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# Rheumatoid Arthritis Bone Fragility Is Associated With Upregulation of IL17 and DKK1 Gene Expression

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**Abstract** Our aim was to compare bone gene expression in rheumatoid arthritis (RA) and primary osteoporosis (OP) patients. Secondary aims were to determine the association of gene expression of the Wnt/β-catenin signaling pathway with inflammatory cytokines in the bone microenvironment and to assess the serum levels of Wnt/β-catenin proteins in both groups. RA patients referred for hip replacement surgery were recruited. Primary OP patients were used as controls. Gene expression of Wnt pathway mediators, matrix proteins, and pro-inflammatory cytokines were analyzed in bone samples. Bone turnover markers, inflammatory cytokines, and Wnt mediators were measured in serum. Twenty-two patients were included: 10 with RA and 12 with primary OP. The expressions of Wnt10b ($p$=0.034), its co-receptor LRP6 ($p$=0.041), and its negative regulator DKK1 ($p$=0.008) were upregulated in RA bone. IL17 gene expression in bone was upregulated in RA patients ($p$=0.031) and correlated positively with Wnt10b ($r$=0.810, $p$=0.015), DKK2 ($r$=0.800, $p$=0.010), and RANKL/OPG ratio ($r$=0.762, $p$=0.028). DKK2 ($p$=0.04) was significantly decreased in RA serum compared with primary OP. In conclusion, bone fragility in RA patients is induced by an unbalanced bone microenvironment and is associated with a specific gene expression pattern, namely, the upregulation of IL17 and DKK1, suggesting that the modulation of these two pathways might prevent RA systemic bone loss.

**Keywords** Rheumatoid arthritis · Bone turnover · Osteoblast · Wnt pathway · IL17

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## Introduction

Rheumatoid arthritis (RA) is a chronic immune-mediated inflammatory disease of unknown etiology, characterized by synovial inflammation leading to cartilage destruction and marginal bone erosions [1]. Evidence suggests that bone remodeling disturbances in RA contribute not only to bone erosions but also to the development of systemic osteoporosis (OP). In fact, RA patients have an increased risk of vertebral fractures, which is independent of bone mineral density (BMD) and corticosteroid use [2]. Thus, RA itself seems to predispose to fractures, although the underlying mechanisms are not completely understood, but might be dependent on the complex interference of the inflammatory environment with bone remodeling mechanisms.

Tumour necrosis factor (TNF) [3, 4], interleukin (IL) 1 [5, 6], and IL6 [7, 8] are among the classic mediators of RA pathogenesis that can contribute to increased bone resorption. More recently, evidence has implicated Th17 cells as additional crucial effectors since inhibition of IL17 or its overexpression in mouse joints suppresses or worsens joint inflammation and damage, respectively [9]. Th17 cells were identified as a typical pro-osteoclastogenic Th subset [10], and IL17 overexpression in collagen-induced arthritis mice not only enhances receptor activator of nuclear factor κ-B ligand (RANKL) expression but also upregulates RANKL/osteoprotegerin (OPG) ratio in the synovium [11].

Recently, some authors have pointed out that one possible effect of RA on bone is interference with the Wnt signaling pathway, a major regulator of osteoblast activity. In fact, inhibition of dickkopf 1 (DKK1, an endogenous inhibitor of Wnt/β-catenin signaling) was able to reverse bone destruction observed in a mouse model of arthritis and to induce new bone formation [12]. The Wnt signaling pathway depends on the Wnt co-receptor low-density lipoprotein receptor-related protein 5 (LRP5) or 6 (LRP6) and mutations that inactivate LRP5 cause OP [13], while mutations that enhance its function result in a high bone mass phenotype [14]. Activation of the Wnt/β-catenin pathway occurs with the binding of Wnt to LRP5 (or LRP6) and to the co-receptor Frizzled [15, 16]. In the absence of a Wnt ligand, the cytosolic levels of β-catenin are kept low by its phosphorylation and subsequent degradation, thereby suppressing the expression of Wnt-responsive genes [17]. Moreover, recent studies have shown that osterix (OSX) is able to inhibit Wnt pathway activity during osteoblast differentiation either through disrupting the binding of T cell factor to DNA and inhibiting β-catenin transcription or by activating the DKK1 promoter [18, 19]. Like DKK1, sclerostin (SOST) inhibits the Wnt/β-catenin pathway by binding to the LRP5/6 co-receptor; however, it binds to a different region of LRP5/6 and does not mediate receptor internalization [20]. SOST knockout mice have increased bone mineral density, bone volume, bone formation, and bone strength [21], while overexpression of SOST leads to osteopenia [22].

In a recent study, we used the SKG mouse model of arthritis to demonstrate the weakening effect of chronic arthritis on bone mechanical behavior [23, 24]. We observed that this effect was due to inflammation-driven derangement of the bone matrix, caused by disturbed bone remodeling and increased collagen turnover [25]. Therefore, we hypothesize that bone fragility in RA patients is due to the presence of a chronic inflammatory microenvironment, which might be associated with disturbances in the Wnt/β-catenin signaling pathway. In this study, we aim to compare bone gene expression in RA and primary OP patients. Secondary aims were to determine the association of gene expression of the Wnt/β-catenin signaling pathway with inflammatory cytokines in the bone microenvironment and to assess the serum levels of Wnt/β-catenin proteins in both groups.

## Methods

### Study Population

This is a nested case–control study from a cohort of 387 consecutive patients undergoing total hip replacement surgery at Lisbon Academic Medical Centre, with bone samples kept in a biobank (Biobanco-IMM) in Lisbon. Patients older than 50 years who were diagnosed with RA according to the 2010 ACR/EULAR revised classification criteria [26] and submitted to total hip replacement surgery between 2007 and 2010 were selected from the biobank collection and included in this study. Patients were excluded if other causes of secondary OP such as malignancies, untreated thyroid disease, terminal renal disease, or hypogonadism were present. Premenopausal women were also excluded. Another group of patients undergoing hip arthroplasty due to hip fragility fracture, matched to gender, age, body mass index (BMI), femoral neck BMD, and without any secondary causes for OP, was selected from the biobank to be used as the control. None of the patients reported a history of other metabolic bone diseases, bone metastasis, primary bone tumors, or osteomyelitis.

All patients were asked to complete a clinical questionnaire at the time of surgery in order to assess clinical risk factors associated with OP, such as age, gender, BMI, prior fragility fracture, family history of hip fracture, long-term use of oral corticosteroids (≥3 months), current smoking and alcohol intake (≥3 units/day), and past and current medication. The probability of fracture at 10 years was calculated using the FRAX tool available online adapted to the Portuguese population (www.shef.ac.uk/FRAX) [27]. Femoral neck BMD of the contralateral hip was measured by dual-

emission X-ray absorptiometry scan using a Lunar Prodigy densitometer (GE Healthcare, UK) within 1 week after surgery.

For RA patients, disease duration, age at disease onset, rheumatoid factor, C-reactive protein (CRP), disease activity score (DAS28 ESR3V), presence of erosions, and RA therapy were also assessed.

Written informed consent was obtained from all patients. The study was conducted in accordance with the regulations governing clinical trials such as the Declaration of Helsinki, as amended in Seoul (2008), and was approved by the Santa Maria Hospital Ethics Committee.

## Micro-computed Tomography

The femoral epiphyses were immediately stored at −80 °C after surgery. A small cylinder of trabecular bone (diameter, 5 mm) was drilled at the principal compressive force direction of the femoral epiphysis. The sample was processed through fixation, dehydration, impregnation, and embedding in polymethylmetacrylate.

Sequential transaxial images through the entire specimen were obtained using micro-computed tomography (μCT; μCT 40, Scanco Medical AG, Brüttisellen, Switzerland) at an isotropic voxel size of 20 μm, integration time of 250 ms, and beam energy and current of 55 keV and 0.145 mA, respectively. A three-dimensional Gaussian filter ($\sigma$=0.8) with a limited finite filter support was used to suppress the noise. The images were binarized to separate bone from other tissues or background using an adaptive thresholding procedure. After thresholding, bone volume fraction (BV/TV), bone surface density (BS/TV), specific bone surface (BS/BV), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular spacing (Tb.Sp), degree of anisotropy (DA=length of the longest mean intercept length (MIL) tensor divided by the shortest MIL tensor), and connectivity density (Conn.D) were assessed for all samples.

## Mechanical Compression Tests

Each bone cylinder was obtained by drilling parallel to the cylinder used for μCT imaging using a perforating drill with a diameter of 15 mm [28]. The cylinders (with only trabecular bone) were defatted for 3 h using a chloroform and methanol solution and were hydrated overnight in a phosphate-buffered saline solution prior to testing.

Compression tests were performed in a universal testing machine (Instron 5566™, Instron Corporation, Canton, USA) with a 10-kN load cell and a cross-head rate of 0.1 mm/s. Stress–strain curves were obtained for each specimen using the Bluehill 2 software (Instron, copyright 1997–2007). This software has the ability to build stress–strain representations from load displacement points, normalized for the dimensions of the specimen. Analyses of the curves were performed in order to obtain the mechanical bone parameters: stiffness (Young's modulus), strength (yield stress), and toughness (energy absorbed until fracture).

## RNA Extraction

Small trabecular bone pieces were collected from the central area of the femoral epiphysis and pulverized using a mortar and pestle. RNA was extracted using TRIzol reagent (Invitrogen, UK) with proteinase K (Bioline, UK) digestion [29] to better dissolve extracellular matrix. Bone powder (80 mg) was placed in TRIzol reagent and homogenized. Lipids were solubilized with 0.2 volumes of chloroform and the fraction containing RNA was preserved. Proteinase K digestion (3.25 μg proteinase K per milligram bone) was performed at 55 °C. RNA was precipitated with 1 volume of ice-cold isopropyl alcohol. RNA pellet was dissolved in RNase/DNase-free water. RNA was cleaned using a commercial kit (RNeasy mini kit, Qiagen, Germany) and genomic DNA contaminants were removed with DNaseI treatment (Qiagen; adapted from [30]). RNA concentration was determined spectrophotometrically (Nanodrop ND-1000 Spectrophotometer, Thermo Fisher Scientific, USA) and its integrity was assessed by a lab-on-a-chip technology (Agilent RNA 6000 Nano Kit, Agilent Technologies, USA) according to the manufacturer's instructions. RNA was stored at −80 °C until further use.

## Quantitative Real-Time PCR

Reverse transcription cDNA synthesis was performed on 60 ng of RNA from each sample using the DyNAmo cDNA synthesis kit (Finnzymes, Finland) and 300 ng of random hexamer primers according to the manufacturer's instructions.

Each cDNA template (3 ng/μl) was amplified in duplicate with a DyNAmo Flash SYBR green qPCR kit (Finnzymes) on

**Table 1** Clinical and biochemical characteristics of the study populations

| | RA | Primary OP | *p* value |
|---|---|---|---|
| *N* | 10 | 12 | |
| Clinical characteristics | | | |
| Age (years) | 67±10 | 74±5 | 0.057 |
| Women (%) | 90 | 92 | 1 |
| BMI (kg/m$^2$) | 26.7±4.3 | 24.8±4.5 | 0.328 |
| BMD (g/cm$^2$) | 0.68±0.06 | 0.71±0.08 | 0.466 |
| *T* score | −2.7±0.8 | −2.4±0.6 | 0.340 |
| FRAX major fracture (%) | 17.9±10.4 | 15.3±14.6 | 0.516 |
| FRAX hip fracture (%) | 9.0±7.1 | 8.8±14.8 | 0.301 |

Values represent the mean±standard deviation or frequencies ($p<0.05$)
*RA* rheumatoid arthritis, *BMD* bone mineral density, *BMI* body mass index

**Table 2** Micro-computed tomography of the trabecular bone specimens

| | RA | Primary OP | *p* value |
|---|---|---|---|
| BV/TV (%) | 0.25±0.09 | 0.25±0.09 | 0.858 |
| BS/BV ($mm^{-1}$) | 13.8±2.6 | 13.8±2.8 | 0.974 |
| Conn.D ($mm^{-3}$) | 6.10±2.75 | 6.01±2.36 | 0.949 |
| Tb.N ($mm^{-1}$) | 1.73±0.42 | 1.75±0.36 | 0.906 |
| Tb.Th (mm) | 0.20±0.04 | 0.20±0.04 | 0.932 |
| Tb.Sp (mm) | 0.57±0.14 | 0.58±0.18 | 0.924 |
| DA | 1.55±0.14 | 1.49±0.22 | 0.486 |

Values represent the mean±standard deviation

*RA* rheumatoid arthritis, *BMD* bone mineral density, *BV/TV* percent bone volume, *BS/BV* bone specific surface, *Conn.D* connectivity density, *OP* osteoporosis, *Tb.N* trabecular number, *Tb.Th* trabecular thickness, *Tb.Sp* trabecular separation, *DA* degree of anisotropy

a Rotor-Gene thermocycler (Qiagen) according to the manufacturer's instructions. Reactions were incubated at 50 °C for 2 min and at 95 °C for 7 min, followed by denaturation at 95 °C for 10 s and annealing/extension at 60 °C for 10 s. The reactions were validated by the presence of a single peak in the melt curve analysis.

Primers for the housekeeping and target genes (Electronic supplementary material Table) were designed using the software Probefinder (http://qpcr.probefinder.com; Roche, Switzerland) in order to anneal in separate exons, preventing amplification of contaminating genomic DNA.

Real-time PCR results were analyzed using the standard curve analysis. The cycle threshold ($C_T$) is defined as the number of cycles required for the fluorescent signal to cross the threshold and exceed the background level. The efficiency of the PCR should be 100 %, meaning that for each cycle, the amount of product doubles. A good reaction should have an efficiency of 90–100 %, which corresponds to a slope between −3.58 and −3.10. The conversion of the $C_T$ value in relative expression levels was performed with the slope and the *Y* intersect extracted from the standard curve and applying the equation $10^{(Y\ \text{intersect}-\text{CT/slope})}$ [31, 32]. The values obtained were normalized with the housekeeping gene phosphomannomutase 1.

**Table 3** Comparison between the relative gene expression of bone remodeling biomarkers and cytokines between RA and primary OP patients

| | RA patients | Primary OP | *p* value |
|---|---|---|---|
| Osteoblast-specific genes | | | |
| RUNX2 | 0.19±0.16 | 0.19±0.15 | 0.947 |
| OSX | 0.16±0.10 | 0.07±0.08 | 0.429 |
| ALP | 0.18±0.18 | 0.24±0.20 | 0.356 |
| OCL | 0.18±0.25 | 0.01±0.01 | 0.226 |
| RANKL | 0.86±1.07 | 1.70±1.83 | 0.231 |
| OPG | 0.35±0.39 | 0.20±0.13 | 0.762 |
| RANKL/OPG | 2.67±2.89 | 11.04±12.97 | 0.118 |
| Osteoclast-specific genes | | | |
| TRAP | 0.15±0.14 | 0.75±1.22 | 0.137 |
| ITGB3 | 0.06±0.04 | 0.18±0.10 | 0.003* |
| CTSK | 5.99±6.95 | 52.41±89.54 | 0.065 |
| Pro-inflammatory cytokines | | | |
| IL1β | 0.023±0.027 | 0.064±0.066 | 0.102 |
| IL6 | 0.240±0.314 | 0.921±0.861 | 0.060 |
| IL17 | 83.59±127.30 | 2.85±3.72 | 0.031* |
| TNF | 0.072±0.060 | 0.154±0.121 | 0.083 |

Values represent the mean±standard deviation

*$p<0.05$

*ALP* alkaline phosphatase, *CTSK* cathepsin K, *IL1* interleukin-1β, *IL6* interleukin-6, *IL17* inteleukin-17, *ITGB3* subunit β3 of the integrin αvβ3, *OCL* osteocalcin, *OPG* osteoprotegerin, *OSX* osterix, *RANK* receptor activator of nuclear factor κB, *RANKL* RANK ligand, *RUNX2* runt-related transcription factor 2, *TNF* tumor necrosis factor, *TRAP* tartrate-resistant acid phosphatase

We studied the bone gene expressions of IL1β, IL6, TNF, IL17, runt-related transcription factor 2 (RUNX2), OSX, alkaline phosphatase (ALP), osteocalcin (OCL), RANKL, OPG, tartrate-resistant acid phosphatase (TRAP), β3-subunit of αvβ3 integrin (ITGB3), and cathepsin K (CTSK). The gene expressions of several components of the Wnt signaling pathway—WNT10B, LRP4, LRP5, and LRP6—as well Wnt inhibitors—secreted frizzled-related protein 1, Wnt inhibitory factor 1 (WIF1), DKK1, DKK2, and SOST—were analyzed.

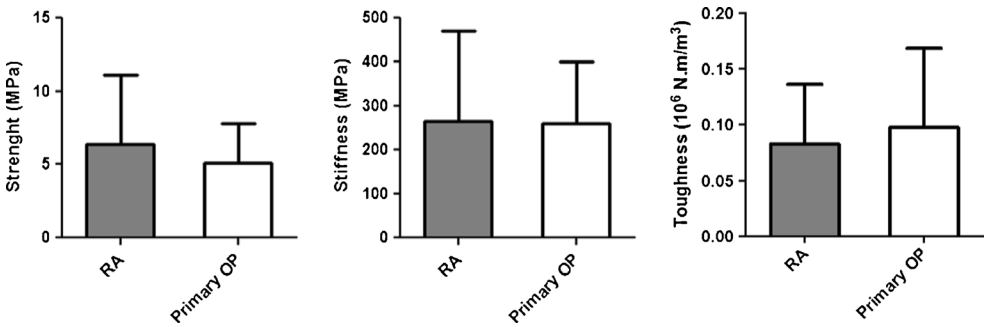


**Fig. 1** The mechanical behavior of bone was analyzed by compression tests of trabecular bone cylinders. Strength, stiffness, and toughness were considered to characterize bone mechanics

### Biochemical Assays

Blood samples were collected before surgery and were stored in the biobank. For this study, assessment of serum calcium and phosphorus, ALP, bone-specific ALP (BSALP), and OCL were performed. Serum carboxy-terminal cross-linked telopeptides of type I collagen (CTX-I) and serum amino-terminal propeptides of type I procollagen (P1NP) were measured using a fully automated Elecsys® electro-chemiluminescent immunoassay analyzers (Roche Diagnostics). DKK2 (USCN, China), RANKL, and SOST (Biomedica, Germany) were measured using ELISA according to the manufacturers' recommendations. IL1β, IL6, IL17, OPG, and DKK1 were evaluated with protein profiling performed by Tebu-bio Laboratories (France).

### Statistical Analysis

Results are presented as the mean and standard deviation or frequencies for continuous and categorical variables, respectively. The RA patient group was compared with the primary OP control group. For continuous variables, such as the clinical characteristics, the serum biomarkers, μCT, and the biomechanical results and gene expression data, normality was confirmed using Shapiro–Wilk test; groups were compared either with Student's *t* test or the non-parametric equivalent Mann–Whitney test. For categorical data, chi-squared or Fisher's exact test was used. Correlations between inflammatory cytokines and Wnt/β-catenin gene expression were performed with Spearman's test. Statistical significance was set for two-sided *p* values at 0.05.

Analysis was conducted using the Statistical Package for Social Sciences manager software, version 19.0 (SPSS, Inc., Chicago, IL, USA).

## Results

### Study Population

Twenty-two patients were recruited for this study: 10 with RA and 12 with primary OP (Table 1).

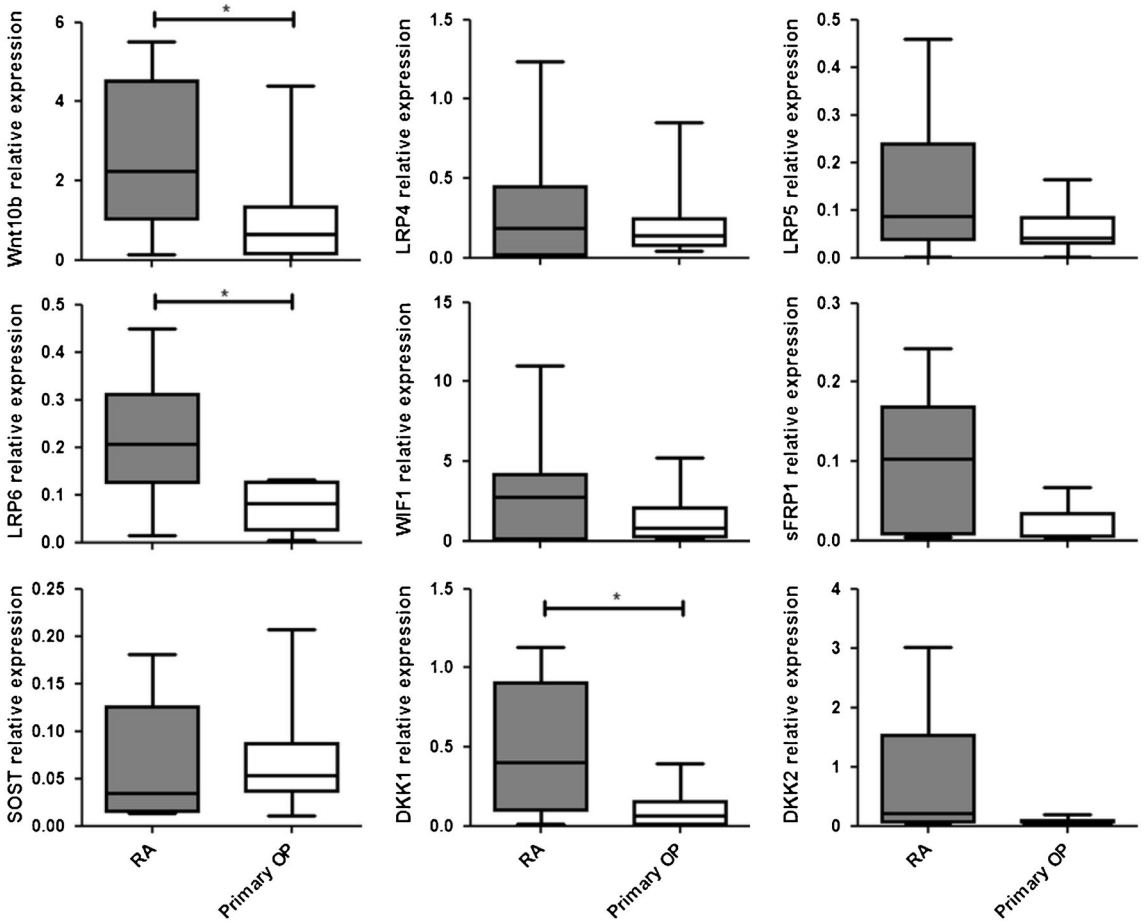


**Fig. 2** Relative expression levels of Wnt/β-catenin pathway genes in RA and primary OP patients (*$p<0.05$). *WNT10B* Wingless-type MMTV integration site family, member 10B, *LRP4*/5/6 low-density lipoprotein receptor-related protein 4/5/6, *sFRP1* secreted frizzled-related protein 1, *WIF1* Wnt inhibitory factor 1, *DKK1* dickkopf 1 homolog, *DKK2* dickkopf 2 homolog, *SOST* sclerostin)

RA patients had a mean age of 68±11 years; 90 % were women, with disease duration of 4±3 years. CRP was 2.1± 2.1 mg/dl. The mean DAS28 3V was of 4.05±2.21, and 44 % of the patients had active disease (DAS28 3V above 3.2). Fifty percent were positive for rheumatoid factor and 50 % had erosive disease. All RA patients were on a low corticosteroid dose (<10 mg/day) and synthetic disease-modifying anti-rheumatic drug monotherapy (nine on methotrexate and one on leflunomide). None of the patients had been treated with biological therapy.

Clinical risk factors for fracture were comparable between groups. FRAX scores (either for major and hip fractures) were similar between RA and primary OP patients.

### RA and Primary OP Bone Is Structurally Similar

To assess bone microstructure, sequential transaxial images were obtained for each specimen (213±44 slices per sample) using μCT. No significant differences were found regarding the trabecular structure between groups (Table 2). Concordantly, RA bone had similar mechanical properties (strength, stiffness, and toughness) to primary OP bone (Fig. 1).

### IL17, WNT10B, LRP6, and DKK1 Gene Expressions Are Upregulated in RA Bone

IL17 bone gene expression was upregulated ($p$=0.031) in RA bone (Table 3) as compared to primary OP. The ligand WNT10B and its receptor LRP6 were upregulated in RA bone ($p$=0.034 and $p$=0.041, respectively). In addition, the Wnt inhibitor DKK1 was upregulated in RA bone ($p$=0.008; Fig. 2). We did not find differences between RA and primary OP gene expression in the gene expression of trabecular bone turnover markers (Table 3), with the exception of the $\beta_3$-subunit of $\alpha_v\beta_3$ integrin, which was upregulated in primary OP patients ($p$=0.003).

### IL17 Gene Expression Is Correlated with WNT10B and DKK2 Gene Expression in RA Bone

In RA patients, IL17 expression was significantly positively correlated with WNT10B ($r$=0.810, $p$=0.015) and DKK2 ($r$=0.800, $p$=0.010). A positive correlation between IL17 and the RANKL/OPG ratio ($r$=0.870, $p$=0.002) was also observed, as determined by gene expression. These associations were not observed for the primary OP group.

### Serum Bone Turnover Markers Are Similar in RA and Primary OP, But Serum Levels of DKK2 Are Significantly Lower in RA Patients

No differences were found between groups on serum bone turnover markers (Table 4). However, RANKL ($p$=0.042) and DKK2 ($p$=0.040) were significantly lower in RA patients.

At serum level, no differences were found between RA and primary OP patients regarding the pro-inflammatory cytokines IL1, IL6, IL17, and TNF (Table 4).

## Discussion

In this study, we found that RA bone microenvironment, when compared with primary OP patients who had similar FRAX scores, BMD, trabecular bone biomechanical behavior, and microstructural organization, has a gene expression profile characterized by the upregulation of the Wnt pathway genes and also of one of its inhibitors, DKK1. Moreover, we also found the upregulation of IL17 bone expression in RA patients, which was positively correlated both with WNT10B expression and with one of its inhibitors, DKK2. Finally, a significantly positive correlation between IL17 expression and RANKL/OPG was observed as well. Therefore, our findings

**Table 4** Serum levels of inflammatory cytokines, Wnt regulators, RANKL/OPG, and bone remodeling markers

| | RA | Primary OP | $p$ value |
|---|---|---|---|
| Pro-inflammatory cytokines | | | |
| IL1β (pg/ml) | 1.32±1.32 | 0.64±0.70 | 0.309 |
| IL6 (pg/ml) | 9.77±6.88 | 6.62±4.19 | 0.396 |
| IL17 (pg/ml) | 11.68±2.03 | 3.38±0.82 | 0.152 |
| Wnt/β-catenin pathway inhibitors | | | |
| DKK1 (pg/ml) | 1169.8±801.4 | 771.0±475.8 | 0.356 |
| DKK2 (ng/ml) | 19.67±13.25 | 46.78±20.82 | 0.040* |
| SOST (pg/ml) | 298.9±217.7 | 438.6±224.3 | 0.323 |
| Osteoclastogenesis markers | | | |
| OPG (pg/ml) | 22.52±18.43 | 11.14±12.13 | 0.269 |
| RANKL (pg/ml) | 1.04±0.86 | 2.72±1.29 | 0.042* |
| RANKL/OPG | 0.19±0.27 | 0.37±0.26 | 0.345 |
| Serum bone remodeling markers and calcium metabolism | | | |
| Calcium (mg/ml) | 9.4±3.5 | 8.3±0.6 | 0.901 |
| Phosphorus (mg/dl) | 3.4±0.4 | 3.0±0.8 | 0.169 |
| Osteocalcin (ng/ml) | 9.0±10.7 | 11.5±9.8 | 0.633 |
| ALP (u/l) | 99.4±51.9 | 87.7±39.8 | 0.770 |
| BSALP (μg/l) | 11.7±5.0 | 10.4±5.4 | 0.728 |
| P1NP (ng/ml) | 43.0±32.4 | 41.9±33.0 | 0.952 |
| CTX-I (ng/ml) | 0.40±0.26 | 0.38±0.21 | 0.854 |

Values represent the mean±standard deviation or frequencies

*$p$<0.05

*RA* rheumatoid arthritis, *DKK* dickkopf, *IL* interleukin, *OP* osteoporosis, *OPG* osteoprotegerin, *RANKL* receptor activator of nuclear factor kappa-B ligand, *SOST* sclerostin, *ALP* alkaline phosphatase, *BSALP* bone-specific ALP, *OP* osteoporosis, *P1NP* procollagen type I amino-terminal propeptide, *CTX-I* carboxy-terminal collagen type I cross-links, *PTH* parathormone

suggest that bone IL17 expression contributes to disturbed bone remodeling in RA.

The increased expression of DKK1 in RA was paralleled by an upregulation of LRP6, which is coherent with the fact that DKK1 induces LRP6 accumulation both at the cell surface and in endosomes [33]. Although not reaching statistical significance like DKK1 did, DKK2, which has a role in the regulation of late-stage osteoblast differentiation into mineralized matrices [34], showed a similar pattern in RA patients. Therefore, in these patients, low bone formation during inflammation may arise from inhibition of Wnt signaling by its endogenous inhibitors DKK and via DKK1-mediated internalization of LRP6.

The increased bone IL17 expression in RA patients had a positive correlation with the RANKL/OPG ratio and WNT10B and DKK2 bone expression. Recently, it was reported that sFRP1 promotes Th17 cell differentiation [35], pointing to a close relationship between the Wnt/β-catenin pathway and IL17. IL17 also inhibits adipogenesis and promotes osteogenesis of mesenchymal stem cells [36], which might be mediated by Wnt signaling. On the other hand, it is well established that IL17 acts as a potent stimulator of osteoclastogenesis by inducing RANKL expression on osteoblasts [37], reinforcing the pivotal role of this cytokine on bone remodeling in RA.

Curiously, WNT10B, which positively regulates bone formation by inducing the osteoblast transcription factors RUNX2, DLX5, and OSX [38], was upregulated in RA patients. This apparently paradoxical result might be explained by the coupling of bone resorption to bone formation, leading to a partially compensatory pro-osteoblastic response that we have previously observed in a mouse model of arthritis [25, 39].

Our observations are relevant as they indicate that bone fragility in RA may arise from bone biologically specific mechanisms, different from those in primary OP. In addition, these differences in gene expression occurred in RA patients with relatively short disease duration. One aspect that cannot be completely clarified solely based on this study is the impact of low-dose corticosteroid used by all enrolled RA patients (a standard practice in the management of RA in Portugal) on the expression of the analyzed genes. In fact, to our knowledge, this is the first study where bone quality and gene expression were assessed in human samples in the context of a chronic inflammatory condition.

The effect of environmental exposure on epigenetic determinants such as DNA methylation should be taken into consideration in the context of the phenotypic expression of a disease such as RA [40–45]. Given the characteristics of this study, this aspect could not be explored, but it is likely that several environmental factors, difficult to identify and control, might influence the expression of the genes that we have studied.

In conclusion, bone fragility in RA patients might be induced by an unbalanced bone microenvironment, which is different from the one occurring in primary OP. RA bone is associated with a specific pattern of gene expression, namely, increased IL17 and DKK1 bone expression, suggesting that the modulation of these two factors might prevent systemic bone loss in RA.

**Disclosure** The authors state that they have no conflict of interest.

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