Uncoupling of Collagen II Metabolism in Newly Diagnosed, Untreated Rheumatoid Arthritis Is Linked to Inflammation and Antibodies Against Cyclic Citrullinated Peptides

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ABSTRACT. ***Objective.*** To investigate the relationship between markers of collagen II synthesis and degradation with disease activity measures, autoantibodies, and radiographic outcomes in a 4-year protocol on patients with early rheumatoid arthritis (RA) who are naïve to disease-modifying antirheumatic drugs.

Methods. One hundred sixty patients with newly diagnosed, untreated RA entered the Cyclosporine, Methotrexate, Steroid in RA (CIMESTRA) trial. Disease activity and radiograph status were measured at baseline and 4 years. The N-terminal propeptide of collagen IIA (PIIANP) and the cross-linked C-telopeptide of collagen II (CTX-II) were quantified at baseline by ELISA. PIIANP was also assayed at 2 and 4 years. Anticyclic citrullinated peptide (anti-CCP) was recorded at baseline. An uncoupling index for cartilage collagen metabolism was calculated from PIIANP and CTX-II measurements.

Results. PIIANP was low at diagnosis and 4 years on ($p < 0.001$), irrespective of treatment and disease activity. PIIANP was lowest in anti-CCP positive patients ($p = 0.006$), and there was a negative correlation between PIIANP and anti-CCP titers ($\rho = -0.25$, p 0.002). CTX-II was increased ($p < 0.001$) and correlated positively with disease activity and radiographic progression, but not with anti-CCP ($p = 0.93$). The uncoupling index was not superior to CTX-II in predicting radiographic changes.

Conclusion. These results suggest that cartilage collagen formation and degradation are unbalanced when RA is diagnosed. The different associations of collagen II anabolism (PIIANP) and collagen II degradation (CTX-II) with anti-CCP, synovitis, and radiographic progression indicate that at this early stage of RA, cartilage collagen degradation is mainly driven by synovitis, while anti-CCP antibodies may interfere with cartilage regeneration by inhibiting collagen IIA formation. Trial registration j.nr NCT00209859. (First Release May 1 2010; J Rheumatol 2010;37:1113–20; doi:10.3899/jrheum.091265).

Key Indexing Terms:
RHEUMATOID ARTHRITIS   COLLAGEN   CARTILAGE   AUTOANTIBODIES   SYNOVITIS

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Rheumatoid arthritis (RA) is a chronic inflammatory disorder that leads to irreversible joint deformities, extraarticular manifestations, and death if not sufficiently treated[1]. Inflammation and cartilage degradation are key features of the disease. The disease outcome is highly variable and therefore identification of patients at particular risk of an aggressive course is important in order to prevent longterm physical disability[2]. Disease activity scores, sensitive imaging techniques, and laboratory measures such as C-reactive protein (CRP), IgM rheumatoid factor, anticyclic citrullinated peptide (anti-CCP), and shared epitopes (SE) are well-established prognostic factors[3,4]. However, in an era of evolving possibilities for targeted therapies, there is a need to develop seromarkers that reflect specific aspects of joint pathology, e.g., cartilage, bone, and soft tissue metabolism[5].

Collagen type II is the major structural protein in cartilage. There is evidence that soluble fragments of collagen II released into the systemic circulation as a result of specific enzymic cleavage are useful in the assessment of cartilage synthesis and breakdown in RA and osteoarthritis (OA)[6]. High levels of the cross-linked C-telopeptide of collagen type II (CTX-II) in urine have been linked to rapid and progressive joint destruction in RA[7-9]. The C- and N-propeptides of collagen II are believed to reflect the rate of collagen II formation because they are cleaved from the parent procollagen in a stoichiometric manner[10]. As a result of alternative splicing there are 2 forms of procollagen II[11]. Type IIA procollagen is synthesized by precartilage and noncartilaginous epithelial and mesenchymal cells and contains an additional cystein-rich domain of 69 amino acids in the N-terminus[12-15]. As cartilage matures, procollagen IIA expression decreases in favor of procollagen IIB[12,16]. Type IIA procollagen is reexpressed in damaged cartilage, e.g., in OA[17]. Since type IIA procollagen seems to be expressed in diseased cartilage in particular, the N-terminal propeptide of collagen IIA (PIIANP) is suggested to reflect a chondroprogenetic and anabolic capacity by chondrocytes[17]. An uncoupling index calculated from PIIANP and CTX-II z-scores has recently been proposed as predictor of radiographic progression in knee OA[18]. We and others have reported that PIIANP in serum is decreased in longstanding RA compared to healthy subjects[19,20]. In addition, in a 1-year prospective study on patients with newly diagnosed RA, PIIANP decreased temporally despite excellent synovitis control[19]. The mechanism underlying subnormal serum PIIANP in RA is unknown. It is well-documented, however, that anti-CCP seropositivity implies an increased risk of future joint destruction, and evidence from murine arthritis suggests that anti-CCP antibodies may have proinflammatory properties[21].

Our aims, in a prospective 4-year protocol, were to study the relationship of PIIANP with autoantibodies, disease activity measures, and radiographic outcome; and to assess the feasibility of an uncoupling index calculated from baseline z-scores of PIIANP and CTX-II to predict radiographic progression at 4 years[18].

MATERIALS AND METHODS

One hundred sixty patients with newly diagnosed and untreated RA were included in the Cyclosporine, Methotrexate, Steroid in RA (CIMESTRA) trial, as reported[22,23]. Patients fulfilled the American College of Rheumatology 1987 revised criteria for RA[24]. Further inclusion criteria were disease duration < 6 months, ≥ 2 swollen joints at baseline, and age 18-75 years[22,23]. Health Assessment Questionnaire (HAQ score, 0-3)[25], visual analog scale (VAS pain, global, and doctor, 0–10), and Disease Activity Score in 28 joints (DAS28)[26] were calculated. Radiographs of hands, wrists, and forefeet were obtained in 155 patients at baseline and in 137 after 4 years, but only 133 patients had radiographs available at both baseline and at 4 years. Total Sharp/van der Heijde score (TSS), joint space narrowing (JSN), and erosion score (ES) were recorded by an independent senior radiologist, who was blinded to treatment group assignment[27]. Also, the estimated annual progression rate before diagnosis was calculated based on disease duration and baseline TSS for each patient as described by Bathon, *et al*[22,23,28].

The trial was approved by the local ethics committee (j. nr M1959-98) and fulfilled the Declaration of Helsinki and the International Conference on Harmonisation 1996 Revised Guidelines for Good Clinical Practice (j.nr NCT00209859)[22,23].

One hundred twenty blood donors aged 20-65 years, stratified according to age groups (decades) and sex served as healthy controls for PIIANP measurements. There was an equal number of men and women. The PIIANP controls were younger (p = 0.001) and included fewer women than the RA population (p = 0.003). Six hundred thirty-six healthy volunteers aged 20-70 years served as controls for CTX-II measurements[6]. This group consisted of 427 women (67%) and 209 men (33%). The CTX-II control population did not differ from the RA population with respect to sex distribution and age (data not shown). CTX-II and PIIANP were measured at baseline, and in addition PIIANP was studied after 2 and 4 years.

Patients were treated with methotrexate (MTX) and cyclosporine or MTX and placebo-cyclosporine, respectively. In addition, swollen joints were injected with betamethasone (IAB)[22]. During the second year, hydroxychloroquine was added and cyclosporine/placebo was tapered to 0, while MTX and IAB were continued, aiming at maximal synovitis suppression.

Laboratory measures. Serum was obtained from routinely drawn nonfasting blood samples collected between 8 AM and 2 PM. Samples were allowed to clot at room temperature, followed by centrifugation at 3000 g for 10 min. Sera were stored at –80˚C. Spot urine samples were collected as the 2nd, nonfasting urine void in the morning and kept frozen at –20˚C until analyzed.

Serum PIIANP was measured by a competitive ELISA (Millipore/ LINCO Research, Billerica, MA, USA) as described[29]. Interassay coefficients of variation were 17.1% and 10.8% for low (64-134 µg/l) and high (371-771 µg/l) concentration controls, respectively. Intraassay coefficients of variation were below 5%. All analyses were done in duplicate using kits with the same lot number and serial samples from the same patient were analyzed simultaneously.

Urinary collagen type II C-telopeptide (CartiLaps) was analyzed at baseline using a commercial ELISA (Nordic Bioscience Diagnostica A/S, Herlev, Denmark) as described[6,30]. Intraassay and interassay variation were 7.6% and 8.3%.

C-reactive protein (CRP, mg/l) was assayed at each visit by standard methods. IgM-rheumatoid factor (IgM-RF, cutoff level: 16 IU/ml) and anti-

cyclic citrullinated peptide antibodies (CCP; cutoff level: 24 U/ml; Euro Diagnostica AB, Malmö, Sweden) were measured at baseline by ELISA as described[31-33].

EDTA-stabilized blood was used as a source of genomic DNA. HLA-DRB1 genotyping for SE was performed by polymerase chain reaction-based sequence-specific oligonucleotide probing, as described[34,35]. Here, we define the SE as the presence of HLA-DRB1*04 and/or HLADRB1*01, and/or HLADRB1*10.

Statistical analysis. All analyses were performed in STATA/9.2 (StataCorp., College Station, Texas, USA). Nonparametric tests were used because data were not normally distributed. The prospective analysis of PIIANP was done using a linear regression model with adjustment for time, sex, and age at baseline. At baseline, year 2 and 4 comparison between patients and controls was done by linear regression models with adjustment for sex and age using PIIANP or CTX-II as the dependent variable. PIIANP and CTX-II were logarithmically transformed to approximate normal distribution in the linear regression analysis.

Z-scores on PIIANP and CTX-II were calculated from logarithmically transformed data using the mean and SD from controls. The z-score expresses the number of SD that patients with RA differ from control mean value[36]. An uncoupling index based on markers of collagen synthesis and degradation (z-score CTX-II minus z-score PIIANP) was calculated to estimate net cartilage metabolism[18,36].

Logistic regression was used to assess the risk of radiographic progression per SD according to baseline levels of these molecular markers. Progression was defined as the smallest detectable difference from baseline. The analyses were done with adjustment for sex, age, anti-CCP status, DAS28, and radiographic status at baseline.

Since PIIANP and CTX-II did not differ between the 2 treatment arms at any time, data from all patients were pooled. Analysis was by intention-to-treat (n = 142). Completers' analysis was also performed and gave similar results (data not shown). All values are presented as median (95% CI) if not otherwise stated. P values < 0.05 were considered as statistically significant except in case of multiple comparisons, where only p values ≤ 0.01 were accepted.

RESULTS

Patients and controls. Sixty-one of the 160 patients (38%) failed to complete 4 years of followup. The reasons for discontinuation were adverse events[11], lack of efficacy[10], patient request[13], and other[27]. Fifty-six (35%) dropped out during the first 2 years. Patients who dropped out did not differ from completers with regard to clinical variables at baseline (data not shown).

In calculations including radiographic data, there were no baseline differences regarding clinical measures between patients who were excluded because of missing radiographs at 4 years and those with radiographs available (data not shown).

Baseline characteristics of the population with RA have been presented[22]. At 4 years followup, 104/134 (78%), 88/134 (66%), and 92/134 (69%) achieved ACR50, ACR70, and DAS28 < 2.6, respectively. Among patients with radiographs available at baseline and after 4 years (n = 133), 53%, 23%, and 49% progressed according to TSS, JSN, and ES score, respectively. Radiographic progression at the 4-year followup was small in terms of Sharp/van der Heijde units [median (interquartile range): TSS 2 (0-7) to 5 (0-11), JSN 0 (0-2) to 0 (0-4), and ES 2 (0-5) to 3 (0-8)].

PIIANP at baseline and followup. PIIANP in controls ranged from 284-2356 µg/l (median 895 µg/l) and was slightly higher in healthy men than in women [941 (883;1021) µg/l vs 870 (797;930) µg/l, p = 0.03]. There was no difference between age groups (p = 0.81). In patients with RA, there was no difference in PIIANP between sex and age groups (p = 0.1 and p = 0.55, respectively). At baseline and after 2 and 4 years, PIIANP was significantly lower than in controls even when adjusted for age group and sex [baseline: 740 µg/l (658;806), 2 years: 720 µg/l (657;798) and 4 years: 740 µg/l (676;809) vs controls 895 µg/l (870;950), p < 0.001 at each time point]. Baseline PIIANP adjusted for age and sex did not differ significantly from PIIANP at 2 years (p = 0.24) and 4 years (p = 0.76).

PIIANP association with clinical and laboratory variables. Subjects who were anti-CCP positive had significantly lower PIIANP than those who were anti-CCP negative at baseline [663 µg/l (586;758) vs 818 µg/l (708;912)], p = 0.006 (Figure 1A). In addition, PIIANP was inversely correlated with anti-CCP titer (ρ = –0.25, p = 0.002). The same trend was observed with respect to IgM-RF [688 µg/l (610;775) vs 810 µg/l (695;897), p = 0.06]. The association between PIIANP and clinical variables at baseline is outlined in Table 1. PIIANP was not associated with any of the clinical variables at any time (data not shown). Forty-eight patients were current smokers and SE carriers. This subset did not differ with respect to PIIANP when compared to nonsmoking SE carriers (n = 67; p = 0.73). Likewise, PIIANP did not differ between smoking (n = 8) and nonsmoking (n = 32) SE noncarriers (p = 0.91).

CTX-II association with clinical and laboratory variables. CTX-II ranged from 0.02-1.59 µg/mmol creatinine (median 0.14 µg/mmol) in the control population. CTX-II exhibited significant differences between healthy age groups (p < 0.001) with the highest levels occurring in patients aged 20-29 years. There was no sex difference (p = 0.46).

Patients with RA had significantly higher CTX-II excretion than control subjects [0.30 µg/mmol creatinine (0.25;0.33) vs 0.14 µg/mmol creatinine (0.13;0.15), p < 0.001)]. This difference remained even after adjustment for sex and age. The association between CTX-II and clinical and demographic data in the population with RA is shown in Table 1. In contrast to PIIANP, CTX-II did not differ between anti-CCP positive versus anti-CCP negative subjects (Figure 1B). There was no correlation between CTX-II and PIIANP (ρ = 0.06, p = 0.44).

Z-scores, uncoupling index, and radiographic progression. CTX-II and PIIANP z-scores were mean (SD) 1.29 (1.05) and –0.62 (1.23), respectively, yielding an uncoupling index at 1.89 (1.58). The difference (i.e., number of SD) from 0 (the mean of controls) was assessed by unpaired t-test and showed that the PIIANP z-score was significantly lower than in controls (p < 0.001), and the CTX-II z-score and the uncoupling index were higher than in controls (p < 0.001 and p < 0.001, respectively).

A.

PIIANP (ng/ml)
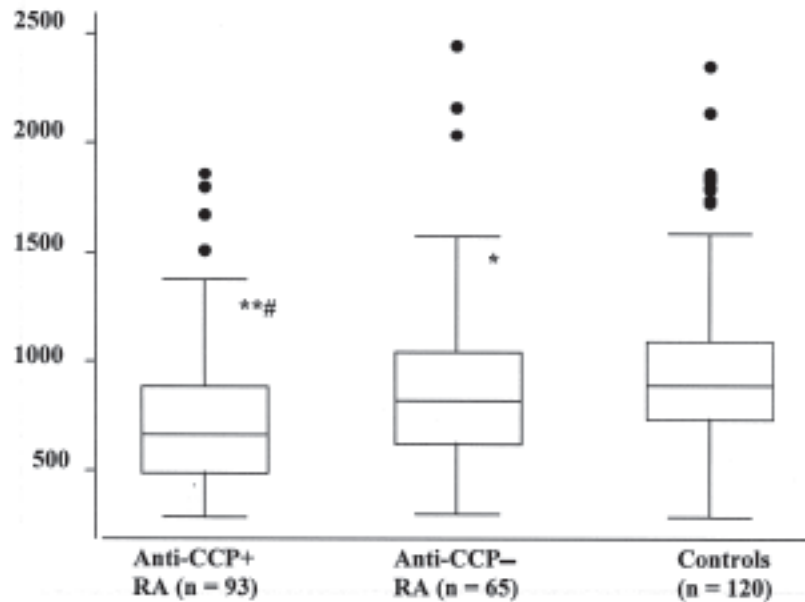
B.

CTX-II (ug/mmol

Figure 1. A. PIIANP in serum according to anti-CCP status in early, untreated rheumatoid arthritis. * p = 0.049 versus controls adjusted for sex and age. ** p < 0.001 versus controls adjusted for sex and age. # p = 0.006 versus anti-CCP negative individuals. PIIANP: N-terminal propeptide of collagen IIA; CCP: cyclic citrullinated peptide. B. Urinary CTX-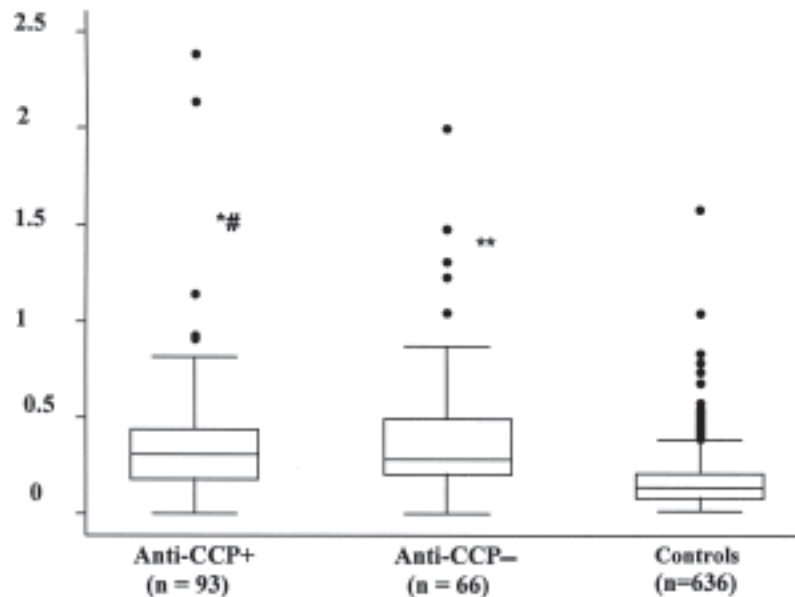II according to anti-CCP status in early, untreated rheumatoid arthritis. * p < 0.001 versus controls adjusted for sex and age. ** p < 0.001 versus controls adjusted for sex and age. # p = 0.93 versus individuals who are anti-CCP negative. CTX-II: cross-linked C-telopeptide of collagen II; CCP: cyclic citrullinated peptide.

Table 1. The associations among baseline PIIANP, CTX-II, the uncoupling index, and clinical and demographic variables. Values are median (95% CI) unless otherwise indicated.

Variables	PIIANP (μg/l)	CTX-II (μg/mmol creatinine)	Uncoupling Index
Sex (women vs men)	692 (615; 777) vs 818 (684; 887), p = 0.13	0.31 (0.26; 0.37) vs 0.26 (0.23; 0.31), p = 0.16	2.14 (1.75; 2.46) vs 1.29 (0.90; 1.58), p = 0.01
Age	rho = 0.09, p = 0.27	rho = 0.18, p = 0.02	rho = 0.09, p = 0.92
Current smokers vs nonsmokers	709 (623; 820) vs 743 (639; 830), p = 0.52	0.32 (0.26; 0.36) vs 0.28 (0.24; 0.32), p = 0.34	1.8 (1.54; 2.77) vs 1.72 (1.35; 2.34), p = 0.40
IgM-RF positive vs negative	688 (610; 775) vs 810 (695; 897), p = 0.06	0.31 (0.26; 0.35) vs 0.27 (0.23; 0.34), p = 0.83	2.1 (1.58; 2.47) vs 1.3 (0.97; 1.97), p = 0.04
Anti-CCP positive vs negative	663 (586; 758) vs 818 (708; 912), p = 0.006	0.31 (0.26; 0.36) vs 0.29 (0.24; 0.33), p = 0.93	2.1 (1.6; 2.6) vs 1.29 (1.04; 1.86), p = 0.01
Any SE vs no SE	739 (618; 803) vs 751 (653; 977), p = 0.11	0.3 (0.25; 0.34) vs 0.29 (0.23; 0.38), p = 0.79	2.03 (1.55; 2.48) vs 1.40 (0.88; 2.02), p = 0.07
No. of swollen joints (0–28)	ρ = 0.09, p = 0.26	ρ = 0.17, p = 0.03	ρ = 0.04, p = 0.61
No. of tender joints (0–28)	ρ = 0.06, p = 0.48	ρ = 0.07, p = 0.37	ρ = 0.0008, p = 0.99
CRP (mg/l)	ρ = 0.0003, p = 1.00	ρ = 0.32, p < 0.001	ρ = 0.24, p = 0.003
DAS28	ρ = 0.02, p = 0.81	ρ = 0.22, p = 0.005	ρ = 0.15, p = 0.06
VAS pain (0–10)	ρ = 0.02, p = 0.81	ρ = 0.12, p = 0.12	ρ = 0.09, p = 0.28
VAS global (0–10)	ρ = –0.05, p = 0.55	ρ = 0.10, p = 0.22	ρ = 0.15, p = 0.07
VAS doctor (0–10)	ρ = 0.03, p = 0.74	ρ = 0.19, p = 0.02	ρ = 0.12, p = 0.13
HAQ score (0–3)	ρ = –0.04, p = 0.60	ρ = 0.17, p = 0.03	ρ = 0.17, p = 0.04
TSS*	ρ = 0.09, p = 0.28	ρ = 0.36, p < 0.001	ρ = 0.16, p = 0.05
JSN*	ρ = 0.09, p = 0.27	ρ = 0.17, p = 0.04	ρ = 0.05, p = 0.56
ES*	ρ = 0.06, p = 0.45	ρ = 0.34, p < 0.001	ρ = 0.15, p = 0.06

* According to Sharp/van der Heijde. PIIANP: N-terminal propeptide of collagen IIA; CTX-II: cross-linked C-telopeptide of collagen II; RF: rheumatoid factor; CCP: cyclic citrullinated peptide; SE: shared epitopes; CRP: C-reactive protein; DAS28: Disease Activity Score (28-joint count); VAS: visual analog scale; HAQ: Health Assessment Questionnaire; TSS: total Sharp/van der Heijde score; JSN: joint space narrowing; ES: erosion score.

The uncoupling index was higher in anti-CCP positive and IgM-RF positive individuals as compared with seronegative subjects, and was higher in women than in men (Table 1). There was a positive correlation between baseline CTX-II and the estimated radiographic progression before diagnosis, including TSS (ρ = 0.35, p < 0.001), ES (ρ = 0.33, p < 0.001), and JSN (ρ = 0.16, p = 0.052). In addition, a weak correlation was recorded between estimated TSS progression and uncoupling index (ρ = 0.16, p = 0.05). Such correlations were not observed for PIIANP (data not shown).

By stratifying according to baseline PIIANP and CTX-II into high (above median) and low (below or equal to median), the high CTX-II subset comprised significantly more erosive progressors than the low CTX-II subset (40/65 vs 25/68, p = 0.006). The same trend was observed among JSN (20/65 vs 11/68, p = 0.064) and TSS progressors (40/65 vs 30/68, p = 0.056). No difference with respect to radiographic progression could be detected when comparing the PIIANP high and low group (data not shown).

CTX-II z-score predicted JSN progression and erosive progression at 4 years (Table 2). Neither the PIIANP z-score nor the uncoupling index could predict JSN or erosive progression (Table 2).

DISCUSSION

We demonstrate a possible link between anti-CCP autoantibodies and cartilage collagen metabolism. Thus, while collagen degradation reflected by CTX-II was increased and correlated positively with disease activity, collagen II anabolism assessed by PIIANP was decreased at baseline and 4 years on, irrespective of treatment and unrelated to disease activity, particularly in the anti-CCP positive subset. Further, there was a negative correlation between PIIANP and anti-CCP values at baseline. No correlations were recorded between these cartilage marker molecules, SE, or smoking.

Our findings of persistent low levels of the anabolic cartilage collagen marker PIIANP for up to 4 years without any correlation with conventional disease activity markers suggest that cartilage collagen regeneration is compromised at a very early stage of the disease, and that this antianabolic state is not primarily driven by local or systemic inflammation. Conversely, in accordance with other reports, CTX-II was significantly increased in our patients with RA at baseline[6] and CTX-II was positively correlated with disease activity measures, suggesting that cartilage collagen degradation is coupled with synovitis activity.

While there is ample evidence for an association between baseline CTX-II and longterm erosive progression in early RA[7,9], the possible correlation between PIIANP and late structural joint damage has not been studied in detail. The association between low PIIANP and anti-CCP status is of

Table 2. The predictive value of baseline PIIANP z-score, CTX-II z-score, and the uncoupling index on radiographic progression at 4 years. OR for radiographic progression were calculated using logistic regression with adjustment for sex, age, anti-CCP status, DAS28, and radiographic status at baseline. Progressors were defined as the smallest detectable difference after 4 years compared to baseline. Values are OR (95% CI).

Variable	PIIANP z-score	CTX-II z-score	Uncoupling Index
OR TSS progression	0.92 (0.62; 1.27)	1.52 (0.93; 2.49)	1.18 (0.91; 1.53)
OR JSN progression	1.05 (0.73; 1.50)	1.95 (1.13; 3.36)	1.25 (0.89; 1.77)
OR ES progression	0.95 (0.69; 1.30)	1.81 (1.14; 2.89)	1.24 (0.97; 1.60)

PIIANP: N-terminal propeptide of collagen IIA; CTX-II: cross-linked C-telopeptide of collagen II; TSS: total Sharp/van der Heijde score; JSN: joint space narrowing; ES: erosion score.

particular note, because antibodies against citrullinated proteins have recently been implicated in the pathogenesis of murine collagen-induced arthritis. Thus, in arthritis induced by citrullinated collagen II, Lundberg, *et al*, found a positive correlation between the amount of citrullinated proteins and the severity of inflammation in affected joints[37]. Kuhn, *et al* reported that murine monoclonal antibodies directed toward citrullinated fibrinogen enhanced arthritis in mice with collagen-induced arthritis, and that mice made tolerant for citrullin-containing peptide developed milder arthritis when challenged with collagen II[21]. The negative correlation between anti-CCP titer and cartilage anabolism observed in our study indicates that anti-CCP antibodies may be involved in human RA pathogenesis, e.g., by local immune-complex formation in the synovium leading to chondrocyte suppression[21,38]. The potential role of humoral immunity in the chain of events leading to arthritis and cartilage depletion is further emphasized by the demonstration of arthritogenic autoantibodies directed against collagen II and glucose-6-phosphate isomerase in different mouse strains[39-41].

The notion of a dual mechanism behind cartilage depletion in RA associated with inflammation and anti-CCP, respectively, is intriguing from a clinical and an interventional point of view. Evidence from animal experiments and human RA supports that synovitis and joint destruction are at least partially dissociated and independent processes[21,42,43]. Cartilage depletion may occur as a result of increased degradation or reduced rate of biosynthesis[44]. Differential linking of these processes with inflammation and autoantibodies may account for the dissociation between inflammation and erosive progression observed in some patients with RA[43]. The well-established correlation between cumulated CRP response and longterm radiographic progression and the similarly remarkable correlation between anti-CCP autoantibodies and joint destruction support that inflammation and humoral autoimmunity may both contribute to cartilage damage in human RA and that these processes are partially independent as also reflected by the lack of a correlation between CTX-II and PIIANP in our study. The possible significance of anti-CCP antibodies is emphasized by the higher uncoupling index in anti-CCP positive versus anti-CCP negative patients. The well-documented antierosive and synovitis suppressive effects by tumor necrosis factor-α and B-cell inhibitors accord with the basic thesis that cartilage metabolism in early RA is affected by different pathways, including systemic inflammation and humoral autoimmunity[45,46].

An uncoupling index based on z-scores of PIIANP and CTX-II has recently been proposed as a useful tool to identify patients with OA at risk of radiographic progression[18]. In our study population of untreated, newly diagnosed patients with RA, this composite measure of cartilage metabolism was not superior to CTX-II alone in predicting progression. This discordance between OA and RA probably reflects that the synovitis-driven type II collagenolysis in RA is more aggressive than in OA. Thus, CTX-II was associated with cartilage depletion as assessed by the estimated as well as the observed radiographic progression. Accordingly, although decreased PIIANP may reflect a disease pathway in human RA pathogenesis, its potential as a biomarker for clinical use awaits future studies using more sensitive imaging techniques than conventional radiographs.

When interpreting these results, certain issues should be considered. First, this is a 4-year prospective study on a well-characterized RA population with symptom duration < 6 months and recruited before initiation of treatment with disease-modifying antirheumatic drugs. This implies that confounding factors related to different disease duration and drug effects on cartilage collagen metabolism at baseline were avoided. An effect by DMARD on subsequent PIIANP analyses cannot be excluded. But PIIANP did not differ between treatment arms and was not correlated with DAS28 score or CRP in the entire study population. Comparisons between patients and controls were adjusted for age and sex. It is well known that the prevalence of radiographic OA increases with advancing age. This would tend to diminish the differences between patients with RA and controls since PIIANP and CTX-II exhibit changes that are qualitatively similar to those reported in our patients with RA[18]. Well-established disease modifiers such as smoking and SE[3] did not influence PIIANP or CTX-II. Finally, about 40% of the patients dropped out during the 4-year followup period, but completers did not differ from noncompleters regarding clinical measurements.

Cartilage collagen formation and degradation is unbalanced when RA is diagnosed as assessed by molecular markers. This uncoupling of collagen II metabolism was most pronounced in patients who were anti-CCP positive. Cartilage collagen degradation as reflected by CTX-II was increased and positively correlated to synovitis, while collagen II anabolism as reflected by PIIANP was decreased and negatively correlated with anti-CCP titers, suggesting a chondrocyte-suppressing effect of these autoantibodies. The collagen II uncoupling index at baseline was not superior to CTX-II in predicting radiographic progression.

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