Early effects of IL-6 receptor inhibition on bone homeostasis: a pilot study in women with rheumatoid arthritis

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Abstract

Objective

A critical role of interleukin-6 (IL-6) in bone homeostasis has been suggested in experimental studies. We examined whether inhibition of IL-6 receptor in patients with rheumatoid arthritis (RA) results in early alterations of circulating markers of bone remodelling.

Methods

Circulating levels of osteoprotegerin, receptor activator of nuclear factor-kappaB ligand (RANKL), Wnt signalling pathway inhibitors Dickkopf-1 (Dkk-1) and sclerostin, markers of bone resorption (C-terminal cross-linking telopeptide of collagen type-I (CTX), tartrate-resistant acid phosphatase isoform-5b) and bone formation (bone-specific alkaline-phosphatase, osteocalcin) were examined in 22 women with active RA before and after two monthly infusions of tocilizumab (8mg/kg each); 'healthy', non-osteopenic, 1:1 age-matched women served as controls.

Results

At baseline, osteoprotegerin/RANKL ratio in patients was lower than controls by 5-fold; circulating osteoprotegerin correlated negatively with corresponding 28-joint-count disease activity scores and circulating RANKL correlated positively with C-reactive protein. Also, Dkk-1, sclerostin, CTX and osteocalcin levels were higher in RA than controls. After two months, osteoprotegerin/RANKL ratio increased, Dkk-1 decreased and sclerostin increased comparing to baseline; other markers did not change significantly. Increases of osteoprotegerin/RANKL ratio were more prominent in 10 patients who achieved remission or low disease activity after tocilizumab than in 12 patients who did not. In contrast, the significant alterations of both Wnt inhibitors were comparable between these patient subgroups.

Conclusions

Anti-IL-6 therapy induced suppression of the inflammatory response affects rapidly the disrupted bone homeostasis in active RA. An additional, possibly specific, effect of IL-6 receptor inhibition on bone remodelling in humans should be further examined.

Key words

tocilizumab, receptor activator of nuclear factor kappaB ligand (RANKL), dickkopf-1, sclerostin

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Introduction

Although human data is scarce, several lines of experimental evidence suggest a critical role of IL-6 in bone homeostasis. Interleukin-6-transgenic mice develop osteopenia due to increased osteoclastogenesis and reduced osteoblast activity, secondary to decreased precursor proliferation and osteoblast function (1). On the other hand, mice deficient in IL-6 have fewer osteoclasts at sites of bone erosion and reduced severity of antigen-induced arthritis; T-lymphocytes from these mice produce decreased levels of receptor activator of nuclear factor-kappaB ligand (RANKL), a potent factor of osteoclastogenesis, relative to its decoy receptor osteoprotegerin (2). Moreover, administration of an-IL-6 receptor antibody in the TNF-transgenic mouse model of inflammatory polyarthritis was found to directly block TNF- and RANKL-mediated osteoclastogenesis, suggesting a direct and specific inhibitory effect on osteoclasts independently of its anti-inflammatory effects (3). Importantly, *in vitro* stimulation of fibroblast-like synoviocytes derived from patients with rheumatoid arthritis (RA) with IL-6/soluble IL-6 receptor was able to directly induce expression of RANKL (4).

Osteoblast function can be suppressed by natural inhibitors of the canonical Wingless-type and Integrase-1 (Wnt) signalling pathway, including Dickkopf-1 (Dkk-1) and sclerostin. Inhibition of Dkk-1 in TNF-transgenic mice attenuates bone erosion by increasing osteoprotegerin expression and promoting bone formation (5). Sclerostin is another Wnt inhibitor which decreases the life span of osteoblasts by stimulating their apoptosis; overexpression of sclerostin *in vivo*, using the osteocalcin promoter, results in gene-dose dependent osteopenia in mice (6).

By extrapolating experimental evidence, the disruption of IL-6 regulation present in patients with RA could be directly associated with the generalised bone loss, typical of this disease, through specific effects of IL-6 on osteoclastogenesis and/or osteoblast function. Thus, we tested the hypothesis that therapeutic inhibition of IL-6 receptor in patients with RA affects bone homeostasis by reducing resorption and/or increasing formation. Since such effects could be reflected by early changes on osteoprotegerin/RANKL axis and Wnt inhibitors Dkk-1 and sclerostin, we evaluated the circulating levels of these molecules before and after a short course of tocilizumab administration for active disease. Tocilizumab inhibits IL-6 binding to its receptors, leading to the blockade of the IL-6 signalling through both receptors, but not to the blockade of the signalling of other IL-6 family cytokines (7).

Patients and methods

Twenty-two women (mean±SD age of 43.1±13.7 years) with active RA were treated with two infusions of tocilizumab 8 mg/kg, 4 weeks apart. Mean disease duration was 10.3±9.2 years. Concomitant treatment with oral prednisolone (11 patients, 4±4.3 mg daily) and oral methotrexate (18 patients, 11.3±6.1 mg, weekly) had been stable during at least 3 months prior to baseline and remained unchanged up to 8 weeks. Disease activity was assessed using the 28-joint-count disease activity score (DAS28).

The following soluble markers of bone remodelling were measured by ELISA at baseline and after 2 months: i) osteoclast regulators: RANKL and osteoprotegerin (Biomedica Medizinprodukte GmbH, Vienna, Austria); ii) Wnt inhibitors: Dkk-1 (Biomedica) and sclerostin (as described in ref. 8); iii) bone resorption markers: C-terminal cross-linking telopeptide of collagen type-I (CTX), and tartrate-resistant acid phosphatase isoform-5b (TRACP-5b) (Immunodiagnostic Systems Ltd, Boldon, Tyne & Wear, UK); iv) bone formation markers: bone-specific alkaline phosphatase (bALP) (Quidel, San Diego, CA, USA) and osteocalcin (Nordic Bioscience Diagnostics A/S, Herlev, Denmark). Sera derived from 'healthy' non-osteopenic women 1:1 age-matched with patients, served as controls. The study protocol was approved by Laikon Hospital Ethics Committee and all subjects gave informed consent.

Mann-Whitney two-sample statistics and Wilcoxon matched pairs signed-ranks test were used for group or pairwise comparisons between baseline and
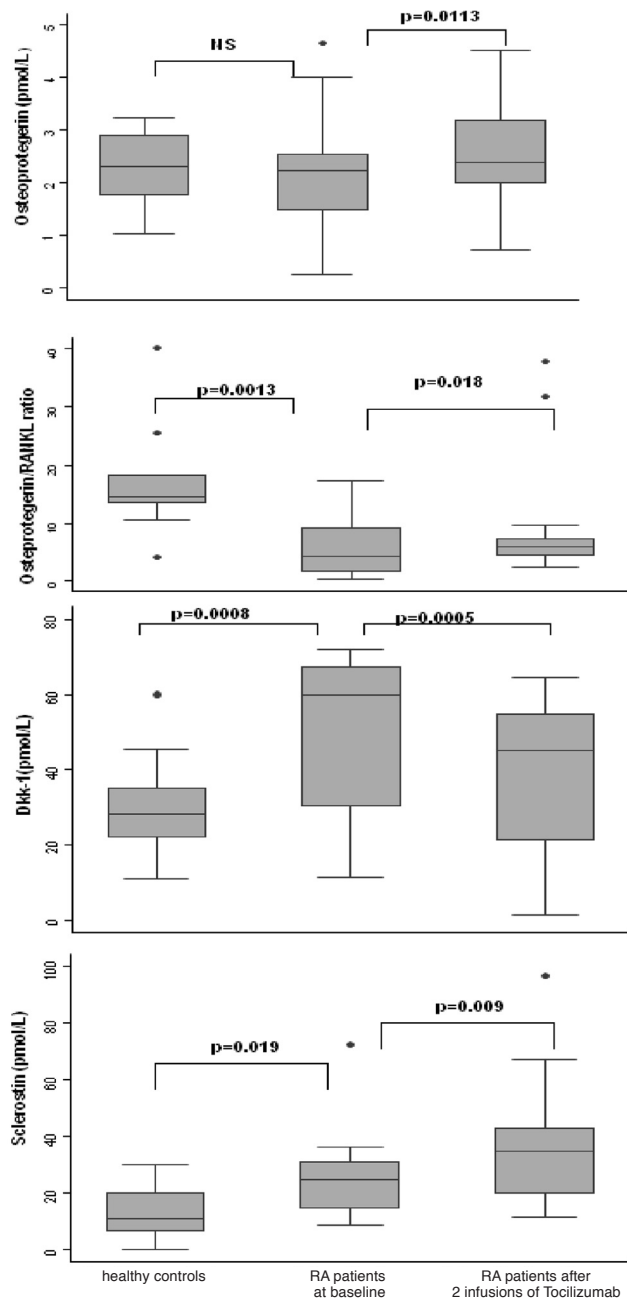
Fig. 1. Abnormal circulating levels of osteoclast regulators and Wnt inhibitors in patients with active RA change after tocilizumab. Box plots representing levels of osteoprotegerin, osteoprotegerin/RANKL ratio, Dkk-1 and sclerostin in 'healthy', non-osteopenic, women 1:1 age-matched with 22 women with active RA at baseline. Measurements were repeated in patients after two monthly infusions of tocilizumab (8 mg/kg each). Differences using Mann-Whitney two sample statistics and Wilcoxon matched pairs signed-ranks test are depicted.

were also found for both Wnt inhibitors Dkk-1 and sclerostin (Fig. 1), as well as for CTX (0.64 ± 0.42 vs. 0.18 ± 0.13 ng/ml, respectively, $p<0.0001$), and osteocalcin (7.3 ± 5.7 vs. 4.3 ± 2.4 ng/ml, $p=0.018$), but not for TRACP-5b (0.98 ± 0.83 vs. 1.04 ± 0.54 U/l) and bALP (16.4 ± 16.3 vs. 13.9 ± 2.9 IU/l).

Comparing to baseline, patients with RA displayed significant increases of osteoprotegerin, osteoprotegerin/RANKL ratio and sclerostin, but reductions of Dkk-1, after tocilizumab (Fig. 1). Reductions of CTX (0.64 ± 0.42 vs. 0.59 ± 0.49 ng/ml) and increases of osteocalcin (7.3 ± 5.7 vs. 9.4 ± 7.0 ng/ml) and bALP (16.4 ± 16.3 vs. 20.2 ± 12.9 IU/l) did not reach significance.

Possible associations between treatment responses and the alterations in circulating osteoclast regulators and/or Wnt inhibitors post-tocilizumab were then examined. Ten patients (Group A) achieved remission or low disease activity, as defined elsewhere (9), having a DAS28 reduction of -2.49 ± 0.81 from baseline. The remaining 12 patients (Group B) had moderate disease activity at two months, albeit having a rather similar to Group A reduction of DAS28 of -2.59 ± 1.40. As shown in Figure 2, osteoprogerin/RANKL ratio increased significantly after tocilizumab only in Group A, suggesting that a state of higher disease activity *per se* precludes normalisation of osteoclast regulators. In contrast, alterations of Wnt inhibitors Dkk-1 and sclerostin were significant in both groups and comparable between patients who achieved remission or low disease activity after tocilizumab and those who did not.

Discussion

RANKL is the most potent factor of osteoclastogenesis; since circulating levels are gender-dependent, only female patients were examined (10). The low osteoprotegerin/RANKL serum ratio found in our patients with active RA indicates increased osteoclast stimulation which leads to increased bone resorption, as evidenced by increased CTX, as well as high bone turnover, as evidenced by increased osteocalcin. The osteoprotegerin/RANKL system was influenced by RA disease activity.

two months, respectively. To calculate osteoprotegerin/RANKL ratio for samples with non-detectable RANKL concentration a value 0.08 pmol/L (lowest detectable) was used. Spearman's coefficients were used to test for correlation between variables. A *p*-value of less than 0.05 was considered significant.

Results

Baseline RA disease activity was either high (DAS28>5.1) or moderate (DAS28>3.2 and ≤5.1) in 14 and 8 patients, respectively. All patients had increased ESR values (49.2 ± 30.2 mm/1h, mean±SD) and/or serum CRP levels (17.2 ± 19.8 mg/l). As shown in Figure 1, osteoprotegerin/RANKL ratio was reduced by almost 5-fold in RA compared to controls, presumably due to highly increased RANKL levels in patients than controls (0.32 ± 0.36 vs. 0.06 ± 0.08 pmol/l, respectively, $p=0.005$). A negative correlation between individual osteoprotegerin levels and DAS28 (Spearman $r=-0.47$, $p=0.025$), and a positive correlation between RANKL and CRP ($r=0.44$, $p=0.039$) were noted. Significant increases in RA patients *versus* controls
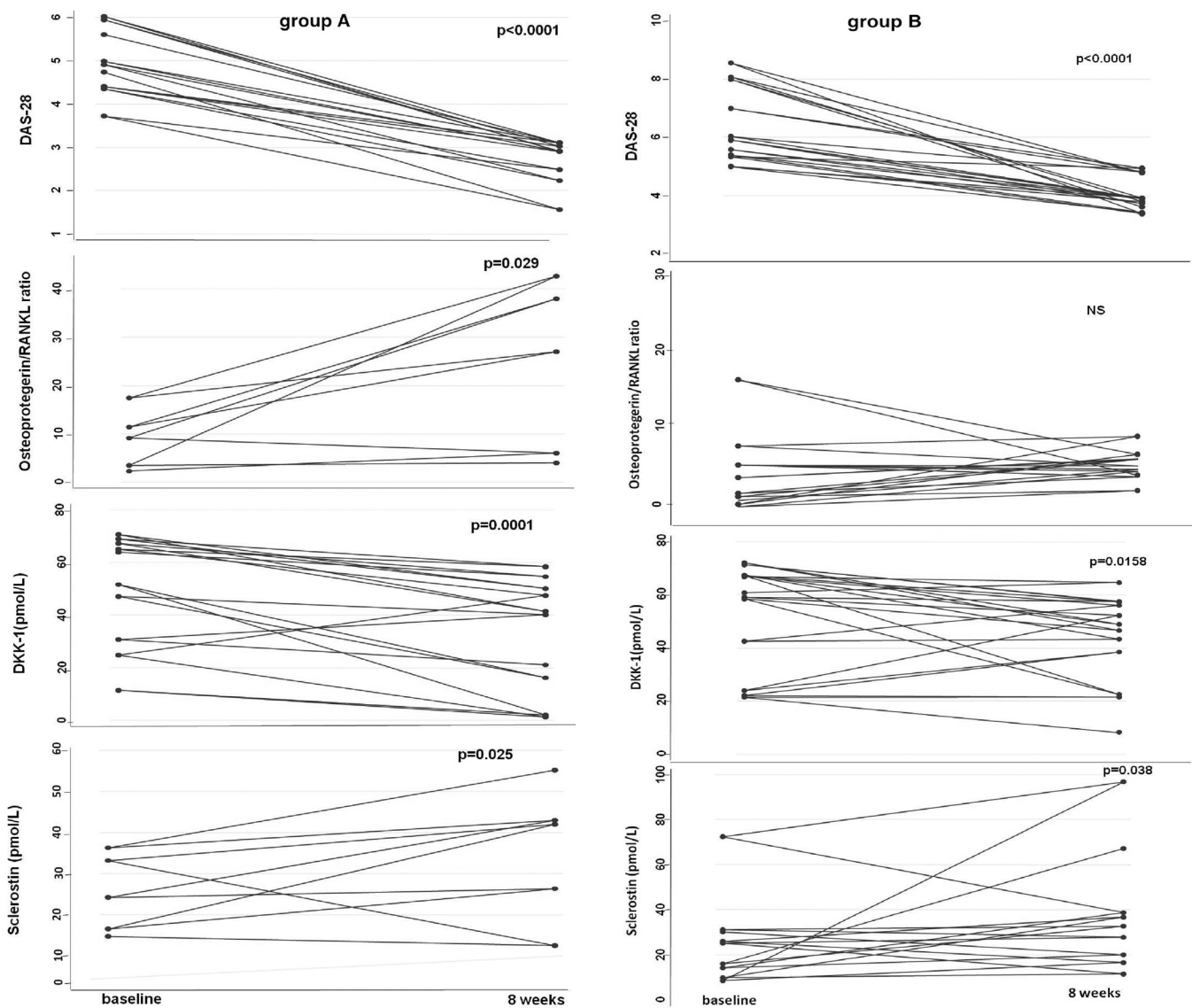
Fig. 2. Changes of circulating levels of osteoclast regulators and Wnt inhibitors in relation to RA disease activity status achieved after tocilizumab. "Spaghetti" presentation of 28-joint count disease activity scores (DAS28), and levels of osteoprotegerin/RANKL ratio, Dkk-1 and sclerostin at baseline and after treatment with two monthly infusions of tocilizumab (8 mg/kg each). Twenty-two women with active RA at baseline were grouped according to disease activity status after two monthly infusions of tocilizumab (8 mg/kg each); group A comprises of 3 and 7 patients who achieved remission (DAS28≤2.6) or low disease activity (DAS28≤3.2), respectively, and group B comprises of 12 patients who did not. Differences using Wilcoxon matched pairs signed-ranks test are depicted.

Despite the relatively small number of patients examined, significant correlations were indeed noted between osteoprotegerin and RANKL with DAS28 and CRP, respectively. Low osteoprotegerin/RANKL circulating levels in early, active RA predicts later joint destruction (11), while efficient treatment-induced increased osteoprotegerin/RANKL ratio expression at the synovial tissue level has a significant impact on osteoclast formation and joint damage in active RA (12). In line with *in vitro* findings showing induction of RANKL in RA patient-derived synoviocytes after stimulation by IL-6/soluble IL-6 receptor (4), we found that inhibition of IL-6 receptor affected the osteoprotegerin/RANKL axis *in vivo*. Early significant increases of serum osteoprotegerin/RANKL may be responsible for later reductions in markers of bone resorption with tocilizumab plus methotrexate in RA patients with an inadequate response to methotrexate (13). Moreover, further studies should examine whether baseline serum RANKL levels may serve to predict remission in RA patients treated with tocilizumab, as it may happen with anti-TNF treatment (9).

In contrast to the low osteoprotegerin/RANKL serum ratio in patients with active RA, corresponding Dkk-1 and sclerostin levels were found increased compared to healthy controls, and did not correlate with individual levels of disease activity. While sclerostin serum levels have not been previously measured in RA patients, serum Dkk-1 have been found increased (14, 15) and associated with a higher risk of subsequent progression of bone erosion, independently of CRP and disease activity (14). Decreased circulating Dkk-1 following tocilizumab administration in our

patients, accompanied by increases in osteoprotegerin/RANKL ratio, suggest a role of IL-6 signalling in the regulation of Wnt pathway. This is in line with findings showing that inhibition of Dkk-1 attenuates bone erosion by increasing osteoprotegerin expression in experimental inflammatory polyarthritis (5). Anti-TNF therapy in RA patients also leads to reduction of serum Dkk-1 (15) and increased synovial osteoprotegerin/RANKL ratio expression (16).

Opposite changes of Dkk-1 and sclerostin levels after tocilizumab in our patients was rather unexpected since addition of recombinant Dkk-1 to mouse osteoblast cell culture dramatically increased sclerostin levels and this was prevented by the addition of an anti-Dkk-1 antibody (5). However, increased sclerostin after tocilisumab may be explained by a balance effect to the reduced osteoclast function and/or to the reduction of Dkk-1. Along this line, we have indeed found that effective anti-resorptive therapies, including risedronate in post-menopausal osteoporosis and zoledronic acid in thalassemia-induced osteoporosis, resulted in reduced Dkk-1 and increased circulating sclerostin levels (8).

Finally, the observed significant alterations of both Wnt inhibitors seemed independent of the disease status achieved at two months of tocilizumab treatment, in contrast to osteoprotegerin/RANKL ratio changes, which were clearly more prominent in those patients who achieved remission or low disease activity than in patients who did not.

Tocilizumab has been reported to be safe, tolerable, and clinically effective for patients with inadequate responses to anti-TNF therapy and for those who were biologics-naïve (17). The results of this pilot study indicate that tocilizumab induced suppression of inflammation response and affects rapidly the disrupted bone homeostasis in active RA. These results can not be entirely explained by an anti-inflammatory treatment effect *per se* and this is in line with the specific blocking effect that anti-IL-6 receptor therapy exerts on osteoclast formation in experimental polyarthritis (3). Thus, a possibly specific, beyond inflammation, effect of anti-IL-6 therapy on bone remodelling in humans should be further examined.

References

1. DE BENEDETTI F, RUCCI N, DEL FATTORE A *et al.*: Impaired skeletal development in interleukin-6-transgenic mice: a model for the impact of chronic inflammation on the growing skeletal system. *Arthritis Rheum* 2006; 54: 3551-63.
2. WONG PK, QUINN JM, SIMS NA, VAN NIEUWENHUIJZE A, CAMPBELL IK, WICKS IP: Interleukin-6 modulates production of T lymphocyte-derived cytokines in antigen-induced arthritis and drives inflammation-induced osteoclastogenesis. *Arthritis Rheum* 2006; 54: 158-68.
3. AXMANN R, BÖHM C, KRÖNKE G, ZWERINA J, SMOLEN J, SCHETT G: Inhibition of interleukin-6 receptor directly blocks osteoclast formation *in vitro* and *in vivo*. *Arthritis Rheum* 2009; 60: 2747-56.
4. HASHIZUME M, HAYAKAWA N, MIHARA M: IL-6 trans-signalling directly induces RANKL on fibroblast-like synovial cells and is involved in RANKL induction by TNF-alpha and IL-17. *Rheumatology* (Oxford) 2008; 47: 1635-40.
5. HEILAND GR, ZWERINA K, BAUM W *et al.*: Neutralisation of Dkk-1 protects from systemic bone loss during inflammation and reduces sclerostin expression. *Ann Rheum Dis* 2010; 69: 2152-9.
6. LOOTS GG, KNEISSEL M, KELLER H *et al.*: Genomic deletion of a long-range bone enhancer misregulates sclerostin in van Buchem disease. *Genome Res* 2005; 15: 928-35.
7. MIHARA M, KASUTANI K, OKAZAKI M *et al.*: Tocilizumab inhibits signal transduction mediated by both mIL-6R and sIL-6R, but not by the receptors of other members of IL-6 cytokine family. *Immunopharmacol* 2005; 5: 1731-40.
8. POLYZOS SA, ANASTASILAKIS AD, BRATENGEIER C *et al.*: Serum sclerostin levels positively correlate with lumbar spinal bone mineral density in postmenopausal women - The six-month effect of risedronate and teriparatide. *Osteoporosis Int* 2011 Jan 11 (Epub ahead of print).
9. GONZÁLEZ-ALVARO I, ORTIZ AM, TOMERO EG *et al.*: Baseline serum RANKL levels may serve to predict remission in rheumatoid arthritis patients treated with TNF antagonists. *Ann Rheum Dis* 2007; 66: 1675-8.
10. KERSCHAN-SCHINDL K, WENDLOVA J, KUDLACEK S *et al.*: Serum levels of receptor activator of nuclear factor kappaB ligand (RANKL) in healthy women and men. *Exp Clin Endocrinol Diabetes* 2008; 116: 491-5.
11. GEUSENS PP, LANDEWÉ RB, GARNERO P *et al.*: The ratio of circulating osteoprotegerin to RANKL in early rheumatoid arthritis predicts later joint destruction. *Arthritis Rheum* 2006; 54: 1772-7.
12. HAYNES D, CROTTI T, WEEDON H *et al.*: Modulation of RANKL and osteoprotegerin expression in synovial tissue from patients with rheumatoid arthritis in response to disease-modifying antirheumatic drug treatment and correlation with radiologic outcome. *Arthritis Rheum* 2008; 59: 911-20.
13. GARNERO P, THOMPSON E, WOODWORTH T, SMOLEN JS: Rapid and sustained improvement in bone and cartilage turnover markers with the anti-interleukin-6 receptor inhibitor tocilizumab plus methotrexate in rheumatoid arthritis patients with an inadequate response to methotrexate: results from a substudy of the multicenter double-blind, placebo-controlled trial of tocilizumab in inadequate responders to methotrexate alone. *Arthritis Rheum* 2010; 62: 33-43.
14. GARNERO P, TABASSI NC, VOORZANGER-ROUSSELOT N: Circulating dickkopf-1 and radiological progression in patients with early rheumatoid arthritis treated with etanercept. *J Rheumatol* 2008; 35: 2313-5.
15. DAOUSSIS D, LIOSSIS SN, SOLOMOU EE *et al.*: Evidence that Dkk-1 is dysfunctional in ankylosing spondylitis. *Arthritis Rheum* 2010; 62: 150-8.
16. CATRINA AI, AF KLINT E, ERNESTAM S *et al.*: Anti-tumor necrosis factor therapy increases synovial osteoprotegerin expression in rheumatoid arthritis. *Arthritis Rheum* 2006; 54: 76-81.
17. WAKABAYASHI H, OKA H, NISHIOKA Y *et al.*: Do biologics-naïve patients with rheumatoid arthritis respond better to tocilizumab than patients for whom anti-TNF agents have failed? A retrospective study. *Clin Exp Rheumatol* 2011; 29: 314-7.