

# Cartilage and Bone Biomarkers in Rheumatoid Arthritis: Prediction of 10-year Radiographic Progression

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**ABSTRACT. Objective.** As current predictors of joint destruction have low specificity, serological biomarkers reflecting bone and cartilage destruction have been proposed as tools in assessing prognosis of rheumatoid arthritis (RA). We examined whether serum concentrations of a panel of biomarkers could predict radiographic progression in patients with RA.

**Methods.** A cohort of 238 patients with RA was followed longitudinally for 10 years with collection of clinical data and serum samples. These analyses focus on the 136 patients with radiographs of the hands available at baseline and at 5 and/or 10 years. Radiographs were scored according to the van der Heijde-modified Sharp score (SHS). Baseline sera were analyzed for receptor activator of nuclear factor- $\kappa$ B ligand (RANKL), osteoprotegerin (OPG), human cartilage glycoprotein-39 (YKL-40), C2C, collagen cross-linked C-telopeptide (CTX-I), and cartilage oligomeric matrix protein (COMP). Multivariate linear and logistic regression analyses were used to identify predictors of radiographic progression.

**Results.** Baseline CTX-I levels were higher in progressors [0.41 ng/ml (interquartile range 0.31–0.75)] than in nonprogressors [0.32 ng/ml (IQR 0.21–0.49)], and were independently associated with 10-year change in radiographic damage score [ $\beta = 16.4$  (IQR 5.7–27.1)]. We found no association between radiographic progression and baseline serum levels of RANKL, OPG, C2C, YKL-40, or COMP.

**Conclusion.** This longterm followup study of patients with RA indicates a relationship between elevated CTX-I levels in serum and subsequent joint destruction. This association was, however, weak, and our study does not support that serum CTX-I or any of the other tested biomarkers will serve as more useful prognostic markers than current predictors such as anti-cyclic citrullinated peptide, radiographic damage early in the disease course, and signs of inflammation. (First Release Dec 15 2008; J Rheumatol 2009;36:266–72; doi:10.3899/jrheum.080180)

## Key Indexing Terms:

RHEUMATOID ARTHRITIS

RADIOGRAPHIC PROGRESSION

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Rheumatoid arthritis (RA) is characterized by synovitis, progressive erosions, and cartilage destruction, but the disease course and progression rate shows pronounced variation between patients. New effective therapies are available, and prevention of joint destruction with aggressive treatment has become a key treatment goal.

When treating patients with RA both with early or more advanced disease, it is crucial to identify those with rapid progressive joint destruction since they will benefit most from aggressive treatment regimens. Current predictors of joint destruction, including early presence of radiographic signs of damage, elevated erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) over time, and the presence of rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibodies (anti-CCP), have low specificity<sup>1-3</sup>. Extensive efforts have therefore been made to identify markers that directly reflect the bone and cartilage turnover rate. These turnover products enter serum or urine, where they can be detected by immunoassays as biomarkers.

Collagen cross-linked C-telopeptide (CTX-I), receptor activator of nuclear factor- $\kappa$ B ligand (RANKL), osteoprotegerin (OPG), C2C, cartilage oligomeric matrix protein (COMP), and human cartilage glycoprotein-39 (YKL-40) have all been suggested to be candidate biomarkers to distinguish between RA patients with rapidly and those with slowly progressive joint destruction<sup>4-12</sup>. Elevated concentrations of CTX-I<sup>13</sup>, a degradation product of C-terminal cross-linking telopeptide of type I collagen, have been found in urine and serum of patients with RA<sup>14,15</sup>. RANKL is essential for development and activation of osteoclasts, and OPG is its soluble decoy receptor<sup>16,17</sup>. Both RANKL and OPG have been found elevated in serum of patients with RA<sup>18</sup>. Cartilage loss is a hallmark of RA, and increased levels of a C-terminal cross-linking telopeptide of type II collagen degradation product (CTX-II) have been suggested to predict RA progression<sup>4,19,20</sup>. CTX-II can only be measured in urine, but recently an immunoassay for measurements of collagen type II degradation in serum, a neoepitope formed by collagenase cleavage, C2C, has become available<sup>21</sup>. C2C concentrations are elevated in RA<sup>21</sup>. COMP, a noncollagenous protein abundantly present in the extracellular matrix of cartilage, and YKL-40 secreted by chondrocytes and synovial fibroblasts are thought to reflect cartilage destruction, but higher levels in RA patients than in controls have not been confirmed. YKL-40 is also secreted by macrophages and leukocytes and may be a marker of inflammation.

Sensitive, specific, and precise immunoassays for serum measurements are commercially available, but the evidence for a contribution in assessing prognosis is conflicting and limited and can only be tested in well characterized longitudinal cohorts. We have previously shown in the same cohort that the presence of the genetic marker PTPN22<sup>22</sup>, high anti-CCP levels, and an algorithm including anti-CCP, IgM RF, ESR and female sex<sup>23</sup> predicted radiographic progression. The aim of our study was to examine whether a panel of serum biomarkers reflecting cartilage and bone degradation could further improve the prediction of longterm radiographic progression in patients with RA.

## MATERIALS AND METHODS

**Patients.** The Norwegian arm of the EURIDISS study (European Research on Incapacitating Disease and Social Support), which started in 1992, consecutively enrolled 238 patients with RA<sup>24</sup>. Patients were followed longitudinally for 10 years and assessed with standard clinical examinations. All patients had RA<sup>25</sup> with relatively short disease duration (maximum 4 yrs, mean 2.3 yrs) at baseline. Procedures for inclusion and patient disposition have been described<sup>24,26</sup>. The regional ethics committee approved the study, and all enrolled patients gave informed consent. The patients were treated according to clinical judgment by their rheumatologist. At baseline, 52% (at 10-yr followup 41%) were using disease modifying antirheumatic drugs (DMARD), 25% (36%) used prednisolone, and 52% (17%) used nonsteroidal antiinflammatory drugs (NSAID). In addition, none (12%) of the patients were treat-

ed with tumor necrosis factor (TNF)-blocking agents. Nineteen percent of the completers used no DMARD or TNF-blocking agents during the entire study.

**Radiographic evaluation.** Baseline anteroposterior radiographs of the hands were available for scoring in 163 patients, 150 (147) patients had radiographs available after 5 (10) years, and the 136 (125) patients with radiographs available both at baseline and at 5 (and/or 10) years were included in these analyses. Radiographs of the feet were not included. The hand radiographs were scored by one experienced reader by the van der Heijde-modified Sharp score (SHS)<sup>27</sup>. Radiographs were scored with known time order, and the reader was blinded for clinical data and study purpose. The potential maximum total score for both hands was 280 [16 areas scored for erosions (score 0–5) and 15 areas for joint space narrowing (score 0–4) in each hand].

**Biomarker analyses.** Serum was collected at baseline and at 5 and 10 years and stored at  $-70^{\circ}\text{C}$ . IgM RF, anti-CCP, CRP, and ESR were analyzed as described<sup>23</sup>. The 6 cartilage and bone biomarkers were measured in serum by commercial ELISA kits according to the manufacturers' recommendations. C2C was measured by a competitive inhibition ELISA (Ibex, Montreal, Quebec, Canada). Reported intra- and interassay coefficients of variation (CV) were 3% and 10%. YKL-40 was measured by a sandwich ELISA (intra- and interassay CV < 7%; Metra YKL-40, Quidel Corp., San Diego, CA, USA). COMP was analyzed by a 2-site ELISA (intra- and interassay CV < 8%; AnaMar Medical AB, Lund, Sweden). CTX-I was measured by ELISA (Crosslaps; Nordic Bioscience, Herlev, Denmark) that used 2 monoclonal antibodies raised against the  $\beta$ -isomerized EKAH  $\beta$  DGGR sequence of the C-telopeptide of  $\alpha$ 1 chains of human type I collagen<sup>28</sup> (intra- and interassay CV < 6% and 9%). RANKL and OPG were measured by capture ELISA with 2 antibodies detecting different epitopes [intra-/interassay CV < 5%/< 9% (RANKL) and < 10%/< 8% (OPG)] (Biomedica Medizinprodukte, Vienna, Austria).

**Statistical analyses.** Statistical analyses were performed using SPSS 14 (SPSS Inc., Chicago, IL, USA). Data are presented by means [standard deviation (SD)] or medians [interquartile range (IQR)] in case of skewed data. Nonparametric tests were used since the data were not normally distributed. Continuous data were compared by Mann-Whitney test and correlations were examined by the Spearman rank-correlation method. Comparisons between proportions were by chi-square test. Associations between the baseline biomarkers (independent variables) and radiographic progression (dependent variable) were examined by univariate and multivariate linear and logistic regression analysis in the whole cohort and in 3 different subgroup analyses: (1) patients with early disease (< 2 yrs,  $n = 72$ ); (2) DMARD-naive patients ( $n = 65$ ); and (3) prednisolone-naive patients ( $n = 101$ ). For the multivariate analysis the independent variables were selected from univariate analyses if  $p < 0.25$ . In the logistic regression analyses a change in SHS of hands from baseline to 5 years of more than 5 units, and from baseline to 10 years of more than 10 units was regarded as radiographic progression. The minimally detectable change of the method based on interobserver reliability is around 4–5 units (hands and feet), although a smaller change is already considered meaningful by clinicians<sup>29</sup>. As we used radiographs only of hands, a progression of more than 5/10 units in 5 and 10 years, respectively, represented real radiographic progression. Analyses with other cutoffs, includ-

ing the median change, were also performed to prove consistency. The biomarkers (independent variables) were treated as continuous variables in the analyses as they did show linearity. However, analyses with dichotomized variables (median and quartiles) were also performed. Log-transformed variables were tested, but not used in the final analyses as they did not improve the model fit. All tests were 2-sided, with the 0.05 significance level.

## RESULTS

*Demographic, clinical, and biologic characteristics of the cohort.* The baseline characteristics of the whole cohort (n = 238) and the 125 patients with complete radiograph sets at 10-year followup are given in Table 1. No important or statistically significant differences were observed between the baseline characteristics of the whole cohort (n = 238, Table 1) and the patients with radiographs available for scoring at baseline and at 5 (n = 136, data not shown) or 10 years (n = 125, Table 1).

*Radiographic outcome over 10 years.* At baseline, 55.2% (n = 69/125) of the patients had erosive disease, and at 10 years 84.4% (n = 105/125). The mean (SD) SHS (hands) at baseline/10 years was 6.8 (11.8)/36.2 (36.6). Radiographic progression (> 10 units) was seen in 59.2% (n = 74) of the patients during the study. The mean (SD) yearly progression rate (SHS hands/disease duration) at baseline and during followup was 3.1 (5.0) and 2.8 (2.9), respectively.

*Levels of serological biomarkers.* Baseline levels of the biomarkers (median) and the variability (IQR) are presented in Table 2.

Anti-CCP-positive patients had higher levels of CTX-I than anti-CCP-negative patients: 0.42 ng/ml (IQR 0.28–0.68) compared to 0.33 ng/ml (IQR 0.23–0.49) (p < 0.05), but such differences were not found for the other biomarkers tested. YKL-40 correlated moderately with ESR and CRP, whereas only weak correlations were observed

Table 1. Baseline characteristics of the 238 patients included in the study and the 125 patients with radiographs available both at baseline and at 10 years.

	All Patients, n = 238	Patients with 10-yr followup Radiographs, n = 125
Female, %	73.5	75.2
Mean (SD) disease duration, yrs	2.3 (1.2)	2.2 (1.2)
Mean (SD) age, yrs	51.9 (13.0)	50.7 (12.7)
Anti-CCP-positive, %	60.5	59.2
IgM RF positive, %	47.9	44.0
Mean (SD) HAQ score	0.9 (0.6)	0.9 (0.6)
Erosive disease, %	55.2*	54.4
Mean (SD) progression rate at baseline, SHS units/yr	2.9 (4.6)*	3.1 (5.0)
DMARD use, %	52.1	52.8
Steroid use, %	25.1	25.5

\* n = 163.

Table 2. Baseline levels of biomarkers in the whole cohort (n = 238) with separate values for patients with progression (n = 74) and no progression (n = 51) at 10 years.

	Baseline, median (IQR)	Progression <sup>†</sup> , median (IQR)	No Progression, median (IQR)
OPG, pmol/l	3.0 (2.2–4.0)	2.9 (2.2–3.8)	2.9 (2.3–4.2)
RANKL, pmol/l	0.03 (0.01–0.11)	0.03 (0.00–0.10)	0.03 (0.00–0.10)
RANKL/OPG	0.01 (0.001–0.04)	0.01 (0.00–0.04)	0.01 (0.00–0.04)
YKL-40, ng/ml	62 (42–94)	66* (39–109)	50 (33–75)
COMP, units	10.0 (8.3–11.8)	9.8 (7.8–12.0)	10.5 (8.2–12.2)
CTX-I, ng/ml	0.38 (0.25–0.62)	0.41** (0.31–0.75)	0.32 (0.21–0.49)
C2C, ng/ml	59 (51–68)	59 (53–70)	59 (50–69)
CRP, mg/l	5 (2–14)	6** (3–16)	3 (1–8)
ESR, mm/h	21 (10–36)	26** (16–45)	13 (7–26)

<sup>†</sup> Change in van der Heijde-modified Sharp score for hands > 1 unit/year.

\* p < 0.05 Mann-Whitney test; \*\* p < 0.01 Mann-Whitney test. OPG: osteoprotegerin, RANKL: receptor activator of nuclear factor-κB, YKL-40: human cartilage glycoprotein-39, COMP: cartilage digomeric matrix protein, CTX-I: collagen cross-linked C-telopeptide, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate.

between the other biomarkers. Patients with a decrease in the biomarker concentration from baseline to 5 years had progression similar to that of the patients with increases in concentrations.

*Associations between serological biomarkers and radiographic progression.* Patients who developed radiographic progression during 10-year followup had significantly (p < 0.05) higher baseline levels of YKL-40 and CTX-I (Table 2). Associations between the baseline biomarker levels and radiographic progression (change in SHS from baseline to 5 and 10 years) were examined by univariate linear regression as shown in Table 3. Baseline CTX-I levels were found to be associated to the change in SHS at 10 years, but not at 5 years (Table 3). OPG, RANKL, OPG/RANKL ratio, COMP, YKL-40, and C2C were not associated to a change in SHS at either 5 or 10 years (Table 3). Univariate logistic regression analyses with radiographic progression as dependent variable were also performed. The levels of ESR and CRP, but of none of the other biomarkers, were significantly (p < 0.05) associated with radiographic progression (Table 3). Confirmatory results were found when the median SHS change was used as a cutoff in the logistic regression. Moreover, the analyses in the 3 subgroup analyses of patients with no DMARD/prednisolone at baseline and in patients with early RA (< 2 years) showed consistent results (data not shown). Analyses of associations between biomarker levels at 5 years and the change in radiographic damage from 5 to 10 years also showed similar results (data not shown). The changes in the levels of the biomarkers from baseline to 5 years were also included as independent variables in the regression analyses, both as continuous variables and dichotomized into positive and negative change, but none of these variables came out as significant predictors of radiographic progression (data not shown). Different



Table 3. Associations between the baseline biomarker level and change in van der Heijde-modified Sharp score from baseline to 5 and 10 years (dependent variable), results from univariate linear and logistic regression analyses.

	Univariate Linear Regression				Univariate Logistic Regression			
	5 Years		10 Years		5 Years		10 Years	
	B (95% CI)	p	B (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
OPG	-0.49 (-1.59 to 0.62)	0.37	-0.50 (-1.52 to 1.41)	0.94	0.99 (0.88 to 1.12)	0.87	1.06 (0.92 to 1.21)	0.42
RANKL	1.1 (-2.2 to 4.3)	0.65	0.51 (-3.85 to 4.88)	0.81	1.48 (0.61 to 3.57)	0.38	1.51 (0.65 to 3.49)	0.41
RANKL/OPG	2.4 (-22.5 to 27.3)	0.80	2.3 (-18.5 to 19.4)	0.84	1.43 (0.54 to 149.1)	0.09	1.43 (0.12 to 99.3)	0.46
COMP	0.33 (-0.82 to 1.50)	0.73	0.54 (-1.30 to 2.38)	0.56	1.08 (0.95 to 1.24)	0.23	0.94 (0.82 to 1.07)	0.36
YKL-40	0.02 (-0.04 to 0.07)	0.68	0.01 (-0.01 to 0.10)	0.77	1.00 (0.99 to 1.01)	0.51	1.00 (0.99 to 1.01)	0.16
CTX-I	5.4 (-1.9 to 12.6)	0.14	20.2 (7.4 to 33.0)	0.05	1.97 (0.72 to 5.36)	0.19	2.76 (0.83 to 9.14)	0.10
C2C	-0.09 (-0.35 to 0.17)	0.31	-0.16 (-0.58 to 0.26)	0.46	0.99 (0.96 to 1.02)	0.47	0.99 (0.97 to 1.02)	0.67
CRP	0.51 (0.26 to 0.75)	< 0.01	0.73 (0.30 to 1.15)	< 0.01	1.06 (1.01 to 1.10)	0.01	1.09 (1.03 to 1.12)	< 0.01
ESR	0.29 (0.15 to 0.43)	< 0.01	0.12 (0.08 to 0.57)	< 0.01	1.03 (1.01 to 1.05)	< 0.01	1.05 (1.02 to 1.08)	< 0.01

ratios and combinations of the markers were tested, with no change in the results.

*Independent predictors of radiographic progression from multivariate analyses.* Variables with p values lower than 0.25 from univariate analyses were included in multiple linear regression models in which the change in SHS at 5 or 10 years was the dependent variable.

CRP, anti-CCP, IgM RF, and progression rate at baseline were independently associated with a change in SHS over both 5 and 10 years. Baseline CTX-I levels were independently associated with a change in SHS over 10 years ( $\beta = 16.4$ , 95% CI 5.7–27.1). The percentage of the change in SHS explained by the model (the  $R^2$ ), increased from 0.30 to 0.35 with CTX-I included in the model. The beta results in the linear regression can be interpreted as the average increase in SHS change that corresponds to one unit change in the baseline variable, i.e., an increase in baseline CTX-I of 0.1 ng/ml (0.1 units) will on average increase the 10-year change in SHS of hands with 1.64 units.

In the multivariate logistic regression analysis, anti-CCP, IgM RF, ESR, and female sex, but none of the cartilage or bone biomarkers, were independent predictors of radiographic progression (data not shown). The biomarkers entered into the linear and logistic regression analysis were also dichotomized into high versus low level according to the median and quartile values since “normal values” were not available. By this approach, CTX-I levels also showed an association to 10-year change (data not shown) in the logistic regression analysis. None of the other cartilage and bone biomarkers were significantly associated with radiographic progression. Consistent results were found when the median was used as a cutoff level for progression in the logistic regression, and when the biomarkers were log-transformed (data not shown). Subgroup analyses in patients with no DMARD/prednisolone at baseline and in patients with early RA (< 2 years) also showed consistent results (data not shown). Looking at radiographic progression from 5 to 10 years, none of the biomarkers predicted radiographic pro-

gression significantly (data not shown). In addition to the total SHS, separate analyses with changes in the joint space narrowing score and erosion score as endpoints were also performed. Results from the total score as dependent variable are presented, since results were similar for the joint space narrowing and erosion scores.

## DISCUSSION

Identification of patients at high risk of radiographic progression is a challenge in daily clinical practice as the currently used predictors have low sensitivity in both early and more advanced disease. We studied whether a panel of biological markers could predict longterm joint destruction in a well described prospective cohort of patients with RA. Our results showed no association between serum levels of RANKL, OPG, C2C, YKL-40, or COMP and subsequent radiographic progression over 5 and 10 years. Baseline CTX-I levels were higher in progressors than in non-progressors, and were independently associated with 10-year change in radiographic damage score. However, an increase in baseline CTX-I of 0.1 ng/ml on average increased the change in SHS of hands by only 1.64 units over 10 years, and adding CTX-I only increased the  $R^2$  of the model by 5%. Further, the association between CTX-I levels at baseline and radiographic progression was not evident for the 5-year change. Since the clinician needs to identify patients at risk of progression rather than to predict the absolute rate of progression, logistic regression analyses were also performed. These analyses did not confirm the association between CTX-I and radiographic progression. Moreover, a whole set of sensitivity analyses with a different cutoff level for radiographic progression, joint space narrowing, and erosions separately in various subgroups of patients (early disease only, DMARD-naive, prednisolone-naive), with combinations of biomarkers confirmed the results.

Although all the biomarkers tested in our study have previously been suggested to be predictors of radiographic progression, the literature is limited and shows conflicting

results. A Swedish prospective study showed that baseline COMP levels were an independent predictor of 5-year but not 10-year radiographic progression<sup>7</sup>. These patients had higher disease activity and shorter disease duration, but the COMP levels and progression rate were comparable to our study. A recent British study on patients with early RA found no association between COMP levels and 2-year radiographic progression<sup>30</sup>. Some smaller studies show conflicting results<sup>8,9,31,32</sup>.

Johansen, *et al* found that persistently elevated YKL-40 levels were associated with radiographic progression<sup>10,12</sup>. However, in agreement with our findings, no association was found by Peltomaa, *et al*<sup>11</sup> or Combe, *et al*<sup>33</sup>.

An association between the RANKL/OPG ratio and 5-year radiographic progression was found by Geusens, *et al* in 92 patients from the COBRA trial<sup>5</sup>. The discrepancy with our study might be related to differences in patient characteristics, but the median yearly progression rate was comparable to our cohort.

An association between collagen type II degradation products (CTX-II) in urine and radiographic progression has been suggested<sup>4,19,20</sup>. As we had not stored urine, we used a new C2C kit that enables serum measurements of type II collagen degradation. Verstappen, *et al* found a weak, but statistically significant association between this serum marker and radiographic progression in a cohort of 87 patients with RA followed for 3 years<sup>6</sup>. The patients had RA of very recent onset and their C2C levels were higher than in our study<sup>6</sup>.

Our study indicates, although not very robustly, an association between serum CTX-I levels and radiographic progression. In the COBRA patients, Garnero, *et al* showed an association between urine levels of CTX-I and radiographic progression<sup>4</sup>. To our knowledge this is the first study to show a relationship between serum CTX-I levels and radiographic progression, as Jansen, *et al*<sup>34</sup> and Visvanathan, *et al*<sup>35</sup> in the ASPIRE study did not find such an association. A recent study has found that short-term changes in collagen type II degradation products after initiation of biologic treatment are predictive of longterm radiographic progression and thus might indicate treatment response<sup>36</sup>. Our study design did not allow conclusions regarding biomarkers and treatment response.

Conflicting results in longitudinal studies might be due to differences in study design and patient population. In biomarker studies, results across studies are difficult to compare due to analytical and pre- and post-analytical differences. There is, for example, no international standardization or normal values of the different biomarker measurements. In addition, some of the biomarkers show significant diurnal variation, such as CTX-I<sup>37,38</sup> and OPG<sup>39</sup>. None of the biomarkers we tested are specific for RA, and serum levels can be affected by comorbidity. As collagen type I degradation also is important in osteoporosis, which is prevalent

in patients with RA, we adjusted for bone mineral density in the analyses, with no alterations of the results. As all analyses should be done simultaneously, samples in all epidemiological studies and clinical trials are frozen for different lengths of time. This might lead to alterations in measured plasma protein concentrations, as indicated for RANKL<sup>40</sup>. However, such alterations were not found in another study of RANKL stability, or for YKL-40 or CTX-I<sup>41-43</sup>. This problem makes comparisons of levels across studies difficult.

Our study has limitations. Patients were treated according to clinical judgment before inclusion and during the study. Fifty-two percent of the patients received DMARD at baseline. It can be questioned whether treatment might be a confounding factor in the relationship between the biomarkers and radiographic progression. Subgroup analyses in patients with no DMARD and prednisolone at baseline, however, showed consistent results. Moreover, if a biomarker were to monitor treatment efficacy, there should still be a relationship in patients when on treatment, but showing radiographic progression. A cohort study like this also suffers from confounding by indication: patients with active, severe disease will be treated more intensively by their doctors. As a consequence, radiographic progression may become a determinant of DMARD therapy rather than of baseline prognostic markers. So there is a possibility that the biomarkers measured may have some prognostic capacity, but that this effect was disguised by differences in treatment over time. The patients in our study were recruited before the era with a focus on early aggressive treatment, and 19% of the patients in this cohort did not receive any DMARD or TNF-blocking agents during the study period. Those patients had significantly lower CRP, ESR, and anti-CCP levels at baseline and a lower Health Assessment Questionnaire score. The modified Sharp Score at baseline and the progression rate during the 10 years were also lower, despite the lack of DMARD treatment.

Radiographs of feet were not available at baseline and could not be used in the present analyses. Missing cases are always a challenge in longterm observational studies; in our study the attrition rate among living patients was 27%<sup>26</sup>. We cannot rule out the possibility that loss of patients might have affected the results, although the baseline characteristics of the patients with complete radiograph sets ( $n = 125/136$ ) were similar to those of the whole cohort ( $n = 238$ ; Table 1).

Serum samples were collected at the same time during the day, but not fasting. We cannot exclude that this might have influenced the CTX-I values and the strength of the association<sup>37,38</sup>. It could be hypothesized that mean values of the biomarkers during treatment could be more closely related to progression in joint destruction than a single baseline measurement. However, analyses of both mean values and 5-year levels and associations with subsequent progression also showed similar results.

The enrollment to this study started in 1992. The cohort was at that time considered to have “early RA,” which no longer holds true according to the current understanding of early disease<sup>44</sup>. The potential usefulness of a prognostic marker should, however, not be considered only for patients with early, untreated RA.

Even with increasing clinical needs of patient selection to different treatment regimens, the methods of assessing joint damage and prognosis in RA have remained largely unchanged over the years. Biological markers reflecting the bone and cartilage turnover rate are thus tempting as new tools, and there is increasing scientific and commercial interest in this field. The OMERACT initiative (Outcome Measures in Rheumatology) has developed validation criteria for biomarkers<sup>45</sup> and has started a process to review the biomarker literature. We believe that this 10-year observational study, where a panel of biomarkers was tested in the same cohort that previously revealed information on prognostic associations between genetic markers, autoantibodies, and radiographic progression<sup>22,23</sup>, adds important information to this validation.

This longterm followup study of patients with RA indicates a relationship between elevated CTX-I levels in serum and subsequent joint destruction. The strength of this association, however, was weaker than for current predictors such as anti-CCP, radiographic progression in early disease, and inflammation markers. Although we cannot exclude such associations, we did not observe any relationship between the other biomarkers measured and radiographic progression. Our study thus does not support that serum measurement of CTX-I, RANKL, OPG, C2C, YKL-40, or COMP will further improve the prediction of longterm radiographic progression in RA.

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