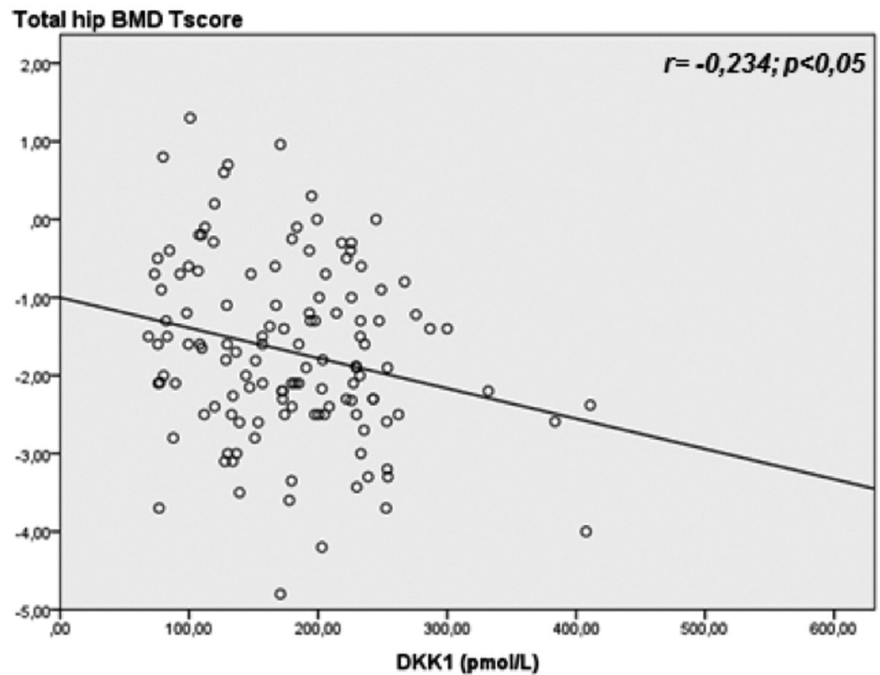


Fig. 3. Correlation between DKK1 and total hip BMD T score in RA patients.

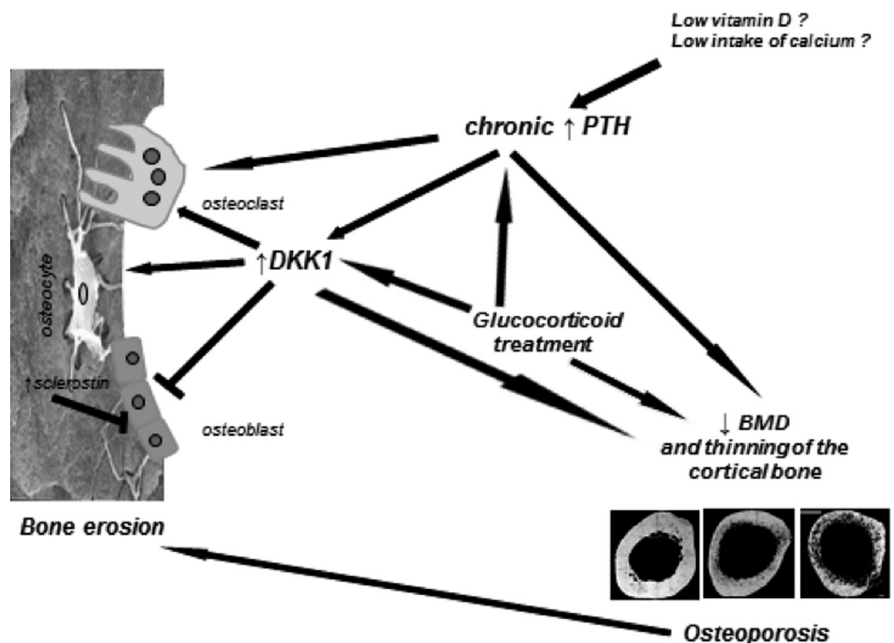


Fig. 4. Common mechanisms for bone erosions and osteoporosis: hypothesized relationship between DKK1, PTH, glucocorticoid treatment and bone involvement. Chronic increase of PTH induces osteoclastogenesis, osteoporosis in cortical bone, and expression of DKK1. DKK1 itself leads to inhibition of osteoblast differentiation, induces expression of another anti-anabolic molecule, sclerostin, by osteocytes, and consequently reduces focal and systemic bone formation. Osteoporosis of cortical bone increases the risk of bone erosions.

The risk for secondary hyperparathyroidism is not infrequent in patients with RA as a consequence of aging, inadequate calcium intake, glucocorticoid use or vitamin D insufficiency (32). In our patients mean daily intake of calcium was lower than rec-

ommended (33), and the large majority was on glucocorticoid treatment. Vitamin D deficiency is very common in patients with RA (34, 35). This is a general problem linked to ageing but it may be even worse in RA patients in whom the disability decreases sun ex-

posure (34, 35). It has been recently reported that in RA patients both the onset of the disease and its radiographic progression is more common during winter or spring, seasons characterized by lower and higher serum levels of 25OHD and PTH, respectively (36). We have recently reported that (12) the risk of typical joint erosions is related with PTH levels more than with serum 25OHD. We hypothesised an impaired 1- α -hydroxylase activity, which is able to convert 25OHD into the active vitamin D metabolite 1,25(OH)₂D. The consequence might be a sparing effect on vitamin D deposits but higher PTH levels for similar 25OHD levels.

The main limitations of our study are the relatively small number of patients, the binary erosion score used, and its cross-sectional nature, which prevents any conclusion on the causality of the associations we found. Another important limitation of the study is the heterogeneity of the investigated population on a great number of factors (demographic, clinic, therapeutic) that can increase or decrease both serum PTH and DKK1 levels and affect bone density in RA. For instance, most patients are on chronic glucocorticoids, one third of them are currently being treated with anti-TNF- α agents, and 44% receive bisphosphonates for bone protection: what we know is GC can enhance expression of DKK1 and chronic treatment induces increased levels of DKK1 in a dose- and time-dependent manner (37); anti-TNF drugs are reported to reduce DKK1 levels in RA patients (2); effects of bisphosphonates on DKK1 serum levels are somewhat contradictory (38, 39). At the same time, glucocorticoids and bisphosphonates can alter PTH serum levels, by inducing a relative secondary hyperparathyroidism, which is even more common when patients do not have an appropriate daily calcium intake, or have inappropriate vit D plasma levels, a very usual finding in RA (34, 35). Distinct populations (that is, not taking vs. taking glucocorticoids, as well as bisphosphonates or anti-TNF- α agents) should be investigated to address appropriately the question regarding the correlation between DKK1 and PTH.

Conclusion

In patients with RA serum PTH and DKK1 are significantly increased, correlated each other and are associated with the presence of typical erosions and lower BMD. This might open new scenarios for the management of RA. However, this finding deserves confirmation in a larger and more selected population.

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