Circulating Dickkopf-1 and Radiological Progression in Patients with Early Rheumatoid Arthritis Treated with Etanercept

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ABSTRACT. ***Objective.*** Dickkopf-1 (Dkk-1) regulates bone remodeling in animal models of inflammatory arthritis, but its role in patients with rheumatoid arthritis (RA) remains unclear.

Methods. Baseline circulating Dkk-1 was measured in 113 patients with RA (< 3 yrs) who received etanercept (10 or 25 mg twice/week, n = 63) or methotrexate alone (n = 40) for 1 year. Progression was assessed by changes in radiological Sharp score.

Results. Increased Dkk-1 was associated with a higher risk of progression of bone erosion, independently of age, sex, baseline radiological damage, C-reactive protein, and disease activity in patients treated with etanercept.

Conclusion. Dkk-1 may be an important mediator of bone erosion in patients with RA. (First Release Oct 1 2008; J Rheumatol 2008;35:2313–5; doi:10.3899/jrheum.080356)

Key Indexing Terms:
DICKKOPF-1 RHEUMATOID ARTHRITIS BONE EROSION

One of the hallmarks of rheumatoid arthritis (RA) is progressive bone erosion. Recent studies in animal models of RA have suggested that Dickkopf-1 (Dkk-1), a soluble inhibitor of the Wnt signaling pathway, plays a major role in joint remodeling[1]. Circulating Dkk-1 decreases after treatment with anti-tumor necrosis factor (anti-TNF), this cytokine being a key stimulator of Dkk-1 expression[1]. The biological relevance of Dkk-1 in relation to progression in patients with RA remains unclear.

We tested the hypothesis that in patients with early RA, circulating Dkk-1 is associated with radiological progression.

MATERIALS AND METHODS

A total of 113 patients (80% women, mean age 49 yrs, mean (SD) disease duration 12 ± 12 mo, 88% rheumatoid factor (RF)-positive, 35% current corticosteroid users) who met the American College of Rheumatology criteria for RA[2] were included. Patients were a random subset, as described[3], from a larger randomized study[4] comparing the efficacy of etanercept and methotrexate (MTX) alone in 632 patients[4]. Randomization from the original total population was performed using the RANUNI function from the Statistical Analysis Software (SAS). Patients were at least 18 years of age, had had RA for not more than 3 years, and had not previously been treated with MTX or biologics. Forty patients received MTX at a dose that was escalated from 7.5 to 20 mg/week over the first 8 weeks, 35 received etanercept 10 mg twice weekly, and 38 received etanercept 25 mg for 1 year[3,4].

Radiographs of hands, wrists, and feet at baseline and 6 and 12 months were scored by 2 physicians who were blinded to patient's treatment assignment and chronological sequence of examinations. They were 2 musculoskeletal physicians trained in the modified Sharp scoring method[5,6]. The interreader correlation was good (r = 0.85)[4]. Each radiograph was scored for both bone erosion and joint space narrowing (JSN) Sharp score, and the mean value of the 2 readers' scores was used in the analysis. The radiographs of the subset of patients used in this analysis were scored by the same readers who read the radiographs of the initial total study population[4]. Linear regression using the 3 scores at baseline and at 6 and 12 months was applied in each patient to get the most accurate estimate of the average yearly progression rate (expressed as Sharp-units/year).

Dkk-1 was measured in fasting blood samples by a sandwich ELISA (Biomedica, Vienna, Austria) using 2 antibodies recognizing synthetic peptides of human Dkk-1[7] with intra- and interassay variation < 12%.

The associations of Dkk-1 with progression were investigated by continuous and categorical approaches. For categorical analyses, patients were separated into quartiles or tertiles of Dkk-1 levels, and progression was defined by an increase of the Sharp scores of ≥ 0.5 unit/year as reported[3]. Relative risks (RR) were estimated by logistic regression analyses.

RESULTS

As shown in Table 1, patients in the 3 treatment groups had similar age, sex distribution, number of swollen and tender joints, baseline C-reactive protein (CRP), and radiological Sharp scores. Baseline characteristics in this random subset were also similar to those of the total population as described[4].

In all patients, each standard deviation (SD) increase of Dkk-1 was associated with a RR (95% confidence interval) of progression of bone erosion (≥ 0.5 unit Sharp score/year) of 1.65 (95% CI 1.06–2.54). When the analysis was performed in each treatment group separately, increased Dkk-1 was associated with a higher risk of progression in patients

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Table 1. Baseline characteristics of patients with early RA, by treatment group, in the study subset and in the initial total population. Results are shown as mean (± SD) for quantitative variables.

Characteristic	Study Subset				Whole-Study Population* (n = 632)
	Methotrexate, n = 40	Etanercept 10 mg, n = 35	Etanercept 25 mg, n = 38	p	
Female, %	85	77	71	0.32	75
Age, yrs	46 ± 10	50 ± 13	52 ± 12	0.10	50 ± 13
Disease duration, mo	13.9 ± 11.2	13.1 ± 10.9	11.3 ± 12.5	0.61	11.7 ± 13
No. tender joints	31 ± 13	30 ± 16	30 ± 18	0.92	31 ± 16
No. swollen joints	23 ± 10	25 ± 12	23 ± 12	0.78	24 ± 12
C-reactive protein, mg/dl	3.8 ± 4.8	5.9 + 8.0	3.0 ± 4.5	0.09	3.8 ± 4.9
Radiological Sharp score					
Bone erosion score	7.3 ± 9.2	7.4 ± 10.2	5.8 ± 11.5	0.76	6.7 ± 9.1
Joint space narrowing score	6.7 ± 8.0	8.0 ± 9.0	5.1 ± 6.8	0.43	5.5 ± 7.3

* Described in Bathon, *et al*[4].

receiving etanercept at 10 mg [RR per SD increase: 2.0 (95% CI 0.9–4.3)] or 25 mg [RR 3.1 (95% CI 1.1–8.7)], but not in the MTX group [RR 1.0 (95% CI 0.6–1.9)]. Subsequent analyses were thus performed in the combined 10 or 25 mg etanercept group. As shown on Table 2, increased Dkk-1 levels were associated with a higher risk of progression of bone erosion whether values were considered as a continuous variable, in quartiles, or in tertiles, although RR did not reach significance in the quartile analysis because of limited number of subjects. Because the proportion of progressors was similar in patients with Dkk-1 in tertile 1 and in tertile 2 (Table 2), these 2 groups were combined in further analyses. In multiple logistic regression including age, sex, baseline joint damage, CRP, and Disease Activity Score, Dkk-1 remained a significant predictor of progression (Table 3). When progression was defined by an increase of ≥ 1 unit Sharp score/year, very similar results were obtained (data not shown). There was no significant association between Dkk-1 and progression of JSN Sharp scores.

DISCUSSION

Increased circulating Dkk-1 was associated with a greater risk of progression of bone erosion in patients with early RA receiving etanercept alone independently of other conventional risk factors. The associations were consistent whether Dkk-1 levels were expressed as a continuous or a categorical variable. These data generated from circulating measurements provide clinical relevance to immunohistological studies showing that Dkk-1 is produced by synoviocytes in patients with RA[1], and with data indicating that blockage of Dkk-1 abolished bone erosion in an inflammatory mouse model[1]. Dkk-1 levels were associated with progression of bone erosion, but not progression of JSN — a surrogate for cartilage loss — in agreement with the main role of this factor in regulating bone remodeling.

Dkk-1 was associated with risk of progression in patients receiving etanercept, but not in those treated with MTX for reasons that were unclear. The specific relationship observed in patients treated with etanercept could be related to limitation of statistical power resulting from subgroup

Table 2. Association between Dkk-1 levels and 1-year radiological progression of bone erosion Sharp score* in 73 patients treated with etanercept.

Classification of Dkk-1 Levels	Relative Risk (95% CI)	Patients with Progression, %
Continuous, per SD increase	2.43 (1.25–4.71)	—
Categorical		
Quartile (Q)		
Q1 (lowest)	1 (Referent)	11.7
Q2	1.32 (0.19–9.02)	15.0
Q3	3.75 (0.64–22.0)	35.3
Q4 (highest)	5.25 (0.90–30.6)	41.2
Tertile (T)		
T1 (lowest	1 (referent)	12.5
T2	1.47 (0.29–7.45)	17.3
T3 (highest)	5.50 (1.30–23.3)	44.0
T3 vs 2 lower T	4.60 (1.46–13.8)	—

* Progression was defined as an increase in bone erosion Sharp score of 0.5 unit/year or more.

Table 3. Multivariate logistic regression model for prediction of 1-year progression of radiological bone erosion Sharp score* in 73 patients treated with etanercept.

Baseline Variables	Cutoff Value	Relative Risk (95% CI)	p
Dkk-1	Highest vs 2 lower tertiles	6.19 (1.76–21.7)	0.0044
Age	Per year	1.02 (0.97–1.07)	0.38
Sex	Female	2.00 (0.48–8.36)	0.35
Bone erosion Sharp score	Highest vs 2 lower tertiles	3.09 (0.81–11.7)	0.098
CRP	Highest vs 2 lower tertiles	0.59 (0.15–2.25)	0.44
Disease Activity Score	Highest vs 2 lower tertiles	1.71 (0.43–6.75)	0.44

* Progression was defined as an increase in bone erosion Sharp score of 0.5 unit/year or more. CRP: C-reactive protein.

analysis and/or genuine underlying biological mechanisms such as common regulation of Dkk-1 production and bone erosion by TNF. Further larger studies should investigate the relationship between Dkk-1 and joint destruction in patients with RA receiving anti-TNF or MTX, alone or combined. The relationship may differ according to treatment regimens, as shown for the association between inflammation and progression[8].

The balance between receptor activator of nuclear factor-κB ligand (RANKL) and osteoprotegerin (OPG) plays an important role in bone erosion[9]. An association between circulating RANKL/OPG ratio and progression was observed in patients with early RA[10]. Because animal data indicate a possible crosstalk between Dkk-1 and the RANKL/OPG system[1], it would have been interesting to analyze these factors in our study. However, accurate measurement of circulating RANKL remains challenging because of uncertainties about which forms are the most biologically relevant and the limited sensitivity of available assays[11].

This study has strengths and limitations. We report for the first time an association of circulating Dkk-1 with prospectively assessed progression in patients with RA. Although a larger proportion of patients with high Dkk-1 values showed progression, a significant number of subjects with low values did progress. Thus, one cannot recommend measuring Dkk-1 alone for identifying individual patients with rapid progression. Because Dkk-1 was associated with progression independently of disease activity and baseline radiological damage, however, it may be useful in combination with other risk factors to improve prediction. We did not measure other soluble antagonists of Wnt, including frizzled-related protein-3, which has been suggested to be involved in joint destruction in osteoarthritis[12], although its role in RA remains unclear. Infliximab has been shown to reduce circulating Dkk-1 in patients with active RA[1]. Because stored followup samples were not available in this study, we could not analyze the effect of MTX or etanercept on circulating Dkk-1.

Elevated Dkk-1 is associated with a higher risk of radiological progression in patients with early RA receiving etanercept. These data support a role of Dkk-1 in bone remodeling in patients with RA.

REFERENCES

1. Diarra D, Stolina M, Polzer K, et al. Dickkopf-1 is a master regulator of joint remodeling. Nature Med 2007;13:156-63.
2. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
3. Garnero P, Gineyts E, Christgau S, Finck B, Delmas PD. Association of baseline levels of urinary glucosyl-galactosyl-pyridinoline and type II collagen C-telopeptide are associated with progression of joint destruction in patients with early rheumatoid arthritis. Arthritis Rheum 2002;46:21-3.
4. Bathon JM, Martin RW, Fleischmann RM, et al. A comparison of etanercept and methotrexate in patients with early rheumatoid arthritis. N Engl J Med 2000;343:1586-93.
5. Sharp JT, Lidsky MD, Collins LS, Moreland J. Methods of scoring the progression of radiological changes in rheumatoid arthritis: correlation of radiologic, clinical and laboratory abnormalities. Arthritis Rheum 1971;14:706-20.
6. van der Heijde DM, van Riel PL, van Leeuwen MA, van Hof MA, van Rijswijk MH, van de Putte LB. Prognostic factors for radiographic damage and physical disability in early rheumatoid arthritis. A prospective study of 147 patients. Br J Rheumatol 1992;31:519-25.
7. Voorzanger-Rousselot N, Journe F, Maitzen S, et al. Evaluation of a new two site-Elisa for serum Dkk-1 in patients with breast cancer and bone metastases [abstract]. Calcif Tissue Int 2007;80:S71.
8. Landewé R, van der Heijde D, Klareskog L, van Vollenhoven R, Fatenejad S. Disconnect between inflammation and joint destruction after treatment with etanercept plus methotrexate: results from the trial of etanercept and methotrexate with radiographic and patient outcomes. Arthritis Rheum 2006;54:3119-25.
9. Gravallese EM, Goldring SR. Cellular mechanisms and the role of cytokines in bone erosions in rheumatoid arthritis. Arthritis Rheum 2000;43:2143-51.
10. Geusens P, Landewé R, 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.
11. Kearns AE, Khosla S, Kostenuik P. Receptor activator of nuclear factor-κB ligand and osteoprotegerin regulation of bone remodeling in health and disease. Endocr Rev 2008;29:155-92.
12. Lane NE, Nevitt MC, Lui LY, de Leon P, Corr M. Wnt signaling antagonists are potential prognostic biomarkers for the progression of radiographic hip osteoarthritis in elderly Caucasian women. Arthritis Rheum 2007;56:3319-25.