

Basal anti-cyclic citrullinated peptide (anti-CCP) antibody levels and a decrease in anti-CCP titres are associated with clinical response to adalimumab in rheumatoid arthritis

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Abstract

Objective

To investigate the effect of adalimumab treatment on anti-cyclic citrullinated peptide antibodies (anti-CCP) in patients with rheumatoid arthritis (RA).

Methods

70 RA patients who failed treatment with disease modifying antirheumatic drugs (DMARDs) received 40 mg adalimumab subcutaneously every other week during 24 weeks. Serum samples were collected at baseline and at weeks 8, 16 and 24 before the corresponding adalimumab dose. The serum anti-CCP levels were tested by enzyme linked immunosorbent assay.

Results

At baseline, 52 of the 70 patients (74.3%) were positive for anti-CCP antibodies. 60 % of the anti CCP positive patients and 44.4% of the anti CCP negative patients were ACR 20 responders at week 24 ($p < 0.049$). The serum levels of anti-CCP antibodies decreased significantly after 24 weeks of adalimumab treatment only in those patients who met ACR 20 response criteria at week 24 ($p < 0.00044$). Differences between baseline anti-CCP titers and those at 8, 16 and 24 weeks were all statistically significant ($p < 0.014$, 0.003 and 0.019 respectively). No statistically significant changes in the anti-CCP levels were observed in patients who did not meet the ACR 20 response criteria.

Conclusion

Basal anti-CCP antibodies levels correlate with clinical response to adalimumab. A decrease in anti-CCP levels on time was observed in patients showing also clinical improvement, suggesting that serum anti-CCP antibodies determination may be useful in assessing treatment efficacy in RA patients.

Key words

Rheumatoid arthritis, adalimumab, anti-CCP, anti-TNF.

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Introduction

Rheumatoid arthritis (RA) is a chronic, inflammatory autoimmune disease that leads to progressive joint destruction and disability. Tumor necrosis factor α (TNF- α) plays a key role in associated pathological events and has been identified as a therapeutic target. In particular, treatment with adalimumab – a human anti-TNF- α monoclonal antibody – often results in high clinical efficacy and delay in radiological progression (1, 2). However about one quarter of patients still have a poor response to this treatment. The identification of genetic and other predictors of treatment response would provide valuable information for therapeutic decisions (3, 4). Anti-cyclic citrullinated peptide antibodies (anti-CCP) have been shown to be useful diagnostic tools-particularly in the early stages of the disease-and to be predictive of disease progression and radiological damage (5-7).

Recent reports suggest that a decrease in anti-CCP antibodies titres might be a useful adjunct in assessing the efficacy of anti-TNF- α treatment (8-10), although this finding was not confirmed by other groups (11, 12). The present 24 weeks study was planned to evaluate in a prospective manner the effect of adalimumab treatment in serum anti-CCP antibody levels in a group of 70 RA patients who had an inadequate response to methotrexate or to other DMARDs. We also investigated if baseline anti-CCP antibodies levels were associated with clinical response on time.

Patients and methods

Patients

Seventy patients fulfilling the 1987 revised ACR criteria for classification of RA were studied (13). All had active disease despite treatment with methotrexate, leflunomide or sulfasalazine, defined by the presence of six or more swollen joints, nine or more tender joints, and morning stiffness greater than 45 minutes. The dosages of disease-modifying antirheumatic drugs (DMARDs) must have been stable for at least 8 weeks before enrolling in the study.

All patients received 40 mg adalimumab (Abbot Laboratories, Chicago,

IL, USA) subcutaneously every other week for 24 weeks. Patients were allowed to continue the same dose of non-steroidal anti-inflammatory drug, oral glucocorticoid and DMARDs they had been taking at the beginning of this study. The study was approved by the Ethical Committees at each study site, and all patients gave their written informed consent.

Clinical and laboratory assessment

The number of tender joints (TJC) (68) and swollen joints (SJC) (66) were evaluated by an assessor at baseline and at weeks 8, 16 and 24, immediately before the corresponding adalimumab dose. We evaluated the clinical status using: Patient's global assessment of disease activity (PGADA), Physician global assessment of disease activity (PhGADA), Patient's assessment of pain (PAP), and Health Assessment Questionnaire score (HAQ). All patients were scored to assess whether or not they achieved the ACR 20 criteria for improvement at weeks 8, 16 and 24.

Laboratory analyses included determinations of erythrocyte sedimentation rate (ESR), blood count, and blood chemistry at each visit. All clinical evaluations and laboratory tests were performed blindly, with respect to the anti-CCP status. Blood samples for studies were drawn at 9-10 am on weeks 0, 8, 16, and 24 immediately before the corresponding adalimumab dose was given and stored at -70°C until use.

Anti-CCP antibodies

All serological determinations were performed at the Immunology Laboratory, of the Las Condes Clinic, Santiago, Chile. Anti-CCP antibodies were detected using a commercial anti-CCP2 enzyme linked immunosorbent assay kit (Euro-diagnostica, Sweden), following the manufacturer's instructions. Briefly this is a quantitative ELISA that uses citrullinated synthetic peptides and read it with horseradish peroxidase labeled anti-human IgG. For reading purpose we used semiautomatic Triturus ELISA reading equipment. The results of the anti-CCP test were considered positive if the antibody level was ≥ 25 IU/ml (cut-off value).

Competing interests: none declared.

IgM rheumatoid factor (RF) measurement

IgM RF was assayed by ELISA on a 96-well plate coated with highly purified Fc fragments of human IgG, prepared by digestion of human IgG with papain and then purification by chromatography on Sephadex G-75 and protein A-Sepharose. For the assays, serum aliquots (200 µl, 1:100 dilution) were added in triplicate in a 200 µl final volume of PBS-Tween and incubated 2 h at 37°C, followed by rinsing in PBS-Tween and incubation with specific anti-human IgM F(ab')₂ fragments conjugated to alkaline phosphatase for 1 h at 37°C. Plates were rinsed with PBS-Tween and incubated with 200 µl alkaline phosphatase substrate (1 mg/ml p-nitro-phenylphosphate) in 0.1 M glycine, 1 mM MgCl₂, 1 mM ZnCl₂, pH 10.4. Absorbance was monitored at 405 nm.

Statistical analysis

The ACR 20 criteria of response at week 24 was defined as the main outcome variable. The DAS28 criteria of improvement was considered as secondary outcome. Patients were grouped according to their serum anti-CCP titers at the beginning of the study as negative (below 25 IU/ml) and positive (≥25 IU/ml). All the study variables were tested for normality with the Shapiro-Wilks test. Since the serum anti-CCP titers were not normally distributed, all comparisons were made by means of non parametric statistics: Kruskal-Wallis ANOVA for comparison between groups and post hoc analyses using the Mann-Whitney U test when appropriate. Friedman ANOVA was used to test changes over time and post hoc analyses were performed using the Wilcoxon matched pairs test. Normally distributed variables were analyzed by means of one-way ANOVA or ANCOVA when appropriate. Post hoc analyses were performed using *t*-test. To assess changes over time a repeated measurement ANOVA was used and paired *t*-test for post hoc analyses. Differences in proportions were analyzed with the Pearson chi square test, the Fisher exact test or the McNemar test according to the number of events/cases involved and the nature of the comparison.

Table I. Baseline clinical and demographic characteristics of patients with rheumatoid arthritis*.

Characteristic	Patients with anti-CCP (+) (n=52)	Patients with anti-CCP (-) (n=18)	<i>p</i> -value
Age (years)	50.2 ± 9.5	45.3 ± 14	0.0506
Sex (female/male)	45/7	16/2	0.06
Duration of disease (months)	147.7 ± 143.3	117.7 ± 99.9	0.2
Rheumatoid Factor (+) n	48	15	0.25
Methotrexate (mg/week)	11.5 ± 6.7	9.4 ± 7.2	0.13
Prednisone (mg/day)	7.9 ± 4.5	7.6 ± 5.9	0.41
NSAID therapy (yes/no)	22/30	7/11	0.06
SJC	16.4 ± 9.2	15.5 ± 7.8	0.34
TJC	21 ± 11.9	17.1 ± 13.4	0.1
VAS	6.9 ± 2	5.6 ± 2	0.01
PGADA	6.6 ± 1.7	5.6 ± 1.7	0.02
PhGADA	6.6 ± 1.8	5.5 ± 1.6	0.01
HAQ	1.3 ± 0.5	1 ± 0.5	0.01
ESR (mm/h)	42.9 ± 28.2	31.2 ± 29.9	0.07

SJC: number of swollen joints; TJC: number of tender joints; PGADA: patient's global assessment of disease activity; PAP: patient's assessment of pain; PhGADA: physician global assessment of disease activity; HAQ: Health Assessment Questionnaire score. *Data represents the means±SD.

In order to evaluate the contribution of serum anti-CCP antibody titres to predict the ACR 20 response at week 24, positive and negative predictive values, positive and negative likelihood ratios, post-test probabilities and odds ratios were calculated reclassifying the patients with regards to basal levels of anti-CCP antibodies at increasing cut-off values. Reference values for sensitivity, specificity, negative and positive predictive values, likelihood ratios and pre-test probability (baseline prevalence of response) were derived from the study data when using 25 IU of serum anti-CCP antibodies as a starting cut-off point.

To deal with missing data, two approaches were used: last observation carried forward and multiple imputations but none of them produced modifications of the results with actual data. Reported results are from actual data.

Results

Patients

Fifty-nine patients completed 24 weeks of therapy. From the eleven patients that withdrew from the study: seven were anti-CCP positive and 4 were anti-CCP negative at baseline. Five patients dropped out because of side effects; in four patients adalimumab was stopped because of lack of clinical response; one patient because of lost to

follow up and one because of protocol violation. One patient discontinued at week seven, three between weeks nine and sixteen, and seven between weeks seventeen and twenty-four.

The baseline demographical and clinical characteristics of the patients are shown in Table I. Even though no significant differences were found in the duration of the disease between anti-CCP positive and anti CCP negative patients, the anti CCP positive group of patients had a basal PGADA, PhGADA, PAP and HAQ score significantly higher than the anti CCP negative group of patients.

Clinical response

ACR 20 criteria of improvement:

An ACR 20 response was achieved by 66% of patients at 24 weeks regardless of their initial serum anti-CCP levels. Patients were grouped according to their basal anti-CCP status. 52 out of 70 (74.3%) patients were anti-CCP positive at the beginning of the study. Among those anti-CCP positive at baseline and with complete follow-up data (48/52), a 60% met ACR 20 response criteria at week 24, while 44.4% of those anti-CCP negative at baseline met ACR 20 response criteria at week 24 (*p*<0.049).

Figure 1 shows the changes in the serum levels of anti-CCP antibodies

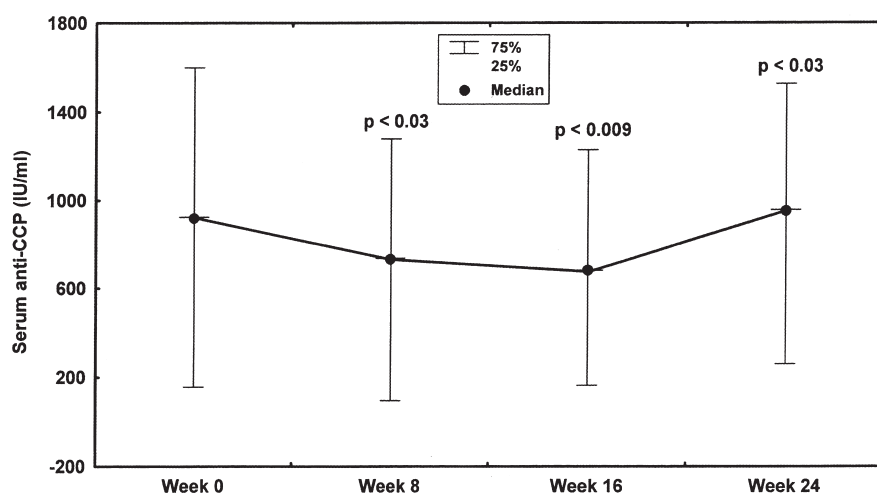


Fig. 1. Changes of serum anti-cyclic citrullinated peptide antibodies in rheumatoid arthritis patients at different times during 24 weeks of adalimumab treatment. The solid circles indicate median values, and vertical bars indicate the interquartile range.

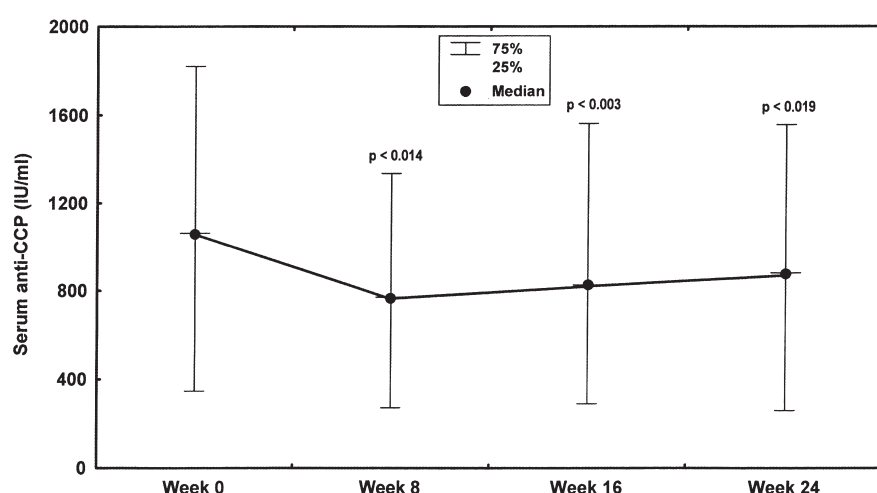


Fig. 2. Changes of serum anti-cyclic citrullinated peptide antibodies in rheumatoid arthritis patients who met ACR 20 response criteria at week 24. The solid circles indicate median values, and vertical bars indicate the interquartile range.

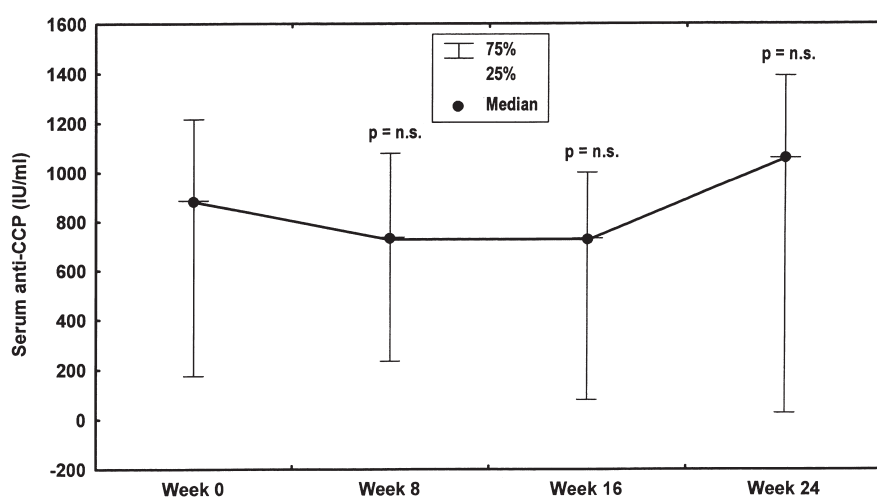


Fig. 3. Changes of serum anti-cyclic citrullinated peptide antibodies in rheumatoid arthritis patients who did not meet ACR 20 response criteria at week 24. The solid circles indicate median values, and vertical bars indicate the interquartile range.

among patients who were anti-CCP positive at the beginning of the study. The anti-CCP sera titers decreased over time from 920 (157-1600) IU at baseline to 731 (95.75-1279.5) IU at week 8, to 679 (163.5-1230) IU at week 16, but slightly increased to 950 (260-1527) IU at week 24 (median, inter-quartile range; $p < 0.03$, $p < 0.09$, $p < 0.03$ respectively). This reduction was mainly accounted for the decrease observed among patients who reached the ACR 20 response criteria, as shown later.

Figures 2 and 3 show the changes in the serum levels of anti-CCP antibodies on time according to the ACR 20 response criteria at week 24 in patients who were anti-CCP positive at the beginning of the study. The serum level of anti-CCP antibodies decreased significantly only in those patients who met ACR 20 response criteria at week 24 ($p < 0.00044$). Differences between baseline anti-CCP titers and those at 8, 16 and 24 weeks were all statistically significant ($p < 0.014$, 0.003 and 0.019 respectively) (Fig. 2). No statistically significant changes in the anti-CCP levels were observed in patients who did not meet the ACR 20 response criteria (Fig. 3).

Predictive value of basal anti-CCP levels

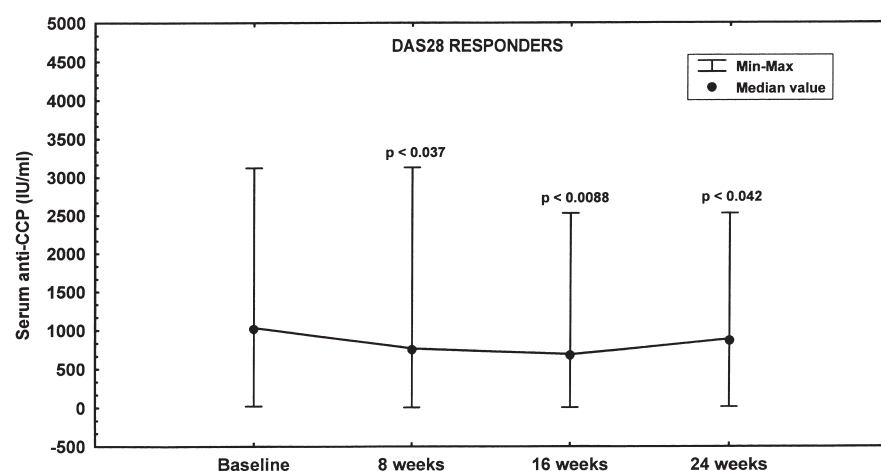
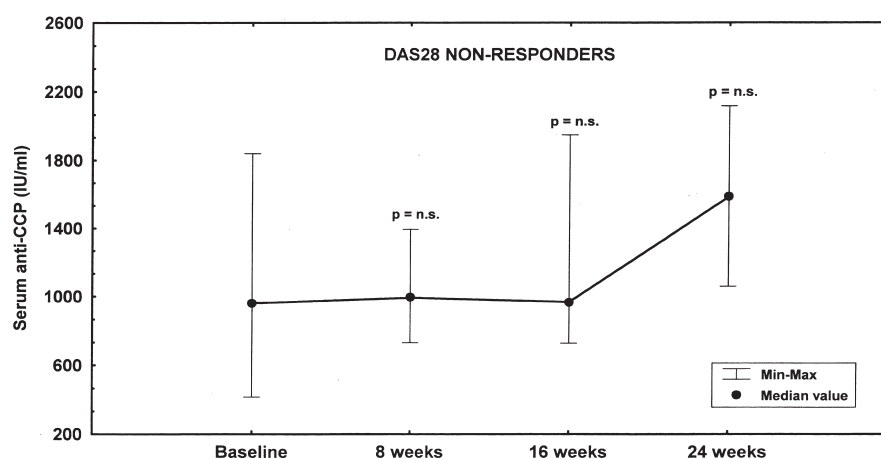
Further analysis was done to investigate whether a cut-off point of anti CCP titer at baseline could better predict an ACR 20 response at week 24. The basal anti-CCP titers were higher among ACR 20 responders (median=626 IU/ml, range 2.29-3123) than non-responders (median 65.3 IU/ml range=14-2212) at week 24 ($p < 0.036$).

Table II shows predictive values of response to treatment, as well as odds ratios at different basal anti-CCP cut-off titers.

The pre test probability to achieve an ACR 20 response at week 24 for the whole group was 66%. As shown, higher basal anti-CCP levels predict a better clinical response to treatment. An anti-CCP basal serum level at about 400 IU/ml was found to increase the odds of improvement by 2.97, while the 66% overall pre-test probability of improvement

Table II. Predictive values of response to adalimumab treatment at different basal anti-CCP cut-off titers.

Anti-CCP Titers (IU)	Positive Likelihood ratio	Negative Likelihood ratio	Post test Probability (%)	Odds ratio
50	1.30	0.63	72	2.08
100	1.31	0.60	72	2.18
200	1.31	0.60	72	2.18
300	1.39	0.51	73	2.40
400	1.45	0.49	74	2.97
500	1.38	0.52	73	2.72
1000	1.40	0.45	73	3.09

**Fig. 4.** Changes of serum anti-cyclic citrullinated peptide antibodies in rheumatoid arthritis patients who met DAS28 criteria of response at week 24. The solid circles indicate median values, and vertical bars indicate the maximum and minimum values.**Fig. 5.** Changes of serum anti-cyclic citrullinated peptide antibodies in rheumatoid arthritis patients who did not meet DAS28 criteria of response at week 24. The solid circles indicate median values, and vertical bars indicate the maximum and minimum values.

under adalimumab rose to 74% at that level. The negative likelihood ratio was the lowest at this cut-off point.

DAS28 criteria of improvement

Clinical response in terms of percentage of patients who improved with treatment was also studied according

to DAS28 criteria of improvement: 86.2% of patients were DAS28 responders at week 24; 87.8% of patients who were anti CCP (+) at baseline, were DAS28 responders at week 24; 82.4% of those who were anti-CCP (-) at baseline also reached DAS28 response criteria ($p=n.s.$).

Basal anti-CCP (+) patients were followed up in order to study antibodies levels on time.

Figures 4 and 5 show the anti-CCP titers on time. The decrease in anti-CCP levels was progressive throughout the course of the adalimumab treatment only in the DAS28 responder group of patients. Differences between baseline anti-CCP titers and those at 8, 16 and 24 weeks were all statistically significant ($p<0.037$, $p<0.009$ and $p<0.042$) respectively. No statistically significant changes in the anti-CCP levels were observed in the DAS28 non responder group of patients.

RF

IgM RF was measured in 32 patients who completed 24 weeks of therapy.

The RF titers exhibited a progressive and significant reduction from baseline to 8, 16 and 24 weeks for the whole group of patients. No difference was found among responders and non responders (according to ACR 20 criteria) in RF titers either at baseline or at 8, 16 and 24 weeks (Fig. 6).

Discussion

TNF- α blockade has emerged as an important therapy for patients with RA. One of the characteristics of RA is the presence of autoantibodies in the circulation. Some of them may contribute to the inflammatory response. Autoantibodies directed against citrullinated peptides are of diagnostic and predictive value in RA (5-7). It has been demonstrated that healthy control subjects and RA patients have a pool of precursor B cells in the circulation that are capable of producing anti-CCP upon activation. However only synovial fluid and bone marrow B cells from anti-CCP-positive RA patients spontaneously produce anti-CCP antibodies (14). These data confirm the local presence of anti-CCP producing cells at the site of inflammation and supports the notion of a local mechanism of activation in RA.

A decrease in anti-CCP antibodies titers has been recently correlated with clinical improvement after anti TNF- α therapy in RA patients (8-10), however this finding was not confirmed by others

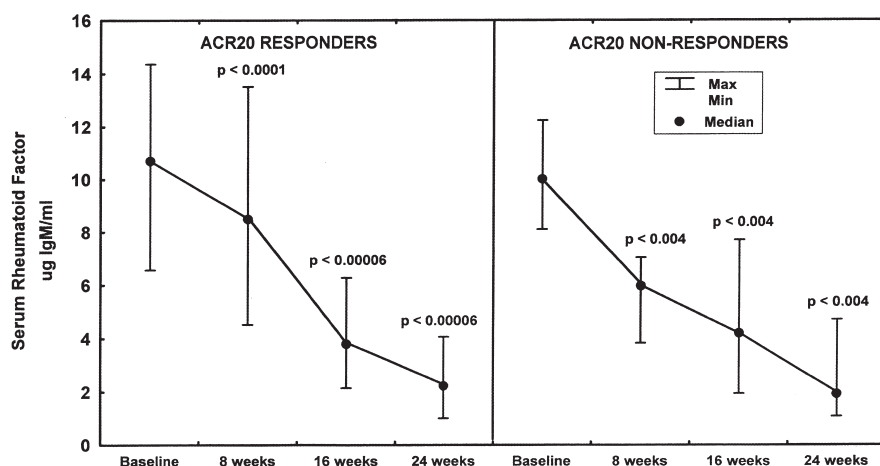


Fig. 6. Rheumatoid Factor levels in RA patients who achieved or not the ACR 20 criteria of response during adalimumab therapy. Medians (range) of serum IgM RF levels were 10.7 (6.5-14.4), 8.5 (4.5-13.5), 3.8 (2.1-7.5), and 2.2 (1.01-4.06) μ g IgM/ml serum at baseline, 8, 16, and 24 weeks respectively for ACR 20 responder group of patients. Medians of serum IgM RF levels were 10.02 (8.1-12.2), 5.9 (3.8-7.04), 4.2 (1.9-7.7) and 1.9 (1.08-4.7) μ g IgM/ml serum at baseline, 8, 16, and 24 weeks respectively for ACR 20 non responder group of patients.

groups (11, 12). This may be explained by the methods used to measure antibody levels, by the number of patients studied, by the different periods of follow-up and by the statistical analysis performed in the different studies.

The present study was planned to evaluate whether the presence and anti-CCP levels on time correlate with adalimumab efficacy in a group of RA patients who had an inadequate response to methotrexate or to other DMARDs. Our results show a significant decrease in the anti-CCP autoantibodies after 24 weeks of adalimumab therapy. To our knowledge, there is one more report that studies the adalimumab effect in anti-CCP titers and they also found a correlation between clinical response and a decrease in anti-CCP antibody levels (9). We also found that patients who were anti-CCP-positive before treatment were also better responders than anti-CCP negative patients. It is possible that anti-CCP-positive RA patients might display a more active disease associated with a higher response to adalimumab therapy in comparison with patients negative for anti-CCP antibodies. In fact we have found that the anti-CCP positive group of patients had a basal PGADA, PhGADA, PAP, and HAQ score significantly higher than the anti CCP negative group of patients. In a recent study, Del Val Del Amo E. *et al.* found a significant association

between the presence and the levels of anti-CCP antibodies and greater RA activity, with higher values of DAS28 and C-reactive protein (15).

The role of serum anti-CCP antibodies testing as diagnostic and prognostic tools for different conditions involving RA patients is already well established (6, 7). Its role as predictive test for therapy response or improvement has not yet been fully explored. We have found that a higher cut-off level than those used for diagnostic purposes could help clinicians to predict response to treatment as assessed by the ACR 20 criteria. Nevertheless, it must be kept in mind that a negative or low serum anti-CCP level does not rule out the possibility of a patient to improve. The mechanisms whereby blocking of TNF- α by adalimumab could lead to a decrease in the generation of autoantibodies such as anti-CCP and RF are not well understood. However, it has been shown that adalimumab and infliximab can down regulate the production of several inflammatory cytokines and mediators (16, 17) and may reduce the synovial infiltration by macrophages, T cells, and plasma cells (18). So, it can be hypothesized that the reduction in inflammatory lymphoplasmacytic infiltrate in rheumatoid synovium by adalimumab will lead to a reduced production of anti-CCP antibodies and RF. However, our results suggest that

the generation of anti-CCP antibodies and RF may be controlled in a different manner in RA, because the inhibition of RF appears to be more dependent on TNF- α blockade and more persistent than the inhibition of anti-CCP antibodies. Anti-CCP antibodies seem to be more specific as a marker of clinical response, because their decrease correlated with ACR 20 criteria of improvement. RF levels decreased in ACR 20 responders and non-responders. It has also been reported that IgM RF, but not anti-CCP antibodies are associated with changes in acute phase reactants during anti- TNF- α treatment (19). The differential effect of adalimumab treatment on IgM RF and the anti-CCP antibodies add support to the existing evidence that RF and anti-CCP antibodies are two different, independent autoantibody systems in RA.

In conclusion our results show that there is a correlation between the clinical efficacy of adalimumab and a decrease in serum anti-CCP antibody levels. The effect of adalimumab on anti-CCP production may reflect an important therapeutic action of this agent.

Thus our findings suggest that basal and serial evaluation of these antibodies could be useful in monitoring the clinical course of the disease during adalimumab treatment.

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