EXTENDED REPORT

Diurnal variation in serum levels of cartilage oligomeric matrix protein in patients with knee osteoarthritis or rheumatoid arthritis

M L E Andersson, I F Petersson, K E Karlsson, E N Jonsson, B Månsson, D Heinegård, T Saxne

Ann Rheum Dis 2006;**65**:1490–1494. doi: 10.1136/ard.2005.051292

See end of article for authors' affiliations

Correspondence to: M Andersson, Spenshult's Hospital for Rheumatic Diseases, SE-313 92 Oskarström, Sweden; maria.andersson@spenshult.se

Accepted 1 May 2006
Published Online First
17 May 2006

Objective: To monitor changes in serum concentrations of cartilage oligomeric matrix protein (COMP) during a 24-h period to determine any diurnal variation, and to estimate the half life of COMP in the circulation in patients with symptomatic knee osteoarthritis and in those with rheumatoid arthritis.

Methods: Serum samples were drawn every 4 h (7 samples/patient over 24 h) in 10 patients with knee osteoarthritis and 14 patients with rheumatoid arthritis. Osteoarthritis was defined radiographically and clinically (American College of Rheumatology (ACR) criteria) and rheumatoid arthritis according to the 1987 ACR criteria. Serum COMP was measured by sandwich ELISA. A statistical model for the diurnal variation in the COMP levels was developed using the computer program NONMEM.

Results: No considerable changes in COMP levels were observed during the day between 08:00 and 21:00 in either group. A significant decrease in serum COMP was apparent during bed rest at night, reaching the lowest levels between 04:00 and 05:00 ($p < 0.03$ or better v all other time points) in patients with osteoarthritis and in those with rheumatoid arthritis. From the rate of decreasing serum COMP levels, a putative half life of COMP in the circulation was estimated to be 7.4 h.

Conclusion: During normal daytime activities, serum COMP levels are constant. The decrease during the night indicates a rapid elimination of COMP once it has reached the circulation. The stable COMP levels during the day suggest that it is not necessary to further standardise the time of serum sampling in clinical practice.

Processes affecting joint cartilage can be monitored by measuring specific matrix proteins or their fragments released and eventually reaching the circulation.^{1–3} Such biomarkers also represent potential tools for monitoring effects of treatment on the tissue.⁴ One such biomarker is cartilage oligomeric matrix protein (COMP). COMP is a 435-KDa non-collagenous protein composed of five subunits primarily identified in cartilage.^{5–6} COMP interacts with collagen and is suggested to have a role in the regulation of fibril assembly as well as a structural role in the maintenance of the mature collagen network.^{7–8} Results from experimental arthritis studies indicate that changes in serum levels of COMP reflect processes in cartilage.^{9–11} Supportive evidence from human arthritis is also emerging. For instance, therapeutic interventions that aim at retarding joint damage—for example, blockade of tumour necrosis factor α in patients with rheumatoid arthritis—normalise serum COMP levels.¹² In other studies, the feasibility of serum COMP as a prognostic indicator of future joint damage and as a marker of ongoing joint damage—for example, in osteoarthritis and rheumatoid arthritis—has been suggested.^{13–16} Thus, although small amounts of COMP are present in other tissues (synovium and tendon), the evidence in favour of serum COMP being primarily derived from cartilage structures is compelling.

A prerequisite for correct interpretation of changes in serum levels of a molecular marker is the knowledge of the variation in its serum levels during the day in people with different joint disorders. In our study, to determine the diurnal variation, we monitored serum levels of COMP during a 24-h period in patients with symptomatic knee osteoarthritis and in those with rheumatoid arthritis.

PARTICIPANTS AND METHODS

Participants

Osteoarthritis group

Ten patients were recruited from the Spenshult cohort. Initiated in 1990, this is an ongoing population-based prospective study on patients with knee pain, with or without knee osteoarthritis, in a southern county of Sweden. A detailed description of the recruitment procedure and characteristics of the patients has been published.¹⁷

The patients were provided overnight accommodation during the study. The whole group took a 30-min walk at 14:00. The group had food together and they were told to go to bed at 22:00, but their activities were otherwise not standardised. During their stay at the hospital, they had their meals at the same time of the day as the patients with rheumatoid arthritis included in the study. The patients were not participating in any treatment and rehabilitation activities at the hospital during the study. The group consisted of five men and five women, median age 62.4 years range (60–64). They all had clinically verified knee osteoarthritis according to American College of Rheumatology (ACR) criteria.¹⁸ Seven of the patients also had radiographic knee osteoarthritis, Kellgren–Lawrence Score ≥ 2 or osteophytes.¹⁹ There were no considerable differences in characteristics such as age, sex and body mass index, between the patients with and without radiographic knee osteoarthritis. The participants were examined by a rheumatologist to exclude inflammatory arthritis.

Abbreviations: ACR, American College of Rheumatology; COMP, cartilage oligomeric matrix protein; DMARD, disease-modifying antirheumatic drug

Rheumatoid arthritis group

In all, 14 consecutively enrolled inpatients with erosive rheumatoid arthritis were included. All patients fulfilled the ACR 1987 criteria for rheumatoid arthritis.²⁰ The group consisted of two men and 12 women, median (range) age 66.0 (45–82) years. The median (range) disease duration was 20 (1–30) years. The patients with rheumatoid arthritis underwent structured individualised training, which lead to a considerable improvement in lower extremity function from hip to toe as measured by the Rheumatoid and Arthritis Outcomes Score.²¹ The patients with rheumatoid arthritis rose at 06:30, had breakfast at 07:30 and trained for 1–2 h both in the morning and in the afternoon. After leisure activities (low physical activity) in the evening, they went to bed at 22:00 and were in bed for the whole night. The meals were served at the same time of day as for the patients with osteoarthritis. Eight of the patients were treated with disease-modifying antirheumatic drugs (DMARDs), two had only prednisolone (2.5–5 mg/day) and four were treated with both DMARDs and low-dose prednisolone. The DMARDs used were methotrexate (six patients), sulfasalazine, azathioprine, ciclosporin and hydroxychloroquine. One patient was treated with etanercept. No intra-articular glucocorticoid injections were given within 1 week before the study.

COMP analyses

Venous blood samples were obtained seven times during 24 h—that is, every 4 h. The time point of the first sampling varied in the two groups depending on the sampling routines for inpatients and outpatients. The sampling in the osteoarthritis group started at 09:00 and in the rheumatoid arthritis group at 08:00. The blood samples were allowed to clot for 60 min at room temperature. They were centrifuged at 2000 g for 10 min at 4°C. The serum was stored at –80°C. Serum COMP levels were analysed with a sandwich ELISA (AnaMar Medical, Lund, Sweden). The detection limit is <0.1 U/L, and the intra-assay and interassay coefficient of variation is <5%. Samples were analysed in duplicate according to the recommendations of the manufacturer. All analyses were carried out without the knowledge of patient characteristics and all samples from each patient were tested on the same plate. Standard curves and two controls were included in each plate.

Data analyses

The observed COMP concentrations were subjected to two different statistical analyses. The first was based on Friedmann's test and Wilcoxon's matched pair test for within-group comparisons of serum COMP concentrations at different observation time points in the osteoarthritis and rheumatoid arthritis groups, respectively.

The second analysis was based on non-linear fixed effects modelling, as implemented in the computer program NONMEM with the first-order conditional estimation method with interaction, aiming to further explore the diurnal variation as well as to compute the fractional turnover rate of COMP.²² The COMP levels were described using a model consisting of a sum of cosine functions.²³ Equation 1 displays a general form of this model,

$$\frac{dC_{ij}}{dt} = k_{in,i} \cdot \sum_{k=1}^n \left(1 + A_{k,i} \cdot \cos \left((t_j - P_{k,i}) \cdot \frac{2\pi}{\tau_{k,i}} \right) \right) - k_{out,i} \cdot C_{ij}$$

where subscripts i, j and k denote patient, time point and cycle; C, predicted COMP level; k_{in} , turnover rate (which for

identifiable reasons was fixed to unity); A, amplitude of the cosine cycle; P, peak time of the cycle; τ , cycle period; and k_{out} , fractional turnover rate.²⁴ From k_{out} , the half life of the fractional turnover rate can be computed as $t_{1/2} = \ln 2/k_{out}$.

The number of cycles (n) was selected such that it was as small as possible, while still providing a good representation of the data. The model was developed by adding cycles with decreasing cycle periods—starting with a 24-h period and adding cycles of 12, 8 and 6 h until no further improvement was achieved.

The parameters of the model can be expressed as varying between patients (interindividual variability), as well as between different groups of patients (as indicated by predictor variables such as patient type), which can be used to test for statistical significance between the groups by comparing the goodness of fit between contending models.²² The predictor variables tested for influence were patient type (rheumatoid arthritis and osteoarthritis) on half time, peak times and turnover rate; radiographic knee osteoarthritis on the turnover rate and the cosine amplitudes; and prednisolone treatment on the turnover rate and the cosine amplitudes.

Ethics procedure

The Ethics Committee, Lund University, Sweden, approved the protocol. The study followed the guidelines from the Helsinki Declaration. Participants gave written consent.

RESULTS

Diurnal variation in patients with osteoarthritis

In patients with knee osteoarthritis, there were no significant changes in COMP levels during daytime between 09:00 and 21:00. A significant decrease in serum COMP was apparent during bed rest at night reaching the lowest levels around 05:00, $p < 0.03$ or better versus all other time points (fig 1). No difference in the diurnal variation between the patients with or without radiographic knee osteoarthritis was observed.

Diurnal variation in patients with rheumatoid arthritis

We found no significant changes in the COMP levels during daytime between 08:00 and 20:00. A significant decrease in serum COMP was apparent during bed rest at night reaching the lowest levels around 04:00, $p < 0.001$ versus all other time points (fig 2). We found no significant differences in the variations in serum COMP levels over the 24-h period between patients treated with low-dose prednisolone ($n = 6$) and those without such treatment (fig 3,4).

Figure 1 Diurnal variation in serum cartilage oligomeric matrix protein (S COMP) levels in 10 patients with knee osteoarthritis.

Figure 2 Diurnal variation in serum cartilage oligomeric matrix protein (COMP) levels in 14 patients with rheumatoid arthritis.

Modelling

The model that best described the observed COMP levels consisted of two cosine cycles, one with a 24-h period and the other with a 12-h cycle. The only significant predictor ($p < 0.05$) was patient type on k_{in} , indicating that patients with rheumatoid arthritis on average had COMP levels that were 25% lower than that of patients with osteoarthritis (fig 5). The half life of COMP for patients with rheumatoid arthritis and those with osteoarthritis was estimated to be 7.4 (SD 0.1) h.

Predicted 24-h COMP curves for the patients with typical rheumatoid arthritis and osteoarthritis are displayed in fig 5, together with the observed COMP levels. Table 1 shows the parameter estimates from the final model.

DISCUSSION

Serum markers that reflect processes in cartilage show a potential useful means of assessing the activity of the disease in this tissue, both in patients with osteoarthritis and in those with rheumatoid arthritis. Several such molecular markers are currently being investigated.¹⁻⁴ The advantage of this approach is the potential for detecting pathological changes early in the disease course, facilitating early intervention. Furthermore, effects of treatment may be discerned by repeated measurements of such markers—for example, COMP—and these effects seem not to be directly linked to inflammation.¹⁰⁻²⁵ To optimise the use of such markers in clinical practice, it is necessary to document any confounding factors. For instance, physical activity may influence serum levels of COMP.²⁶⁻²⁷ Another factor, which is of importance in evaluating levels in relation to sampling, is the diurnal variation in the serum levels of a marker. Diurnal variations of different molecules in rheumatoid arthritis has been described, but, as far as is known, without any relation to the diurnal variation in serum COMP.²⁸

We now show that the serum concentration of COMP is stable during daytime—that is, when serum samples are normally obtained. Our study corroborates and extends a study by Vilim *et al*,²⁹ who studied COMP levels in patients with rheumatoid arthritis and osteoarthritis, in serum samples obtained at two time points, in the morning before breakfast and 2 h after lunch. These authors found that the serum levels of COMP did not change considerably between the two sample occasions in either group. In the study by Vilim *et al*, like our study, patients with osteoarthritis had considerably higher serum COMP levels than those with rheumatoid arthritis.

Figure 3 Diurnal variation in serum cartilage oligomeric matrix protein (COMP) levels in eight patients with rheumatoid arthritis not treated with systemic glucocorticoids.

We have shown for the first time that there is considerable diurnal variation in serum COMP, with the lowest values at night during bed rest. Furthermore, we were able to calculate a half life of COMP in the circulation, which was similar in patients with osteoarthritis and in those with rheumatoid arthritis. It is notable that the estimated half life is only in the order of 7 h, which is surprisingly short. This means that the high levels observed in certain patient groups reflect a high level of influx of COMP to the circulation from tissues. Taken together, the findings support the use of COMP as a serum marker for joint diseases. The stable levels during daytime should facilitate testing in a clinical setting, as samples may be obtained at any time during office hours. Furthermore, the considerable turnover of COMP in the circulation makes it possible for the serum levels to change owing to a pathological process in cartilage or owing to therapeutic interventions.

The finding of lower serum levels of COMP in patients with rheumatoid arthritis than in those with osteoarthritis may be due to patient selection, and does not reflect a different characteristic of these diseases in general.

We found no considerable difference in serum COMP changes between patients with rheumatoid arthritis treated with or without glucocorticoids. However, the variations over

Figure 4 Diurnal variation in serum cartilage oligomeric matrix protein (COMP) levels in six patients with rheumatoid arthritis treated with systemic glucocorticoids.

Figure 5 Predicted 24-h cartilage oligomeric matrix protein (COMP) level curves typical for patients with osteoarthritis (dashed line) and rheumatoid arthritis (solid line). Also displayed are the observed COMP levels for patients with osteoarthritis (crosses) and rheumatoid arthritis (open circles).

the 24-h period seemed to be less marked in the patients treated with prednisolone (figs 3, 4). Although serum levels did not differ, this may reflect the treatment, as it is well established that glucocorticoids lower serum levels of COMP.^{11–30} This difference in variability could not be confirmed in the modelling, which may be owing to the small group sizes.

The results from this study show that in patients with osteoarthritis and rheumatoid arthritis, serum COMP levels are stable in daytime, when blood sampling normally occurs. The variations in COMP levels over the 24-h period were remarkably similar in patients with osteoarthritis and in those with rheumatoid arthritis. The levels were stable during daytime in both groups, although the level of activity

somewhat varied, which supports the conclusion that it is not necessary to further standardise the time of day for serum sampling in clinical practice.

ACKNOWLEDGEMENTS

We thank Dr Torsten Boegård, Helsingborg, Sweden, for analysing the x rays.

Authors' affiliations

M L E Andersson, I F Petersson, Spenshult's Hospital for Rheumatic Diseases, Halmstad, Sweden
B Månsson, T Saxne, Department of Rheumatology, Lund University, Lund, Sweden
K E Karlsson, E N Jonsson, Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden
D Heinegård, Department of Experimental Medical Science, Section for Cell and Matrix Biology, Lund University

Funding: This study was supported by the Swedish Medical Research Council, NIAMS, National Institute of Health Grant U01-AR050926, King Gustaf V 80-year Found, The Swedish Rheumatism Association, Spenshult's Research Foundations, the Österlund and the Kock Foundations.

Competing interests: None declared.

REFERENCES

- 1 **Saxne T, Månsson B.** Molecular markers for assessment of cartilage damage in rheumatoid arthritis. In: Firestein GS, Panayi GS, Wollheim FA, eds. *Rheumatoid arthritis—new frontiers in treatment*. New York: Oxford University Press, 2000:291–304.
- 2 **Garnero P, Rousseau JC, Delmas PD.** Molecular basis and clinical use of biochemical markers of bone, cartilage, and synovium in joint diseases. *Arthritis Rheum* 2000;**43**:953–68.
- 3 **Poole AR.** Biochemical/immunochemical biomarkers of osteoarthritis: utility for prediction of incident or progressive osteoarthritis. *Rheum Dis Clin North Am* 2003;**29**:803–18.
- 4 **Kraus VB.** Biomarkers in osteoarthritis. *Curr Opin Rheumatol* 2005;**17**:641–6.

Table 1 Parameter estimates from the final statistical model of the diurnal variation		
	Parameter estimate	RSE
$t_{1/2}$ (h)	7.36	0.072
A_1 (U/l)	0.253	0.25
P_1 (h)	7.03	0.11
A_2 (U/l)	0.247	0.14
P_2 (h)	10.9	0.026
k_{in} OA (U/l)/h	0.75	0.095
k_{in} RA (U/l)/h	1*	–
IV k_{in} (%)	23	0.24
IV P_1 (%)	22	0.57
IV A_1 (%)	54	0.74
Residual error (U/l)	0.84	0.25

A, amplitude of the cosine cycle; IV, interindividual variability; k_{in} , turnover rate; OA, osteoarthritis; P, peak time of the cycle; RA, rheumatoid arthritis; RSE, relative standard error of the estimate; $t_{1/2}$, half life of fractional turnover rate.
*Fixed parameter.

- 5 **Hedborn E**, Antonsson P, Hjerpe A, Aeschlimann D, Paulsson M, Rosa-Pimentel E, *et al*. Cartilage matrix proteins. An acidic oligomeric protein (COMP) detected only in cartilage. *J Biol Chem* 1992;**267**:6132–6.
- 6 **Saxne T**, Heinegård D. Cartilage oligomeric matrix protein: a novel marker of cartilage turnover detectable in synovial fluid and blood. *Br J Rheumatol* 1992;**31**:583–91.
- 7 **Rosenberg K**, Olsson H, Mörgelin M, Heinegård D. Cartilage oligomeric matrix protein shows high affinity zinc-dependent interaction with triple helical collagen. *J Biol Chem* 1998;**273**:20397–403.
- 8 **Heinegård D**, Lorenzo P, Saxne T. Matrix glycoproteins and proteoglycans in cartilage. In: Harris ED, *et al*. *Kelley's textbook of rheumatology*. Philadelphia: Elsevier Saunders, 2005:48–62.
- 9 **Joosten LA**, Helsen MM, Saxne T, van De Loo FA, Heinegård D, van den Berg WB. IL-1 alpha beta blockade prevents cartilage and bone destruction in murine type II collagen-induced arthritis, whereas TNF-alpha blockade only ameliorates joint inflammation. *J Immunol* 1999;**163**:5049–55.
- 10 **Larsson E**, Erlandsson-Harris H, Lorentzen JC, Larsson A, Månsson B, Klareskog L, *et al*. Serum concentrations of cartilage oligomeric matrix protein, fibrinogen and hyaluronan distinguish inflammation and cartilage destruction in experimental arthritis in rats. *Rheumatology (Oxford)* 2002;**41**:996–1000.
- 11 **Larsson E**, Erlandsson-Harris H, Larsson A, Månsson B, Saxne T, Klareskog L. Corticosteroid treatment of experimental arthritis retards cartilage destruction as determined by histology and serum COMP. *Rheumatology (Oxford)* 2004;**43**:428–34.
- 12 **Crnkic M**, Månsson B, Larsson L, Geborek P, Heinegård D, Saxne T. Serum cartilage oligomeric matrix protein (COMP) decreases in rheumatoid arthritis patients treated with infliximab or etanercept. *Arthritis Res Ther* 2003;**5**:R181–5.
- 13 **Petersson IF**, Boegård T, Svensson B, Heinegård D, Saxne T. Changes in cartilage and bone metabolism identified by serum markers in early osteoarthritis of the knee joint. *Br J Rheumatol* 1998;**37**:46–50.
- 14 **Månsson B**, Carey D, Alini M, Ionescu M, Rosenberg LC, Poole AR, *et al*. Cartilage and bone metabolism in rheumatoid arthritis. Differences between rapid and slow progression of disease identified by serum markers of cartilage metabolism. *J Clin Invest* 1995;**95**:1071–7.
- 15 **Sharif M**, Kirwan JR, Elson CJ, Granell R, Clarke S. Suggestion of nonlinear or phasic progression of knee osteoarthritis based on measurements of serum cartilage oligomeric matrix protein levels over five years. *Arthritis Rheum* 2004;**50**:2479–88.
- 16 **Lindqvist E**, Eberhardt K, Bendtzen K, Heinegård D, Saxne T. Prognostic laboratory markers of joint damage in rheumatoid arthritis. *Ann Rheum Dis* 2005;**64**:196–201.
- 17 **Petersson IF**, Boegård T, Saxne T, Silman AJ, Svensson B. Radiographic osteoarthritis of the knee classified by the Ahlback and Kellgren & Lawrence systems for the tibiofemoral joint in people aged 35–54 years with chronic knee pain. *Ann Rheum Dis* 1997;**56**:493–6.
- 18 **Altman R**, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, *et al*. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. *Arthritis Rheum* 1986;**29**:1039–49.
- 19 **Kellgren JH**, Lawrence JS. Radiological assessment of osteo-arthritis. *Ann Rheum Dis* 1957;**16**:494–502.
- 20 **Arnett FC**, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, *et al*. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;**31**:315–24.
- 21 **Bremander AB**, Petersson IF, Roos EM. Validation of the Rheumatoid and Arthritis Outcome Score (RAOS) for the lower extremity. *Health Qual Life Outcomes* 2003;**1**:55.
- 22 **Beal SL**, Sheiner LB. *NONMEM users guides*. MD: Globomax, 1989–1998.
- 23 **Brynnel L**, Paalzow LK, Karlsson MO. Consequence of exercise on the cardiovascular effects of l-propranolol in spontaneously hypertensive rats. *J Pharmacol Exp Ther* 2000;**294**:1201–8.
- 24 **Gabrielsson J**, Weiner D. *Pharmacokinetic and pharmacodynamic data analysis: concepts and applications*. Stockholm: Swedish Pharmaceutical Press, 2000:109–22.
- 25 **Saxne T**, Lindell M, Månsson B, Petersson IF, Heinegård D. Inflammation is a feature of the disease process in early knee joint osteoarthritis. *Rheumatology (Oxford)* 2003;**42**:903–4.
- 26 **Andersson M**, Thorstensson C, Roos E, Petersson IF, Heinegård D, Saxne T. Serum-COMP levels increase during physical exercise in patients with knee osteoarthritis. *Osteoarthritis Cartilage* 2003;**11**(Suppl A):10.
- 27 **Mundermann A**, Dyrby CO, Andriacchi TP, King KB. Serum concentration of cartilage oligomeric matrix protein (COMP) is sensitive to physiological cyclic loading in healthy adults. *Osteoarthritis Cartilage* 2005;**13**:34–8.
- 28 **Cutolo M**, Serio B, Cravio C, Pizzorni C, Sulli A. Circadian rhythms in RA. *Ann Rheum Dis* 2003;**62**:593–6.
- 29 **Vilim V**, Voburka Z, Vytasek R, Senolt L, Tchetverikov I, Kraus VB, *et al*. Monoclonal antibodies to human cartilage oligomeric matrix protein: epitope mapping and characterization of sandwich ELISA. *Clin Chim Acta* 2003;**328**:59–69.
- 30 **Weitoft T**, Larsson A, Saxne T, Rönblom L. Changes of cartilage and bone markers after intra-articular glucocorticoid treatment with and without postinjection rest in patients with rheumatoid arthritis. *Ann Rheum Dis* 2005;**64**:1750–3.