# Biochemical markers of joint tissue turnover in early rheumatoid arthritis

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**ABSTRACT**

*The progression of rheumatoid arthritis (RA), a disease characterized by synovitis, cartilage degradation and bone erosion, is highly variable from patient to patient. New specific biological markers reflecting quantitative and dynamic changes in joint tissue turnover have been recently developed and include assays for type II collagen synthesis and degradation and synovitis. Increasing evidence from prospective studies in early RA indicate that some of these markers may be useful to predict the progression and identify patients at risk for rapid joint damage, before any damage is detected by radiography. Although studies on their value in assessing the efficacy of treatments are still limited, preliminary data in early RA suggest that biological markers will play an important role in the development and the early monitoring of disease modifying antirheumatic drugs with respect to future radiographic progression.*

## Introduction

Rheumatoid arthritis (RA) is an inflammatory disease characterized by synovitis, cartilage degradation and subchondral bone erosion (1). The progression of joint damage is highly unpredictable and variable from patient to patient, ranging from self-limited disease to rapid progressive destruction. Several retrospective and prospective studies have identified potential predictors of radiographic progression (2-7), including clinical signs of disease activity, systemic indices of inflammation such as the erythrocyte sedimentation rate (ESR) and serum C-reactive protein (CRP), rheumatoid factor (RF) positivity, HLA-DBR1 genotypes and radiographic damage. Consequently, most of the current predictors are based on either clinical signs of disease activity and/or inflammation or radiographic damage.

Predictive factors based on disease activity or radiographic damage have some limitations. Indeed, it has been suggested that the pathophysiological processes of joint inflammation and destruction may be partially independent, and erosions may continue despite partial suppression of inflammation and clinical improvement (8). Radiographs of the hands and feet provide a semi-quantitative measure of bone erosion and indirectly of cartilage loss (9, 10). This technique, however, has poor sensitivity and there is often already significant joint damage when the radiological diagnosis is established. These considerations suggest a need for accurate, precise, biologically plausible assays reflecting the dynamics of tissue metabolism in RA, which can be used for prognostication, i.e. predicting which patients will have (un)favorable long-term outcomes and for monitoring the effects of therapies. As conventional radiography is currently considered to be an established standard for measuring joint damage in clinical trials with RA patients, these new tests should ideally reflect structural damage measured by radiography, and should be able to predict forthcoming imaged damage at its earliest evidence.

In this paper we will briefly review recent developments in biological markers of bone, cartilage and synovium tissue, and explore their potential clinical use in early RA

## Candidate biological markers for RA

The joint is a complex organ, and RA alters the metabolism of different tissues including bone, cartilage and synovial membrane. To evaluate the mechanisms involved in joint destruction, the simultaneous use of markers for these three tissues could prove to be

useful. The extracellular matrix of these tissues is composed primarily of collagens including type I (bone and synovium), type II (cartilage) and type III (synovium) associated with proteoglycans (e.g. aggrecan in cartilage) and other glycoproteins (reviewed in 11). Potential biological markers for RA include matrix components (and/or their breakdown products), cytokines, and proteases [*e.g.* matrix metalloproteases (MMPs)] (Table I).

**Bone markers**

For bone formation, the most specific markers are serum osteocalcin, skeletal alkaline phosphatase and the amino-terminal propeptide of type I collagen (PINP). The most sensitive resorption markers are collagen crosslinks including deoxypyridinoline (DPD) and the amino- and carboxy-terminal cross-linked telopeptide of type I collagen (CTX-I, NTX-I). In a large cross-sectional study involving 318 patients with established RA, we found that, compared to healthy controls, bone formation assessed by serum osteocalcin was decreased whereas bone resorption measured by serum CTX-I was increased, suggesting an imbalance of bone remodeling in patients with RA (12). Interestingly, we found that patients presenting evidence of radiographic erosive disease had serum CTX-I levels which were 35% higher compared to patients with no joint damage, but the levels of serum osteocalcin were similar in the two groups. However, since systemic levels of bone turnover markers are believed to reflect the overall rate of bone turnover, which is affected by different conditions such as menopausal status, osteoporosis and other bone diseases, it remains unclear whether they would predict the progression of local juxta articular bone erosion in patients with early RA (13). Consequently, most recent studies have concentrated on the development of markers of cartilage and synovium turnover.

**Table I:** Molecular markers of bone, cartilage and synovium turnover

| | Synthesis | Degradation |
|---|---|---|
| *Bone* | | |
| Type I collagen | *N and C-propeptides (PICP, PINP) | * Pyridinoline (PYD),<br>* Deoxypyridinoline (DPD)<br>* C et N- Telopeptide (CTX-I, NTX-I, ICTP) |
| Non-collagenous proteins | *Osteocalcin<br>*Bone alkaline phosphatase | *Bone sialoprotein (BSP)<br>*Tartrate resistant acid phosphatase (TRAP, 5b isoenzyme) |
| *Cartilage* | | |
| Type II collagen | *N and C-propeptides (PIICP, PIIANP and PIIBNP) | * PYD<br>* Type II collagen C-telopeptide (CTX-II)<br>* Type II collagen alpha chains fragments (COL2-3/4 long mono and COL2-3/4C Short) |
| Aggrecan | *Chondroïtin sulfate (epitopes 846, 3B3, 7D4) | * Core protein fragments<br>* Keratan sulfate (epitopes 5D4, ANP9) |
| Non-aggrecan and non-collagen proteins | *Glycoprotein 39 (YKL-40)<br>*Cartilage-derived retinoic acid sensitive protein (CD-RAP) | * COMP |
| *Synovium* | | |
| Type III collagen | *Type III N propeptide (PIIINP) | * PYD * CTX-I, NTX-I<br>*Glucosyl-galactosyl-pyridinoline (Glc-Gal-PYD) |
| Non-collagenous proteins | *Hyaluronan<br>*YKL-40<br>*COMP | |
| Proteases and inhibitors | *MMP-1, 2, 3, 9,<br>*TIMP 1, 2 | |

**Cartilage markers**

The level of cartilage synthesis can be evaluated by assaying epitopes located on the chondroïtine sulfate chains of the aggrecan (e.g., the 846 epitope), propeptides of type II collagen (PIICP and PIINP) or other proteins such as YKL-40 . PIICP and PIINP are particularly interesting because they are direct indicators of the most abundant protein of cartilage. PIINP exists as two variants, PIIANP and PIIBNP, which reflect the production of IIA and IIB, collagen, respectively. Collagen IIA is expressed mainly during development, but can be re-expressed in osteoarthritic cartilage, whereas the IIB variant is the main form of adult cartilage (14). Using specific antibodies, we developed an ELISA for PIIANP and found decreased serum levels in patients with established RA as compared to controls, suggesting depressed cartilage synthesis in RA which is likely to result from the inhibitory effects of inflammatory cytokines such as IL-1 and TNF-α on matrix synthesis (15)

Cartilage breakdown can be evaluated by measuring the fragments of the aggrecan protein moiety, keratan sulfate epitopes (e.g epitope 5D4), type II collagen fragments, or non-collagenous protein such as cartilage oligomeric matrix protein (COMP). Serum COMP is the most extensively studied cartilage marker so far, and some researchers have found an association between increased COMP levels and progression in RA (reviewed in 11). The biological significance of COMP levels remains unclear, however, because COMP is produced not only by chondrocytes, but also by synovial cells, tendon fibroblasts, and osteoblasts (16). Thus high circulating COMP levels may indicate increased cartilage breakdown and/or inflammation of the synovial membrane. After many years of unsuccessful research, the recent development of assays spe-

cific for type II collagen breakdown probably represents a breakthrough in the field of biological markers for RA, given that degradation of collagen fibers is associated with irreversible cartilage destruction. Antibodies recognizing different type II collagen fragments have been developed. Those directed against the neo-epitopes generated by the collagenases have been mainly used to demonstrate increased type II collagen cleavage in cartilage explants. Two different assays recognizing a sequence – which may be either un-nitrated or nitrated – of the triple helix of type II collagen have also been reported. Preliminary data suggest increased serum levels of these markers – especially the nitrated form – in patients with RA. Most of the clinical data reviewed below have, however, been generated using urinary CTX-II, a fragment of the C-telopeptide of type II collagen (17).

**Markers of synovitis**

Synovitis plays a predominant role in the pathophysiology of RA. Several markers have been proposed to assess synovitis, including serum hyaluronan, N-propeptide of type III procollagen and other non-collagenous proteins such as YKL-40 (Table I). However, these markers are not specific to synovial tissue (11). A glycosylated pyridinoline derivative, glucosyl-galactosyl-pyridinoline (Glc-Gal-PYD), which is found in large amounts in human synovium and at very low levels in the cartilage and other soft tissues, has recently been described (18). Urinary excretion of Glc-Gal-PYD has been reported to be markedly increased in patients with early levels correlating with clinical disease activity (19) and the extent of synovitis of the wrists evaluated by magnetic resonance imaging.

**Clinical uses of biological markers for rheumatoid arthritis**

*Prediction of progression*

Progression shows considerable variation across patients with RA, some of them being characterized by self-limiting disease and others by rapidly progressive destruction. Identifying RA patients at high risk for progression of joint damage at a very early stage is of particular importance for the management of the disease. Indeed such patients may be candidates for treatment with appropriate aggressive disease-modifying antirheumatic drugs (DMARDs) which have been shown to reduce the progression of joint damage (20-22) but may not be optimal for all patients.

Several studies have reported associations between increased levels of some of the biochemical markers of synovitis, and bone or cartilage degradation and a more rapid progression of the disease (reviewed in 11). However, most of these earlier studies included a limited number of patients generally with advanced disease, the estimation of radiographic progression was often based on non-standardized methods and the analyses did not control for the level of disease activity. These limitations probably account for the inconsistent findings (11). More recently, several larger, well-designed studies have been undertaken to assess the potential values of biological markers in early RA.

Cunnane *et al.* (23) have shown in 85 patients with early RA (< 2 years, mean 7 months) followed for 18 months, that time integrated serum levels of matrix metalloproteinase (MMP) 3 were highly correlated with the acute phase response but not with the number of new joint erosions, contrasting with time integrated serum MMP-1 levels which were associated with new bone erosion, but not systemic inflammation. These biochemical marker findings emphasize the dissociation between inflammation and the progression of joint damage in early RA (23). In another study of 127 patients with RA of less than 2 years' duration (mean 11 months) a significant relationship was found between increased baseline serum COMP levels and radiographic progression assessed over the following two years by changes in the Larsen score, independently of ESR (24).

More recently, we evaluated the relationship between baseline levels of specific markers of synovium, cartilage and bone degradation and radiographic progression in large populations of well characterized patients with very early disease involved in randomized clinical trials. In a study comprising 116 methotrexate naïve patients with early RA (median disease duration 1 year),it was found that higher baseline levels of urinary Glc-Gal-PYD, serum CTX-I and urinary CTX-II – which are specific markers of synovium, bone and cartilage degradation, respectively – were associated with a 3- to 3.5-fold increased risk of progression as assessed by changes in the total Sharp score over 1 year (19, 25). After adjustment for baseline levels of disease activity, serum CRP levels and radiographic damage, these markers were still highly predictive of the progression. Combining the assessment of joint damage by X-ray and molecular markers of cartilage/-synovium turnover allowed the better identification of patients with an increased risk of progression compared to those who were identified by one assessment alone.

These results were confirmed in another study of 110 patients with early RA (median disease duration 4 months) included in the COBRA study and followed prospectively for a median of 4 years. In that study, we found that baseline values of CTX-I and CTX-II above the limit of controls were associated with a 8- and 11-fold increased risk of total joint progression, respectively. This predictive value was again independent of baseline disease activity (assessed by the DAS), rheumatoid factor (RF), erythrocyte sedimentation rate (ESR) and radiographic joint damage at baseline (26). In a multivariate model including these well established risks factors, we found that urinary CTX-I and CTX-II were the strongest predictors of progression, with a likelihood ratio for a positive test of 3.8 and 8, respectively. When patients were separated into those with joint damage (baseline Sharp score $\geq 4$, n = 49) and without joint damage (n = 61) at baseline, CTX-I and CTX-II were even more predictive of progression in those with no joint damage, with odds ratios of 14.9 and 25.7 respectively. In the same cohort we also analyzed the relationships between baseline serum levels of the receptor activator of nuclear factor κB-ligand (RANKL) and osteo-

protegerin (OPG) which have been shown in animal studies to play a major role in the process of bone erosion in early RA (27, 28). We found that increased serum RANK-L levels and decreased OPG levels were significantly associated with a higher risk of progression. The ratio between OPG and RANK-L at baseline appeared to be a strong predictor of progression independently of other known predictors of radiographic progression, including CTX-II assessed in the urine. Collectively these two studies indicate that both bone and cartilage degradation are increased very early in RA and that the increase in osteoclastic bone resorption is, at least in part, mediated by the OPG-RANK-L system. These findings also have important clinical implications. They suggest that the measurements of bone and cartilage degradation using specific systemic molecular markers could be useful to detect patients at high risk of progression of joint damage very early in the disease, before radiography can detect any abnormality. Thus, in patients with increased bone/cartilage degradation, even in the absence of joint damage, early intervention with treatments aimed at reducing both bone and cartilage degradation – in combination with anti-inflammatory therapy – may help to prevent subsequent joint damage.

## Monitoring the efficacy of disease-modifying antirheumatic drugs

Prediction of treatment efficacy on progression remains a challenge for the clinician, because of the relatively low sensitivity of radiographic changes. Since clinical trials with DMARDs have shown that radiographic progression at the group level can be slowed if disease activity is suppressed, measures of disease activity, such as ESR, swollen joint count, and disease activity score (DAS), are considered to be adequate monitoring tools for guiding therapy. This approach is probably valid if DMARDs adequately suppress disease activity. However, complete disease remission in RA is rare, and monitoring disease activity does not allow for differentiation in the choice and timing of DMARDs. Therefore, additional tools that may predict long-term radiographic progression in individual patients would be of great value. Molecular markers that directly and specifically reflect structural damage in cartilage and bone are likely to be useful for monitoring the effects of DMARDs with respect to future radiographic progression

In the longitudinal analysis of the COBRA study discussed above, we found that therapy with sulfasalazine, methotrexate and a pulse dose of prednisolone – which was more efficient than sulfasalazine alone in reducing progression over 5 years – also produced a significantly higher decrease in urinary CTX-II which could be detected within 3 months of therapy (29). The magnitude of the changes in CTX-II at 3 months were predictive of the changes in the radiological scores after 5 years. Thus, the measurement of specific biochemical markers of joint tissue turnover before initiating treatment and within a few months after is likely to yield useful information with respect to the radiographic prognosis in patients with RA, which cannot be obtained by measuring parameters of disease activity alone. In the current context of expanding therapeutic possibilities, monitoring cartilage and bone degradation using specific molecular markers could be used to judge the efficacy of a therapy or a strategy at an early stage, so that therapy can be changed if necessary.

## Conclusion

Work on biological markers for RA has recently made important progress, in particular with regard to the development of assays specific for the production and breakdown of type II collagen and of synovial membrane activity. Further research is needed to develop new assays based on fully characterized reagents, with the goal of improving sensitivity and specificity. An increasing body of evidence suggests that a combination of biological markers could provide useful adjuncts to radiography and disease activity indices for identifying early RA patients at risk of rapid joint destruction, potentially before any joint damage is detected by radiography. The rapid responsiveness of biological markers should prove valuable in the clinical development of drugs for preventing joint destruction, and in monitoring their treatment effects in patients with early RA.

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