IgM-Rheumatoid Factor, Anti-Cyclic Citrullinated Peptide, and Anti-Citrullinated Human Fibrinogen Antibodies Decrease During Treatment with the Tumor Necrosis Factor Blocker Infliximab in Patients with Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** To investigate the effect of treatment with infliximab on serum levels of rheumatoid factor (IgM-RF), antibodies against cyclic citrullinated peptide (anti-CCP), and antibodies against deiminated human fibrinogen, a specific citrullinated peptide (ACF), and their association with disease activity and disease duration in patients with rheumatoid arthritis (RA).

Methods. The study sample included 62 consecutive patients who were treated with infliximab for at least one year. IgM-RF, anti-CCP, and ACF were measured at 0, 14, 30, and 46 weeks.

Results. Patients had a mean age of 54 years and median disease duration of 10 years and were predominantly female (81%). At baseline 63%, 77%, and 82% of patients were positive for IgM-RF, anti-CCP, and ACF, respectively. In terms of percentages, the levels of IgM-RF were reduced by 64% at 46 weeks, while anti-CCP and ACF levels were reduced by roughly 25%. The decrease in serum levels of these autoantibodies was not associated with the decrease in disease activity. The change in ACF was significantly related to disease duration, while the changes in IgM-RF or anti-CCP were not.

Conclusion. In a cohort of patients with RA who responded to infliximab therapy, all autoantibodies decreased significantly, but IgM-RF showed a larger decrease than anti-CCP or ACF. These changes in levels of autoantibodies are not directly related to the change in disease activity. Early in the disease, ACF levels were best influenced by treatment with infliximab. (J Rheumatol 2008; 35:425–8)

Key Indexing Terms:
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Rheumatoid arthritis is a chronic polyarticular inflammatory disease that may lead to cartilage destruction and bone erosions. One of the characteristics of the disease is the presence of autoantibodies. Rheumatoid factor (IgM-RF) is an antibody that targets the Fc fragment of IgG. IgM-RF is observed in about 75% of patients with RA, but it is also frequently observed in other inflammatory diseases[1,2]. Antibodies against cyclic citrullinated peptide (anti-CCP) target multiple citrullinated proteins and are highly specific for RA[3-5]. The experimental first-arthritis model used fibrin injections[6]. Antibodies against deiminated fibrinogen, a specific citrullinated peptide (ACF), previously described as filaggrin antibodies, are as sensitive and specific for RA as the anti-CCP antibodies[7]. Deiminated fibrinogen has been detected in inflamed joints of patients with RA, but also in non-RA inflamed joints[7,8]. This could suggest that the antibody response against deiminated fibrinogen, but not the target, is specific for RA. These findings and their presence in early and even preclinical disease[9] suggest a role for anti-CCP and ACF antibodies in the pathogenesis of RA.

Anti-CCP and ACF appear to provide a useful diagnostic tool and are predictive of disease progression and radiological damage[10,11]. Thus, it is important to understand potential factors influencing these antibody responses. Several studies have described a decrease in serum concentration of

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IgM-RF after use of disease modifying therapies[12-18]. Only a few studies have examined the effect of aggressive RA treatment [anti-tumor necrosis factor (TNF)] on the level of anti-CCP antibodies, and these studies show conflicting results: 4 studies showed a decrease whereas 2 studies showed no change in anti-CCP titers during anti-TNF treatment[12-18].

Because of these conflicting results and because, to our knowledge, the effect of treatment on serum levels of ACF antibodies has not been described, we investigated changes in IgM-RF, anti-CCP, and ACF in a cohort of patients with RA treated for one year with infliximab.

MATERIALS AND METHODS

Patients. Consecutive patients with RA who were treated with infliximab for at least one year were studied. All patients fulfilled the American Rheumatism Association (American College of Rheumatology) criteria for RA[19], had active disease [Disease Activity Score of 28 joints (DAS28) ≥ 3.2], and had previously failed at least 2 disease modifying antirheumatic drugs (DMARD) including methotrexate. Previous infection with tuberculosis or cardiac failure (New York Heart Association class III and IV) were contraindications for treatment with infliximab. In total, 80 patients were included into the cohort, but 18 patients dropped out within the first year of treatment, 13 because of nonresponse to treatment and 3 because of side effects, and 2 patients died due to infliximab unrelated events. Thus, 62 patients were eligible for analyses. Baseline characteristics of the dropout patients were comparable to the patients included in the study (data not shown).

Methods. Infusions with infliximab were given at 0, 2, 6, and 14 weeks and subsequently with an 8-week interval. Infliximab was administered intravenously in a starting dose of 3 mg/kg. In patients with inadequate response as judged by the patient's rheumatologist the dosage of infliximab could be increased to 7.5 mg/kg.

Demographic data. The demographic data collected at baseline were recorded from medical history and patients' medical records. The following variables were collected: age, sex, disease duration, presence or absence of bony erosions, IgM-RF status (positive if IgM-RF > 30 u/l), current and previous use of DMARD and corticosteroids. Disease activity was measured at each visit using the DAS28 score[17].

IgM-RF, CCP, and ACF. Serum was collected on the morning before each infusion and stored immediately at –20°C or lower until analyses. A total of 62 patients had serum available for evaluation. IgM-RF, anti-CCP, and ACF were measured at 0, 14, 30, and 46 weeks. Samples for IgM-RF, anti-CCP, and ACF were considered positive when above the cutoff values of 30, 50, and 150 U/ml, respectively. Anti-CCP and IgM-RF were measured at the Jan van Breemen Institute, while ACF was measured at Sanquin Research, Amsterdam. IgM-RF was measured using an in-house ELISA[10]. The anti-CCP level was measured using the anti-CCP-2 ELISA according to the manufacturer's recommendations (Axis-Shield). ACF was measured as described[11]. Briefly, microtiter plates were incubated 1 h with 10 µg/ml deiminated fibrinogen *in vitro* at room temperature. After 5 washes with phosphate buffered saline (PBS)/0.2% Tween, plates were incubated with serum samples diluted 1:50 in PBS, 0.05% (vol/vol) Tween-20, 0.2% gelatine (PTG) and were incubated for 1 h. All assays were done in duplicate. After 5 more washes plates were incubated with a horseradish peroxidase (HRP) labeled mouse monoclonal antibody to IgG for 1 h. The plates were developed with tetramethyl benzidine (TMB) in 0.11 M NaAc and H_2O_2, and the reaction was stopped with H_2SO_4. The plates were read at 540 nm wavelength.

Statistical analysis. We analyzed only the patients positive for each test.

A Mann-Whitney test was used to compare changes in RF, anti-CCP, and ACF between the different timepoints.

As distributions of RF, anti-CCP, and ACF were not normal, a log-transformation was performed before statistical analyses. Then, generalized estimating equation (GEE) analyses were performed. GEE is a regression analysis for longitudinal data modulating the course of the independent variables and dependent variables over time. GEE were used to investigate the course of these autoantibodies over time during treatment with infliximab and their longitudinal relationship with disease activity, DAS, CRP, and ESR, and with disease duration. To analyze the influence of the effect of disease duration on change in autoantibodies we calculated an interaction-term: disease duration × timepoint.

These analyses were performed using Stata version 7. A p value < 0.05 was considered statistically significant.

RESULTS

In total, 62 patients were included into the study: 50 (81%) women with a mean age of 54 years and median disease duration of 10 years (Table 1). The mean DAS28 decreased from 5.4 (1.3) to 3.7 (1.6) at 14 weeks and 3.6 (1.5) at 30 and 46 weeks. Median ESR decreased from 27 mm/h (2–85) at baseline to 16 mm/h (3–105) at 46 weeks, and the median CRP from 11 mg/l (0–175) at baseline to 5 mg/l (0–178) at 46 weeks.

IgM-RF, anti-CCP, and ACF. At baseline, 63%, 77%, and 82% of the patients were positive for IgM-RF, anti-CCP, and ACF, respectively. All serum markers decreased significantly from baseline during 1-year treatment with infliximab (Table 2).

In terms of percentages, levels of IgM-RF were reduced by 64% at 46 weeks and anti-CCP and ACF levels were reduced by roughly 25% (Figure 1). The percentage decrease in IgM-RF was statistically greater than the decrease in anti-CCP and ACF ($p < 0.001$).

Nineteen of the 39 patients (49%) who were positive for

Table 1. Baseline characteristics of the 62 patients.

Characteristic	Data
Demography	
Age, yrs, mean (SD)	54 (12)
Sex female, n (%)	50 (81)
Disease duration, yrs, median (range)	10 (1–49)
Erosive disease, n (%)	57 (91)
Use of corticosteroids, n (%)	16 (25)
Use of methotrexate, n (%)	56 (90)
Disease activity	
DAS-28, mean (SD)	5.4 (1.3)
ESR, mm/h, median (range)	27 (2–85)
CRP, mg/l, median (range)	11 (0–175)
Serum markers	
IgM-RF, U/ml, median (range)	53 (5–804)
Positive, n (%)	39 (63)
Anti-CCP, U/ml, median (range)	500 (0–16640)
Positive, n (%)	48 (77)
ACF, U/ml, median (range)	616 (63–13463)
Positive, n (%)	51 (82)

DAS: Disease Activity Score; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; CCP: cyclic citrullinated peptide; ACF: anti-citrullinated human fibrinogen.

Table 2. Changes in IgM-RF (n = 39), anti-CCP (n = 48), and ACF (n = 51) during one-year treatment with infliximab. Data are median (range).

	Baseline	14 Weeks	30 Weeks	46 Weeks
IgM-RF, U/ml	110 (34–804)	62 (13–627)*	51 (7–490)*	38 (8–465)*
Anti-CCP, U/ml	1232 (83 –16640)	1220 (42–10500)	1050 (26–12920)*	960 (30–11880)*
ACF, U/ml	754 (163–13463)	687 (66–13097)*	652 (69–10289)*	539 (58–13783)*

* $p < 0.01$ vs baseline calculated using generalized estimating equations. RF: rheumatoid factor; CCP: cyclic citrullinated peptide; ACF: anti-citrullinated human fibrinogen.

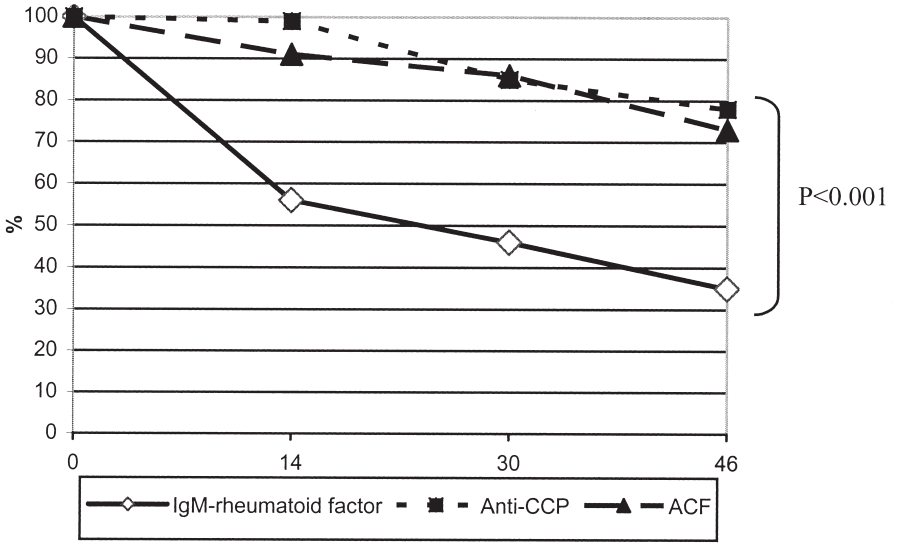

Figure 1. Percentage changes from baseline in serum concentrations of IgM-RF, anti-CCP, and ACF at 0, 14, 30, and 46 weeks during one-year treatment with infliximab.

IgM-RF at baseline had a negative IgM-RF titer at 46 weeks. Only one of the 48 anti-CCP-positive patients (2%) and 3 of the 51 ACF-positive patients (6%) had a negative titer at 46 weeks.

There was no difference in change in autoantibodies between patients treated with different doses of infliximab (from 3 to 7.5 mg/kg).

The decrease in IgM-RF, anti-CCP, and ACF levels during treatment with infliximab was compared to the course of the disease activity markers corrected for disease duration, sex, and age: the analysis showed only a marginal association between ESR and anti-CCP levels; a 1-mm/h decrease in ESR is associated with a decrease of anti-CCP of 1.03 (SE 0.6) ($p < 0.05$). We could not establish any other relationship between any of the disease activity markers (DAS28 and CRP) and autoantibody levels.

Similarly, we also compared the course of autoantibodies corrected for disease duration. This analysis showed that the change in ACF was significantly related to disease duration, but to neither IgM-RF nor anti-CCP. Interestingly, the ACF decreased more in patients with shorter disease duration ($p < 0.05$).

DISCUSSION

In our study of patients with RA who received infliximab for a year, IgM-RF, anti-CCP, and ACF concentrations all decreased significantly. The decrease in IgM-RF was substantially greater than those of anti-CCP and ACF. Moreover, almost 50% of the patients who were IgM-RF-positive at baseline turned negative during treatment with infliximab, while seroconversion of anti-CCP and ACF occurred in fewer than 10%.

Our finding that anti-CCP decreased is different from results of 2 other studies that reported unchanged anti-CCP levels during anti-TNF treatment. This difference could be due to the fact that we studied patients with RA from a cohort treated with infliximab for at least 1 year: these patients can be classified as responders. Other studies observing responders showed a decrease in anti-CCP levels or showed a decrease in their responder subgroup[12,14,16]. On the other hand, studies investigating both responders and nonresponders found no significant changes in anti-CCP[15].

Remarkably, we did not find a direct relationship between disease activity and changes in levels of autoantibodies, except a marginal relationship between ESR and

anti-CCP. This was surprising, as the pattern of mean change in disease activity markers was almost identical to that of the change in autoantibodies. However, only one of the previous studies investigating the effect of antirheumatic treatment on autoantibody titers could not establish a consistent relationship between disease activity and changes in IgM-RF or anti-CCP[18]. An explanation could be that autoantibody levels decrease in patients who respond to antirheumatic therapy, but there is no direct relationship with disease activity[12,14]. It could also be because we studied only patients who continued treatment for 1 year (responders), and therefore had too little contrast in our study population.

The effects of anti-TNF treatment in RA have been attributed to the induction of apoptosis of inflammatory cells[21]. Citrullination is a post-translational modification of proteins in the apoptotic process and therefore infliximab could influence the formation and presence of antibodies against citrullinated proteins by directly interfering with the regulation of the apoptotic process.

To our knowledge, our study was the first to investigate the response of ACF levels during treatment with a TNF-blocking agent. The ACF concentration showed a significant decrease during treatment with infliximab. Decreases in ACF levels were negatively associated with disease duration, meaning that ACF decreased more in patients with shorter disease duration, possibly implying a window of opportunity for the treatment of RA. Lowering ACF levels through antirheumatic treatment may cause a disruption of the cycle of the proposed immunological conflict between citrullinated fibrin and its antibodies leading to self-maintenance of RA synovitis[6].

Our study showed that in a cohort of RA patients responding to infliximab treatment during one year of therapy, IgM-RF underwent a larger decrease than anti-CCP or ACF, but all 3 autoantibodies decreased significantly. This decrease was not directly related to the change in disease activity, but was probably indirectly related to a good clinical response. Early in the disease, ACF levels were best influenced by treatment with infliximab.

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