Mod Rheumatol (2004) 14:356–360
DOI 10.1007/s10165-004-0323-4

ORIGINAL ARTICLE

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Cartilage oligomeric matrix protein in serum and synovial fluid of rheumatoid arthritis: potential use as a marker for joint cartilage damage

Received: January 26, 2004 / Accepted: June 15, 2004

Abstract This study examined the serum and synovial fluid concentrations of cartilage oligomeric matrix protein (COMP) in relation to the evolution of joint cartilage damage and the requirement for surgery in 125 patients with rheumatoid arthritis (RA). We compared the erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) level, and matrix metalloproteinase-3 (MMP-3) levels with COMP levels determined by specific enzyme-linked immunosorbent assay (ELISA). Patients were divided into three groups: (1) patients with least erosive disease (LES); (2) patients with more erosive disease (MES); and (3) patients with mutilating disease (MUD). In addition, synovial fluid samples were collected from patients undergoing arthroscopic synovectomy of the knee joint (ASS) and total knee arthroplasty (TKA). Serum COMP levels correlated with the ESR ($P < 0.0001$, $r = 0.374$, $n = 125$) and the CRP level ($P = 0.0014$, $r = 0.281$, $n = 125$). COMP levels did not correlate with the MMP-3 level ($P = 0.182$, $r = 0.114$, $n = 125$). The COMP levels of the LES group were significantly lower than those of the MES or MUD groups. Lastly, synovial fluid COMP levels in the TKA group were higher than in the ASS group. Therefore, these findings suggest that serum and synovial fluid COMP levels in patients with RA may reflect cartilage destruction and are correlated with the ESR and the CRP level, which are indicators of the acute-phase response.

Key words Biomarker · Cartilage oligomeric matrix protein (COMP) · Matrix metalloproteinase-3 (MMP-3) · Rheumatoid arthritis (RA)

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Introduction

Rheumatoid arthritis (RA) is a chronic condition that leads to varying degrees of functional impairment and disability. Symptoms reflecting the inflammatory process often predominate in early disease, whereas the symptoms and consequences related to the extent of joint destruction increase later.[1]

Cartilage oligomeric matrix protein (COMP) is a member of the thrombospondin family and is a noncollagenous extracellular matrix protein found predominantly in cartilage but also in tendon, ligament, and meniscus. COMP is a calcium-binding protein of high molecular weight (>500 kDa) and is composed of five identical subunits.[2,3] At the current time the biological function of COMP is still unclear. The carboxy-terminal globular domain of native COMP binds to collagens I, II, and IX.[4–6] Mutations in the domains of the type 3 calcium-binding repeats that affect calcium binding result in the skeletal dysplasia pseudoachondroplasia.[5,6] Furthermore, mutations in the carboxy-terminal globular domain that affect collagen type IX binding result in some forms of multiple epiphyseal sdysplasia.[5,6] Bovine and human COMP protein contains an RGD sequence suggesting that COMP interacts with type I and type II collagen, but not with proteoglycans, in the presence of Zn^{2+}.[3,7] These findings suggest that COMP may be involved in regulating fibril formation and maintaining the integrity of the collagen network.[4] As a result, COMP has been considered a marker of cartilage metabolism and is reported to be one of the most used markers in joint diseases.[8]

The first assay facilitating its measurement in serum and synovial fluid was described in 1992 and was based on a polyclonal antiserum; it was performed as an inhibition enzyme-linked immunosorbent assay (ELISA).[9] Indeed, most of the studies investigating the potential utility of COMP in RA and osteoarthritis (OA) have been performed using this assay. In this study, we assessed whether COMP might be used as a marker for the evolution of joint cartilage damage and an indication for surgery in patients with RA.

Patients and methods

Patients

The protocol included serum and synovial fluid sampling. The samples were stored at −80°C until assayed. All patients fulfilled the American College of Rheumatology criteria for RA. Only patients who were not receiving glucocorticoids or who were on a stable dose of prednisolone (<10 mg daily) were included in the study. The synovial fluids were aspirated carefully without contaminating the blood.

There were 125 patients in the study, including 18 men and 107 women. The mean age of the patients was 58.9 ± 12.1 years (range 23–85 years) at the time of the study, and the mean duration of RA disease was 12.3 ± 7.8 years (range 3–25 years). COMP levels were measured and compared with the C-reactive protein (CRP) level, the erythrocyte sedimentation rate (ESR), and the matrix metalloproteinase-3 (MMP-3) level. Synovial fluid COMP levels were also measured.

Quantification of serum COMP

Cartilage oligomeric matrix protein was measured by a sandwich ELISA using two monoclonal antibodies directed against separate antigenic determinants on the human COMP molecule (Wielisa Kit; Wieslab, Lund, Sweden). The serum concentrations of COMP obtained by this assay are highly correlated with serum levels obtained by the original inhibition assay ($r > 0.9$ in RA samples). MMP-3 was also determined by a sandwich enzyme immunoassay system.[10]

Statistical analysis

Comparisons were performed by Wilcoxon's matched-pairs test, the Mann-Whitney U-test, or the chi-square test, as indicated. Correlations were calculated using Spearman's rank correlation coefficient. $P < 0.05$ was considered significant.

Results

We initially analyzed serum COMP levels. Serum COMP levels were comparable between men and women (2.4 ± 1.8 vs. 1.9 ± 0.7 μg/ml; male vs. female; $P = 0.826$). The relation between age and COMP levels is shown in Table 1. COMP levels of patients under 50 years of age were significantly lower than in patients older than 50 years of age ($P = 0.011$). However, no significant difference was evident among patients older than 50 years of age stratified into age categories of 50–60, 60–70, and ≥70 years of age (Table 1).

Table 1. Age distribution

Age (years)	Patients (no.)	COMP (μg/ml)	*P*
<50	19	1.58 ± 0.54	
50–59	43	2.11 ± 0.79	0.011*
60–69	39	2.05 ± 1.29	NS
≥70	24	2.08 ± 0.76	NS

*With respect to serum cartilage oligomeric matrix protein (COMP) levels in patients less than 50 years of age

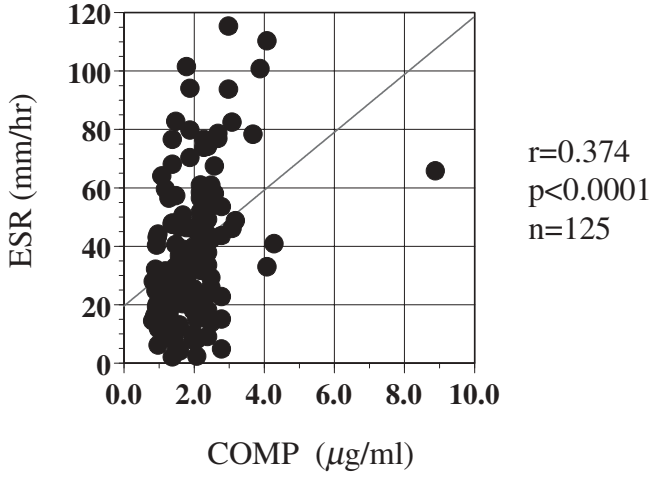

Fig. 1. Correlation between serum cartilage oligomeric matrix protein (*COMP*) and erythrocyte sedimentation rate (*ESR*)

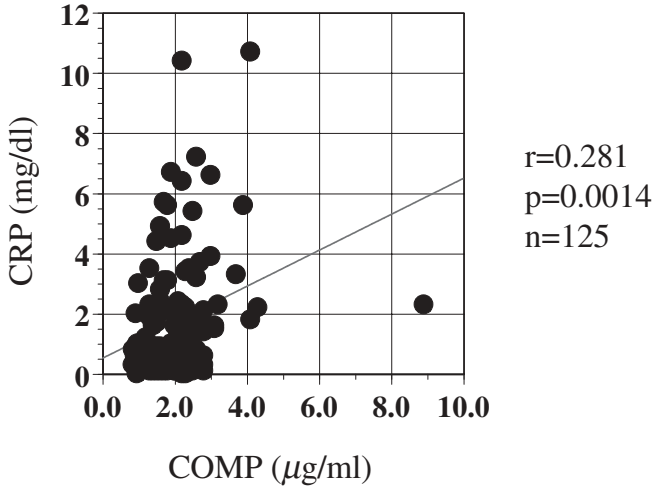

Fig. 2. Correlation between serum COMP and C-reactive protein (*CRP*)

We then analyzed the correlation between serum COMP and the ESR and CRP level. As shown in Fig. 1, COMP levels correlated with the ESR ($P < 0.0001$, $r = 0.374$, $n = 125$). They also correlated well with CRP levels ($P = 0.0014$, $r = 0.281$, $n = 125$) (Fig. 2). These data therefore suggested some relation between COMP levels and ESR and CRP levels. However, assessment of the relation between MMP-3 and COMP levels indicated no significant correlation

Table 2. Grouping of patients

Condition	Age (years)	Male/female	Duration (years)
Total (n = 125)	58.9 ± 12.1	18/107	12.3 ± 7.8
Least erosive disease (LES) (n = 42)	60.0 ± 12.7	6/36	10.6 ± 6.7
More erosive disease (ME) (n= 56)	59.3 ± 12.5	7/49	10.8 ± 7.8
Mutilating disease (MUD) (n = 27)	57.5 ± 10.2	5/22	16.3 ± 8.7

Results are the mean ± SD

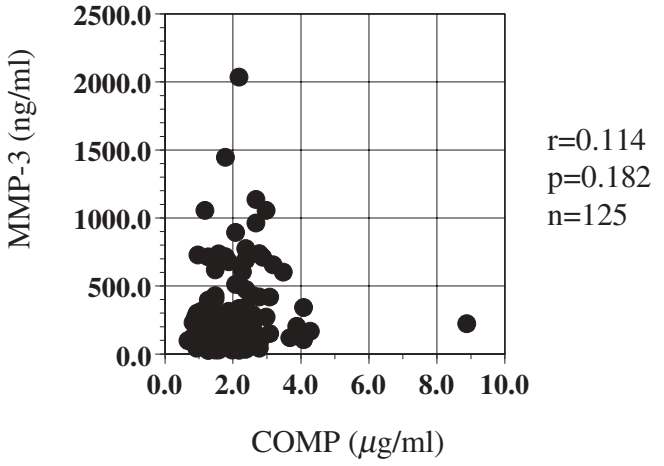

Fig. 3. Correlation between serum COMP and serum matrix metalloproteinase-3 (*MMP-3*)

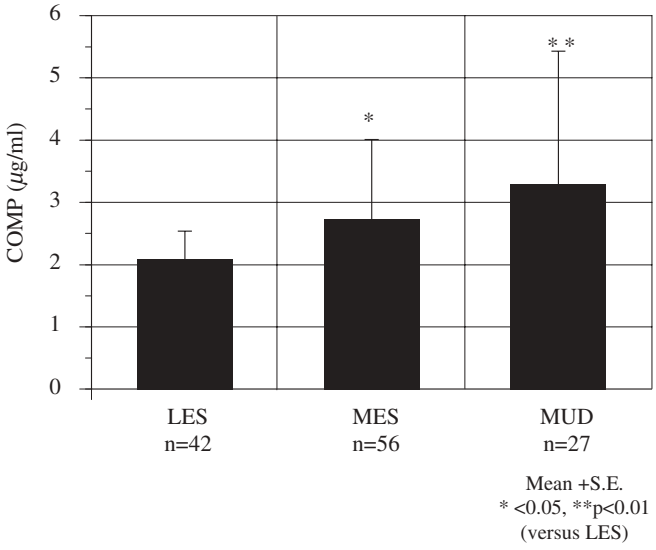

Fig. 4. Serum COMP levels in three groups. *LES*, least erosive disease; *MES*, more erosive disease; *MUD*, mutilating disease

between COMP and MMP-3 levels (P = 0.182, r = 0.114, n = 125) (Fig. 3).

We classified patients into three groups: (1) the subset with least erosive disease (LES); (2) the subset with most erosive disease (MES); and (3) the subset with mutilating disease (MUD).[11] There was no significant difference in age, disease duration, or gender ratio among the groups (Table 2). The results showed that COMP levels of the LES group were significantly lower than those of the MES and MUD groups, and those of the MES group were lower than those of the MUD group (Fig. 4).

In addition to serum COMP levels, we measured synovial fluid COMP levels from the knee joint. These patients were outpatients who had severe swelling and effusion in their knee joints. We found a strong correlation between the serum and synovial fluid COMP levels, with the synovial fluid COMP levels always being much higher than the serum COMP levels (Fig. 5). This result therefore indicated that serum COMP levels reflected synovial fluid COMP levels. We then collected synovial fluid samples from the patients who underwent arthroscopic synovectomy of the knee joint (ASS) and total knee arthroplasty (TKA). In our institute, we use the Larsen classification for radiological evaluation of destructive arthritic change of the knee joint.[12] We found that ASS is indicated for those with grades I, II, and III, but TKA is indicated for those with grades IV and V. Synovial fluid COMP levels in the TKA group were significantly higher than those in the ASS group (Fig. 6).

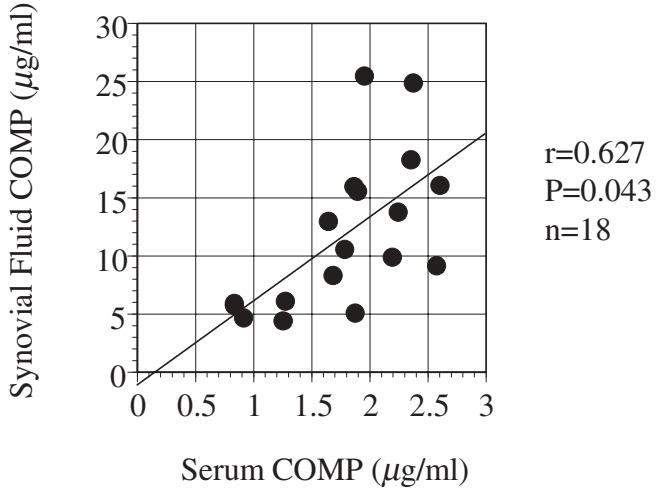

Fig. 5. Correlation between serum COMP and synovial fluid COMP in the knee joint

Discussion

Cartilage oligmeric matrix protein is reported to be one of the most frequently used markers in joint diseases. Saxne and Heinegård[9] developed an ELISA to detect COMP in synovial fluid and serum. Forslind et al.[13] reported that the

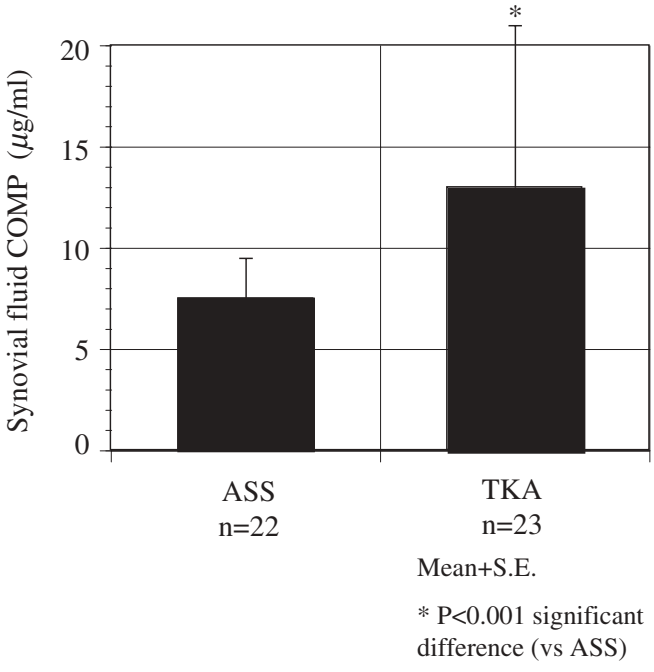

Fig. 6. Synovial fluid COMP levels in patients undergoing arthroscopic synovectomy of the knee joint (*ASS*) and in patients undergoing total knee arthroplasty group (*TKA*). ASS is usually indicated for cases of grade I, II, and III, and TKA is indicated for cases of grade IV and V in the Larsen classification of radiological evaluation[12]

measurement of serum COMP during the early stage of RA held promise as a prognostic marker for the future development of joint destruction. Skoumal et al.[14] also reported that serum levels of COMP reflected increased cartilage turnover and suggested that serum COMP might be used as a marker of cartilage degradation in patients with established RA. Crnkic et al.[15] studied the changes in the serum COMP level during a 6-month period from the initiation of treatment of RA patients with either infliximab or etanercept. They concluded that COMP was a potentially useful marker for evaluating the effects of novel treatment modalities for RA.[15] In contrast, Roux-Lombard et al.[16] reported that COMP did not reflect the inflammatory CRP-related component or the destructive aspect of the disease. Therefore, we evaluated the usefulness of COMP levels in the assessment of the condition of RA joints. We assessed the correlation between serum COMP and the ESR and CRP and MMP-3 levels. Although our data indicated a significant correlation of COMP levels with the ESR and CRP, we found no correlation between COMP levels and MMP-3.

One of the main questions about RA is whether COMP reflects inflammation or structural deterioration of the joint (or both), as COMP is also secreted by synovial cells. There is some evidence that the serum COMP level primarily reflects cartilage turnover in the presence of RA. Therefore, although COMP levels correlate with the ESR and CRP levels in patients with RA, we believe that COMP levels are not increased in conditions of generalized inflammation and that the serum MMP-3 concentration is a useful marker of generalized inflammation and a predictor of bone damage in RA.[10]

Fex et al.[17] indicated that serum COMP concentrations were not useful for identifying patients prone to small-joint destruction. In contrast, Mansson et al.[18] reported that the quantification of COMP did contribute to the assessment of the extent of tissue destruction and might help with the early identification of patients at risk of rapidly progressing destruction of knee and hip joint. They analyzed the influence of COMP levels that were higher in patients with aggressive RA (characterized by the requirement for hip replacement within the following 4 years) than in those with nonaggressive RA and suggested that COMP might be a valuable prognostic factor for large joint destruction.[18] Wollheim et al.[19] compared DRB1* typing and serum COMP levels in a prospective study of a group of RA patients with or without early hip joint destruction. They concluded that serum COMP levels were more informative predictors of aggressive disease than HLA DRB1* typing.[19]

We classified our patients into three groups: LES, MES, and MUD.[11] Erosive articular changes were primarily limited to the peripheral smaller joints in the LES group, whereas larger axial joints were also involved in the MES group.[11] Patients in the MUD group exhibited extensive damage of almost all joints.[11] Our data indicated that the COMP levels of the LES group were significantly lower than those of the other two groups. Furthermore, the highest COMP levels were found in the MUD group. These data suggest that serum COMP levels do reflect the number of joints, the area of degenerative change within joints, or both.

We also measured the COMP levels in the knee synovial fluid of patients with RA. From the clinical point of view, we collected synovial fluid from patients undergoing ASS or TKA. In our institute, ASS is indicated for patients whose radiograph exhibits some joint space between the femur and tibia, whereas TKA is indicated for patients whose radiograph exhibits a severe destructive arthritis. That is, ASS is indicated for patients with grades I, II, and III; and TKA is indicated for patients with grades IV and V of the Larsen classification of radiological evaluation.[12] The synovial fluid COMP levels of patients undergoing TKA were significant higher than those of patients undergoing ASS. This indicates that COMP levels can indicate the degree of degenerative change in articular cartilage.

Conclusions

Our findings suggest that COMP levels in serum and synovial fluid of RA patients reflect the degree of cartilage destruction. However, because these data are cross-sectional and obtained from a small group of patients, further prospective studies in larger populations are required. It may be that the lack of specificity of COMP for cartilage limits its use in assessing the severity of joint damage in RA. Neidhart et al.[20] reported that the absolute levels of COMP in serum and synovial fluid together with its fragmentation pattern in synovial fluid were promising markers of joint

tissue metabolism. Therefore, attention should be focused on developing assays for COMP and other cartilage-derived macromolecules or their fragments, as the release of these proteins into the circulation from the joint may reflect disturbances in joint tissue turnover.

Acknowledgment We thank Daiichi Fine Chemical Co. for technical assistance.

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