COMP-C3b Complexes in Rheumatoid Arthritis with Severe Extraarticular Manifestations

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ABSTRACT. ***Objective.*** To investigate biomarker patterns in rheumatoid arthritis (RA) with extraarticular manifestations.

Methods. Cartilage oligomeric matrix protein (COMP), COMP-C3b, and soluble terminal complement complexes (sTCC) were measured by ELISA.

Results. COMP-C3b levels were higher in patients with RA than in healthy controls and lower in extraarticular RA (ExRA) than in RA controls. In patients with ExRA, sTCC levels were higher than in RA controls, and correlated inversely with serum COMP-C3b levels in the ExRA group.

Conclusion. Patients with ExRA had lower levels of COMP-C3b. This may be a consequence of complement consumption or a lower potential for COMP from these patients to activate complement. (J Rheumatol First Release Nov 1 2013; doi:10.3899/jrheum.130613)

Key Indexing Terms:
COMPLEMENT SYSTEM
CARTILAGE OLIGOMERIC MATRIX PROTEIN
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Rheumatoid arthritis (RA) is a systemic disease characterized by progressive joint damage, which may be complicated by extraarticular manifestations contributing to an increased mortality[1]. Severe extraarticular RA (ExRA) manifestations are accompanied by an increase in rheumatoid factor (RF), antibodies against cyclic citrullinated peptide (anti-CCP), and C-reactive protein (CRP)[2], and are associated with a more severe disease[3]. Such manifestations occur with an incidence of about 1/100 person-years in patients with RA[1]. In patients with RA in southern Sweden, the predominant ExRA manifestations were serositis and cutaneous vasculitis[4].

Activation of complement is closely associated with disease activity in RA[5] resulting in complement consumption during disease flares. Because extraarticular features in RA reflect a more aggressive disease, complement consumption is often more pronounced in patients with ExRA. Low C4 and CH50 levels, high complement consumption, and extensive vascular C3 deposition were reported in extraarticular RA[6,7,8,9], possibly due to the presence of circulating immune complexes[9,10]. In RA patients with vasculitis-related neuropathy, low C4 is a marker of poor prognosis[11]. Further, soluble terminal complement complexes (sTCC), the end-product of local or systemic complement activation, are elevated in pleural fluid from RA patients with pleuritis[12], and there is local complement consumption in RA-associated pericarditis[13]. Hypocomplementemia due to consumption was also described in Felty syndrome[14], where it may contribute to neutropenia and joint destruction[15]. In an inception cohort of patients with RA, those who subsequently developed severe ExRA manifestations had lower C4 levels at baseline, suggesting that complement activation is part of the early pathogenesis of ExRA[16].

Cartilage oligomeric matrix protein (COMP), an abundant component of the articular cartilage, is found at elevated levels in serum and synovial fluid of patients with RA[17], likely resulting from excessive cartilage breakdown. COMP activates complement and may propagate the inflam-

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matory cycle[18]. As an indication of COMP-induced complement activation *in vivo*, complexes between the complement activation product C3b and COMP were found in the serum of patients with RA and correlated with disease activity[19]. To further the understanding of these processes, we determined whether the levels of COMP-C3b differ between RA patients with and without extraarticular manifestations, and estimated the degree of complement activation in the patients by measuring serum levels of sTCC.

MATERIALS AND METHODS

Patients. Thirty-four consecutive patients from a single center, with recently diagnosed severe ExRA manifestations (cutaneous vasculitis, pericarditis, pleuritis, Felty syndrome and neuropathy) according to predefined criteria[1], were enrolled as described[2]. For each patient with ExRA, 2 control patients with RA but no evidence for current or previous ExRA, individually matched to extraarticular subjects for age, sex, and disease duration, were selected from a community-based research database[1]. Blood was drawn and physical examinations were performed directly after ExRA was diagnosed and before any new treatment was started. Serum was available from 34 ExRA cases and 67 RA controls, and EDTA-plasma from 24 ExRA cases and 65 RA controls. Further, 81 healthy individuals with no history of rheumatologic disease were used as a control group. All samples were collected in a standardized fashion (nonfasting) with informed consent. Samples were stored at –80°C after centrifugation. The study was approved by the Ethical Review Board at Lund University.

Measurement of antibodies and CRP. Anti-CCP was detected with ELISA (Eurodiagnostica; second-generation test;[2]). IgM RF[2] and high-sensitivity CRP were quantified using nephelometry (Beckman Image).

Measurement of COMP-C3b. Microtiterplates (Maxisorp, Nunc) were coated with a monoclonal antibody against COMP (12A11, homemade) at 5 µg/ml in 50 mM Hepes pH 7.4, 2 mM $CaCl_2$ or with buffer only overnight at 4°C. Between each step of the assay, the plates were washed with 50 mM Tris-HCl, 150 mM NaCl, 0.1% Tween-20, pH 8.0. The wells were blocked using 1% bovine serum albumin (BSA; Millipore) in 50 mM Hepes, pH 7.4 with 2 mM $CaCl_2$. Serum was diluted 1:70 in 50 mM Hepes pH 7.4, 150 mM NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$ with 50 µg/ml BSA, added to the wells and incubated for 2 h at RT. A biotinylated anti-C3 antibody (CC7761, Sigma) was diluted in blocking buffer and incubated in the wells for 1 h at room temperature followed by a streptavidin-HRP conjugate (21130, Pierce). The plates were developed with o-phenylenediamine substrate (Dako) and H_2O_2 and the absorbance at 490 nm was measured using a Cary 50 MPR microplate reader (Varian). Each sample was analyzed in duplicate and values obtained from the uncoated wells were subtracted from values obtained from antibody-coated wells. Obtained readings were then normalized against an internal control sample rendering data presented as arbitrary units. No interference of RF was seen in the assay.

Measurement of sTCC. ELISA with neoepitope-specific monoclonal antibodies was used to measure sTCC in plasma, recognizing activated C9 as the capture antibody and anti-C6 as the detection antibody, as described[20].

Statistical analysis. Differences in COMP-C3b, COMP, or sTCC levels between groups were evaluated using a Mann-Whitney U test or Kruskal-Wallis test with a Dunn's multiple comparison posttest. Two-measurement correlation analysis was performed according to Spearman.

RESULTS

COMP-C3b is lower in patients with ExRA compared to RA controls. Patients with ExRA had significantly higher disease activity/severity by all measures compared to RA controls (Table 1). Further, patients with ExRA were more

Table 1. Description of patients and controls.

	ExRA*	RA Controls	Healthy Controls
N	34	67	81
Male/female	13/21	29/38	25/56
Age, yrs, mean (SD)	64.6 (12.1)	63.9 (11.4)	49.4 (10.8)
Disease duration, yrs, mean (SD)	12.1 (11.6)	11.5 (9.7)	n/a
RF-positive	30/31 (97%)	45/65 (69%)	n/d
Anti-CCP-positive	25/31 (81%)	38/66 (58%)	n/d
Rheumatoid nodules**	15/32 (47%)	12/67 (18%)	n/a
HAQ, mean (SD)	1.32 (0.88)	0.72 (0.74)	n/d
Swollen joints, mean (SD)	7.1 (5.8)	2.9 (3.4)	n/d
Ritchie's index, mean (SD)	10.5 (8.4)	8.0 (7.4)	n/d
Current DMARD, n (%)	17 (50)	52 (72)	0
Current corticosteroids, n (%)	12 (35)	18 (27)	0
CRP, mg/L, median (IQR)	21.5 (9.0–52.5)	6.9 (2.2–14.0)	n/d
COMP-C3b, AU, median (IQR)	1.98 (1.49–2.65)	2.55 (2.02–3.29)	0.59 (0.49–0.81)
COMP, U/l, median (IQR)	8.85 (4.90–11.68)	8.80 (6.25–10.50)	7.02 (5.79–8.65)
sTCC, AU/ml, median (IQR)	3.00 (1.67–7.50)	2.40 (0.75–3.41)	0.01 (0–0.09)

* One or more of the following: major cutaneous vasculitis (n = 11; leg ulcers or purpura, verified by biopsy or dermatologist; nailfold lesions alone do not qualify), pericarditis (n = 9; symptomatic and verified by echocardiography), pleuritis (n = 8, symptomatic and verified by radiograph), Felty syndrome (n = 5; persistent neutropenia and splenomegaly), neuropathy (n = 4; symptomatic mononeuropathy/polyneuropathy, verified by electroneurography/electromyography). ** Current, or previous, based on medical records. COMP: cartilage oligomeric matrix protein; RA: rheumatoid arthritis; ExRA: extraarticular RA; AU: arbitrary units; CRP: C-reactive protein; HAQ: Health Assessment Questionnaire; n/a: not applicable; n/d: not determined; RF: rheumatoid factor; anti-CCP: antibodies against cyclic citrullinated peptide; DMARD: disease-modifying antirheumatic drug; sTCC: soluble terminal complement complex; IQR: interquartile range.

often positive for anti-CCP and RF. Compared with RA controls, fewer patients with ExRA were currently treated with disease-modifying antirheumatic drugs (Table 1). Confirming earlier results, COMP-C3b complexes were significantly elevated in all patients with RA combined compared to healthy controls (Figure 1A). Interestingly, patients with ExRA had significantly lower COMP-C3b levels than RA control patients (Figure 1B). Serum COMP levels were slightly but significantly elevated in all patients with RA combined compared to healthy controls (Figure 1C); however, there was no difference in the COMP levels between the ExRA and the RA control groups (Figure 1D). As observed in other patient cohorts[18,19], there was no correlation between the COMP-C3b and the COMP levels in either patients with ExRA (r_s = 0.12, p = 0.51) or RA controls (r_s = –0.18, p = 0.15), indicating that only a subset of the liberated COMP has the capacity to activate complement. Serum COMP-C3b levels did not correlate with anti-CCP or RF levels in either of the RA groups, and there was no difference in COMP-C3b between RF-positive and RF-negative patients, or between patients with versus without rheumatoid nodules (not shown). In contrast to

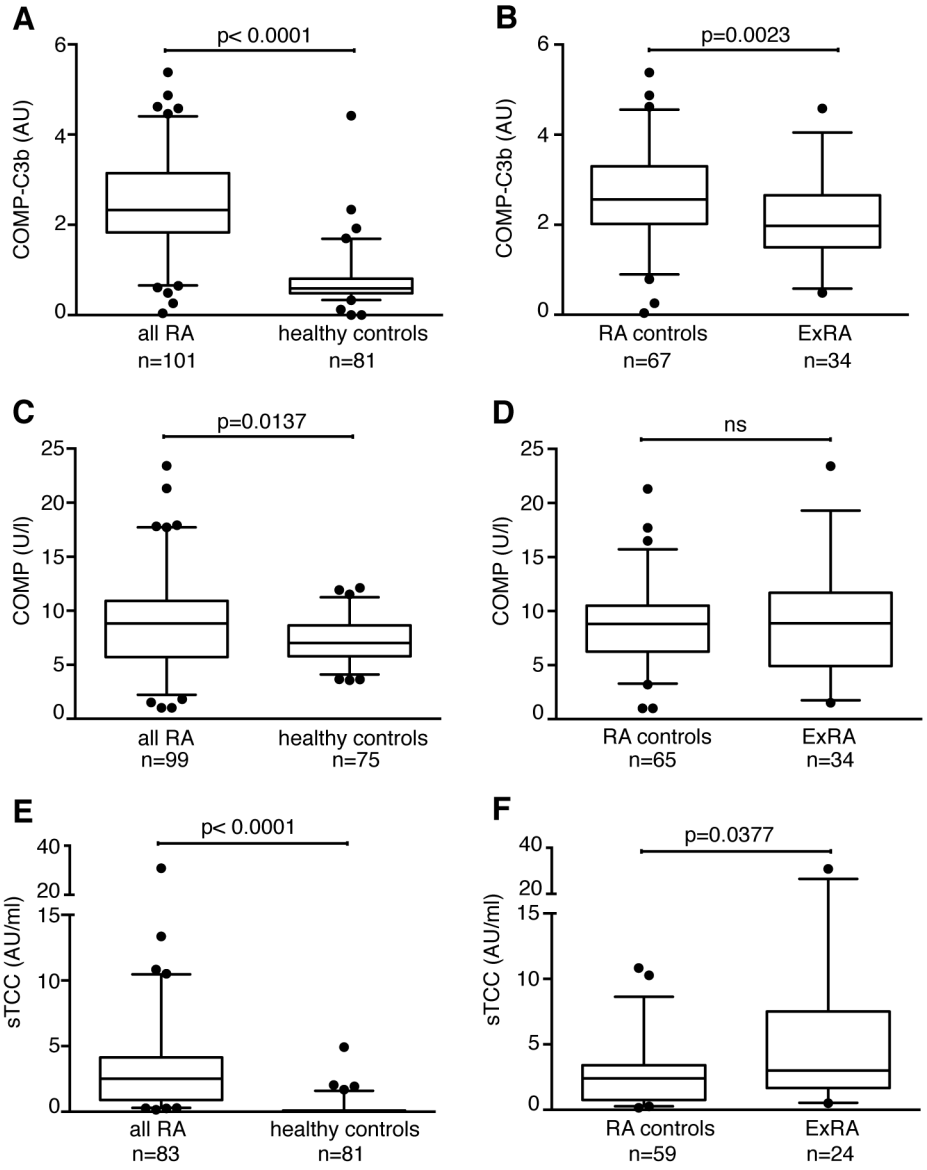

Figure 1. COMP-C3b, COMP, and sTCC in patients and controls. Serum levels of COMP-C3b (A and B) or COMP (C and D) were measured in healthy controls, RA controls with uncomplicated disease, or in RA patients with extraarticular disease (ExRA). Levels of sTCC (E and F) were measured in plasma. The statistical significance of differences between the groups was measured using a Mann-Whitney U test. COMP: cartilage oligomeric matrix protein; sTCC: soluble terminal complement complexes; RA: rheumatoid arthritis; ns: not significant.

patient cohorts analyzed in an earlier study[19], there was no correlation between COMP-C3b and CRP in the currently analyzed RA cohort (r_s = 0.07, p = 0.72 for ExRA patients; r_s = 0.01, p = 0.91 for RA controls). Further, the swollen joint count and Ritchie Index did not correlate with COMP-C3b in ExRA cases or RA controls (not shown).

COMP-C3b levels are lower in patients with complement consumption. Since COMP-C3b levels in serum were previously correlated to inflammatory measures and reflected disease activity in RA, we investigated whether the surprising finding of lower COMP-C3b levels seen in the ExRA group was related to increased complement consumption and therefore a decreased amount of available complement. As a measure of complement activation whether local or systemic, we determined sTCC in plasma.

Healthy controls had mostly undetectable levels of sTCC in plasma, whereas such complexes were present in most patients with RA (Figure 1E). Patients with ExRA had higher sTCC levels than RA controls, indicating a more substantial complement activation (Figure 1F). This could lead to reduced levels of complement, which in turn systemically decreases the potential to form COMP-C3b complexes once COMP is liberated into the circulation. This is supported by the finding that sTCC levels correlated inversely with serum COMP-C3b levels in the ExRA group (r_s = –0.45, p = 0.029), whereas there was no significant correlation in the RA control group (r_s = 0.15, p = 0.26). However, serum COMP correlated positively with sTCC in the RA control group (r_s = 0.41, p = 0.002), but no correlation was seen in the ExRA group (r_s = –0.24, p = 0.25).

Because of the small sample size, power was limited for analyses of individual manifestations. However, individuals with cutaneous vasculitis and/or vasculitis-related neuropathy combined had lower COMP-C3b levels and higher sTCC levels compared to RA controls, whereas there was no significant difference in COMP levels (Figure 2A-C). Because patients with RA who present with non-mechanical mononeuropathy or polyneuropathy often have underlying necrotizing vasculitis[11], there is a rationale for pooling these with cases of cutaneous vasculitis.

DISCUSSION

We found that RA patients with extraarticular manifestations have lower amounts of circulating COMP-C3b, possibly because of higher complement consumption. The

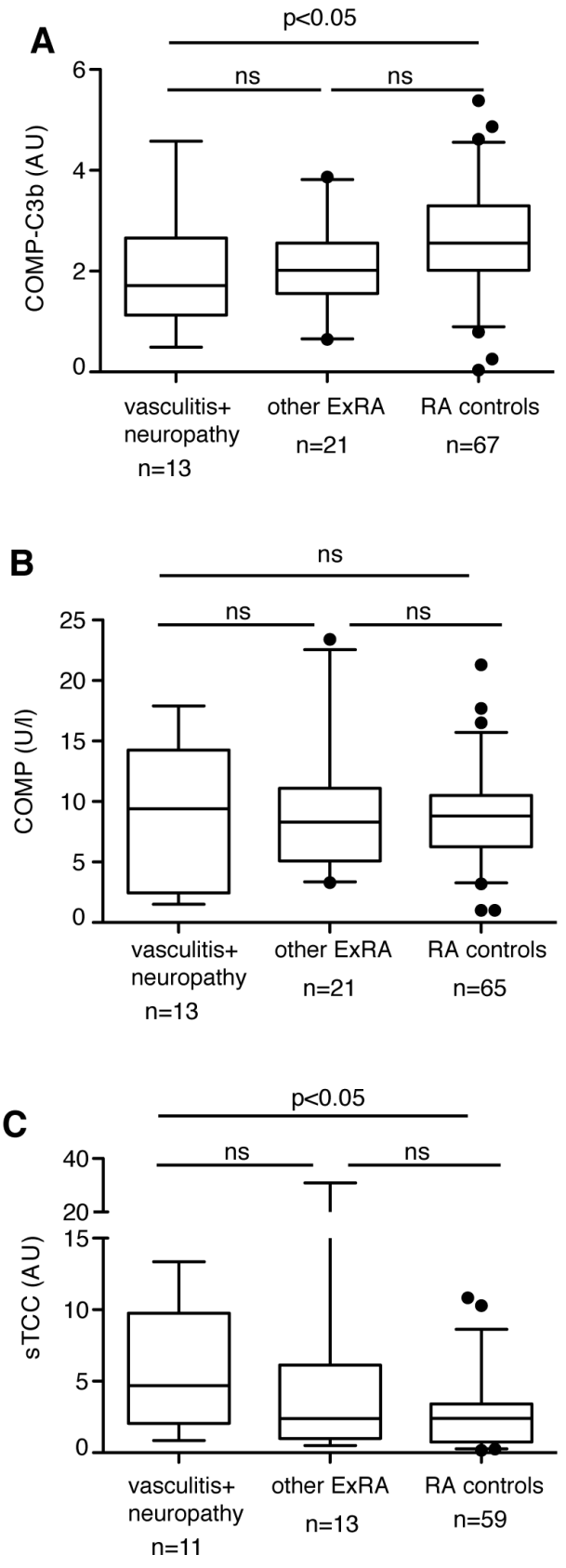

Figure 2. COMP-C3b is decreased while sTCC is increased in RA-associated vasculitis and neuropathy. Serum levels of COMP-C3b (A) and COMP (B) and plasma levels of sTCC (C) were compared in RA patients with vasculitis and/or neuropathy combined, in patients with other types of ExRA, and in RA controls. Statistical significance of differences between the groups was measured using a Kruskal-Wallis test with a Dunn's multiple comparison posttest. COMP: cartilage oligomeric matrix protein; sTCC: soluble terminal complement complexes; RA: rheumatoid arthritis; ExRA: extraarticular RA; ns: not significant; AU: arbitrary units.

results also support the concept that complement activation is particularly important in patients with RA-associated severe vasculitis.

In the current patient cohort we did not see the previously observed correlation between COMP-C3b and CRP. This discrepancy should be interpreted with caution, owing to the unusual case population in the present study and the limited sample size. In the previous study the correlation was significant but weak[19], and can most likely only be observed in a larger patient cohort.

The lower levels of COMP-C3b in ExRA might in addition to complement consumption be a reflection of different complement-activating properties of COMP in ExRA compared to uncomplicated RA. This might be due to altered protease activity within the joint related to more severe inflammation in ExRA. In addition, complexes of smaller COMP-fragments and C3b might not be detected in the sandwich ELISA, which is based on monoclonal antibodies recognizing only 1 epitope in COMP. We found that while COMP correlated positively with sTCC in the RA control group, no such correlation was found in the ExRA group. One explanation for this discrepancy is that the sTCC found in serum in uncomplicated RA might be related mainly to the ongoing joint-inflammation releasing COMP, whereas in ExRA the sTCC may be formed as a consequence of inflammation in extraarticular tissues.

COMP-C3b complexes are lower in patients with RA displaying severe extraarticular manifestations than in RA control patients, which might be a consequence of fewer complement components available or a lower complement-activating potential of COMP from patients with ExRA.

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