

SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

**EFFECTS OF DISEASE-MODIFYING ANTIRHEUMATIC DRUG
TREATMENT ON PERIARTICULAR BONE REMODELING IN
RHEUMATOID AND PSORIATIC ARTHRITIS**

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**UNIVERSITY OF DEBRECEN
DOCTORAL SCHOOL OF CLINICAL MEDICINE**

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Members of the Examination Committee: Prof. Péter Lakatos, MD, PhD, DSc
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The Examination takes place at the Library of Department of Ophtalmology,
Faculty of Medicine, University of Debrecen
on 30th May 2018 at 10.30 am

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The PhD Defense takes place at the Faculty Conference Centre (Kenézy Villa),
Faculty of Medicine, University of Debrecen
on 30th May 2018 at 2.00 pm

1 INTRODUCTION

1.1 Inflammation and bone loss

Rheumatoid arthritis (RA) and psoriatic arthritis (PsA) are chronic inflammatory joint diseases characterized by bone destruction, progressive damage and impaired joint function. Inflammatory arthritides have been associated with both localized bone resorption and/or pathologic bone formation, as well as generalized bone loss. Inflammation is the key trigger for local bone erosions affecting the cortical bone margin, which is in direct contact with the inflamed synovium, and it also induces periarticular bone loss or “juxta-articular osteoporosis” characterized by resorption of the cortical and trabecular bone close to inflamed joints. Moreover, PsA is characterized by inflammation at enthesal sites, where bone is also directly exposed to inflammatory tissue. Inflammation interferes with bone homeostasis at a systemic level that results in osteoporosis and increased fracture risk at both axial and appendicular skeletal sites. This tight relationship between inflammation and bone loss clinically reflects the molecular and cellular interactions between the immune system and the bone, also known as osteoimmunology. Recent studies on the pathomechanisms of bone destruction in RA have extended current concepts on the interactions of immune system, inflammation and bone suggesting that bone loss mediated by antibodies against citrullinated proteins (ACPA) may even precede the onset of inflammation.

1.2 Bone resorption and the RANK/RANKL/osteoprotegerin pathway

Bone loss results from an imbalance between bone-resorbing osteoclasts, large multinucleated cells from hematopoietic derivation, and bone-forming osteoblasts of mesenchymal origin. Synovial inflammation is a source of pro-inflammatory cytokines like $\text{TNF-}\alpha$, interleukin (IL)-1, IL-6, IL-17 and IL-23, which either directly trigger osteoclast differentiation and bone resorption, or indirectly increase the expression of receptor activator

of nuclear factor-kappa B ligand (RANKL) in mesenchymal cells. The RANK-RANKL system is the master regulator of osteoclastogenesis, thus the major driver of bone destruction in inflammatory arthritis. Osteoprotegerin (OPG) produced by osteoblasts and bone marrow stromal cells is a naturally occurring decoy receptor of RANKL that inhibits the RANKL-RANK interaction. OPG not only prevents formation of local bone erosions, but it has been shown to protect against systemic bone loss in human TNF-transgenic mice. Low OPG/RANKL ratio has been associated with future progression of radiographic damage in RA.

1.3 Bone formation and mediators of the Wnt pathway

Inflammatory arthritides show profound differences in joint architecture and structural integrity of periarticular bone. These cover the whole spectrum; from an almost purely erosive disease like RA to a prominently bone forming disease like axial spondyloarthritis (SpA). PsA shows mixed pattern of bone erosions and new bone formation. Bony spurs in PsA may develop along joints, at the insertion sites of the entheses, and at intervertebral spaces. The molecular determinants responsible for the different patterns of joint remodeling are not yet elucidated, however differences are at least partly based on the variable capability to form new bone, which may reflect a skeletal response to inflammation.

Wingless-related integration site (Wnt) proteins emerge as key promoters of osteoblastogenesis, hence new bone formation in inflammatory arthritis. The Wnt/ β -catenin pathway not only increases bone formation, but also inhibits bone resorption by blocking apoptosis of osteoblasts and upregulating OPG. Dickkopf-1 (DKK-1) and sclerostin are natural inhibitors of Wnt signaling and they block the differentiation and function of osteoblasts. DKK-1 increases the expression of macrophage-colony stimulating factor and RANKL, enhances RANKL-RANK interaction and decreases OPG expression. DKK-1 also induces sclerostin expression by osteocytes, and promotes synovial angiogenesis. It has been shown that TNF- α impairs bone formation by inducing DKK-1 and sclerostin expression. These data suggest a

cross-talk between bone catabolic RANKL and bone anabolic Wnt pathways, which are both influenced by TNF- α .

1.4 Biomarkers of bone remodeling

Bone is a rigid, yet dynamic organ. In order to maintain its structural integrity, and to fulfill its role in mineral homeostasis, bone is continuously formed and repaired, a process termed remodeling. Bone remodeling is a complex, tightly regulated process that is the result of two opposite activities, the production of new bone matrix by osteoblasts and the destruction of old bone by osteoclasts. The rates of bone formation and destruction can be monitored either by measuring predominantly osteoblastic or osteoclastic enzyme activities, or by assaying bone matrix components released into the bloodstream and excreted in the urine. Bone matrix is mainly composed of type I collagen, while the main collagen in articular cartilage is type II. Serum procollagen type-I N-propeptide (PINP), and non-collagen proteins like bone-specific alkaline phosphatase (bone ALP) and intact osteocalcin (OC {1-49}) produced by osteoblasts, are markers of bone formation. Type I collagen fragments such as serum C-terminal cross-linking telopeptides (CTX-I), and urinary N-terminal cross-linking telopeptide of type-I collagen (NTX-I) and free deoxypyridinoline crosslinks (fDPD) are sensitive markers of bone resorption. Several biochemical bone turnover markers (BTM) were found to be useful in assessing joint damage in RA. Yet, there are fewer publications devoted to the evaluation of bone and cartilage metabolism in PsA. To date, no single bone turnover marker has been found to reflect joint destruction with sufficient accuracy to use in the clinic.

1.5 Effects of TNF- α inhibition (TNFi) on bone turnover markers

TNFi agents were shown to increase levels of bone formation markers, such as OC and P1NP, and suppressed CTX-I, NTX-I, fDPD and RANKL levels, which are markers of bone resorption, in RA and SpA. TNFi therapy was also shown to increase OPG/RANKL, OC/CTX and P1NP/CTX ratios. On the contrary, other studies with TNFi showed no effects on OC, P1NP and

CTX levels. More recent studies showed that TNFi also suppress DKK-1 leading to increased bone formation in RA. On the other hand, a single study in patients with SpA showed that TNFi paradoxically increased DKK-1 production, whilst others found increased sclerostin production in RA after a short-term treatment with TNFi. Our group demonstrated that in a mixed cohort of RA and SpA patients TNFi decreased DKK-1, but increased sclerostin levels.

These findings suggest that the effects of TNFi on BTMs are heterogeneous, but in general TNFi therapy is associated with a modest increase in formation and decline in resorption, thus beneficial on local and systemic bone remodeling.

1.6 Impact of conventional DMARDs on bone

Among conventional disease-modifying antirheumatic drugs (DMARDs), a structure-sparing effect has been documented for methotrexate (MTX), sulphasalazine, and leflunomide used as monotherapy or in combination treatments. It is not yet clear whether conventional DMARDs have specific effects on bone and cartilage damage, or whether their protective effect is secondary, resulting from the reduction of inflammation. It has been shown that MTX supports the structure-sparing effects of biologics by acting synergistically with the biologic agents. When used as monotherapy, the structure-sparing effect of MTX is quite moderate compared with that of TNFi, even if MTX is used in DMARD naive patients. Nonetheless, MTX has been shown to reduce the expression of RANKL in synovial fibroblast cultures, which may indicate a specific effect on osteoclasts.

1.7 Quantifying periarticular bone density in RA and PsA

Detection and quantification of bone erosions are important outcome measures given that their presence likely reflects subsequent functional impairment of the affected joint. Periarticular bone loss however may precede the development of erosions, therefore its recognition at an early stage of the disease is essential in preventing irreversible joint damage. Several imaging methods have been developed to quantify periarticular bone

density. One such technique is dual-energy x-ray absorptiometry (DXA), which is known as an objective and precise method for monitoring bone loss, including that in the hands. Measures of periarticular osteoporosis are more suitable for assessment of bone damage in RA, because hand bone loss occurs earlier than generalised bone loss. The rate of periarticular bone loss is higher than that in central sites and the precision of the DXA is also superior at the hand compared with the hip and spine. In RA, disease-related bone loss detected by DXA occurs in the very early phase of the disease process and hand bone loss is greater in patients with active disease.

Another technique for quantifying periarticular bone density is digital X-ray radiogrammetry (DXR). Previous research has shown that metacarpal BMD loss as measured by DXR is associated with disease activity, and BMD loss in the first year after diagnosis is predictive of radiologic progression up to 20 years later in patients with early RA. Far less is known about periarticular bone changes in early PsA. Hand bone loss in PsA as measured by DXR was studied in only one clinical trial, and no study to date has compared changes in cortical bone mineral density of RA and PsA using DXR.

2 AIMS

This thesis consists of 3 studies investigating different aspects of bone remodeling in response to anti-TNF- α therapy along with conventional DMARDs in RA and PsA patients. We evaluated early and long-term effects of TNFi treatment on bone turnover markers (Study 1) and mediators of bone remodeling (Study 2), along with changes in hand and axial BMD in a cohort of RA and PsA patients with established disease. Thereafter we assessed periarticular BMD of the hands in patients with early RA and PsA prior to and after introducing DMARDs (Study 3).

Our specific aims were as follows:

Study 1

Evaluation of early and long-term effect of TNFi treatment on bone turnover markers and BMD in RA and PsA

- To study the relationship between disease activity, BTMs and BMD prior to TNFi treatment.
- To assess the early (1 month) and long-term (1 and 3 years) effect of TNFi treatment on disease activity measures, levels of BTMs, and measures of hand, spine and hip BMD in patients with RA and PsA.

Study 2

Assessment of serum bone remodeling mediators in RA and PsA patients prior to and following TNFi therapy

- To examine the early (1 month) and more long-term (12 months) effects of TNFi treatment on serum OPG, RANKL, DKK-1 and sclerostin levels in patients with RA and PsA.
- To study associations between circulating mediators of bone remodeling and disease activity along with hand BMD measures prior to and following TNFi in RA and PsA.

Study 3

Comparison of hand BMD as measured by DXR between early, treatment-naïve RA and PsA patients 3 and 12 months after introducing DMARDs

- To compare changes in hand DXR-BMD and radiographic progression between early (disease duration <12 months), treatment-naïve RA and PsA patients 3 and 12 months after introducing an antirheumatic treatment.
- To compare demographic and clinical parameters for patients with normal hand DXR-BMD to those with bone loss.
- To identify predictors for early hand bone loss at the time of disease presentation in RA and PsA.

3 METHODS

3.1 Study 1

3.1.1 Patients

Patients were recruited from the Biologic Clinic at St. Vincent's University Hospital (SVUH) having been referred there for screening prior to initiation of TNFi therapy. Inclusion criteria were diagnosis of RA or PsA according to ACR or CASPAR criteria respectively and age between 18 and 80 years. All patients had on-going active joint inflammation and had failed to respond adequately to at least one disease-modifying drug including maximum tolerated (up to 25 mgs/week) doses of MTX. Exclusion criteria included previous treatment with biologic agents 3 months prior to entering the study, withdrawing from the biologic treatment during the study period, treatment with anti-resorptive medications, parathyroid hormone (teriparatide) or strontium ranelate 6 months prior to or during the study, diseases of bone metabolism and pregnancy. The use of calcium and vitamin D supplements and a stable dose of prednisolone of less than 10 mg/day were permitted. Following screening, patients had their baseline clinical assessments performed and these were repeated after therapy at 3 (n=57), 12 (n=47) and 36 months (n=51). Clinical assessments included a recording of the following parameters: tender joint count (TJC), swollen joint count (SJC), patients' visual analogue scale for global health (GVAS), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), health assessment questionnaire (HAQ), duration of early morning stiffness (EMS), pain and fatigue scores VAS. A Disease Activity Score 28 with four variables (DAS28-CRP) was calculated and EULAR response criteria were applied. Informed, written consent was obtained according to the Declaration of Helsinki. The study was approved by St. Vincent's Healthcare Group Ethics and Medical Research Committee.

3.1.2 Biochemical measurements

Serum and urine samples were collected at baseline and after 1, 12 and 36 months of TNFi therapy according to our laboratory protocol: following an overnight fast, morning blood was collected in serum tubes containing a clot activator, and a second void urine sample was provided. Serum 25-hydroxyvitamin D (25OHD), parathyroid hormone (PTH), bone ALP, PINP, OC {1-49}, CTX-I, urinary NTX-I and fDPD were measured as previously described. NTX-I and fDPD were expressed as a ratio with urine creatinine concentration which was measured by a kinetic Jaffe method.

3.1.3 BMD measurements

BMD measurements were recorded using a Hologic Discovery A Model with software version 12.6 (Waltham, MA). BMD assessments were obtained at left total hip, lumbar spine and hands at baseline, 12 and 36 months. T-scores and Z-scores were calculated at hip using NHANES III database and at spine using manufacturer's database. Periarticular BMD of hands was measured as previously described. In brief, global hand BMD included all hand bones distal from the wrist joint; 7 sub-regions of interests (ROI) were selected for analysis. These included the carpus and the periarticular regions of the second, third and fourth MCP and PIP joints. The periarticular ROIs included 10 mms of bone proximal and distal to the joint line excluding the joint space incorporating both the proximal and the distal juxta-articular components of the joints. The mean BMD of the 14 regions in total (termed hand BMD) and the average BMD at the two periarticular regions, MCP and PIP, were calculated.

3.1.4 Statistical methods

At baseline differences between means were tested by either independent t-test, t-test for unequal variances, or Mann-Whitney-test where appropriate. Differences between categorical variables were tested by chi-squared-test. Associations between variables were tested by either Pearson or Spearman correlations. In order to identify associated variables,

BMD at hand was divided at the median into two equal groups. Independent variables were tested by univariate single logistic regression analysis. Those variables with a $p < 0.25$ were entered into a multivariable logistic regression model. Variables with a Wald statistic > 0.25 were removed from the model; the remainder were retained based on testing differences between models using the likelihood ratio test. Given that hand BMD is a continuous variable, a multiple linear regression analysis was performed with independent variables being selected based on correlation analysis ($p < 0.05$).

In order to assess the response to TNFi therapy, repeated measures analysis of variance (ANOVA) was performed using a split-plot design, which tested between-subject and within-subject factors. The between-subject variable was the disease category (RA or PsA) and the within-subject variables included either 3 or 4 levels of the repeated measure factor as follows: 3 time points for BMD at hand ($n=45$), spine ($n=45$), and hip ($n=44$); 4 time points for BTMs ($n=48$); and 4 time points for clinical and inflammatory variables. In addition to testing the main effects, the interaction between each within-subject variable and disease category was tested. If significant differences were noted in the main effects, then pairwise comparisons of within-subject variables were performed, and significance testing was adjusted for multiple comparisons according to Bonferroni-correction. Results with $p < 0.05$ were considered statistically significant. Statistical analysis was performed using SPSS for Windows, Version 18 (Chicago, IL).

3.2 Study 2

3.2.1 Patients

Study population was the same as described in 3.1.1.

3.2.2 Testing of mediators of bone remodeling

Serum samples were collected at baseline and after 1 and 12 months of TNFi therapy according to our laboratory protocol: following an overnight fast, morning blood was collected in serum tubes containing a clot activator. Serum total OPG, sRANKL, DKK-1 (Biomedica Gruppe, Wien, Austria) and sclerostin (USCN Life Science Inc, Wuhan, China) were measured using commercial sandwich enzyme-linked immunosorbent assays (ELISA) in the Department of Laboratory Medicine, University of Debrecen as previously described. The OPG assay detects monomeric, dimeric and ligand bound OPG employing a mouse monoclonal anti-OPG antibody (Ab) as capture Ab and a biotinylated goat polyclonal Ab for detection. The sRANKL assay measures soluble, uncomplexed human RANKL using a human recombinant OPG for capture and a biotin-labeled goat polyclonal Ab for detection. The DKK-1 assay detects total DKK-1 employing an Ab specific for human DKK-1 and a biotin-labeled anti DKK-1 Ab for detection. The sclerostin assay measures human sclerostin employing a monoclonal Ab specific for human sclerostin and a biotinylated polyclonal anti-human sclerostin Ab for detection. The inter-assay coefficients of variation (CV) was <8 % for OPG (lower detection limit: 0.14 pmol/L, upper detection limit: 30 pmol/L), <6 % for sRANKL (lower detection limit: 0.02 pmol/L, upper detection limit: 2 pmol/L), <9% for DKK-1 (lower detection limit: 0.38 pmol/L, upper detection limit: 50 pmol/L), and <12% for sclerostin (lower detection limit: 0.007 pmol/L, upper detection limit: 0.45 pmol/L).

3.2.3 BMD measurements

BMD assessments were obtained at hands at baseline and 12 months as described in 3.1.3.

3.2.4 Statistical methods

Differences between RA and PsA in mediators of bone remodeling levels and BMD measures were compared using Mann-Whitney U-test. Wilcoxon tests were used to compare response of bone remodeling regulators and BMD to TNFi treatment in RA and PsA. To assess the

response to TNFi therapy, repeated measures analysis of variance (ANOVA) was performed (within-subject factor: 0, 1, 12-month time points, between-subject factors: RA and PsA groups, and TNFi treatment). $P < 0.05$ was considered as statistically significant. Correlations between clinical parameters, mediators of bone remodeling and BMD measures were analyzed using Spearman's Correlation. Given that hand BMD is a continuous variable, a multiple linear regression analysis was performed with independent variables being selected based on correlation analysis ($p < 0.05$). Statistical analysis was performed using SPSS for Windows version 20.0 (Armonk, NY).

3.3 Study 3

3.3.1 Patients and study design

A total number of 64 patients (32 RA, 32 PsA) were recruited from the Rheumatoid Arthritis and Spondyloarthropathy Clinics at the Department of Rheumatology, St. Vincent's University Hospital. Recent-onset (symptom duration < 12 months), treatment naïve PsA and RA patients with active joint inflammation, aged 18 to 80 years were enrolled consecutively. PsA patients fulfilled the CASPAR criteria and patients with RA met the 2010 ACR/EULAR classification criteria for RA. Exclusion criteria were pregnancy, diseases of bone metabolism, previous treatment with disease-modifying anti-rheumatic drugs (DMARDs) or biologic agents, and treatment with anti-resorptive medications, parathyroid hormone or strontium ranelate 6 months prior to the study. The use of calcium and Vitamin D supplements and a stable dose of prednisolone of less than 10 mg/day were permitted during the study. The study was approved by St. Vincent's Healthcare Group Ethics and Medical Research Committee. All subjects were enrolled after agreeing to participate and signing informed consent forms.

3.3.2 Demographic and clinical variables

Clinical assessments were performed at baseline and at 3 (n=60) and 12 (n=58) months. Data collected included age, sex, menopausal and smoking status, current or previous use of oral contraceptive, hormone replacement therapy, corticosteroids (CS), calcium and Vitamin D supplements, and alcohol intake (units/week). Body mass index (BMI) was calculated as the patient's weight in kilograms divided by height in meters, squared and BMI was further divided into normal (<25 kg/cm²) and overweight/obese (≥25 kg/cm²) categories. Clinical variables included TJC, SJC, presence of dactylitis, Psoriasis Area Severity Index (PASI) and the DAS28-CRP. Patients' self-reported parameters such as EMS, GVAS, pain and fatigue scores, and HAQ were recorded. Laboratory assessments included a recording of the following parameters: antibodies against cyclic citrullinated peptides (aCCP) and rheumatoid factor (RF) positivity, ESR and CRP.

3.3.3 Radiological scoring

X-rays of hands and feet were obtained at 0, 3 and 12 months and read by a radiologist blinded to the patient characteristics and the objective of the study. The Sharp-van der Heijde Modified Scoring Method for PsA was used. Erosion (ES) and joint space narrowing scores (JSNS) were calculated and added up for the total score (mSHS). X-rays from PsA patients were also scored for proliferation (PS) according to the Psoriatic Arthritis Ratingen Score.

3.3.4 Hand DXR measurements

Digital X-ray radiogrammetry (DXR) was used to measure hand BMD on the same digital hand X-rays scored for radiographic joint damage. The DXR-BMD technique has been described in detail previously. In short, BMD is estimated through an automated analysis of the cortical bone at the centres of the second, third, and fourth metacarpals on a standard projection digital radiograph as specified by the DXR device manufacturer (Sectra, Sweden). One digital posterior-anterior X-ray image of each hand (palm flat to detector table, focus film

distance of 100 cm, focus centered on the third metacarpal bone) was obtained. All patients were acquired using standard exposure factors of 50 kVp and 3.2 mAs. To avoid biasing dominant and non-dominant hands and to achieve better precision, the mean of both hands was used for the analyses. Mean DXR-BMD (mg/cm^2) values of both hands and changes in DXR-BMD ($\text{mg}/\text{cm}^2/\text{month}$) were calculated and compared between the two groups at the 3 time points. Changes in hand BMD were further analysed by stratifying PsA and RA into 3 subgroups based on previously established cut-offs by the manufacturer for the categories of normal (BMD loss $<0.25 \text{ mg}/\text{cm}^2/\text{month}$), moderately elevated BMD loss ($\geq 0.25 \text{ mg}/\text{cm}^2/\text{month}$) and highly elevated BMD loss ($>2.5 \text{ mg}/\text{cm}^2/\text{month}$).

3.3.5 Statistical analysis

Statistical analysis was performed using SPSS for Windows version 20.0 (Armonk, NY). Baseline characteristics were described using mean (SD), median (IQR) for continuous variables and percentage for counts. Differences between PsA and RA in response of clinical measures to anti-rheumatic treatment were compared using Mann-Whitney U-test. The same test was applied comparing RA and seropositive RA patients to PsA with regards to changes in X-ray scores and DXR-BMD. Categorical variables were compared by chi-square test, and independent sample t test and Mann Whitney U-test were used to compare continuous variables between patients with normal hand DXR-BMD and those with bone loss. Logistic regression analyses were performed to identify demographic and disease related factors associated with hand BMD loss. $P < 0.05$ was considered as statistically significant.

4 RESULTS

4.1 Study 1

4.1.1 Baseline findings

We recruited 62 patients (35 RA, 27 PsA). RA patients were older ($p=0.022$). ESR ($p=0.049$) and CRP ($p=0.003$) were significantly higher in RA compared to PsA. DAS28-CRP reflected high disease activity in both groups at baseline. Clinical subtype of PsA patients were asymmetrical oligoarthritis ($n=15$), symmetrical polyarthritis ($n=11$) and predominant spondylitis ($n=1$).

Serum ionised calcium was within the reference range in all subjects. Median serum 25OHD was 52.6 (18.9-105.7) nmol/L; 16.4% of the patients were at-risk of deficiency (<30 nmol/L) and 54.1% had sufficient levels (≥ 50 nmol/L). There was an inverse correlation between 25OHD and PTH ($r=-0.376$; $p=0.003$). BTMs did not correlate with any clinical disease activity scores. PINP correlated directly with both ESR ($r=0.257$, $p<0.049$) and CRP ($r=0.284$, $p<0.030$). Urine fDPD correlated directly with both ESR ($r=0.493$, $p<0.001$) and CRP ($r=0.621$, $p<0.001$). None of the other markers correlated with ESR or CRP.

According to WHO criteria and based on BMD results expressed as T-scores, osteopenia was evident in the spine in 22% and in the hip in 10%, and osteoporosis was evident in the spine in 12% and in the hip in 2% at baseline.

There were no significant correlations between both hip and spine BMD and any of the clinical measures of disease activity, or with BTMs, even when analysis was repeated with BMD results expressed as T-scores. In contrast, hand BMD correlated significantly and inversely with ESR ($r=-0.217$; $p=0.036$), HAQ ($r=-0.295$; $p=0.023$), bone ALP ($r=-0.340$; $p=0.007$), PINP ($r=-0.334$, $p=0.009$), CTX-I ($r=-0.332$; $p=0.008$), NTX-I ($r=-0.355$; $p=0.008$) and fDPD ($r=-0.351$, $p=0.005$). Hand BMD also correlated with age ($r=-0.337$,

p=0.007) and weight ($r=0.470$, $p<0.001$). Thereafter analysis of variables was confined to the primary outcome measure, hand BMD.

Disease category, gender, body weight, bone ALP, PINP, CTX-I and NTX-I were associated with hand BMD according to univariate single logistic regression analysis ($p<0.05$). After adjustment for disease category, odds ratios for association between BTMs and hand BMD were tested; significant associations were noted for bone ALP, PINP, CTX-I and NTX-I. Multivariable logistic regression analysis identified three associations with low hand BMD: presence of RA, lower body weight, and higher serum bone ALP. The final model increased association with hand BMD from chosen cut-off of 50% up to 77%. The stepwise multiple linear regression analysis for the associations with hand BMD was significant ($r^2=0.446$; $p<0.001$) with the model also including disease category, weight and bone ALP.

4.1.2 Clinical response to TNFi therapy

The choice of TNFi therapy was at the discretion of the treating physician with 32 etanercept, 28 adalimumab and 2 patients receiving infliximab. Sixty-six % of the patients received combination therapy (TNFi in combination with one conventional DMARD therapy) from baseline with MTX ($n=37$), leflunomide ($n=2$), hydroxychloroquine ($n=1$) and sulfasalazine ($n=1$). Eight (22.8%) patients with RA and 4 (14.8%) with PsA received low dose prednisolone at various time points during the study period. We were unable to evaluate statistically the effect of concomitant CS use on BTMs due to small sample size. In all, 26 (66.6%) women were postmenopausal, 19 (79.2%) with RA and 7 (46.6%) with PsA. None of the patients used menopausal hormone therapy.

At 36 months 51 patients completed the study (4 withdrawals, 2 died, 5 other reasons); although varying numbers had complete datasets at all the time points for analysis by split-plot ANOVA. For all the clinical measures, highly significant improvements were noted; disease category had a main effect on ESR only ($p=0.021$) with ESR being lower in

PsA than RA. There was only one interaction effect, which was between disease category and TJC ($p=0.023$). The effect on both ESR and CRP was waning at 3 years.

4.1.3 Response of bone turnover markers to TNFi therapy

For BTMs, significant changes were noted for bone ALP ($p<0.001$) and to a lesser extent for both PINP ($p=0.040$) and OC_{1-49} ($p=0.039$); disease category only had an effect on bone ALP ($p=0.015$) with bone ALP being higher in PsA than RA at all time points; and the only interaction was between disease category and CTX-I, whereby CTX-I drifted downwards in RA but increased in PsA ($p=0.037$).

There were significant correlations between change in urine fDPD and both change in ESR ($r=0.721$, $p<0.001$) and change in CRP ($r=0.748$, $p<0.001$); but no similar changes were noted with the other BTMs. We introduced an index that combined a formation and a resorption marker. In order to choose the best combination, we explored the correlation between changes in markers over the 3 years; the highest correlation was between PINP and CTX-I ($r=0.383$; $p=0.006$). The index was expressed as a ratio ($\mu\text{g}/\mu\text{g}$) identical to a recently reported index. There was no significant change in the index with anti-TNF- α therapy, but there was a definite trend towards positive remodeling balance that ameliorated at the time of the final measurement.

4.1.4 Changes in hand, hip and spine BMD in response to TNFi

Hand BMD showed a steady, but not significant increase in both diseases over 3 years. When comparing the two diseases, hand BMD was higher in PsA at baseline ($p=0.025$) and at 3 months ($p=0.069$), and remained higher at all time points. For BMD, the within-subject effects were seen for PIP BMD that increased ($p=0.002$) and for MCP BMD that declined ($p=0.016$). No effect of disease category was noted; there was an interaction between disease category and MCP BMD ($p<0.001$) with MCP BMD declining at end of first year for RA ($p=0.073$) whereas PsA shows a steady decline between baseline and third year ($p=0.086$).

For axial BMD, the within-subject effects were seen for hip BMD that declined ($p=0.014$). Hip BMD decreased in both diseases over the study period and was significantly lower in RA at 3 years compared to baseline ($p=0.044$) and to 1 year ($p=0.045$). Spine BMD also declined in RA and was lower at both 1 and 3 years of TNFi treatment compared to baseline ($p=0.044$ and $p=0.032$, respectively), whilst it remained stable in PsA. Hip and spine BMD measures were higher in PsA compared to RA over the study period, however differences were not significant.

4.2 Study 2

4.2.1 Descriptive statistics at baseline

Demographic and disease-specific characteristics of the whole group, RA and PsA patients are described in 4.1.1. Serum levels of mediators of bone remodeling were not statistically different between the two diseases prior to TNFi treatment.

4.2.2 Changes in mediators of bone remodeling in response to TNFi

We did not find significant differences in serum levels of mediators of bone remodeling in the entire group between any time points. OPG/RANKL ratio reflecting remodeling balance were also similar. Interestingly, repeated measures analysis of variance revealed that TNFi treatment had significant effect on changes in OPG levels between 1-12 months and that is dependent on the diagnosis (test of within subject contrasts $p=0.04$) with OPG being higher in RA than PsA.

In RA, OPG levels were higher at 1 year compared to 1 month ($p=0.03$), whilst there was no significant difference in OPG observed in PsA during the study. Serum RANKL, DKK-1 and sclerostin levels did not change significantly in either RA or PsA over the treatment period. When comparing the two diseases, OPG levels were higher in RA compared to PsA during the entire period of treatment with a significant difference at 1 year ($p=0.01$). No significant

difference in RANKL, DKK-1 and sclerostin levels was observed between RA and PsA at any time point, though DKK-1 levels were lower in PsA at 12 months approaching significance ($p=0.089$). OPG/RANKL ratios and their change were similar in RA and PsA during the study.

4.2.3 Correlations between mediators of bone remodeling and disease activity

OPG levels correlated with ESR and CRP at both prior to and after TNFi treatment. DKK-1 associated with SJC and DAS28-CRP at 1 year. OPG/RANKL ratio also correlated with CRP and DAS28-CRP at 1 year. No correlations were found between RANKL and markers of disease activity. High serum sclerostin levels were associated with low DKK-1 levels ($r=-0.453$ $p=0.001$) at 12 months.

OPG levels were correlated with ESR ($r=0.42$, $p=0.04$) and CRP levels ($r=0.46$, $p=0.02$) at baseline in PsA, whilst OPG levels were associated with ESR ($r=0.43$, $p=0.04$) and CRP ($r=0.46$, $p=0.03$) at 1 year in RA. High serum sclerostin levels were associated with low DKK-1 levels in PsA ($r=-0.605$ $p=0.04$) after 12 months of TNFi therapy (data not shown).

4.2.4 Associations between mediators of bone remodeling and hand BMD

ESR associated with hand BMD at both prior to and after treatment, and inverse associations were noted for OPG at 1 and 12 months. Decrease of DKK-1 level from 1-12 month was strongly associated with improvement of hand BMD in PsA. In order to identify predictors of periarticular bone gain following TNFi treatment, multiple linear regression analyses (backward method) were performed including variables demonstrating significant association with rank correlations (ESR, OPG and DKK-1). Regression analyses revealed that lower OPG level at baseline was independent predictor of hand bone gain at 12 months in the entire group ($B=-0.44$, $p=0.008$) and in RA ($B=-0.4$, $p=0.037$), approaching significance in PsA ($B=-0.46$, $p=0.055$). Higher DKK-1 prior to treatment associated with lower hand BMD at 1 year in RA ($B=-0.4$, $p=0.047$).

It has been suggested that OPG/RANKL ratio reflects bone remodeling activity and associates with radiographic damage in inflammatory arthritis. We compared disease activity and BMD measures between patients with high OPG/RANKL ratio (n=11) to those with low ratio (n=29) prior to and after TNFi treatment using the mean values of OPG/RANKL at 0 and 12 months as a cut-off. At baseline, patients with high OPG/RANKL were older and had longer disease duration, but we did not find any significant difference in gender, disease category, inflammatory markers or BMD between the two groups at 12 months (data not shown).

4.3 Study 3

4.3.1 Demographic and clinical characteristics of early PsA and RA patients

We recruited 62 patients, 32 with PsA and 32 with RA. All patients were Caucasian with the exception of 1 patient, who was of mixed race (Irish/Indian). The mean age was 44 years; patients with PsA were younger than RA. Clinical subtype of PsA patients were symmetrical polyarthritis (n=17) and asymmetrical oligoarthritis (n=15), 2 patients had axial involvement, but none of the patients had predominant spondylitis. There were 21 ever-smoker patients in both groups, weekly alcohol intake was higher in the PsA group ($p=0.05$). Twenty-six and 25 RA patients were anti-CCP and RF positive, respectively. DAS28-CRP reflected high disease activity in both groups at baseline. ESR, CRP, TJC, SJC and DAS28-CRP were significantly higher in RA compared to PsA ($p=0.0009$; 0.006 ; <0.0001 ; 0.0001 ; 0.0001 , respectively). The mean BMI was 28 kg/cm^2 in both groups. Ten patients with PsA had acute dactylitis at their baseline visit. The majority of PsA patients had mild or moderately severe psoriasis with median PASI 3.35. There were no significant differences between the two groups in patients' self-reported clinical parameters such as EMS, pain, global health, fatigue or HAQ scores.

With regards to anti-rheumatic therapy, 61 patients (29 PsA; 32 RA) were commenced on a DMARD therapy at baseline, the majority (29 PsA; 30 RA) starting methotrexate. Three

PsA patients with fertility concerns at the time of the recruitment were commenced on TNF inhibitor (TNFi) monotherapy. At the time of the recruitment all female patients were asked to stop TNFi therapy after a positive pregnancy test. In each group, 4 (12.5%) patients were started on a TNFi in combination with MTX. There were no patients taking anti-resorptive medications, 11 patients were on oral CSs (6 PsA; 5 RA) less than 10 mg prednisolone per day. At 12 months 55 patients were on a DMARD (25 PsA; 30 RA), 2 patients remained on TNFi monotherapy, 20 (10 PsA; 10 RA) received a TNFi in combination with MTX, and none of the patients took oral prednisolone.

4.3.2 Response of clinical measures to DMARD treatment

60 patients (28 PsA and 32 RA) attended their 3 months visit and 58 patients completed the study (28 PsA and 30 RA). Six patients failed to complete the study due to work commitments. Significant improvements in disease activity scores were noted throughout the study in both groups. ESR, CRP and DAS28-CRP were significantly lower in PsA than in RA at 3 months ($p=0.034$; 0.021 ; 0.005 , respectively) with DAS28-CRP remaining lower at 12 months ($p=0.025$). According to the EULAR response criteria, 68% of the patients (19 PsA; 22 RA) were responders at 3 months and 81% (23 PsA; 24 RA) were responders at 12 months.

4.3.3 Change in hand DXR-BMD

Despite intervention of appropriate antirheumatic drug therapy, mean hand DXR-BMD was lower in both diseases at 3 months compared to baseline. Mean DXR-BMD increased from 3 to 12 months in PsA. In contrast DXR-BMD decreased further in RA and was lower at 1 year compared to baseline ($p=0.043$) and to 3 months ($p=0.018$). Hand BMD as measured by DXR was higher in PsA than in RA throughout the study. Patients started on a TNFi in combination with a DMARD had higher hand DXR-BMD at 3 and 12 months comparing with those who started on a DMARD monotherapy ($p=0.015$ and $p=0.021$, respectively).

There was no significant bone loss in the PsA group throughout the study. In contrast mean Δ DXR-BMD from 0-3, 3-12 and 0-12 months showed elevated bone loss in RA. Highly elevated bone loss at 1 year was present only in the RA cohort (n=2). Changes in hand DXR-BMD from baseline to 12 and from 3 to 12 months in PsA were significantly different compared to RA patients reflecting cortical bone gain in PsA and bone loss in RA.

4.3.4 Radiographic progression

There was no significant change in mean ES, JSNS, mSHS and PS in either group over 1 year. Erosions were present in 22% of PsA and 41% of RA patients at baseline; 25% and 40% at 12 months, respectively. ES was lower in PsA compared to RA. Mean mSHS scores were 1.1 ± 3.2 ; 1.3 ± 3.6 and 1.4 ± 3.7 in PsA, and 1.9 ± 4.3 ; 2 ± 4.3 and 1.4 ± 3.1 in RA at 0, 3 and 12 months. Baseline mSHS did not correlate with mean DXR-BMD scores at any time points, whilst there were significant inverse correlations between mSHS prior to treatment and changes in DXR-BMD from 3-12 months in both groups (PsA $r = -0.45$, $p = 0.024$; RA $r = -0.37$, $p = 0.047$).

4.3.5 Comparison of patients with bone loss to those with normal hand DXR-BMD

We compared disease characteristics at baseline between patients with normal hand DXR-BMD and those with bone loss. Patients with bone loss at 3 months consumed significantly higher levels of alcohol and bone loss was associated with elevated BMI. Hand bone loss at 12 months significantly associated with diagnosis of RA. No significant differences were found between the groups in terms of gender, smoking history, presence of erosions prior to treatment, use of CSs and treatment with a DMARD in combination with a TNFi. Patients with hand bone loss over 12 months were older, presented with lower PASI scores, had significantly higher aCCP and RF levels, TJC, SJC and DAS28-CRP at baseline compared to those with normal BMD.

4.3.6 Logistic regression analyses

To identify independent predictors of hand bone loss multivariate forward stepwise logistic regression analysis was performed. We included variables demonstrating significant association with bone loss at 3 or 12 months with univariate analyses. BMI >25 kg/cm², RA/PsA, age, alcohol, aCCP, RF and SJC28 were entered into the model. Logistic regression analyses revealed that elevated BMI (OR=3.59, p=0.041) and heavier alcohol intake (OR=1.13, p=0.035) were associated with early hand bone loss. Patients with RA (OR=57.48, p=0.008), heavier alcohol intake (OR=1.27, p=0.012) and higher SJC28 (OR=1.5, p=0.036) at baseline had significantly increased risk for hand bone loss over 12 months.

5 DISCUSSION

RA and PsA are chronic inflammatory diseases characterized by progressive destruction of the joints. Despite RA and PsA share similar pathophysiological concepts, there are considerable differences in the anatomical localization of inflammatory lesions and in periarticular bone changes. While RA is considered the prototype of a destructive arthritis with only few signs of repair, PsA combines features of bone erosions and new bone formation. Inflammatory arthritides have been associated with both localized bone loss and generalized osteoporosis mediated by an imbalance in bone remodeling in favor of bone resorption. Local joint destruction has been investigated in depth in RA, but despite periarticular osteoporosis also being a recognized clinical feature in PsA, only few studies on systemic bone loss and bone turnover have been published in PsA patients.

In addition to traditional risk factors, inflammation is the key trigger of bone loss in arthritis. The RANK-RANKL-OPG system is the main driver of bone destruction, whilst in bone formation the Wnt-B-catenin axis and its inhibitors, DKK-1 and sclerostin are the most relevant. TNF- α is cytokine which plays a central role in synovial inflammation and bone resorption being involved in the production of RANKL, and by inducing DKK-1 and sclerostin expression. Anti-TNF agents are very effective in reduction of inflammation but there are only few studies investigating their role in preventing periarticular bone loss and generalised osteoporosis. It is also not fully elucidated whether conventional DMARDs have structure-sparing effects on bone and cartilage damage.

Besides clinical and structural effects on bone, TNFi agents may also influence bone turnover and the production of bone biomarkers. While the effect of TNFi therapy on bone remodeling markers in RA has been a subject of a small number of papers, it is inadequately studied in PsA, and has not been compared between RA and PsA in a prospective study design. Although findings of previous investigations are somewhat inconsistent, in most studies,

changes in bone biomarkers after TNFi therapy were associated with favourable effects on local and central BMD.

There is a growing interest in bone and cartilage biomarkers that could be used predicting and assessing changes in structural damage in inflammatory arthritis. To date, no single biomarker has so far emerged as a reliable predictor of joint damage. Identifying bone biomarkers that could be used predicting response to treatment would be also of interest, and would support more informed clinical decisions on the most appropriate treatment regimens for individual patients.

In this work, we investigated the impact of anti-TNF- α therapy along with conventional DMARDs on bone remodeling in RA and PsA patients. In Study 1 we evaluated early and long-term effects of TNFi treatment on bone turnover markers along with changes in hand and central BMD in a cohort of RA and PsA patients with established disease. In Study 2, using the same cohort of patients, we were focusing on the effect of TNFi treatment on mediators of bone remodeling. In Study 3 we assessed the impact of DMARD treatments on periarticular BMD of the hands in patients with early RA and PsA and investigated whether any disease characteristics at baseline predict hand bone loss as measured by DXR.

5.1 Evaluation of early and long-term effect of TNFi treatment on bone turnover markers and BMD in RA and PsA

At baseline, we identified associations between low hand BMD and the following variables: diagnosis of RA, lower body weight, higher serum bone ALP, higher PINP, higher CTX-I and higher NTX-I. We found that baseline ESR, HAQ and BTMs correlated with hand BMD but not with hip or lumbar spine BMD. Thus, analysis at baseline suggested that joint inflammation is associated with remodeling activity and lower hand BMD. After three years of TNFi therapy, periarticular BMD increased significantly around PIP joints but declined around MCP joints; hip BMD declined, and spine BMD was unchanged. Bone ALP increased steadily over the 3 years and was consistently higher in PsA compared to RA; PINP and OC{1-

49} increased to a lesser degree. Resorption markers were unchanged; but, change in urine fDPD was the only BTM to correlate with change in ESR. This suggests that anti-TNF- α therapy results in an increase in bone formation without a change in bone resorption; this would give a more favorable remodeling balance in periarticular bone.

We observed an inverse relationship between BTMs and hand BMD at baseline on logistic regression analysis suggesting that active joint inflammation alters periarticular bone remodeling with resultant bone loss due to negative remodeling balance. Following TNFi therapy, the steady increase in PIP BMD was contemporaneous with a steady increase in bone ALP and to a lesser extent in the two other formation markers, PINP and OC{1-49}, implying a shift towards positive remodeling balance as a mechanism for periarticular bone gain. Bone ALP, being consistently higher in PsA compared to RA reflects the known effect of PsA on bone formation, but the absence of an interaction effect between disease category and bone formation markers, as demonstrated by the split-plot ANOVA, suggests that the bone formation response occurs regardless of whether a patient has PsA or RA.

Regarding BTMs in general, median levels at baseline of all markers except fDPD were below median levels in healthy Irish adults; and following intervention median values for bone ALP but not PINP or OC {1-49} were above median levels in healthy Irish adults. In our study cohort, both ESR and CRP correlated strongly with fDPD levels at baseline indicating that fDPD is most likely an indicator of joint inflammation. Our results are in agreement with previous observations. Whereas, there were no correlations between both ESR and CRP with the two other resorption markers, CTX-I and NTX-I. In addition there were significant but weaker correlations between ESR and CRP with serum PINP, which is a collagen-based formation marker, but not with the non-collagen based formation markers, namely serum OC{1-49} and bone ALP.

Over 3 years of TNFi therapy, we demonstrated close correlations between changes in both ESR and CRP with change in urine fDPD; but no correlation with change in the other BTMs. Similar associations between ESR and fDPD levels have been reported in patients with

active RA treated with Infliximab. This indicates that urine fDPD is more likely a marker of joint inflammation than a marker of periarticular bone remodeling activity. Our data suggests that the effect of TNFi on bone is principally on suppressing disease activity, which in turn alters bone remodeling activity at sites of joint inflammation.

From studies of bone histomorphometry using in-vivo tetracycline, it is known that bone remodeling entails cycles of resorption coupled to formation. RA is considered as a purely erosive disease with little sign of repair of bone erosions, whilst PsA shows a mixed pattern of destruction and remodeling. Bone remodeling is regulated by local and systemic factors that drive osteoblast and osteoclast differentiation and function. Recent studies in RA have provided insights into the mechanism involved in the uncoupling of bone resorption and formation in this form of inflammatory arthritis.

Recent data suggest that periarticular bone formation is actively suppressed by inflammation; thus, blockade of TNF is highly effective in retarding structural damage in RA. It has been proposed that continued suppression of inflammation via anti-TNF agents may accelerate new bone formation in PsA. Harrison et al. noted in RA, but not in PsA, that periarticular bone loss was strongly related to measures of joint inflammation. We demonstrated that periarticular BMD around PIP joints improved in both diseases following 36 months of anti-TNF- α treatment, but not around MCP joints. This latter observation was rather unexpected. To our knowledge this is the first study comparing periarticular BMD changes assessed in MCP and PIP joints in both RA and PsA as measured by DXA following anti-TNF- α treatment. Finzel et al. have shown limited repair in bone erosions of the MCP joints in RA patients treated with TNFi as measured by high-resolution μ CT. We did not find any marked differences in central BMDs between the two diseases. After three years of TNFi therapy hip BMD decreased and spine BMD remained stable. Anti-TNF- α treatment of mice transgenic for the human TNF gene leads to significant improvement in local bone resorption, but does not prevent generalised bone loss. Future more robust, prospective studies might focus on RA and PsA patients with both early and established disease comparing axial, hand

and periarticular BMDs as these results may have implications for prevention and treatment strategies of osteoporosis in inflammatory arthritis.

5.2 Assessment of serum bone remodeling mediators in RA and PsA patients prior to and following TNFi therapy

Many factors have been identified that increase the ratio of RANKL to OPG expression, that tilting the balance in favour of bone resorption, but TNF- α has emerged as a dominant regulator. TNF- α directly stimulates RANKL production by stromal cells, T and B lymphocytes, and endothelial cells, or indirectly enhances RANKL expression by upregulation of prostaglandins, IL-1 and IL17. TNF- α inhibits new bone formation by inducing the expression of Wnt antagonist DKK-1 and sclerostin. The effect of TNFi treatment on bone remodeling in inflammatory arthritis has been a subject of several papers, however results are not always consistent.

We evaluated early (1 month) and more sustained (1 year) effects of TNFi on serum mediators of bone remodeling in RA and PsA patients. Despite TNF- α is involved in the production of RANKL along with anti-anabolic DKK-1 and sclerostin, we did not observe any early or long-term effect of TNFi treatment on the serum levels of these mediators. In contrast, TNFi showed an effect on OPG levels from 1-12 months in RA with OPG levels increased significantly. This observation further supports our findings in Study 1, suggesting that TNFi therapy results in increase in bone formation without a change in bone resorption; therefore, it has positive effect on bone remodeling in periarticular bone. In agreement with our finding, serum OPG levels increased after 6 months of TNFi treatment in a cohort of RA patients with mean disease duration of 12 years. Catrina et al. demonstrated increased expression of OPG in synovial tissue after 8 weeks of TNFi in patients with RA with no changes in RANKL. Another group, however found steady decrease in serum OPG levels with no change in OPG/RANKL ratio in RA patients after 6 months of TNFi.

When comparing the two diseases, RANKL and sclerostin levels were similar in RA and PsA during the study, whilst DKK-1 and OPG levels were lower in PsA at 1 year. Low DKK-1 levels have been associated with SpA, that shares similar pathogenetic features of new bone formation with PsA.. It has been suggested that patients with SpA and possibly a subset of PsA may have accelerated pathologic new bone formation when treated with anti-TNF- α agents due to lower DKK-1 levels and subsequent disinhibition of Wnt signalling. In line with this, decrease of DKK-1 level from 1-12 month was strongly associated with improvement of hand BMD in our PsA group. We found that OPG levels were higher in RA compared to PsA during the entire period of treatment with a significant difference at 1 year. While our study is the first comparing serum levels of bone remodeling mediators of the RANK-RANKL-OPG network along with the Wnt-pathway inhibitors between RA and PsA patients after a relatively long period of time of TNFi treatment, one study showed no difference for synovial expression of OPG, RANK and RANKL between patients with RA and psoriatic SpA, though the heterogeneous treatment used in that cohort makes difficult to interpret the results.

Data on associations between mediators of bone remodeling and markers of systemic inflammation is rather controversial. In our study, RANKL, DKK-1 and sclerostin levels did not correlate with inflammatory markers at baseline, whereas OPG levels correlated with ESR and CRP both prior to and after treatment, and DKK-1 associated with SJC and DAS28-CRP at 1 year. Serum OPG levels have been shown to correlate with markers of systemic inflammation in PsA patients, whilst OPG did not show associations with disease activity measures such as DAS or RAMRIS (RA magnetic resonance imaging score) in early RA patients of whom only 2.6% received TNFi at 12 months.

Considering that TNFi only showed an effect on OPG levels in RA and that OPG levels strongly correlated with inflammatory markers, and given that both OPG levels and ESR were higher in RA compared to PsA, we can speculate that OPG is more likely a marker of joint inflammation than a marker of periarticular bone remodeling. On the other hand, we noted inverse relationship between OPG levels and hand BMD measures following TNFi treatment in

RA and found that lower OPG levels prior to treatment predicted bone gain after 1 year. These latter findings were rather unexpected in view of the protective role of OPG against bone and joint destruction in RA. An earlier study showed that serum RANKL concentrations were predictive of the therapeutic response to anti-TNF- α therapy in RA patients, while OPG levels were not.

Taken together, our results suggest that TNFi treatment, in addition to suppressing inflammation, restores the balance of bone remodeling and may have a positive net effect on periarticular BMD.

5.3 Comparison of hand BMD as measured by DXR between early, treatment-naïve RA and PsA patients 3 and 12 months after introducing a DMARD therapy

We compared hand DXR-BMD and radiographic changes in early RA to PsA patients 3 and 12 months after initiating a DMARD treatment. We also investigated whether any disease characteristics at baseline predict hand bone loss as measured by DXR during the first year. We demonstrated hand bone gain in PsA but bone loss in RA over 12 months. Diagnosis of RA, heavier alcohol intake and SJC were independent predictors for hand bone loss over 1 year.

Whilst both RA and PsA are associated with bone erosions and the number of erosions are similar in established disease, there are profound differences in structural joint changes demonstrated by high-resolution peripheral quantitative CT (HR-pQCT). Our study used DXR to quantify periarticular bone changes of the hands, as it has been suggested that DXR is a sensitive method for detecting and monitoring cortical osteoporosis in both early and late stages of RA. Another advantage of this technique is that the analysis of cortical bone loss is based on hand radiographs, which are often taken routinely in clinical practice. We found that although patients were started on appropriate systemic treatment early in their disease course, there was hand BMD loss in both diseases in the first 3 months. One possible

explanation of this finding is that the majority of the patients were commenced on MTX which may have had little or only modest structure-sparing effect on periarticular bone. The effect of MTX on bone is still unclear, but has been shown to inhibit osteoclast formation in co-cultures of RA fibroblast-like synoviocytes and peripheral blood mononuclear cells by reducing rank ligand expression and increasing osteoprotegerin secretion. MTX also prevents osteoclastogenesis via direct effects on osteoclast precursors independent of RANKL. In contrast, patients started on a TNFi in combination with a DMARD had higher hand DXR-BMD at 3 and 12 months comparing with those who started on a DMARD monotherapy. This reflects the known beneficial effects of TNF blocking agents, since TNF is the key inducer of osteoclast formation and contributes to the imbalance between bone resorption and formation in arthritis. Interestingly, despite improvement in disease activity measures, having similar number of patients on TNFi and comparable number of responders to treatment in both diseases at 1 year, the observed change in hand DXR-BMD over 12 months was significantly different between PsA and RA with increases in hand BMD in PsA, but further bone loss in RA. Our observation may indicate that repair mechanisms in periarticular bone are different in early PsA with associated increased cortical bone formation compared to RA. This has been suggested previously, but observations were based on patients with established disease. A recent study showed that osteophytes at the MCP joints are increased in number, extent and size in PsA as compared to RA and osteophytes progress in size in PsA regardless of treatment with MTX or TNFi.

Previous research has shown that hand bone loss in inflammatory arthritis is associated with high inflammatory activity, longer disease duration and joint damage at the time of diagnosis. Age, female gender, immobility and presence of autoantibodies are also considered as risk factors. In order to identify predictors for early hand bone loss as measured by DXR, we compared baseline disease characteristics of patients with normal hand DXR-BMD to those with bone loss. Logistic regression analyses revealed that elevated BMI and heavier alcohol intake were associated with hand bone loss in the first 3 months of the study. This

former finding was rather unexpected as increased adiposity has been shown to be protective against radiological progression in RA. Comparing patients with BMI >25 (64%) to those with normal BMI (36%) we found no differences in hand BMD or its change over time. We demonstrated that diagnosis of RA, heavier alcohol intake and higher SJC28 were independent predictors for hand bone loss over 12 months. Presence of RA and SJC were shown to be predictive for radiological damage in both RA and PsA, in fact we also reported association of RA with low hand BMD as evaluated by dual-energy x-ray absorptiometry. Our observation, that heavier alcohol intake predicts hand bone loss as early as 3 months and at 1 year is one of the most interesting findings of this study. To our knowledge, the effect of alcohol on the metacarpal bones has not been previously investigated in the early phase of inflammatory arthritis. While chronic heavy alcohol abuse is established as an independent risk factor for secondary osteoporosis and fractures, very little is known about its influence on periarticular bone. The effects of alcohol on bone remodeling and microarchitecture are linked to the dose ingested and the duration of consumption, and depends on the age, sex, hormonal status as well as the type of alcoholic beverage. Chronic ethanol consumption has been found to increase cortical bone damage in rats in a dose-dependent manner, with greater negative effects proportionate to greater alcohol doses. Reduction in bone mass and strength following alcohol consumption is mainly due to bone remodeling imbalance, with a predominant decrease in bone formation. More recently, new mechanisms of action of alcohol on bone remodeling were reported including osteocyte apoptosis, suppression of the Wnt signalling pathway and the stimulation of oxidative stress.

6 CONCLUSIONS

Inflammatory arthritides, such as RA and PsA have been associated with both localized bone resorption and/or pathologic bone formation, as well as generalized bone loss. Anti-TNF- α agents along with conventional DMARDs are effective in reduction of inflammation, but their structure-sparing effects and role in preventing periarticular bone loss is not fully elucidated. This thesis consists of 3 studies investigating the impact of DMARD treatments on periarticular bone remodeling in RA and PsA patients.

Clinical relevance and new findings of our research are listed below:

Study 1

1. Bone formation markers, in particular bone ALP, are better indicators of bone response to TNFi treatment than resorption markers.
2. In response to 3 years of TNFi treatment, periarticular BMD increased in the PIP region but declined at MCP sites, whilst TNFi showed no effect on central BMD.
3. We showed that there is a much closer association between BTMs with hand BMD than with central BMD, and that BTMs reflect bone remodeling activity mainly at sites of inflammation.
4. Our study demonstrates that in RA and PsA patients with established disease the increase seen in bone formation over time likely reflects the beneficial effect of TNFi treatment on periarticular bone remodeling balance in both diseases.

Study 2

1. TNFi showed an effect on OPG levels in particular in RA, but did not influence serum levels of RANKL, DKK-1 and sclerostin. We found that whilst OPG level at baseline was an independent predictor of periarticular bone gain in the hand at 1 year after TNFi

therapy, it is more likely a marker of joint inflammation than a marker of periarticular bone remodeling.

2. Decrease in DKK-1 level along with improvement of hand BMD in PsA patients may further support previous assumptions that patients with PsA have accelerated bone formation when treated with TNFi due lower DKK-1 levels and subsequent disinhibition of Wnt signalling.
3. Our observations indicate that the effects of TNF inhibition on bone remodeling may be partially independent from its impact on disease activity. In addition to suppressing inflammation, TNFi treatment may restore the balance of bone remodeling resulting in a positive net effect on periarticular BMD.

Study 3

1. Despite intervention of appropriate DMARD therapy and improvement in disease activity measures, we found hand bone gain in PsA but bone loss in RA over 12 months, supporting the hypothesis of different pathomechanisms being involved in hand bone remodeling in PsA.
2. Presence of RA, heavier alcohol intake and SJC at the time of disease presentation were identified as independent predictors for hand bone loss in the first year of the disease course.

7 SUMMARY

Inflammatory arthritides, such as RA and PsA have been associated with both localized bone resorption and/or pathologic bone formation, as well as generalized bone loss. Despite RA and PsA share similar pathophysiological concepts, there are considerable differences in the anatomical localization of inflammatory lesions and in periarticular bone changes. Periarticular bone loss may precede the development of erosions, therefore its recognition at an early stage of the disease is essential in preventing irreversible joint damage. In addition to traditional risk factors, inflammation is the key trigger of bone loss in arthritis. The RANK-RANKL-OPG system is the main driver of bone destruction, whilst in bone formation the Wnt-B-catenin axis and its inhibitors, DKK-1 and sclerostin are the most relevant. TNF- α is a cytokine which plays a central role in synovial inflammation and bone resorption being involved in the production of RANKL, and by inducing DKK-1 and sclerostin expression. Anti-TNF- α agents along with conventional DMARDs are effective in reduction of inflammation but their structure-sparing effects and role in preventing periarticular bone loss is not fully elucidated. In addition to clinical and structural effects on bone, TNF inhibitors may also influence bone turnover and the production of bone biomarkers. To date, no single bone turnover marker has been found to reflect joint destruction that could be used predicting response to treatment.

This thesis consists of 3 studies investigating the impact of anti-TNF- α agents along with conventional DMARDs on periarticular bone remodeling in RA and PsA patients. We used DXA and DXR techniques for quantifying BMD changes in the hand and central sites. In Study 1 we evaluated early and long-term effects of TNFi treatment on bone turnover markers along with changes in hand and central BMD in a cohort of RA and PsA patients with established disease. In Study 2, using the same cohort of patients, we were focusing on the effect of TNFi

treatment on mediators of bone remodeling. In Study 3 we assessed the impact of a DMARD treatment on periarticular BMD of the hands in patients with early RA and PsA, and investigated whether any disease characteristics at baseline predict hand bone loss as measured by DXR.

Our observations are summarized below:

Study 1

The effect of TNFi on bone over 3 years has not been compared previously in RA and PsA in a prospective study design. At baseline, hand BMD was inversely associated with BTMs. Bone ALP increased steadily and was always higher in PsA, but resorption markers did not change, suggesting that bone formation markers show bone response to TNFi better than resorption markers. The collagen-based marker of bone resorption, fDPD seems to be altered by TNFi therapy as part of anti-inflammatory rather than as a bone remodeling effect. The modest improvement seen in PIP BMD but the lack of effect of TNFi on central BMD may provide some evidence that bone turnover markers better reflect bone remodeling activity at sites of inflammation.

Our study demonstrates that in a sample of RA and PsA patients with established disease there is a much closer association between BTMs with hand BMD than with central BMD. The increase in bone formation in both RA and PsA over time likely reflects the beneficial effect of TNFi treatment on periarticular remodeling balance in both diseases.

Study 2

Our study is the first comparing serum levels of bone remodeling mediators of the RANK-RANKL-OPG network along with Wnt-pathway inhibitors following a 1-year TNFi treatment between RA and PsA patients. TNFi showed a long-term effect on OPG levels in particular in RA, but not on RANKL, DKK-1 and sclerostin. We found that whilst OPG level at

baseline was an independent predictor of periarticular bone gain in the hand after 1 year of TNFi therapy, it is more likely a marker of joint inflammation than a marker of periarticular bone remodeling. Our observations indicate that the effects of TNF- α inhibition on bone remodeling may be partially independent from its impact on disease activity. Decrease in DKK-1 level along with improvement of hand BMD in PsA patients may further support previous assumptions that patients with PsA have accelerated bone formation when treated with TNFi due to lower DKK-1 levels and subsequent disinhibition of Wnt signaling.

Results of this study suggest that TNFi treatment, in addition to suppressing inflammation, restores the balance of bone remodeling and may have a positive net effect on periarticular BMD in RA and PsA patients.

Study 3

This is the first prospective study comparing hand BMD changes after introducing an anti-rheumatic treatment in early, treatment-naïve RA to PsA patients using DXR technique. Despite intervention of appropriate anti-rheumatic drug and improvement in disease activity measures, we found hand bone gain in PsA but bone loss in RA over 12 months. Our observations support the hypothesis of different pathomechanisms being involved in hand bone remodeling in PsA. Presence of RA, heavier alcohol intake and SJC at the time of disease presentation were identified as independent predictors for hand bone loss in the first year of the disease course. Since alcohol is widely consumed and may induce cortical bone loss, further studies are required to address the influence of alcohol on periarticular bone in inflammatory arthritis.

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Subject: PhD Publikációs Lista

Candidate: Ágnes Szentpétery
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List of publications related to the dissertation

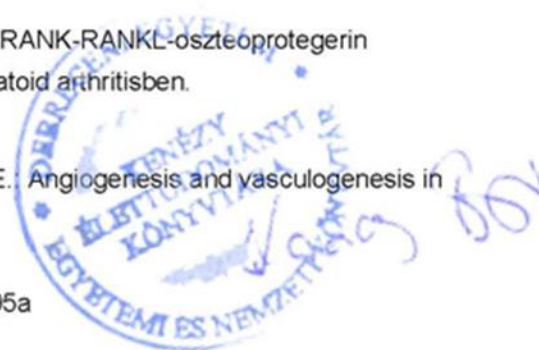
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Total IF of journals (all publications): 40,15

Total IF of journals (publications related to the dissertation): 16,952

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

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