Relation of Doppler ultrasound synovitis *versus* clinical synovitis with changes in native complement component levels in rheumatoid arthritis patients treated with biologic disease-modifying anti-rheumatic drugs

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Abstract

Objective

The complement system plays a fundamental role in mediating the activity of rheumatoid arthritis (RA). Biologic therapy can reduce native complement component levels and its activation. We aimed to study the relation of Doppler ultrasound (US) synovitis versus clinical synovitis with changes in native complement component levels in RA patients on biologic therapy.

Methods

This was a cross-sectional study. Ninety-seven consecutive patients with RA on biologic therapy for at least 3 months were recruited. Clinical, laboratory and Doppler US assessments were performed. The Disease Activity Score in 28 joints (DAS28), Simplified Disease Activity Index (SDAI) and a 12-joint US assessment were carried out. Synovitis was semiquantitatively scored in B-mode and power Doppler (PD) mode.

Results

A significant decrease in native complement (i.e. C3 and C4) and C-reactive protein (CRP) levels was observed. This was highly significant for C3 decrease ($p<0.0005$), and C4 decrease ($p<0.0005$). Synovitis detected by PD US showed significant negative association with C3 change ($p<0.008$), where patients with higher C3 change were more likely to have PD US inactive status on assessment.

Conclusion

Our results suggested that disease inactive status determined by PD US but not by clinical assessment can be related with decrease in complement in RA patients treated with biologic therapy.

Key words

ultrasound, Doppler, complement, rheumatoid arthritis, biologic therapy

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Introduction

The complement system not only forms part of the innate immunity but also contributes to modulating the adaptive responses (1). There is evidence suggesting increased level of complement as acute phase reactant mediated by proinflammatory cytokines important in active rheumatoid arthritis (RA) such as interleukin 1 (IL-1), interleukin 6 (IL-6) and tumour necrosis factor-α (TNF-α) (2-6). Activation of the complement system, by immune complexes or C-reactive protein (CRP), plays an important role in inflammatory activity of RA as shown by significant level of complement degradation products found in the synovial fluid of affected joints in RA (7, 8).

Hietala *et al*. demonstrated in animal model of RA that mice deficient of complement factor C3 were less likely to develop collagen-induced arthritis in comparison to control mice (9). To the best of our knowledge, only one study dealing with native complement levels and clinical response to biologic therapy has been published (10). This study reported that patients with higher baseline native C3 level and lower reduction after 22 weeks of treatment with anti-tumour necrosis factor (anti-TNF) showed poorer European League Against Rheumatism (EULAR) response, in comparison to those with lower baseline level and greater reduction. Biologic therapy may act by reducing native complement levels via reduction of proinflammatory cytokines. Analysis of our cohort of patients on tocilizumab therapy showed reduction of complement levels (*i.e*. C3 and C4) after this treatment and these levels plateau after 3 months ($p<0.05$) (personal communication, Maria Montoro Alvarez, Hospital General Universitario Gregorio Marañón, Madrid, Spain).

Musculoskeletal ultrasound (US) is becoming widely used in this era of RA and it is able to detect synovitis on B-mode and Doppler mode (11-13). There is solid evidence demonstrating that this technique is more sensitive than clinical examination in detecting synovitis (14-18). Pathologic blood flow detected by Doppler US has shown predictive value in relation to structural damage progression in both active RA and RA in clinical remission (19, 20). In addition, Doppler US-detected synovitis has also proven predictive value in relation to flare or relapse in RA patients in clinical remission (21, 22). This study aimed to evaluate the relation of Doppler US assessment *versus* clinical assessment of synovitis with changes in native complement component levels in RA patients on biologic therapy.

Methods

Patients and study protocol

Ninety-seven patients (87 women and 10 men) who attended our intravenous biologic daycare unit and subcutaneous biologic clinic from May 2013 to August 2013 were recruited consecutively for this cross sectional study. All patients fulfilled the American College of Rheumatology (ACR) 1987 Revised Classification Criteria for RA and had been on biologic therapy for at least 3 months. The biologic agent doses received by the patients were as per standard recommendations. Thirty-seven (38.1%) patients were treated with tocilizumab, 24 (24.7%) with abatacept, 13 (13.4%) with infliximab, 10 (10.3%) with etanercept, 5 (5.2%) with adalimumab, 3 (3.1%) with golimumab and 5 (5.2%) with certolizumab. This study was conducted in accordance with the Declaration of Helsinki 2008 and was approved by the local ethics committees (Hospital General Universitario Gregorio Marañón, Madrid, Spain). Informed consent was obtained from all patients prior to study entry.

Clinical and laboratory assessment

The following data were recorded from our Biologic Therapy Unit database: age, sex, duration of RA, rheumatoid factor (RF) status (normal 0–25 IU/mL), anti-citrullinated protein antibody (ACPA) status (normal 0–25 U/mL), medications received (synthetic and biologic disease-modifying anti-rheumatic drugs [DMARDs], oral corticosteroids and non-steroidal anti-inflammatory drugs [NSAIDS]), and serum complement (*i.e*. C3 [normal 91–190 mg/dL] and C4 [normal 18–56 mg/dL]) and CRP levels (normal 0–0.8 mg/dL) before starting current biologic agent.

Each patient was evaluated by a rheuma-

tologist (MMA) for DAS28-erythrocyte sedimentation rate (ESR) and Simplified Disease Activity Index (SDAI) composite scores on assessment. RA clinical activity was defined as DAS28-ESR >2.6 or SDAI >3.3. Blood tests were also obtained on the same day for serum complement (*i.e.* C3 and C4), ESR (normal 2–20 mm/hour) and CRP levels.

Doppler US assessment

Doppler US assessment was carried out with multifrequency (7–12 MHz) linear array transducers (eSaote, MyLab70, Italy). Systematic 12-joint (*i.e.* bilateral 2nd and 3rd metacarphophalangeal, wrists, elbows, knees and ankles joints) US assessment with both B-mode (*i.e.* grey-scale) and power Doppler (PD) mode according to a previously published study (23) was performed by two rheumatologists (O.Y. Chong, I. Janta) experienced in musculoskeletal US. Both rheumatologist scanned together and scored in consensus both B mode and PD synovitis, in each examined joint. Outcome Measures in Rheumatology Clinical Trials (OMERACT) definitions for synovitis components were used (24). Synovitis was semiquantitatively scored by both together in B-mode (*i.e.* synovial hyperthrophy and synovial fluid together) and PD mode (*i.e.* Doppler signal within synovial hyperthrophy) on a scale of 0 to 3 by consensus (23, 25). These rheumatologists were particularly trained in scoring synovitis by a rheumatologist with long experience in US and involvement in the OMERACT Musculoskeletal US group (EN). They were blinded to both clinical and laboratory data, and patients were asked not to reveal their symptoms to the sonographers. A global score for B-mode and PD mode synovitis were calculated for each patient by the sum of the B-mode and PD mode scores, respectively, from each individual joint assessed. PD US activity was defined as a global PD score >0.

Statistical analysis

The analysis was performed using SPSS for Windows version 15.0 (SPSS, Chicago, IL, USA). Data were given as mean and standard deviation in cases of normal distribution, and median and range in cases of non-normal distribution. Student *t*-test for paired samples was performed to demonstrate change in laboratory data. Fisher's test was used to assess association between dichotomised variables. Correlations between laboratory and clinical data were analysed by the Pearson's correlation coefficient. Correlation was considered weak if the correlation coefficient was ≤0.3, moderate if 0.3–0.7 and a strong if >0.7. $p<0.05$ was considered significant.

Results

Patient characteristics

The mean age ±SD of the patients was 57.3±13.3 years (22–87 years), mean disease duration ±SD was 19.2±24.6 years (0.3–33 years) and mean duration ±SD on current biologic DMARDs was 28.9±32.7 months (3–144 months). RF was positive in 53 patients (54.6%) and ACPA was positive in 72 patients (74.2%). Prior to current biologic DMARDs, 51 patients (52.6%) were biologic naïve.

Twenty-eight patients (28.9%) were on oral corticosteroids and 22 patients (22.7%) were on NSAIDs. All patients were either on one synthetic DMARD, 62 patients (63.9%), or none at all, 35 patients (36.1%). Forty-three patients (44.3%) were on methotrexate, 14 (14.4%) on leflunomide, 2 (2.1%) on hydroxychloroquine and 3 (3.1%) on azathioprine.

Clinical, laboratory and US findings

Ninety-four patients and 95 patients had complete data for DAS28(3)-ESR and SDAI composite score, respectively. There were 49 (52.1%) patients with DAS28-ESR ≤2.6, 45 (47.9%) patients with DAS28-ESR >2.6, 9 (9.5%) patients with SDAI ≤3.3 and 86 (90.5%) patients with SDAI >3.3. Four patients (4.1%) had global B-mode score=0.93 patients (95.9%) had global B-mode score >0. Fifty-three patients (54.6%) had global PD score=0 and 44 patients (45.4%) had global PD score >0. The mean±SD DAS28-ESR was 2.7±1.1 (0.5–6.1), mean SDAI±SD was 12.7±8.6 (0.1–52.7), mean global BM score±SD was 6.5±4.7 (0.0–20.0) and mean global PD score±SD was 1.6±2.5 (0.0–14.0).

On assessment, the mean C3±SD was 94.2±22.5 (48.0–159.0), mean C4± SD was 18.1±7.2 (1.7–40.7) and mean CRP±SD was 0.5±1.1 (0.1–7.6). Out of the total of 97 patients, 72 (74.2%) had decreased C3 and 58 (59.8%) had decreased C4. Eighty-seven patients had both CRP levels before and on assessment of which 66 (75.9%) of them had decreased CRP.

The decrease in the complement and CRP levels, after at least 3 months on biologic therapy, were significant as shown in Table I. Particularly, these were highly significant ($p<0.005$) for C3 and C4.

Table II shows the association between the changes in complement and CRP levels (categorised according the median value) and the clinical and PD US activity status. The C3 change showed a negative association with the disease activity determined by US PD ($p=0.008$). A significantly greater percentage of patients with PD US-determined inactive status (68.8%) was found in patients with higher C3 decrease than in those with less reduction of the C3 level (40.8%). By contrast, the C3 change

Table. I. Mean±SD for change and decrease in C3, C4 and CRP levels.

Parameters (mg/dL)	Mean±SD	Min	Max	*p*-value
C3 change (97 patients)	-16.2 ± 24.5	-105.1	62.1	<0.0005
C3 decrease (72 patients)	-25.6 ± 20.2	-105.1	-0.6	<0.0005
C4 change (97 patients)	-1.9 ± 6.6	-18.1	16.3	0.005
C4 decrease (58 patients)	-6.0 ± 4.7	-18.1	-0.2	<0.0005
CRP change (62 patients)	-1.2 ± 3.7	-20.8	4.0	0.003
CRP decrease (66 patients)	-1.9 ± 4.2	- 20.8	-0.01	0.001

SD: standard deviation; C3: complement 3; C4: complement 4; CRP: C-reactive protein; min: minimum; max: maximum.

Table II. Association between the changes in laboratory parameters and US PD activity.

Parameters (mg/dL)	DAS28-ESR ≤2.6 n (%)	DAS28-ESR >2.6 n (%)	*p*-value	SDAI ≤3.3 n (%)	SDAI >3.3 n (%)	*p*-value	Global PD=0 n (%)	Global PD >0 n (%)	*p*-value
C3 change									
≤ -11.8	30 (63.8)	17 (36.2)	0.038	3 (6.4)	44 (93.6)	0.486	20 (40.8)	29 (59.2)	0.008
> -11.8	19 (40.4)	28 (59.6)		6 (12.5)	42 (87.5)		33 (68.8)	15 (31.3)	
C4 change									
≤ -1.3	29 (60.4)	19 (39.6)	0.148	3 (6.3)	45 (93.8)	0.317	25 (51.0)	24 (49.0)	0.543
> -1.3	20 (43.5)	26 (56.5)		6 (12.8)	41 (87.2)		28 (58.3)	20 (41.7)	
CRP change									
≤ -0.15	24 (55.8)	19 (44.2)	0.388	3 (7.0)	40 (93.0)	1.000	24 (54.5)	20 (45.5)	0.830
>-0.15	19 (45.2)	23 (54.8)		4 (9.3)	39 (90.7)		25 (58.1)	18 (41.9)	

C3: complement 3; C4: complement 4; CRP: C-reactive protein; DAS28-ESR: Disease Activity Score (28)-erythrocyte sedimentation rate; SDAI: Simplified Disease Activity Index; PD: power Doppler.

Table III. Correlations between complement levels, CRP levels and their changes with clinical and PD US disease activity.

Parameters (mg/dL)	DAS28-ESR	SDAI	Global BM	Global PD
C3	0.26*	-0.04	-0.09	-0.02
C3 change	0.12	-0.01	-0.08	-0.04
C4	0.21*	-0.16	-0.13	-0.07
C4 change	0.21*	-0.011	-0.03	-0.04
CRP	0.39*	0.40*	0.09	0.19
CRP change	0.18	0.19	0.01	-0.01

*$p<0.05$. C3: complement 3; C4: complement 4; CRP: C-reactive protein; DAS28-ESR: Disease Activity Score (28)-erythrocyte sedimentation rate; SDAI: Simplified Disease Activity Index; BM: B-mode synovitis; PD: power Doppler synovitis.

showed a positive association with the disease activity determined by DAS28-ESR (p=0.038).

Table III displays the correlations between the complement and CRP levels on assessment and their changes and the clinical and PD US scores of disease activity. There was a significant weak positive correlation between C3 and C4 levels on assessment and DAS28-ESR, and also between C4 change and DAS28-ESR. A significant moderate positive correlation was found between CRP on assessment and both clinical scores. No significant correlation was found between the laboratory parameters and the PD US scores.

Representative ultrasound images of B-mode synovitis and synovial Doppler signal of the metacarpophalangeal and elbow joints are shown in Figures 1 and 2.

Discussion

Musculoskeletal US has emerged as an essential bedside tool for rheumatologists in diagnosing and monitoring joint inflammation in patients with RA (11-13, 19, 20). Our study recruited RA patients who have been on biologic DMARDs for at least 3 months and we focused on the relation of PD US with the change in native complement component levels.

Discrepancy in disease activity status occurred when different clinical and Doppler US activity scores were used. (*e.g.* 52% of our patients were in clinical remission when DAS28-ESR was used but only 9.5% of them were in clinical remission according to SDAI remission criterion). Similarly, with US B-mode and PD mode, remission was found in a low percentage and around half of the patients, respectively. These differences were expected and in agreement with previously published studies (19, 20, 22, 26, 27).

Biologic DMARDs received by our patients were able to significantly reduce acute phase reactants (*i.e.* C3, C4 and CRP) involved in active RA. The highly significant decrease and change in C3 (p<0.0005) levels highlight the dominant role of C3 with regard to inflammation in active RA as described in animal models (9).

Our most important result was the negative association found between PD US activity and C3 change, illustrating that patients with PD US remission status were more likely to have greater reduction of C3 level during biologic therapy. However, regarding clinical measures of disease activity, we only found controversial data such a positive association between C3 change and DAS28-ESR and a positive correlation between C4 change and DAS28-ESR. These findings support the capability of Doppler US to detect active joint inflammation in RA and that information on synovitis provided by Doppler US is different to that provided by clinical assessment. Nevertheless, the contradictory relations between DAS28-ESR and C3 and C4 changes could be attributed to limitation of this study, mainly the variability in the current and previous biologic therapy and concomitant treatment and the duration of these therapies.

Conclusion

In conclusion, this is the first study that suggests a link between Doppler US and the complement system involved in RA. This finding may encourage further translational research on Doppler US and the native complement components in relation to biologic therapy in RA patients.

Key messages

- C3 has an important role in joint inflammation in RA and its level can be reduced with biologic therapy.
- Doppler-determined inactive status was associated with decrease in C3 in RA patients on biologic therapy.
- This link suggests further research is needed on Doppler US and complement system in RA.

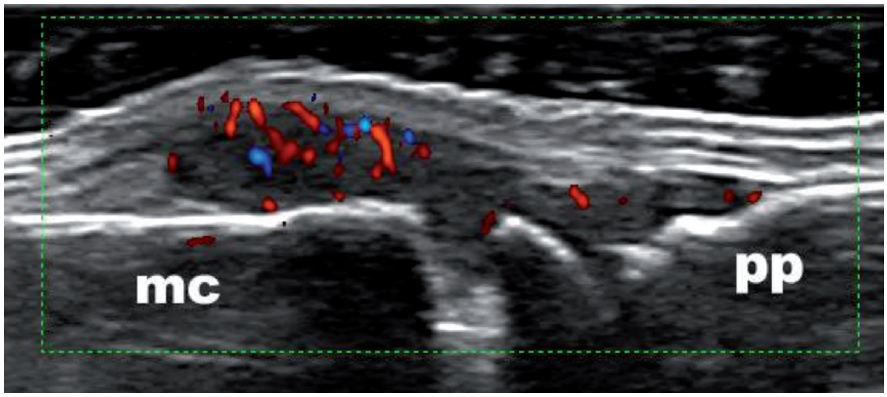

Fig. 1. Longitudinal ultrasound image of the dorsal aspect of a metacarphophalangeal joint showing B-mode synovitis and synovial Doppler signal. **mc**: metacarpal bone; **pp:** proximal phalanx.

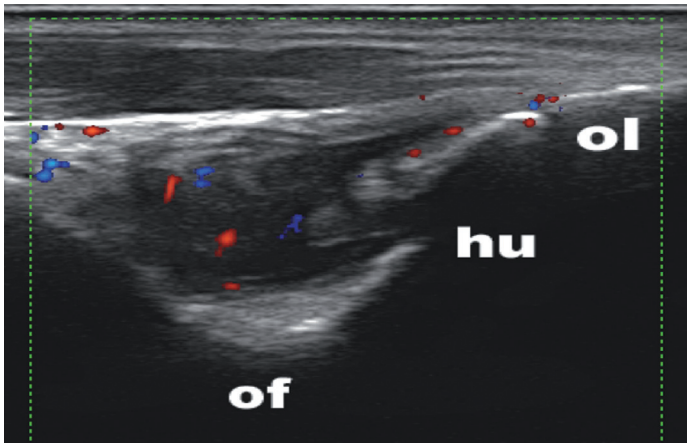

Fig. 2. Longitudinal ultrasound image of the posterior recess of the elbow showing B-mode synovitis and synovial Doppler signal of the olecranon fossa.
of: olecranon fossa;
hu: humerus;
ol: olecranon.

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