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**SYNOVIAL TISSUE RANK LIGAND EXPRESSION AND RADIOGRAPHIC
PROGRESSION IN RHEUMATOID ARTHRITIS.
OBSERVATIONS FROM A PROOF-OF-CONCEPT RANDOMIZED
CLINICAL TRIAL OF CYTOKINE BLOCKADE**

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ABSTRACT

Objective:

To evaluate synovial tissue receptor activator of nuclear factor- κ B ligand (RANKL) and osteoprotegerin (OPG) as biomarkers of disease activity, progressive joint damage, and therapeutic response, during cytokine blockade in rheumatoid arthritis (RA).

Methods:

Patients with active RA entered a randomized open-label 12-month study of anakinra 100 mg/day, administered as monotherapy or in combination with pegsunercept 800 μ g/kg twice weekly. Arthroscopic synovial tissue biopsies were obtained at baseline, at 4 weeks and at the final time-point. Following immunohistochemical staining, RANKL and OPG expression was quantified using digital image analysis (DIA). Radiographic damage was evaluated using the van der Heijde modification of the Sharp scoring system.

Results:

Twenty-two patients were randomized. Baseline expression of RANKL, but not OPG, correlated significantly with baseline CRP levels ($r = 0.61$, $p < 0.01$). While a significant reduction in OPG expression following treatment was observed in clinical responders at the final timepoint ($p < 0.05$ vs baseline), RANKL levels did not change, and the RANKL:OPG ratio remained unaltered, even at the highest levels of clinical response. When potential predictors of radiographic outcome were evaluated, baseline RANKL expression correlated with erosive progression at 1 year ($r = 0.71$, $p < 0.01$).

Conclusions:

Distinct, though related, pathophysiologic processes mediate joint inflammation and destruction in RA. Elevated synovial tissue RANKL expression is associated with

progressive joint erosion, and may be independent of the clinical response to targeted therapy. The potential therapeutic importance of modulating RANKL in RA is highlighted, if radiographic arrest is to be achieved.

INTRODUCTION

Rheumatoid arthritis (RA) is a disabling, inflammatory arthropathy, commonly associated with progressive articular cartilage loss, bone erosion and deformity [1]. In recent years, the use of biologic therapies targeting specific mediators of inflammation and matrix destruction has been associated with enhanced control of joint inflammation and retardation of progressive structural joint damage [2-7].

A critical common pathway leading to localized articular bone erosion in RA involves the activation of RANK by RANKL, promoting differentiation of osteoclast progenitors into mature osteoclasts which effect bone resorption [8]. RANKL is detectable in T-cells and fibroblasts in RA synovial tissue, and its expression is regulated by many proinflammatory cytokines and their inhibitors [9]. OPG is a naturally occurring inhibitor of RANK / RANKL interaction, is also expressed in RA synovial tissue and acts as a potent regulator of osteoclastogenesis [9]. Therapies modulating the OPG / RANKL axis have resulted in retardation of joint destruction in animal models of inflammatory arthritis, and recently in patients with RA [10-14].

Although a number of previous studies have examined synovial tissue OPG / RANKL expression in RA, few have examined the expression of OPG and RANKL in RA synovial tissue before and after biologic therapy [15-22]. The aim of the present study was to evaluate the utility of synovial tissue levels of receptor RANKL and OPG as biomarkers of progressive structural joint damage, disease activity and therapeutic response, during a trial of selective cytokine blockade in RA. It was conducted as a component of the SPECTRA trial, a single centre, phase II open-label synovial tissue biopsy study, involving therapy with anakinra administered either alone or in combination with PEGylated soluble tumour necrosis factor (TNF) receptor type I (pegsunerccept), for a planned duration of 52 weeks [23, 24].

PATIENTS AND METHODS

Patients

As previously described, eligible patients for the SPECTRA study were aged 18 years or older, had active RA of ≤ 10 years duration diagnosed according to the 1987 revised American College of Rheumatology (ACR) criteria, and had ≥ 9 tender joints (of 68 evaluated), ≥ 6 swollen joints (of 66 evaluated), a clinically inflamed knee joint, and at least 2 of the following 3 criteria: morning stiffness lasting ≥ 45 minutes, C-reactive protein (CRP) ≥ 1.5 mg/dl, erythrocyte sedimentation rate (ESR) ≥ 28 mm/h [23, 25].

Major exclusion criteria included prior use of any TNF α antagonist or anakinra, a history of any other major chronic inflammatory disease, malignancy (besides non-melanoma skin carcinoma within 5 years) or demyelinating disorder, a positive tuberculin skin test, history of tuberculosis (TB) infection, or chest radiograph suggestive of prior TB, any infection requiring systemic antibiotics within 12 weeks of screening, any uncontrolled medical condition, pregnancy or breast feeding, and ACR functional class IV disease [26].

Doses of concomitant RA therapies such as non-steroidal anti-inflammatory drugs (NSAIDs) and oral corticosteroids (≤ 10 mg/day of prednisolone or equivalent) must have been stable for 4 weeks prior to screening.

Protocol

Patients were recruited for this 52-week, single-center, open-label study at St. Vincent's University Hospital (SVUH), Dublin. A total of 30 patients were to be recruited. The protocol was approved by the institution's ethics committee, and patients gave their written informed consent. Patients were randomly assigned to

receive self-administered single subcutaneous injections of either anakinra 100 mg/day, or anakinra 100 mg/day plus pegsunercept 800µg/kg twice weekly. Traditional DMARDs were discontinued at least 6 weeks prior to screening. Doses of concomitant NSAID therapy were kept constant throughout the study. If necessary, oral corticosteroid therapy could be temporarily increased for ≤ 2 weeks up to twice during the study. Up to 2 intra-articular corticosteroid injections were also permitted if required.

Clinical assessment

ACR20, ACR50, and ACR70 responses were assessed at week 4, and at the final timepoint [27]. Patients who withdrew from the study prematurely due to active RA or adverse events were classified as non-responders. DAS28 scores were also calculated at baseline, week 4, and at the final timepoint [28].

Radiographic assessment

Radiographs of the hands and feet were obtained at baseline, and at weeks 24 and 52. Radiographic damage was quantified by 2 blinded observers (TR, BB) using the van der Heijde modification of the Sharp scoring system [29]. Modified Sharp scores were then calculated as the mean of the scores obtained by the 2 observers. The minimum clinically important difference (MCID) in radiographic scores over time was calculated as the smallest detectable difference (SDD) for these 2 observers using the limits of agreement method of Bland and Altman [30]. Progression scores below this threshold were classified as non-significant. Using an assumption of linear progression, inferred rates of annual radiographic progression prior to study entry were calculated using damage scores and disease duration at study baseline.

Acquisition and preparation of synovial tissue

Each patient underwent arthroscopy of a clinically inflamed knee joint at baseline, immediately prior to commencing therapy. Arthroscopy of the same knee was repeated following 4 and 52 weeks of therapy, or at the time of withdrawal from the study. Multiple synovial tissue biopsies (at least 6) were obtained throughout the knee joint under local anaesthesia using a 2.7mm arthroscope and 2.8mm universal biopsy forceps (Karl Storz GmbH & Co., Tuttlingen, Germany). The synovial tissue was prepared as previously described [31]. In brief, tissue samples were snap-frozen together en bloc in Tissue-Tek OCT (Miles Inc. Diagnostic Division, Elkhart, IN) by immersion in liquid nitrogen. Frozen blocks were stored in liquid nitrogen until sectioned for staining. Seven μm thick sections were cut in a cryostat and mounted on glass slides. The slides were fixed in acetone at room temperature for 10 minutes and stored at -70°C until immunohistochemical analysis was performed. After thawing for 20 minutes at room temperature, sections were stained with anti-OPG (MAB 805, R&D Systems, Minneapolis, USA) and anti-RANKL (sc-9073, Santa Cruz Biotechnology, Santa Cruz, USA). A standard three-stage immunoperoxidase method was used for each antibody (Vector Laboratories, Lumigen Inc., Southfield, USA), and was followed by counterstaining with haematoxylin. Sections in which the relevant primary antibody was replaced with isotype-matched irrelevant primary antibodies served as negative controls.

Microscopic analysis of synovial tissue.

For each stained tissue section, a single 40X digital image was acquired as previously described [32]. All slides were coded to maintain operator blinding. Image acquisition, modification and analysis were performed using AnalySIS software (Soft Imaging Systems, Denver, Colorado) and a personal computer with Intel Pentium III

800 MHz processor and Windows™ 2000 Professional Version 5.0.2195 environment. For each antibody, separate threshold RGB values were specified for total tissue (blue or brown) and for positive stain (brown). These threshold values remained constant throughout the analysis of sections stained for a given marker. The mean optical density (MOD) was calculated for the positively stained areas of each image and expressed as a % of the maximum possible density. The integrated optical density (IOD) was then calculated as the product of the % tissue occupied by positive stain and the MOD.

Study closure

In February 2003, while recruitment was ongoing, preliminary analyses of the results of 2 larger phase 3 clinical trials evaluating therapy with anakinra plus either etanercept or pegsunercept in RA indicated an increased incidence of serious infection in patients receiving combination therapy [33]. All studies of combination anakinra plus anti-TNF α therapy were suspended. All patients involved in the present study were contacted and informed in person of the reasons behind the study's early closure. Final assessments (including arthroscopic synovial tissue biopsy) were obtained, and patients receiving combination therapy were offered monotherapy by their supervising physician. Following study closure, week 26 and 52 radiographs were obtained, where possible, from all remaining patients who had participated in the study. (Terrance- you did a nice job of writing this---sometime let's discuss the behind the scenes discussions over this. There were actually 2-fold higher infections with the IL1ra+Enbrel versus the IL1ra+PEGsunercept therapy. Amgen did not want these data out there for obvious reasons.)

Statistical analysis

Cross-sectional differences between pairs of patient groups were evaluated using the Mann-Whitney U test. Proportional differences between groups were evaluated using Chi-squared tests. The Wilcoxon signed ranks test was employed to examine longitudinal differences in variables between baseline and subsequent timepoints. Correlations were calculated using Spearman's Rho. For efficacy measures, intent-to-treat analysis was performed for all patients who received at least one dose of study drug. The performance of baseline variables as predictors of radiographic progression (presence or absence of clinically significant progression) was evaluated using receiver operating characteristics (ROC) curves. A ROC curve plots sensitivity against 1-specificity for a range of values of a given independent variable, where that variable is being evaluated as a test for a dependent dichotomous variable, whose 2 possible values are typically taken as either "positive" or "negative". Area under the ROC curve (AUROC) values for a given predictor near 1 or 0 identify it as a good test for a specified outcome, while values near 0.5 indicate a poor test.

To evaluate the relationship between radiographic outcome and sustained expression of synovial OPG / RANKL during the course of the study, AUCs were calculated using baseline, week 4 and final timepoint tissue data. Linear change in these tissue biomarker levels between timepoints was assumed. Since the final timepoint differed between patients, time-averaged AUCs were calculated by dividing the AUC for each patient by the number of weeks from baseline to final biopsy, allowing inter-patient comparison. All statistical calculations were performed using SPSS software (version 11.0). Two-tailed significance testing was employed throughout, and p-values less than 0.05 were taken as significant.

RESULTS

Patient characteristics and outcomes

Twenty-two patients were randomized to the monotherapy or combination therapy groups and consented to serial arthroscopic synovial biopsies. Baseline demographic and clinical characteristics have been described previously [23]. In brief, the median age was 52 years and 18 patients (82%) were female. The median (range) disease duration was 23 (3-87) months, and 10 patients (46%) had symptoms of RA for less than 1 year. Sixteen patients (73%) were rheumatoid factor positive. The patient population had moderate to severe RA, and median tender and swollen joint counts were 20 (of 68) and 16 (of 66), respectively. Of the total patient group, 11 (50%) were DMARD naïve; the median number of previous DMARDs was 1. At the time of study closure, 3 patients (1 monotherapy, 2 combination) had completed 52 weeks of treatment, while 12 patients (6 in each treatment group) were receiving active treatment. These patients were withdrawn from the study at a median (range) timepoint of 31 (12-47) weeks. All patients contributed a 2nd synovial biopsy at 4 weeks and 16 contributed a 3rd biopsy after a median (range) of 32 (12-52) weeks. A total of 17 patients contributed post-baseline radiographic scores.

The clinical and radiographic outcomes are presented in association with the levels of OPG and RANKL expression on an individual patient basis in Table 1. Of the total study group, 11 patients (50%) achieved an ACR20 response or better at the final timepoint. ACR20, 50 and 70 response rates at the final timepoint were 64%, 64% and 46% with combination therapy, and 36%, 9%, and 0% with monotherapy, respectively. Of the 15 patients with 12-month radiographic data, the proportions of responders (ACR20 or better) and non-responders who demonstrated clinically

significant increases in total Sharp score were 28% and 40%, respectively (there were no statistically significant differences between response groups).

Baseline expression of OPG and RANKL in synovial tissue and progressive joint damage

OPG and RANKL expression was observed in all synovial tissue sections evaluated. OPG expression was largely confined to endothelial cells, whereas RANKL was widely expressed by inflammatory cells within the lining layer, sublining layer and in perivascular regions. Levels of expression of both biomarkers varied widely in the total patient group (Table 1) - baseline IODs ranged from 4.2 to 20.9 for OPG and from 3.6 to 35.2 for RANKL expression. At baseline, there was a statistically significant correlation between synovial tissue RANKL expression and serum CRP levels (Spearman's Rho 0.61, $p < 0.01$), but not DAS28. There were no correlations between synovial tissue OPG expression and measures of disease activity.

In order to evaluate the potential utility of synovial tissue OPG and RANKL expression as predictors of progressive joint damage, correlations between baseline tissue biomarker levels, as well as the RANKL:OPG ratio, and changes in modified total Sharp, erosion and narrowing scores at 6 and 12 months were evaluated. ROC curve analysis was also employed to determine the ability of these baseline tissue signals to predict the occurrence of clinically meaningful progressive structural damage. For comparison, known predictors of radiographic outcome (ESR and serum CRP) were subjected to the same analysis. The results are presented in Table 2. Baseline RANKL expression correlated significantly with erosive progression at both the 6 and 12 month time-points and also demonstrated predictive utility in ROC curve analysis. Similar results were observed for the baseline RANKL:OPG ratio and

erosive progression at 1 year. As expected, both ESR and CRP also demonstrated predictive utility for erosive progression at 6 and 12 months. No significant relationship between OPG expression at baseline and radiographic change was observed. Linear regression could not be performed as sufficient variables could not be normalized using transformation. When logistic regression was used to examine the predictive value of multiple baseline variables simultaneously, no variable emerged as independently predictive of significant progression in any measure of joint damage. Neither early changes in, nor sustained expression (time-averaged AUC) of either biomarker demonstrated any significant correlations with radiographic outcome (data not shown).

Cross-sectional analysis of OPG and RANKL expression and disease activity

The relationship between measures of disease activity and synovial tissue OPG / RANKL expression are presented in Table 3. RANKL expression correlated significantly with ESR and CRP levels at baseline and with CRP and DAS28 scores at week 4. Strikingly however, at the final timepoint (median 32 weeks) this relationship was lost, and Spearman's Rho values for correlations between RANKL and disease activity were as low as 0.03. A similar trend was observed for the RANKL:OPG ratio. OPG expression did not correlate significantly with disease activity in cross-sectional analysis at any timepoint.

Effects of biologic therapy on OPG and RANKL expression in synovial tissue

Sixteen of the 22 study patients had baseline, 4 week and final biopsies. Table 4 highlights changes in tissue OPG and RANKL expression after treatment in the total group, by ACR response, and in the individual treatment groups. No significant changes in median OPG and RANKL IOD values were observed after treatment in the

total group, or in either therapeutic subgroup. Ten of the 16 patients demonstrated an ACR20 response. Changes in tissue levels of OPG correlated significantly with Δ DAS28 at both the early and late time-points (Table 3: Spearman's Rho 0.45 and 0.59, respectively, $p < 0.05$). In ACR20 responders, a statistically significant decrease in OPG expression from baseline was observed, which exceeded the minimal, non-significant change observed in patients who did not respond to treatment (-33.7% vs 5.0%, respectively, $p < 0.05$). RANKL expression did not change significantly from baseline following therapy in the total group or in any clinical response subgroup.

DISCUSSION

This paper is the first to describe the utility of OPG and RANKL expression, quantified using computerized digital image analysis, as synovial tissue biomarkers of radiographic progression, disease activity and therapeutic response, exclusively during targeted cytokine blockade. These 2 biomarkers were detected in all synovial tissue sections evaluated. The intensity with which they were expressed varied widely between individual patients and timepoints. Baseline RANKL expression correlated with erosive progression at 6 and 12 months with a high level of statistical significance. In cross-sectional analysis, RANKL expression correlated with measures of disease activity at baseline and week 4, but not at the final timepoint, while OPG levels did not reflect disease activity. In longitudinal analysis following treatment, tissue OPG expression decreased significantly in clinical responders and changed in proportion to the magnitude of the therapeutic response, whereas neither RANKL expression nor the ratio of RANKL:OPG in synovial tissue changed significantly in any response subgroup.

A number of previous cross-sectional studies have evaluated synovial tissue or fluid levels of RANKL and OPG in RA [15-22, 34-36]. While synovial fluid RANKL, but

not OPG levels, correlated with the acute phase response in one study of patients with active RA, the present study provides the first observation of a significant cross-sectional correlation between measures of disease activity and RANKL in synovial tissue [34]. Several cross-sectional studies have highlighted differences in tissue RANKL and OPG expression between active and inactive RA or between RA and other diseases [15, 17, 18]. In 1 study evaluating RANKL expression in RA, SpA, OA and healthy controls, the highest tissue RANKL levels were observed in RA patients with active disease [15]. In a second study, OPG was absent from synovial tissue sections obtained from patients with active RA, in whom, conversely, the highest levels of RANKL expression were observed [17]. A significant inverse relationship between fluid levels of RANKL and OPG has been reported in patients with RA and OA, and when these patients were treated as a signal disease group [36].

In the present study, OPG levels decreased following targeted therapy in proportion to the clinical response, while RANKL remained unchanged from baseline at the final timepoint, despite substantial reductions in disease activity. The effects of therapy on OPG / RANKL expression in RA synovial tissue have been evaluated in 3 previous studies [19, 20, 22]. In the first, tissue OPG and RANKL were quantified in 18 patients before and 8 weeks after commencement of infliximab or etanercept therapy [20]. In keeping with the current findings, RANKL levels did not change significantly following treatment. However, in contrast to the observations arising from the present study, tissue OPG expression increased significantly following successful TNF α blockade. In a second study from the same center, synovial biopsies were obtained from the knees of patients before and 2 weeks after a single intra-articular corticosteroid injection [19]. In this setting, OPG levels remained static, while RANKL expression decreased significantly following the therapeutic intervention. In

a final study, patients receiving conventional DMARD therapy were followed with serial synovial biopsies for 3 years [22]. Longitudinal changes in tissue RANKL, but not OPG expression, reflected clinical and erosive change.

There are a number of possible reasons behind the discrepant OPG / RANKL signals observed among these studies. It is likely that differing therapies, designs, timeframes, staining, and quantification systems all contribute. In a study utilizing synovial tissue obtained from patients with spondylarthropathy, tissue OPG and RANKL signals differed substantially between differing antibodies [37]. The present study involved anakinra therapy in all patients. No previous studies have evaluated the effects of anakinra on synovial tissue OPG. It has been shown that IL-1 β can stimulate OPG production from RA fibroblast-like synoviocytes [38]. It is biologically plausible, therefore, that therapy specifically antagonising this cytokine should result in OPG down-regulation.

A further novel aspect of the present study was the observation that high pre-treatment synovial tissue RANKL levels and the ratio of tissue RANKL:OPG predicted progressive joint erosion, despite clinically effective targeted therapy. Two previous studies, both following observational cohorts, examined the potential utility of tissue RANKL as an early predictor of structural outcome in RA [18, 21]. In one, RANKL and OPG proteins were detected using immunofluorescence, while quantitative reverse transcription polymerase chain reaction was employed to detect RANKL mRNA in the second. In these studies, radiographic follow-up took place at timepoints between 2 and 3 years following the initial assessment. Unlike the present 1-year study, baseline tissue RANKL failed to predict subsequent progressive joint damage in either. It is likely that differing quantification methods, varying therapy,

and the longer follow-up period account for the lack of predictive signal in these studies.

In keeping with a previous study, high baseline ESR and CRP levels, which correlated with RANKL expression, were also associated with subsequent radiographic progression in univariate analysis [39]. Multiple linear regression analysis would be required to examine whether the relationship between RANKL and erosive progression was independent of its correlation with the acute phase response. This was not possible in the present study as the data could not be sufficiently normalized by transformation. A larger sample size would serve to clarify this issue. The association observed in this study between intense RANKL expression at baseline and progressive articular erosion is, however, entirely consistent with the biologic role of this mediator. Failure of RANKL to decrease following clinically efficacious therapy is a possible explanation for the discordance between radiographic progression and the clinical response to targeted therapy that has been observed previously [40]. Moreover, in studies using animal models of RA to evaluate novel therapies targeting the RANKL pathway, radiographic benefit without clinical improvement was observed, in keeping with the hypothesis that distinct, though related mechanisms may underlie synovitis and joint destruction [11-13, 41]. These observations have recently been replicated in humans with denosumab, a monoclonal antibody which targets RANKL [14].

A number of important limitations should prompt caution in interpreting the findings from this study: the patient numbers were small, the study was terminated prematurely, and an anti-TNF monotherapy arm was not included. The study was not specifically powered to detect particular outcomes or inter-group differences. It would be desirable to employ multivariate analysis to evaluate whether the relationship

between RANKL and erosive progression was independent, or reflecting other interactions. The sample size in this study precluded such an approach. Single primary antibodies were used to stain for RANKL and OPG. Ideally the signals observed in the study should be validated using other antibodies. Nevertheless, this is the first study in which a direct relationship between intense baseline synovial tissue expression of RANKL, a pivotal mediator of osteoclastogenesis, and progressive bone erosion has been described in RA. Excellent clinical responses to cytokine-targeted therapy were not necessarily accompanied by RANKL suppression in tissue. The potential therapeutic importance of modulating RANKL in RA is therefore highlighted, if radiographic arrest is to be achieved [9].

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Table 1. Synovial tissue OPG/RANKL expression and radiographic scores in individual patients, arranged according to clinical response.

Patient	ACR response	Treatment group	OPG, IOD			RANKL, IOD		
			Baseline	Week 4	Final	Baseline	Week 4	Final
1	ACR70	2	15.1	7.0	5.2	23.6	8.6	6.8
2	ACR70	2	6.2	3.5	.	11.1	5.4	.
3	ACR70	2	5.7	7.4	5.1	13.6	8.2	14.7
4	ACR70	2	20.9	20.4	8.5	17.4	26.2	26.6
5	ACR70	2	12.7	15.8	14.3	16.7	8.5	16.6
6	ACR50	1	9.5	8.9	6.3	12.7	10.4	9.8
7	ACR50	2	6.9	9.4	1.7	3.6	5.0	5.4
8	ACR50	2	7.1	7.8	5.7	14.6	15.8	4.8
9	ACR20	1	19.4	7.0	9.7	4.4	11.4	5.0
10	ACR20	1	6.6	4.1	.	17.6	17.2	4.7
11	ACR20	1	11.9	9.1	11.7	31.1	23.7	33.0
12	-	2	8.5	1.4	.	18.6	5.5	.
13	-	1	6.0	8.6	.	8.4	6.3	.
14	-	1	10.0	9.1	.	13.9	11.5	.
15	-	2	8.5	6.4	10.1	9.5	8.9	10.5
16	-	1	8.5	12.6	7.3	8.5	20.2	13.6
17	-	1	4.2	10.2	9.0	4.5	14.1	18.7
18	-	1	14.5	10.3	7.5	24.0	19.9	6.0
19	-	2	9.2	17.2	8.5	11.2	20.7	7.4
20	-	2	6.8	4.5	8.0	9.5	13.7	13.8
21	-	1	7.0	7.8	.	22.6	19.5	.
22	-	1	18.0	22.0	.	35.2	19.0	.

OPG = osteoprotegerin, RANKL = receptor activator of NF- κ B. ACR responses are at final timepoint. Treatment group 1 received anakinra, group 2 received anakinra + pegsunercept.

IOD = integrated optical density. Patients with clinically significant radiographic progression are highlighted in grey. See text for further definitions.

Table 2. Baseline synovial tissue RANKL, OPG and progressive structural damage

	Δ Total Sharp score		Δ Erosion		Δ Joint space narrowing	
	6 months	12 months	6 months	12 months	6 months	12 months
	OPG	-0.02	-0.09	0.18	0.14	-0.11
RANKL	0.39	0.45	0.59*	0.73**†	0.12	0.11
RANKL:OPG ratio	0.38	0.57*	0.42	0.61**†	0.19	0.30
ESR	0.52*	0.53**†	0.45	0.60**††	0.49*	0.44
CRP	0.51*	0.58**†	0.50**†	0.78**†	0.34	0.32

Δ denotes change. All values are Spearman's Rho. * $P < 0.05$, ** $P < 0.01$ for correlations.

† $P < 0.05$, †† $P < 0.01$ for areas under ROC curves. ROC curves were constructed using baseline biomarkers as test variables and presence or lack of clinically significant progression in structural damage as outcome. See text and Table 1 for further definitions.

Table 3. Synovial tissue OPG/RANKL expression and disease activity

	ESR		CRP		C
	Cross-section	Change†	Cross-section	Change†	
OPG					
Baseline	0.13	-	0.28	-	
Week 4	0.07	0.04	0.24	0.20	
Final	0.10	0.60*	0.24	0.50	
RANKL					
Baseline	0.44*	-	0.61**	-	
Week 4	0.36	0.03	0.59**	0.10	
Final	-0.05	0.46	0.03	0.33	
RANKL:OPG ratio					
Baseline	0.36	-	0.47*	-	
Week 4	0.18	-0.16	0.14	-0.28	
Final	-0.42	-0.10	-0.20	0.03	

Values are Spearman's Rho. † Refers to correlation between change in synovial tissue marker and change in disease activity variable.

* P < 0.05, ** P < 0.01. See Table 1 for further definitions.

Table 4. Changes in synovial tissue OPG/RANKL by clinical response and treatment group

Total Group	ACR20	
n = 22	n = 11	
(n = 16 w. final biopsy)	(n = 10 w. final biopsy)	(n = 7)

OPG

Baseline IOD	8.5 (6.8, 13.1)	9.5 (6.6, 15.1)	8.
Week 4 % change	-4.3 (-35.7, 31.3)	-6.4 (-43.7, 24.7)	3.
Final % change	-13.6 (-50.3, 13.3)	-33.7 (-62.4, -6.6)*‡	-33.

RANKL

Baseline IOD	13.7 (9.2, 19.6)	14.6 (11.1, 17.6)	14.
Week 4 % change	-15.4 (-41.3, 45.7)	-18.3 (-49.1, 38.9)	-29.
Final % change	7.0 (-58.8, 48.8)	2.8 (-68.1, 23.7)	-0.

RANKL:OPG ratio

Baseline	1.4 (1.1, 2.1)	1.6 (0.8, 2.4)	1.
Week 4 % change	-0.4 (-21.2, 55.6)	-0.5 (-20.6, 54.3)	-12.
Final % change	16.1 (-16.6, 94.9)	16.1 (-14.4, 203.9)	16.

IOD = integrated optical density. * P < 0.05, ** P < 0.01 for change from baseline. ‡

P < 0.05 vs. non-responders. See Table 1 for further definitions.