Anti-Cyclic Citrullinated Protein Antibodies as a Predictor of Response to Anti-Tumor Necrosis Factor-α Therapy in Patients with Rheumatoid Arthritis

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ABSTRACT. ***Objective***. The treatment of rheumatoid arthritis (RA) has changed dramatically with the introduction of anti-tumor necrosis factor (TNF) agents. Unfortunately, a subset of patients have partial or no response. No measurements were found to predict the efficacy of this therapy. Anti-cyclic citrullinated protein antibodies (anti-CCP) are highly specific and sensitive for RA, and their titer correlates with erosive disease. We investigated the correlation between the efficacy of infliximab therapy and the titer of anti-CCP.

Methods. Thirty consecutive seropositive patients with RA were treated with infusion of 3 mg/kg infliximab on Weeks 0, 2, 6, and 14. Clinical assessment and blood withdrawal were done before each treatment, i.e., at the minimal concentration of the drug. Disease activity was assessed by DAS28 score and by interleukin 6 (IL-6) level. Anti-CCP titer was measured by a commercial ELISA at Week 0 and Week 14.

Results. At baseline, 24 patients were positive for anti-CCP antibodies. In most patients there was a significant correlation between clinical response to therapy and anti-CCP titer. The results were especially noteworthy in those patients who showed a sustained and significant decrease in IL-6 levels through the entire period.

Conclusion. Anti-CCP titer and IL-6 levels might be early predictors of the efficacy of anti-TNF therapy in patients with RA. (J Rheumatol 2006;33:497–500)

Key Indexing Terms:
ANTI-CYCLIC CITRULLINATED PEPTIDE ANTIBODIES RHEUMATOID ARTHRITIS
ANTI-TUMOR NECROSIS FACTOR ALPHA THERAPY

Anti-tumor necrosis factor (TNF) agents have dramatically changed the treatment of rheumatoid arthritis (RA) over recent years. Treatment with anti-TNF agents is highly efficient and it delays radiological changes and downregulates inflammatory cytokines stimulated by TNF-α[1-3]. However, despite tremendous overall improvement in the majority of patients with RA, a subset of patients have only partial response, or no response at all, clinically and radiologically[4]. So far no predictors of response to this expensive therapy have been identified.

Rheumatoid factor (RF) is an IgM antibody directed against the constant region of IgG. When RF was described over 60 years ago[5], it was the first known autoantibody in RA and was widely used for diagnostic and prognostic values[6]; however, more recently its relatively low sensitivity (50–80%) and specificity (70–80%) have led to the search for more specific antibodies.

Anti-cyclic citrullinated peptide antibody (anti-CCP), first described by Schellekens and van Jaarsveld and coworkers in 1998[7,8], belongs to the family of antifillagrin autoantibodies[9]. Detectable by a variety of assays, it has many different names (antikeratin antibody, antiperinuclear factor): all are directed against proteins containing citrulline amino acid residues[10]. Anti-CCP antibodies are produced locally in the synovium of RA patients[11] and are highly specific (98%) and sensitive (80%) for RA[12-14]. They are present early in the disease and their titers correlate with erosive disease[15].

However, there is little knowledge regarding the effect of infliximab therapy on the level of anti-CCP antibodies. We investigated the correlation between the efficacy of infliximab therapy and the titer of anti-CCP, RF, and interleukin 6 (IL-6).

MATERIALS AND METHODS

Thirty consecutive patients (22 women, 8 men) meeting the American College of Rheumatology (ACR) criteria for RA[6] were enrolled in the study between June 2002 and June 2003 after giving informed consent. The patients were seen at the Department of Rheumatology, Rambam Medical Center,

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Haifa. All patients had a history of failed treatment with at least 3 disease modifying antirheumatic drugs (DMARD). Patients continued DMARD, steroids, and nonsteroidal antiinflammatory drugs (NSAID) at a stable dose during the first 14 weeks of infliximab therapy. Patients received infliximab at a dose of 3 mg/kg according to the usual protocol (at baseline, after 2 and 6 weeks, and afterwards bimonthly). Twenty-six patients were taking methotrexate (MTX) therapy, as a single drug or as part of combination therapy, 2 patients were taking leflunomide, and 2 patients azathioprine. Sixteen patients were also receiving prednisone (5–10 mg/day).

The main clinical and laboratory variables assessed were tender and swollen joint count, patient's assessment of disease activity, physician global assessment of disease activity, and erythrocyte sedimentation rate (ESR). We calculated the 28 joint Disease Activity Score (DAS28), which incorporates tender and swollen joint counts for 28 joints, ESR, and patient's assessment of disease activity[16] at baseline and after 14 weeks. DAS28 response was defined based on a combination of a significant change from baseline and the level of disease activity attained. Good DAS28 response was defined as a significant decrease in DAS28 score (> 1.2) and a low level of disease activity (≤ 3.2). Nonresponse was defined as a decrease of ≤ 0.6 or a decrease of 0.6–1.2 with a score of > 5.1 on the DAS28. Any other scores were regarded as indicative of moderate responses. DAS > 5.1 represented high disease activity[16,17].

Serum samples collected at baseline and at Weeks 2, 6, and 14 were processed and stored at –80°C until used for this study. Anti-CCP antibodies (IgG) were measured using commercially available second-generation ELISA kits (Diastat™ Axis, Shield Diagnostics, Dundee, Scotland). The assay was performed according to the manufacturer's instructions. Anti-CCP antibody levels were expressed in arbitrary units per milliliter and were considered to be positive at a cutoff value of > 5 U/ml. IgM RF was assayed using quantitative immunonephelometry test (Behring, Marburg, Germany). RF was considered positive when the concentration was higher than the cutoff value of the kit (15 IU/ml).

All serum samples with high concentrations of RF or anti-CCP antibodies were further quantified at a greater sample dilution. IL-6 level was measured by ELISA kits (R&D Systems, Minneapolis, MN, USA) using a monoclonal anti-human IL-6 antibody, according to the manufacturer's instructions. Anti-CCP antibodies and RF were measured at baseline and after 14 weeks. IL-6 level was measured at baseline and at Weeks 2, 6, and 14.

To verify the specificity of our assays, sera from 19 patients with spondyloarthropathy, 5 patients with osteoarthritis, and 6 healthy blood donors were tested for anti-CCP antibodies and RF.

Statistical analysis was performed using SPSS statistical software and the tests used were ANOVA, t test (equal variance), Mann-Whitney rank-sum test, and Kruskal-Wallis one-way analysis of variance on ranks (normality). A p value of 0.05 or less was considered statistically significant.

RESULTS

The sociodemographic and disease-related characteristics of the 30 study participants are shown in Table 1. The mean age of the patients was 50.6 years (range 25–78), two-thirds were women, and the mean duration of the disease was 12 years (range 1–31). At baseline, all patients had active disease, as measured by the mean DAS of 7.35, and the mean number of failed DMARD was 4.

Twenty-four of the 30 patients with RA were positive for anti-CCP antibodies, and an identical number of patients were positive for RF. One patient with new-onset disease (less than a year) was anti-CCP-positive but negative for RF; another patient was RF-positive but negative for anti-CCP. No clinical differences at baseline were observed among the patients with positive anti-CCP antibodies, positive RF, or negative antibodies. None of the control group was positive for anti-CCP antibodies, while low titer RF was detected in 2 blood samples.

Table 1. Demographic and baseline data of patients.

Female/male	22/8
Age, yrs	50.6 ± 13.9
Duration of disease, yrs	12 ± 8.9
DAS score	7.353 ± 0.945
ESR, mm/h	53.3 ± 23.9
RF, IU/ml	212 ± 182
Anti-CCP, u/ml	94 ± 64
IL-6, pg/ml	21.4 ± 18.3

According to the clinical response (a change in DAS score ≥ 1.2), after 14 weeks of infliximab therapy, the patients were divided into 2 groups: responders (24 patients, 20 female) and nonresponders (6 patients, 2 female). The baseline data of the responders versus nonresponders showed no differences regarding disease duration, number of failed DMARD, starting DAS, and levels of RF and IL-6 (Table 2). No significant distinctions were found among DMARD and DMARD doses between the 2 groups (Table 3). Doses of MTX were 10–25 mg/week and prednisone 5–10 mg/day. Erosive disease was found in 20 responder patients and in 5 nonresponders. The only significant difference between the 2 groups was the baseline level of anti-CCP titer (Table 2). Anti-CCP antibodies were positive at baseline in all nonresponders and in 18

Table 2. Baseline characteristics of patients according to their response to treatment.

	Responders, N = 24	Nonresponders, N = 6	p
Age, yrs	50.6 ± 13.9	44.4 ± 11.1	NS
Duration of disease, yrs	11 ± 8.2	12 ± 7.8	NS
DAS28	7.46 ± 0.79	7.00 ± 1.34	NS
ESR, mm/h	55 ± 22	47 ± 30	NS
RF, IU/ml	167.4 ± 128.4	130.5 ± 72.4	NS
Anti-CCP, u/ml	71 ± 68	150 ± 57	< 0.05
IL-6, pg/ml	18.5 ± 15.7	28.7 ± 24.5	NS
Erosive disease, no. patients	20/24	5/6	NS

U: arbitrary units.

Table 3. Concurrent therapy received by the patients.

	Responders	Nonresponders
Combination therapy*	7	5
MTX monotherapy, mean dose 17.5 mg/wk, range 10–25 mg/wk	13	1
Prednisone ≤ 10 mg/day	10	6
Azathioprine, 150 mg/day	2	0
Leflunomide, 10 mg/day	2	0

* Methotrexate (MTX) 15–25 mg/week, salazopyrine 2 g/day, and hydroxychloroquine 400 mg/day.

responders. Five nonresponders had baseline anti-CCP titer higher than 100 units/ml compared to only 4 of the responders. In the remaining 14 out of the 18 anti-CCP-seropositive responders the titer was lower than 100 units/ml. Data for patients' anti-CCP titers are described in Table 4. Generally, there were no apparent differences in baseline variables between the group with anti-CCP titer > 100 and the group with the lower titer, except for evidence of erosive disease and RF titer: the titer of RF was higher in the group with high anti-CCP, and erosive disease was more frequent in this group, but the differences did not reach statistical significance.

After 14 weeks of therapy, a statistically significant decrease in the anti-CCP titer was found only in the responder group. Neither the decrease in RF titer nor the change in ESR reached statistical significance.

IL-6 was measured in 15 patients with positive anti-CCP antibodies. In all these patients there was a marked reduction of IL-6 levels in the second week of therapy (from 18.5 ± 15.7 pg/ml to 2.84 ± 1.95 pg/ml in responders and from 28.7 ± 24.5 pg/ml to 5.4 ± 3.6 pg/ml in nonresponders). In the nonresponders, the level slowly increased afterwards (19.5 ± 17.1 pg/ml), while in the responders it remained low (4.9 ± 4.6 pg/ml) and was not statistically significant.

Throughout 18 months' followup after the first infliximab infusion, 6 patients discontinued the treatment due to lack of efficacy (1 patient from the responders who previously reacted well and 5 patients from the nonresponders). The 5 nonresponders discontinued infliximab after 22–30 weeks of therapy. Seven other patients discontinued due to side effects. Sixteen out of 24 patients of the responder group and 1 of 6 nonresponding patients continued with infliximab. In 3 patients, anti-CCP antibodies became negative after 18 months. In 4 patients with high anti-CCP titer (3 from the responders and one from the nonresponders) the time lag to improvement was longer than 14 weeks.

DISCUSSION

Anti-tumor necrosis factor (TNF) agents have dramatically changed the treatment of RA. However, they may be associated with severe adverse effects and their high cost may be prohibitive. Therefore, identifying early predictors of response is important regarding both the individual patient and health economics.

Few studies have examined the effects of active RA treatments on the level of anti-CCP antibodies[18–21]. Mikuls and coworkers observed declines in levels of both anti-CCP antibodies and RF in RA patients treated with different DMARD, but the changes in anti-CCP antibody level were not associated with treatment response[21]. Alessandri and coworkers and Bobbio-Pallavicini and colleagues investigated the effect of anti-TNF therapy (infliximab) on autoantibody level, and found a significant decrease in serum anti-CCP antibodies and RF, which correlated to clinical improvement[18,19]. Although observing no significant overall effect on median anti-CCP antibody levels, de Rycke and colleagues described a subset of patients who, with infliximab treatment, experienced a marked decline in the anti-CCP antibody levels[20].

The objective of our study was to assess a possible correlation between the efficacy of infliximab therapy and anti-CCP titer. And, indeed, a significant association between the clinical response to therapy and the decrease in anti-CCP titer was found, consistent with the other studies. The positive correlation was noteworthy especially in those patients with a sustained and significant decrease in IL-6 level through the entire period.

The most striking result, however, was the link between the baseline level of anti-CCP antibodies and the clinical response to therapy. The baseline anti-CCP titer was the only statistically significant difference between the responder group versus the nonresponders. Lower levels of anti-CCP were predictive of clinical response to therapy (Table 5). This finding is consistent with an observation of Alessandri and colleagues regarding positive correlation between lower baseline levels of anti-CCP and clinical response to infliximab[18]. This observation is of particular importance, because it may serve as a simple and practical tool to predict response to infliximab. Other noteworthy observations were the seroconversion from positive to negative anti-CCP in 3 patients after more than a year of therapy, and the relatively delayed response to therapy in patients with baseline high titer of antibodies. Patients with high anti-CCP titer tended also to have higher RF titer and erosive disease, but the differences between the 2 groups did not reach statistical significance, possibly due to the small sample size.

The mechanisms by which infliximab could lead to a decrease in titers of autoantibodies such as RF and anti-CCP

Table 4. Baseline characteristics of patients with anti-CCP titer > 100 u/ml and titer < 100 u/ml.

	Anti-CCP > 100, N = 9	Anti-CCP < 100, N = 21	p
Age, yrs	58 ± 13	47 ± 14	NS
Duration of disease, yrs	14 ± 11.2	12 ± 8.6	NS
DAS28	7.14 ± 1.33	7.41 ± 0.77	NS
ESR, mm/h	52 ± 28	54 ± 24	NS
RF, IU/ml	240.4 ± 190.7	162.1 ± 150.7	NS
IL-6, pg/ml	29.4 ± 21.32	17.3 ± 16.2	NS
Erosive disease, no. patients	8/9	17/21	NS

Table 5. Predictive value of anti-CCP titer.

	Responders	Nonresponders	
Anti-CCP < 100 u/ml	20	1	PPV 0.95
Anti-CCP > 100 u/ml	4	5	NPV 0.56
	Sensitivity 0.833	Specificity 0.833	

PPV: positive predictive value; NPV: negative predictive value.

are not understood and any explanation remains speculative. Infliximab therapy has proven to reduce the amounts of synovium-infiltrating cells including plasma cells[22]. Because RF-producing cells are present in inflamed rheumatoid synovium and the local environment may favor synovial RF production, we can speculate that the reduction in inflammatory lymphoplasmacytic infiltrate in rheumatoid synovium will lead to reduced production of RF.

Citrullination represents a post-translational modification of proteins in the apoptotic process[23]. Citrullinated fibrin is one of the major citrullinated proteins in rheumatoid synovium and represents an important antigenic target of anti-CCP antibodies[24]. Indeed, intraarticular fibrin injection was the first experimental model of arthritis developed 40 years ago by Dumonde and Glynn[25]. Infliximab may induce cell type-specific apoptosis in the monocyte/macrophage population in RA synovium that diminishes synovial inflammation and decreases the number of synovial macrophages[26]. Whether changes in serum levels of anti-CCP antibodies during infliximab therapy are related to modulation of apoptosis or to reduction in the number of inflammatory cells in synovium is still an issue for further investigation.

In conclusion, anti-CCP titer might be an early predictor of efficacy of anti-TNF therapy in patients with RA. Lower levels correlate with better response. Clinical response in patients with high titer of anti-CCP might be delayed beyond 14 weeks. High anti-CCP levels predicted poorer early response to infliximab. In view of the association of high anti-CCP titer with erosive disease, a strategy of initially higher and more frequent infliximab doses should be considered for clinical trials.

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