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## Treatment of arthritis with tumour necrosis factor antagonists. Clinical, immunological and biochemical aspects

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## Research article

## Open Access

**Serum cartilage oligomeric matrix protein (COMP) decreases in rheumatoid arthritis patients treated with infliximab or etanercept**Meliha Crnkic<sup>1</sup>, Bengt Månsson<sup>1,2</sup>, Lotta Larsson<sup>1</sup>, Pierre Geborek<sup>1</sup>, Dick Heinegård<sup>2</sup> and Tore Saxne<sup>1,2</sup><sup>1</sup>Department of Rheumatology, Lund University Hospital, Lund, Sweden<sup>2</sup>Department of Cell and Molecular Biology, Section for Connective Tissue Biology, Lund University, Lund, SwedenCorresponding author: Tore Saxne ([tore.saxne@reum.lu.se](mailto:tore.saxne@reum.lu.se))

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*Arthritis Res Ther* 2003, **5**:R181-R185 (DOI 10.1186/ar760)© 2003 Crnkic *et al.*, licensee BioMed Central Ltd (Print ISSN 1478-6354; Online ISSN 1478-6362). This is an Open Access article: verbatim copying and redistribution of this article are permitted in all media for any purpose, provided this notice is preserved along with the article's original URL.**Abstract**

Changes in serum cartilage oligomeric matrix protein (COMP) were studied during a 6-month period from initiation of treatment of rheumatoid arthritis patients with either infliximab or etanercept, to elucidate whether the favourable results of tissue protection reported in clinical trials are corroborated by changing levels of circulating COMP. Rheumatoid arthritis patients commencing treatment with infliximab ( $N=32$ ) or etanercept ( $N=17$ ) were monitored in accordance with a structured protocol. Only patients who were not receiving glucocorticoids or who were on a stable dose of oral prednisolone ( $<10$  mg daily) were included. Serum COMP was measured by a sandwich immunoassay based on two monoclonal antibodies against human COMP in samples obtained at treatment initiation and at 3 and 6 months. Serum

COMP decreased at 3 months in both infliximab- and etanercept-treated patients ( $P<0.001$  and  $<0.005$ , respectively) and remained low at 6 months. There was no significant correlation between changes in or concentrations of serum COMP and serum C-reactive protein at any time point. A decrease in serum COMP was seen both in ACR20 responders (patients meeting the American College of Rheumatology criteria for 20% improvement) and in nonresponders. The pattern of changes of serum COMP, a marker for cartilage turnover, in these patient groups supports the interpretation that infliximab and etanercept have a joint protective effect. Serum COMP has potential as a useful marker for evaluating tissue effects of novel treatment modalities in rheumatoid arthritis.

**Keywords:** cartilage, COMP, etanercept, infliximab, serum**Introduction**

Rheumatoid arthritis (RA) is a chronic condition, which leads to varying degrees of functional impairment and disability. In early stages, symptoms reflecting the inflammatory process often predominate, whereas later the symptoms and consequences related to the extent of joint destruction increase [1]. Traditionally, treatment has focused on ameliorating the inflammation. Although rather effectively alleviating the symptoms related to the inflammatory process, most disease-modifying antirheumatic drugs used until very recently have been less effective in retarding the progressive joint destruction. The coupling between inflammation and subsequent joint destruction has thus been questioned [2].

A new era in antirheumatic therapy began when biologic agents that target specific cells or mediators in the disease process were developed and their feasibility was investigated in clinical trials. The first biologic principle tested in RA patients and shown to be dramatically effective in reducing the signs and symptoms of inflammation was blocking of tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ) [3]. This was accomplished either by a monoclonal antibody, infliximab, or by a soluble receptor binding both TNF- $\alpha$  and TNF- $\beta$ , etanercept [4–6]. Another principle tested was blocking of the effects of interleukin-1 using a receptor antagonist, which seemed somewhat less efficient in ameliorating inflammation [7]. In subsequent trials, the effect of

blocking these cytokines on the progression of joint destruction has been tested. With radiography of hands and feet used as an end point, these treatment modalities show promise in retarding the destructive process [8–10].

Several drawbacks of clinical trials focusing on prevention of joint destruction can be identified. Since the rate of progression is slow, the trials must be conducted over a long period, preferably more than one year, to be able to detect changes. Furthermore, evaluation relies on radiographic assessment, which is time-consuming and, despite improvements during recent years, remains a subject of controversy regarding technical details [11]. Also, radiography is impractical in early stages of drug development, i.e. for proof of drug efficacy in small groups of patients or animals.

Alternative or complementary tools to assess progression of tissue damage should therefore facilitate the evaluation of treatments with the potential to modify structural joint damage. Molecular-marker technology is a tool that may aid in evaluating tissue effects of antirheumatic therapy [12]. One promising candidate is COMP (cartilage oligomeric matrix protein, also called thrombospondin-5), originally isolated and characterized in cartilage [13]. The first assay suitable for its measurement in serum and synovial fluid was described in 1992 and was based on a polyclonal antiserum and performed as an inhibition ELISA [14].

In this communication, we report the use of a novel sandwich ELISA using a combination of two monoclonal antibodies for the detection of COMP in serum. The purpose of the study was to monitor serum COMP changes during a 6-month period from initiation of treatment of RA patients with either infliximab or etanercept. By monitoring serum COMP, we wanted to elucidate whether the favourable results of tissue protection reported for the TNF-blockers could be corroborated by changing levels of a primarily cartilage-derived marker during treatment.

## Materials and methods

### Patients

Patients with RA who are treated with infliximab or etanercept at our unit are monitored in accordance with a structured clinical protocol [15]. All patients have consented to be included in the protocol. No formal approval by the ethics committee is required for this protocol, which is an integrated part of the routine management of these patients. The protocol includes repeated serum sampling. The sera are stored at  $-80^{\circ}\text{C}$  [14]. The patients included in the present study were those who at a given time point were included in the protocol and had been treated for at least 6 months. They all fulfilled the American College of Rheumatology criteria for RA [16]. Only patients who were not receiving glucocorticoids or who were on a stable dose of prednisolone ( $<10\text{ mg}$  daily) were included in the study. Furthermore, no intra-articular glucocorticoid injections

were given during the study period or 3 months prior to enrolment. The reason for the rigorous control of glucocorticoid administration was that previous studies had indicated that glucocorticoid treatment, whether oral or intra-articular, may modify serum COMP levels [12]. Infliximab and etanercept were given in accordance with the standard recommendations, i.e., for infliximab, infusion intravenously of  $3\text{ mg/kg}$  body weight at baseline, week 2, and week 6 and then every 8 weeks, and, for etanercept,  $25\text{ mg}$  subcutaneously twice weekly.

### Quantification of serum COMP

Serum COMP was measured by a sandwich ELISA utilising two monoclonal antibodies directed against separate antigenic determinants on the human COMP molecule (AnaMar Medical, Lund, Sweden). The detection limit is  $<0.1\text{ u/l}$  and the intra-assay and inter-assay coefficient of variation is  $<5\%$ . The assay is not influenced by rheumatoid factors. The serum concentrations of COMP obtained by this assay are highly correlated with serum levels obtained by the original inhibition assay ( $r$  values  $>0.9$  in RA samples) (T Saxne and D Heinegård, unpublished). Samples obtained at baseline and after 3 and 6 months of therapy if available were analysed.

### Statistical analyses

Comparisons were done by Wilcoxon's matched-pairs test, the Mann-Whitney  $U$ -test, or the chi-square test, where appropriate and correlations were calculated using Spearman's rank correlation coefficient. A  $P$  value of  $<0.05$  was considered significant.

## Results

Some characteristics of the two groups of patients are shown in Table 1. There were no significant differences between the groups in the examined variables.

Serum concentrations of COMP decreased in patients treated with infliximab or etanercept (Figs 1 and 2). The decrease was apparent at the 3-month follow-up ( $P<0.001$  and  $<0.005$ , respectively) and levels remained low after 6 months compared with baseline values, though not significant for etanercept. In the etanercept-treated group, serum COMP decreased in 15 of the 17 patients, whereas in the infliximab group, serum COMP decreased in 23 patients and remained unchanged or increased in 9 patients (no significant difference between the groups). The reason why some of the patients were not included after 6 months was in most cases that the prednisolone dosage had been changed and according to the inclusion criteria these patients were then not eligible for follow-up. Three patients had stopped taking TNF-blockers after the 3-month follow-up. The serum sample from the 3-month follow-up was missing for one patient taking etanercept, and sera from the 6-month follow-up were missing for two infliximab-treated patients.

**Table 1****Characteristics of patients with rheumatoid arthritis in the two treatment groups**

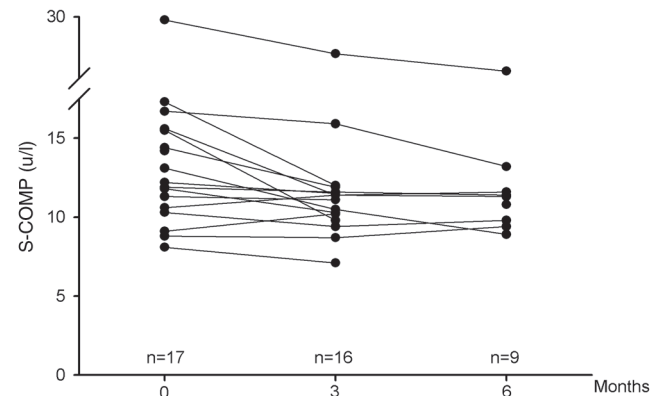
Patients	Treatment	
	Infliximab	Etanercept
No.	32	17
Age (years) <sup>a</sup>	56.7 (23.5–80.9)	55.3 (23.2–70.4)
Female/male	25/7	10/7
Duration of disease (years) <sup>a</sup>	12.9 (3.0–55.5)	13.7 (2.5–41.0)
Serum CRP at baseline (mg/l) <sup>a</sup>	20 (1–84)	36 (1–85)
Serum COMP at baseline (u/l) <sup>a</sup>	11.3 (5.5–19.2)	12.2 (8.1–29.7)
DAS28 at baseline <sup>a</sup>	5.3 (2.4–8.1)	5.9 (3.7–7.5)
HAQ at baseline <sup>a</sup>	1.4 (0.4–2.8)	1.6 (0.3–2.8)
Monotherapy/combination therapy	5/27	3/14
Prednisolone/no prednisolone	20/12	11/6
ACR20 response at 3 months	16 (50%)	11 (65%)

There were no significant differences between the groups. <sup>a</sup>Median (range). ACR20 = 20% improvement according to the American College of Rheumatology criteria; CRP = C-reactive protein; COMP = cartilage oligomeric matrix protein; DAS = disease activity score; HAQ = health assessment questionnaire.

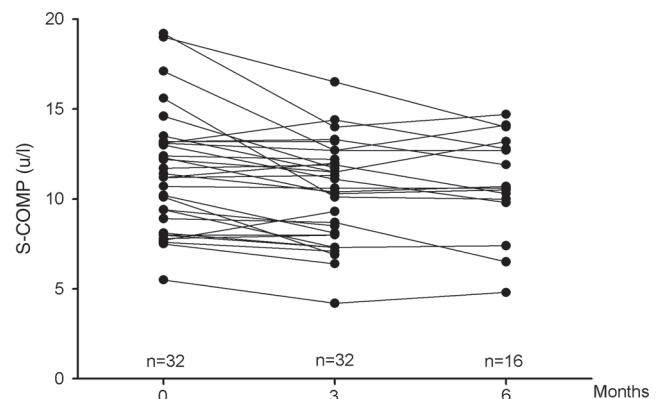
The most marked reduction in serum COMP was seen in the patients with the highest baseline values. The decrease was significant both in ACR20 responders (patients meeting the American College of Rheumatology criteria for 20% improvement) at 3 months and in nonresponders for both drugs ( $P < 0.05$  or better) (Fig. 3). The COMP values did not correlate significantly with the C-reactive protein (CRP) values at any time point, nor did the changes ( $\delta$  values) in CRP between time points correlate with those in COMP. The CRP values decreased significantly in the ACR20 responders in both infliximab- and etanercept-treated patients ( $P < 0.001$  and  $P < 0.03$ , respectively), whereas in the nonresponders, no significant difference in CRP values between baseline and the 3-month follow-up was found. The baseline serum COMP levels did not differ between patients with or without prednisolone treatment.

## Discussion

The COMP levels decreased in both treatment groups during the initial 3 months of therapy. This suggests that TNF blockade modifies the release of COMP from the tissue and supports the interpretation that this treatment modality retards the development of joint destruction, as previously indicated [8,10]. Importantly, levels of COMP decreased both in responders and nonresponders. These observations taken together are consistent with the hypothesis that inflammation and tissue destruction are not directly linked, thus corroborating earlier published

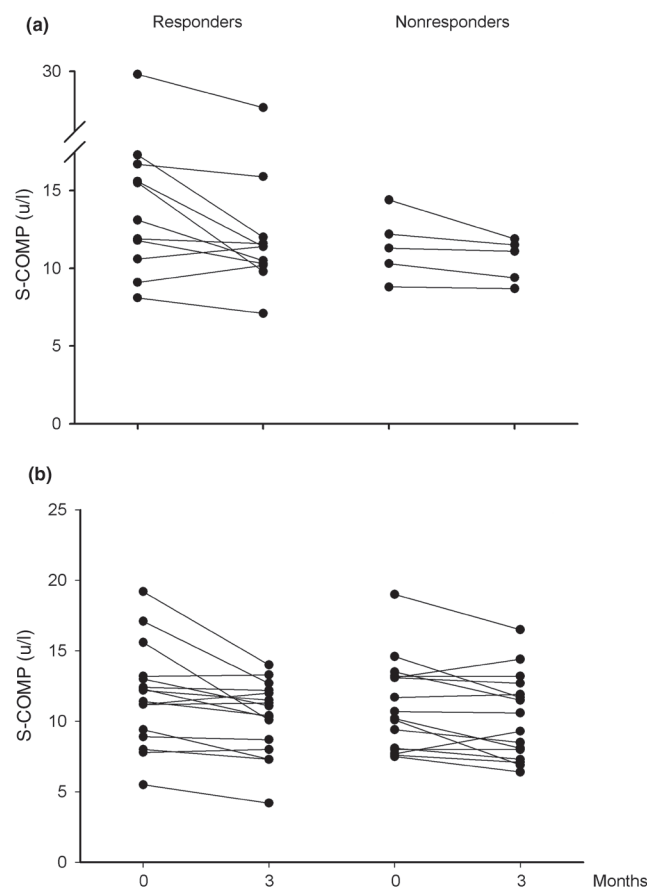
**Figure 1**

Serum COMP (S-COMP) at baseline and after 3 and 6 months in rheumatoid arthritis patients treated with etanercept. Serum COMP was lower than at baseline after 3 months ( $P < 0.005$ ). The levels remained low after 6 months, although not significantly lower than at baseline ( $P = 0.07$ ). See text for details. COMP = cartilage oligomeric matrix protein (thrombospondin-5).

**Figure 2**

Serum COMP (S-COMP) at baseline and after 3 and 6 months in rheumatoid arthritis patients treated with infliximab. Serum COMP was lower than at baseline after 3 months ( $P < 0.001$ ). The levels remained low after 6 months ( $P < 0.001$  compared with baseline). For details, see text. COMP = cartilage oligomeric matrix protein (thrombospondin-5).

radiographic data on TNF blockade in RA [10]. This interpretation is further supported by the lack of correlation between CRP levels and between changes in CRP levels and changes in COMP levels. Although COMP is not unique to cartilage [17–19], the observations in this study clearly support a role for serum COMP as a marker reflecting processes not directly linked to the inflammation in RA. Having this characteristic, COMP adds to the variables that should be useful to include in the evaluation of potentially tissue-protective antirheumatic drugs. A recently published study in which the original inhibition assay was used for detection of serum COMP supports this conclusion

**Figure 3**


Serum COMP (S-COMP) at baseline and after 3 months in rheumatoid arthritis patients treated with etanercept (a) or infliximab (b), grouped according to whether they were ACR20 responders (i.e., met the American College of Rheumatology criteria for 20% improvement) or nonresponders. Serum COMP was lower than at baseline after 3 months in all groups ( $P < 0.05$  or better). For details, see text and Table 1.

[20]. In this study of adalimumab, a fully human anti-TNF- $\alpha$  monoclonal antibody, in RA, it was shown that baseline serum COMP was higher in the patient group whose disease progressed radiographically during a 2-year period. The COMP levels decreased during treatment in patients with radiographic progression but remained low and unchanged in patients with no progression.

We chose to include only patients without glucocorticoid treatment or with stable, low-dose prednisolone treatment, which considerably reduced the number of patients eligible. The reason for this action was, as pointed out above, that glucocorticoids tend to lower serum COMP levels. However, this effect does not seem to be due to the anti-inflammatory effect of glucocorticoids, since the lowering of serum COMP by glucocorticoids does not correspond to a decrease in inflammation, e.g. as measured by CRP

or erythrocyte sedimentation rate (ESR) [12]. Instead, it could be hypothesized that the effect might be due to the joint protective effect suggested for glucocorticoids [21,22]. Somewhat in contrast to previous findings, we did not in this study find any difference in baseline levels of COMP between low-dose prednisolone users and patients not taking prednisolone. We have no explanation for this. However, the possible effect of glucocorticoids on serum COMP needs to be considered when it is used as a biomarker, e.g. in drug trials or for clinical purposes.

A drawback of this study is the lack of radiographs for comparison with the changes in serum COMP levels. When we started to include patients in the protocol, we did not include radiographic follow-up, because we did not anticipate that treatment of patients with advanced RA with TNF blockers could substantially alter the radiographic appearance of the joints. It turns out that we were wrong. Furthermore, because of the observational nature of our protocol, no suitable group for comparison was available. Since our clinical results accord with the results of the pivotal trials of infliximab and etanercept that included radiographs, we believe that our study corroborates the results of these trials and that the findings support the use of COMP as a marker of cartilage processes in RA.

## Conclusion

Serum COMP decreases during the first 3 months of treatment with etanercept or infliximab in patients with RA in a manner different from changes in symptoms and in levels of CRP. Thus, COMP shows promise as a non-inflammation-related marker of the disease process in RA, which should be useful for evaluating novel treatment modalities in the disease.

## Competing interests

TS and DH are share-holders in AnaMar Medical.

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