Original article

Decrement of serum cartilage oligomeric matrix protein (COMP) in rheumatoid arthritis (RA) patients achieving remission after 6 months of etanercept treatment: Comparison with CRP, IgM-RF, MMP-3 and anti-CCP Ab

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Running title: COMP associates with efficacy of etanercept in RA.

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Abstract

Objective: The aim of this study was to evaluate whether serum COMP (cartilage oligomeric matrix protein) can estimate the therapeutic response of rheumatoid arthritis (RA) after 6 months of treatment with etanercept.
Methods: Forty-five RA patients receiving 25 mg of etanercept twice a week for 6 months were registered in this prospective observational study. Clinical response to the therapy was evaluated by disease activity score (DAS) 28. Laboratory variables—COMP, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), IgM-rheumatoid factor (IgM-RF), matrix metalloproteinase-3 (MMP-3), and anti-cyclic citrullinated peptide antibodies (anti-CCP Ab)—were assessed at baseline and after 6 months of treatment. We assessed the correlations between serum COMP and other variables and whether serum COMP is associated with DAS28 remission.
Results: Serum COMP correlated with DAS28-ESR ($p < 0.05$, $r = 0.40$) at baseline. At 6 months of etanercept treatment, 10 patients entered remission (DAS28-ESR < 2.6) whereas the other 35 patients did not (DAS28-ESR > 2.6). The decrement of serum COMP at 6 months was significant in the remission group (N = 10) but not in the non-remission group (N = 35). On the other hand, CRP, ESR and MMP-3 decreased at 6 months regardless of remission status. IgM-RF titer as well as anti-CCP Ab titer did not differ at 6 months.
Conclusions: Serum COMP at baseline reflects clinical disease activity of RA. Serum COMP is a valuable serologic marker to identify the subset of RA patients achieving remission during treatment with etanercept.

Key Words
Rheumatoid arthritis, cartilage oligomeric matrix protein, etanercept

1. Introduction

Cartilage oligomeric matrix protein (COMP) is a member of the thrombospondin family and a noncollagenous extracellular matrix protein found mainly in cartilage, and plays an important role in maintaining the integrity of the collagen network [1, 2]. COMP is released first into the synovial fluid and next diffused into the blood in varying disease conditions of cartilage damage; thus, serum COMP reflects the degree of articular cartilage involvement. Since the amount of articular cartilage is greater in large joints than in small joints, serum COMP is supposed to be a marker of cartilage damage in large joints [3, 4, 5] though controversial observation remained [6]. The biological function of COMP is still unclear at the current time.

In recent years anti-tumor necrosis factor (TNF) therapy has been widely used in the clinical treatment of rheumatoid arthritis (RA), and preferential efficacy toward RA disease activity and joint destruction has been established [7, 8]. Etanercept is a recombinant TNF receptor-Fc fusion protein, and large-scale clinical trials of etanercept have identified a certain subset of patients, including those with lower disease activity at baseline, male sex and better physical functions at baseline, that can be predicted to achieve remission using etanercept [9]; however, the association of its clinical efficacy with serologic variables, especially in Japanese patients, remains unknown. Although COMP was reported as predictive marker of cartilage damage and joint destruction, reports about serum COMP during anti-TNF therapy are few, and rarely found in Japan.

We have examined the efficacy and change of serologic variables in Japanese RA patients treated with 25 mg of etanercept twice a week for 6 months. We have shown for the first time that serum COMP is an excellent marker of clinical disease activity in etanercept-treated RA patients compared with C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), matrix metalloproteinase 3 (MMP-3) and autoantibodies.

2. Materials and Methods

Although this clinical study is based on post-marketing surveillance (PMS), it is a prospective consensus-based analysis, as described below.

Forty-five RA patients were selected to enroll in the present study from the Unit of Translational Medicine, Department of Immunology and Rheumatology, Graduate School of Biomedical Sciences, Nagasaki University and Sasebo Chuo Hospital, Center for Rheumatic Disease. They gave their informed consent to the protocol that was approved by the Institutional Review Board of Nagasaki University and Sasebo Chuo Hospital. All of the patients fulfilled the 1987 criteria of the American College of Rheumatology (ACR) for RA [10]. In brief, 150 RA patients to date had received etanercept in Nagasaki University and Sasebo Chuo Hospital, among whom 45 patients were selected for the present study. Inclusion criteria of the present study were treatment with 25 mg etanercept twice a week for 6 months; treatment with a stable dose of daily prednisolone less than 10 mg throughout the 6-month period; no intra-articular glucocorticoid injections during the 6-month period, and no change in concomitant use of disease-modifying anti-rheumatic drugs (DMARDs) during the 6-month period. The DMARDs introduced in the present study are slightly different from those in the United States and Europe, and are listed in Table 1. Serum samples were collected and stored at -80 ºC at baseline and at 6 months until the assay.

Clinical response to the therapy was evaluated by disease activity score (DAS; DAS28-ESR; high disease activity > 5.1, moderate disease activity < 5.1 and > 3.2, low disease activity < 3.2, remission < 2.6). Laboratory variables―COMP, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), IgM-rheumatoid factor (RF), matrix metalloproteinase-3 (MMP-3), and anti-cyclic citrullinated peptide antibodies (anti-CCP Ab)―were assessed at baseline and after 6 months of treatment. All serologic analyses were performed on the stored samples using commercially available kits in accordance with instructions: COMP (COMP ELISA^{R}, AnaMar Medical AB, Göteborg, Sweden), CRP (Eiken Chemical Co. Ltd., Tokyo, Japan), IgM-RF (Dade Behring, Marburg, Germany; cut-off value was 9.5 U/ml), MMP-3 (Daiichi Pure Chemicals, Fukuoka, Japan) and anti-CCP Ab (DIASTAT Anti-CCP, Axis-Shield, Dundee, UK; cut-off value was 4.5 U/ml).

Statistical analyses: Within-group comparisons were made using Mann-Whitney's U test. The changes from baseline were compared using Wilcoxon's signed rank test. Correlations were assessed with Spearman's correlation coefficient test. The overall significance level for statistical analysis was 5% (two-sided). *P* values less than 0.05 were considered statistically significant.

3. Results

Clinical characteristics of 45 patients at baseline

Demographical and clinical characteristics of 45 patients are shown in Table 1. Most of the patients were established RA patients with high disease activity and classified as advanced stage. Thirty-two of the 45 patients (71%) were taking low-dose predonisolone (mean dose; 6.64 ± 2.74mg daily) at entry. Thirty-one patients (69%) were treated with DMARDs (18 methotrexate, 6 salazosulfapyridine, 3 Cyclosporin A, 2 leflunomid, 2 mizoribine, 1 tacrolimus, 1 Bucillamine: 3 patients were treated with two DMARDs each).

Table 2 shows correlations of COMP with other variables at baseline. Serum COMP levels correlated with DAS28-ESR ($p < 0.05$, $r = 0.40$), ESR ($p < 0.05$, $r = 0.33$) and CRP ($p<0.05$, $r=0.33$) at baseline (Table 2). There was no association between serum COMP and age, disease duration at baseline.

Decrement of COMP at 6 months was significant in the patients achieving remission at 6 months

Change of the variables over the 6-month treatment with etanercept in 45 patients is shown in Table 3. The average value of serum COMP in 45 RA patients at baseline was 12.0 ± 3.4 U/l (Table 3); this value did not differ in patients with and without predonisolone treatment (data not shown). Serum COMP had decreased at 6 months ($p < 0.05$, by Wilcoxon's signed rank test). CRP ($p < 0.0001$), ESR ($p < 0.0001$), MMP-3 ($p <0.001$), DAS28-ESR ($p < 0.0001$), mHAQ ($p < 0.01$) also decreased at 6 months whereas the titers of anti-CCP Ab and IgM-RF did not change (Wilcoxon's signed rank test).

Overall, DAS28-ESR at 6 months significantly improved, but was still evaluated as moderate disease activity (mean DAS28-ESR, 3.66 ± 1.28 as calculated in Table 3 at 6 months); thus, we divided the 45 patients according to achievement of remission. As shown in Table 4, at 6 months, 10 patients had entered remission whereas 35 patients had not. COMP, CRP, ESR and titer of anti-CCP Ab and IgM-RF were not statistically different between the 2 groups at baseline (Data not shown, by Mann-Whitney's U test). The change of serologic variables was evaluated in the 2 groups. CRP, ESR and MMP-3 were reduced in a similar fashion regardless of the achievement of remission; COMP was the only variable to be reduced in the remission group ($N = 10$) compared with non-remission group ($N = 35$) (Wilcoxon's signed rank test).

4. Discussion

A few reports of change in serum COMP during anti-TNF therapy have been reported in Europe and the United States. In adalimumab trials, high serum COMP at baseline was considered to predict a poor outcome for the patients [11, 12]. In PMS data of etanercept treatment of 17 RA patients for 6 months, serum COMP decreased during the treatment [13]; however, there were no reports that showed the association of serum COMP value with the efficacy of etanercept treatment for RA.

We examined 45 RA patients treated with etanercept for 6 months. Serologic variables of COMP, CRP, ESR, MMP-3, anti-CCP Ab, IgM-RF and DAS28-ESR were serially obtained at entry and at 6 months. As shown in previous reports [14, 15], the serum COMP level at baseline correlated with clinical disease activity at baseline. However, this was the first observation that serum COMP decrement at 6 months correlates with disease status at 6 months, i.e., achievement of remission, as compared with other serologic variables. Trial of Etanercept and Methotrexate with Radiographic Patient Outcomes (TEMPO) study predicted the variables at baseline for remission after 3 years. Male sex, low disease activity, low mHAQ and combination with methotrexate at baseline were predictive of remission at 3 years [9]. In the present study, there was a predominant male sex tendency (7/10 in remission vs. 5/35 in non-remission, $p = 0.07$ by Chi-Square test) in the remission group, and DAS28-ESR ($p = 0.003$ by Mann-Whitney's U test) as well as mHAQ ($p = 0.04$ by Mann-Whitney's U test) at baseline were lower in the remission group than in the non-remission group. Combination therapy with DMARDs was also statistically high in the remission group compared with the non-remission group (10/10 in remission vs. 17/35 in non-remission, $p = 0.014$ by Fisher's exact probability test). Therefore, the characteristics of the remission group in the present study are comparable with those in the TEMPO trial, and the COMP level decrement at 6 months may reflect the outcome of patients.

In summary, we focused on the role of serum COMP in etanercept-treated Japanese RA patients. In contrast to various demographic data with prognostic value, serum COMP was shown to be a valuable indicator of the clinical outcome itself.

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Conflicts of interest

We declare that there is no conflict of interest in this paper.

Abbreviations

anti-CCP Ab: anti-cyclic citrullinated peptide antibodies
COMP: cartilage oligomeric matrix protein
CRP: C-reactive protein
DAS : disease activity score
DMARDs: disease-modifying anti-rheumatic drugs
ESR: erythrocyte sedimentation rate
IgM-RF: rheumatoid factor
MMP-3: matrix metalloproteinase
PMS: post marketing surveillance
RA: rheumatoid arthritis
TNF: tumor necrosis factor

Table 1

Demographic data of 45 RA patients at baseline

Characteristics	Data at baseline
Age (years [a])	55.9 ± 13.5
Female/male (n)	33/12
Duration of disease (years [a])	9.9 ± 6.9
Stage (n)	I, 0; II, 10; III, 9; IV, 26
Concomitant steroid (n)	32
Concomitant DMARDs(n)	31
	MTX, 18; SASP, 6; CsA, 3; LEF, 2;
	MZR, 2; TAC, 1; BUC, 1
Tender joints(n [a])	13.2 ± 7.6
Swollen joints(n [a])	6.0 ± 4.6
CRP (mg/dl [a])	28.4 ± 33.4
ESR (mm/hr [a])	61.7 ± 36.1
mHAQ [a]	0.81 ± 0.67
DAS28-ESR [a]	5.86 ± 1.35

[a] M±SD

MTX, methotrexate; SASP, salazosulufapyridine; CsA, cyclosporin A; LEF, leflunomide; MZR, mizoribine; TAC, tacrolimus; BUC, bucillamine

Table 2

Serum COMP at baseline correlates with disease activity of RA

Characteristics	r	*P*
DAS28-ESR	0.402	0.008
ESR	0.331	0.028
CRP	0.332	0.028
MMP-3	0.081	0.622
Anti-CCP	0.155	0.335
IgM RF	0.213	0.158

Correlation of COMP with other variables at baseline was calculated as described in Patients and Methods. COMP correlated with DAS28-ESR at baseline.

Table 3

Change of variables in 45 RA patients after 6-month treatment with etanercept

Characteristics	Baseline	After 6 months	*P*
COMP(U/l [a])	12.0 ± 3.4	11.1 ± 3.0	< 0.05
CRP (mg/dl [a])	28.4 ± 33.4	7.1 ± 12.1	< 0.0001
ESR (mm/hr [a])	61.7 ± 36.1	32.8 ± 29.0	< 0.0001
MMP-3 (U/l [a])	253 ± 236	157 ± 194	< 0.001
Anti-CCP (U/ml [a])	163 ± 166	157 ± 163	NS
IgM-RF(U/ml [a])	279 ± 1030	218 ± 848	NS
DAS28-ESR [a]	5.86 ± 1.35	3.66 ± 1.28	< 0.0001
mHAQ [a]	0.81 ± 0.67	0.55 ± 0.60	< 0.01

[a] M±SD

As compared with baseline, COMP, CRP, ESR, MMP-3, DAS28-ESR and mHAQ were decreased

by etanercept at 6 months.

Table 4

Serum COMP was selectively decreased in remission compared with other serologic variables

	Remission group (N = 10)			No remission group (N = 35)		
	Baseline	After 6 months	*P*	Baseline	After 6 months	*P*
COMP(U/l [a])	12.6 ± 4.5	10.8 ± 3.6	< 0.05	11.6 ± 3.1	11.2 ± 2.9	NS
CRP (mg/l [a])	22.6 ± 33.2	0.7 ± 0.9	< 0.01	30.4±34.0	8.9 ± 13.2	< 0.001
ESR (mm/hr [a])	39.3 ± 30.6	10.0 ± 11.2	< 0.01	68.7±35.5	39.3 ± 29.4	< 0.001
MMP-3 (U/l [a])	197 ± 125	99 ± 58	< 0 .05	270 ± 259	174 ± 215	< 0.01
IgM-RF(U/ml [a])	178 ± 381	122 ± 273	NS	308±1154	245 ± 952	NS
Anti-CCP (U/ml [a])	150 ± 171	171 ± 224	NS	125 ± 161	113 ± 136	NS

[a] M±SD

After 6 months of treatment, the 45 patients were divided into 2 groups: 10 patients who achieved remission and 35 patients who did not. CRP, ESR and MMP-3 were decreased in a similar fashion regardless of remission; the decrement of COMP was found only in the remission group.

NS, not significant