

## EXTENDED REPORT

# The effect of etanercept on anti-cyclic citrullinated peptide antibodies and rheumatoid factor in patients with rheumatoid arthritis

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**Objective:** To evaluate the changes in anti-cyclic citrullinated peptide antibodies (anti-CCP) and rheumatoid factor (RF) following etanercept treatment in patients with rheumatoid arthritis.

**Methods:** The study included 90 patients with rheumatoid arthritis who failed treatment with disease modifying antirheumatic drugs (DMARDs). All patients were allowed to continue treatment with DMARDs; 52 of them received etanercept as a twice weekly 25 mg subcutaneous injection for three months, and the others did not. Serum samples were collected at baseline and one month intervals during the treatment course. The serum levels of anti-CCP and RF were tested by enzyme linked immunosorbent assay and nephelometry, respectively.

**Results:** At baseline, 45 of the 52 etanercept treated patients (86.5%) and 32 of the 38 controls (84.2%) were positive for anti-CCP. Tests for RF were positive in 78.9% and 84.2% of patients with or without etanercept treatment, respectively. The serum levels of anti-CCP and RF decreased significantly after a three month etanercept treatment ( $p=0.007$  and  $p=0.006$ , respectively). The average decrease from baseline calculated for each individual patient in the etanercept treated group was 31.3% for anti-CCP and 36% for RF. The variation in anti-CCP was positively correlated with the variation in disease activity, swollen and tender joint counts, RF, and C reactive protein.

**Conclusions:** Etanercept combined with DMARDs leads to a much greater decrease than DMARDs alone in the serum levels of anti-CCP and RF in rheumatoid arthritis, compatible with a reduction in clinical disease activity.

Rheumatoid arthritis is a systemic autoimmune disease characterised by chronic joint inflammation that eventually results in bone destruction and severe disability. Disease modifying antirheumatic drugs (DMARDs) may retard disease progression.<sup>1</sup> However, not all patients with rheumatoid arthritis can tolerate or respond to the traditional DMARDs.<sup>2</sup> In recent years, advances in molecular technology have contributed to direct specific treatment aimed at relevant proinflammatory cytokines, such as tumour necrosis factor  $\alpha$  (TNF $\alpha$ ) and interleukin 1 (IL1). Etanercept, a soluble TNF $\alpha$  receptor fusion protein, can bind and neutralise extracellular TNF $\alpha$ . It has been shown to have marked clinical efficacy with minimal toxicity in rheumatoid arthritis patients who have an inadequate response to conventional DMARD treatment.<sup>3–5</sup>

The diagnosis of rheumatoid arthritis depends primarily on the clinical manifestations of the disease, with only limited serological support. Rheumatoid factor (RF), the only serologic marker in routine use, is detectable only in ~70% of patients with rheumatoid arthritis, and may be present in other diseases.<sup>6</sup> Another diagnostic test for rheumatoid arthritis, the assay for anti-cyclic citrullinated peptide antibodies (anti-CCP), has been developed in recent years. These antibodies are directed to proteins that contain the unusual amino acid citrulline, which is derived from the post-translational modification of arginine by peptidylarginine deiminases.<sup>7</sup> Anti-CCP is useful in the preclinical and early diagnosis of rheumatoid arthritis.<sup>8–11</sup> It is also important for the prediction of disease severity and radiographic joint damage.<sup>12–16</sup> The recently developed enzyme linked immunosorbent assay (ELISA), used for detecting anti-CCP, has a high specificity (89–96%) and reasonable sensitivity (65–88%) in the diagnosis of rheumatoid arthritis.<sup>17–19</sup> The high

disease specificity and frequent early presence of anti-CCP suggest that this antibody plays an important role in the pathogenesis of rheumatoid arthritis.

It is evident that etanercept is effective in relieving the signs and symptoms and radiological outcome of patients with active rheumatoid arthritis.<sup>20–21</sup> However, the changes in serum anti-CCP levels during etanercept treatment have not been evaluated before. In the present study, therefore, we investigated whether this new biological agent, etanercept, has a significant impact on anti-CCP antibodies as well as RF in rheumatoid patients who have an inadequate response to DMARDs. Complete clinical disease activity and serological indices were evaluated before and after treatment.

## METHODS

### Patients

We studied 90 patients who had rheumatoid arthritis, as defined by American College of Rheumatology criteria.<sup>22</sup> All candidates had failed treatment with methotrexate and another DMARD course (hydroxychloroquine, azathioprine, ciclosporine, sulfasalazine, or lefunomide), based on the British Society for Rheumatology guidelines for prescribing TNF $\alpha$  blockers in adult rheumatoid patients.<sup>23</sup> No biological agents had been used in these patients, and DMARDs, corticosteroids ( $\leq 10$  mg of prednisolone), and non-steroidal anti-inflammatory drugs (NSAIDs) had to have been given at

**Abbreviations:** CCP, cyclic citrullinated peptide; DAS28, 28 joint disease activity score; DMARD, disease modifying antirheumatic drug; ELISA, enzyme linked immunosorbent assay; ESR, erythrocyte sedimentation rate; IL, interleukin; RA, rheumatoid arthritis; RF, rheumatoid factor; TNF $\alpha$ , tumour necrosis factor  $\alpha$

**Table 1** Disease related characteristics of 90 patients with rheumatoid arthritis with or without etanercept

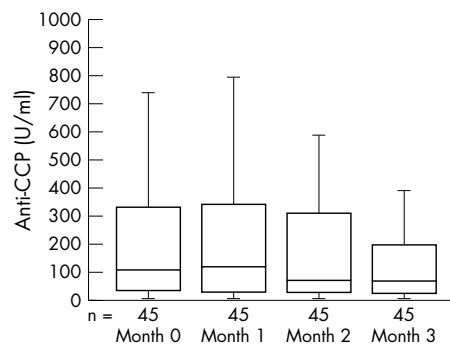
Characteristic	Etanercept group (n = 52)	Control group (n = 38)	p Value
Age (years)	53 (20 to 80)	52 (20 to 69)	0.223
Women (%)	90	87	
Duration of disease (years)	9.5 (1 to 20)	8 (1 to 21)	0.882
Methotrexate (mg)	7.5 (0 to 20)	7.5 (0 to 20)	0.551
DMARDs except methotrexate (n)	1.5 (1 to 3)	1 (1 to 3)	0.954

Values are median (range).  
DMARD, disease modifying antirheumatic drug.

a stable dose for at least four weeks before and during the treatment course. After obtaining informed consent from the patients, we used an unbalanced design which involved a 4:3 random allocation procedure to obtain the most information about the new treatment. Thus all patients were then randomly assigned into two groups: 52 in the etanercept group (DMARDs with etanercept) and 38 in the control group (DMARDs without etanercept). Patients in the etanercept group received a subcutaneous injection of etanercept at a dose of 25 mg twice weekly for three months. The DMARDs received by these rheumatoid patients were similar between those with or without etanercept, and included methotrexate (89% v 92%), sulfasalazine (77% v 79%), hydroxychloroquine (58% v 53%), cyclosporine, leflunomide, and azathioprine (less than 10%). The clinical disease activity of the patients was evaluated before and after treatment by a trained nurse without knowledge of the treatment arm. The results were recorded with a 28 joint disease activity score (DAS28) which included the total number of tender and swollen joints, plus the erythrocyte sedimentation rate (ESR), and the general health status.<sup>24</sup> Serum samples were obtained from all patients at baseline and one month intervals during the treatment, and stored at  $-80^{\circ}\text{C}$  until analysed.

### Measurement of anti-CCP and RF

We used the commercially available second generation ELISA test for anti-CCP (Diastat, Axis Shield Diagnostics, Dundee, UK). The assay was carried out according to the manufacturer's instructions. All assays were done in duplicate. The results of the anti-CCP test were considered positive if the antibody level was greater than the cut off value (5 U/ml). RF was measured by laser nephelometry for the IgM isotype (Date Behring, Marburg, Germany), and a level  $>20$  IU/ml was considered positive. For both assays, in those cases in which the antibody level was too high for the optical densities to fall on a standard curve for the original dilution, samples were further diluted until an acceptable range for detection could be read. Acute phase reactants were measured by ESR (mm/h) and C reactive protein (mg/dl) using standard laboratory methods. We also used the ELISA kit to test for anti-CCP in samples from 30 normal human blood donors to verify the specificity.



**Figure 1** Changes of serum anti-cyclic citrullinated peptide antibodies (anti-CCP) levels during the three month etanercept treatment in 45 anti-CCP positive patients with rheumatoid arthritis. The lines in the boxes indicate median points; the edges of the boxes indicate the lower and upper quartile points.

### Statistical analysis

Data were summarised as the median and range for continuous variables, and as proportions for categorical variables. Comparison of the variables in the control and etanercept treated groups was done using the Mann-Whitney U test (in view of the non-normal distribution of the results). The changes from baseline to follow up of study variables among the control and etanercept treated groups (intragroup analysis) were measured with Wilcoxon's signed rank test. The correlation analysis was made using Spearman's test. Differences were considered statistically significant where  $p < 0.05$ . Statistical analyses were carried out using the SAS statistical package.

## RESULTS

### Patients

The baseline demographic characteristics of the patients were similar between the two groups (table 1). The changes in the main clinical and laboratory indices before and after treatment in the two groups are summarised in tables 2 and 3. The baseline variables were not significantly different between the two groups.

**Table 2** Changes in the main clinical features before and after treatment in the etanercept and control groups

Variable	Etanercept group (n = 52)			Control group (n = 38)		
	Month 0	Month 3	p Value	Month 0	Month 3	p Value
DAS28	6.2 (3.0 to 9.1)	3.2 (0.3 to 7.1)	$<0.001^*$	6.2 (3.1 to 8.4)	5.9 (3.0 to 8.3)	0.053
Swollen joints	6 (1 to 25)	2 (0 to 11)	$<0.001^*$	7.5 (0 to 25)	7 (0 to 27)	0.548
Tender joints	9.5 (0 to 28)	2 (0 to 18)	$<0.001^*$	12 (1 to 26)	11 (1 to 28)	0.667

Values are shown as median (range).  
\*Significant difference.  
DAS28, 28 joint disease activity score.

**Table 3** Changes in the laboratory tests before and after treatment in the etanercept and control groups

Variable	Etanercept group (n = 52)			Control group (n = 38)		
	Month 0	Month 3	p Value	Month 0	Month 3	p Value
Anti-CCP (U/ml)	60 (1 to 3099)	49 (1 to 1438)	0.007*	118 (1 to 3098)	101 (1 to 1200)	0.648
RF (IU/ml)	87 (10 to 3170)	64 (0 to 1440)	0.006*	95 (10 to 2580)	76 (10 to 1290)	0.091
ESR (mm/h)	43 (7 to 123)	11 (1 to 77)	<0.001*	39 (3 to 120)	32 (1 to 124)	0.355
CRP (mg/dl)	1.5 (0 to 11.5)	0.1 (0 to 6.25)	<0.001*	1.7 (0 to 8.39)	1.1 (0 to 12.2)	0.555

Values are median (range).

\*Significant difference.

Anti-CCP, anti-cyclic citrullinated peptide antibodies; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor.

**Clinical response**

Patients treated with three months of etanercept and DMARDs achieved a more substantial clinical response than those treated with DMARDs alone at the end point, as defined by changes in DAS28 (table 2). The reduction of DAS28 in the etanercept group was significant, while there was a borderline decrease in the control group.

**Anti-CCP**

In all, 86.5% and 84.2%, respectively, of those in the etanercept and control groups at baseline were positive for anti-CCP. None of the 30 normal individuals were positive for anti-CCP antibodies. The positive or negative characteristics of anti-CCP remained essentially unaltered during the treatment with etanercept or DMARDs. A strong correlation between the levels of anti-CCP and RF at baseline was observed ( $r = 0.291$ ,  $p = 0.005$ ). The serum level of anti-CCP decreased significantly only in those patients who received a three month etanercept treatment (table 3). After the exclusion of the seven anti-CCP negative patients in the etanercept group, the average decrease from baseline calculated for each individual patient was 31.3% for anti-CCP, and the reduction was progressive throughout the etanercept treatment course (fig 1). The variation in anti-CCP levels was positively correlated with the variation in DAS28 ( $r = 0.378$ ,  $p < 0.001$ ), swollen joint count ( $r = 0.255$ ,  $p = 0.015$ ), tender joint count ( $r = 0.339$ ,  $p = 0.001$ ), RF ( $r = 0.406$ ,  $p < 0.001$ ), and C reactive protein ( $r = 0.389$ ,  $p < 0.001$ ), but not with the variation in ESR ( $r = 0.193$ ,  $p = 0.069$ ).

**RF and acute phase reactants**

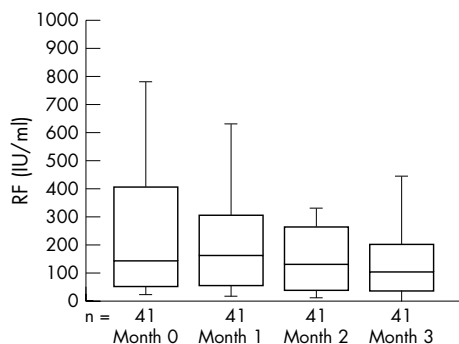
At baseline, 41 of the 52 patients (78.9%) in the etanercept group and 32 of the 38 patients (84.2%) in the control group were positive for RF. Seven patients in the etanercept group and three in the control group with low levels of RF became negative during the treatment course. There was a significant

decrease of RF in the group treated with etanercept (table 3), and the average decline from baseline was 36% for RF positive patients. The decrease in RF levels was also progressive throughout the course of the three month etanercept treatment (fig 2). The inflammatory markers ESR and C reactive protein decreased dramatically, beginning in the first month, and this decrease persisted throughout the etanercept treatment course.

**DISCUSSION**

This three month trial provides evidence for an obvious reduction in the disease activity and autoantibody production of rheumatoid patients in response to DMARDs combined with etanercept. These patients had longstanding rheumatoid arthritis (mean duration, 7.6 years), and all had been treated with methotrexate, the drug of first choice among the current DMARDs. The addition of etanercept provided more benefit without potentiating the toxic effects of DMARDs. Previous studies have shown that the regimen of etanercept plus methotrexate is more effective at reducing clinical activity and retarding radiographic progression than methotrexate alone in rheumatoid patients.<sup>4 25</sup> Etanercept as monotherapy has also been shown to be superior to methotrexate in reducing disease activity and retarding radiographic destruction in patients with early rheumatoid arthritis.<sup>20 21</sup> The clinical response to etanercept is rapid, generally occurring within two weeks, and nearly always within three months after the initiation of treatment.<sup>3 26</sup> Our trial results showed that etanercept with DMARDs offered more advantages in terms of clinical symptoms and signs, inflammatory indices, and autoantibody levels than DMARDs alone.

With its high specificity, anti-CCP plays an important role in the diagnosis of rheumatoid arthritis.<sup>17-19</sup> The presence of anti-CCP is associated with radiographic erosion and damage in rheumatoid arthritis.<sup>12-16</sup> Infliximab, a chimeric anti-TNF $\alpha$  monoclonal antibody, has been reported to reduce the anti-CCP levels in rheumatoid patients who had a good clinical response after six months of infliximab treatment.<sup>27</sup> However, other studies did not obtain the same findings at weeks 22 and 30 of infliximab treatment.<sup>28 29</sup> Even the serological reduction of anti-CCP during infliximab treatment (at week 30) could return to baseline with a longer duration of follow up (at weeks 54 and 78).<sup>30</sup> It has been reported that a shorter disease duration ( $\leq 12$  months) predicts a greater reduction in anti-CCP antibody levels with conventional treatment.<sup>31</sup> The mean level of anti-CCP has also been reported to decline during the three year follow up of DMARDs treatment in early onset rheumatoid arthritis ( $\leq 12$  months).<sup>15</sup> In our three month study, only four and seven rheumatoid patients had a disease duration less than one year in the control and etanercept groups, respectively. The anti-CCP variations were similar between patients with a disease duration longer than or shorter than one year in groups with or without etanercept. The effect of etanercept on anti-CCP antibodies was not consistent with that of most previous studies of infliximab. Except for the difference between infliximab and



**Figure 2** Changes of serum rheumatoid factor (RF) levels during the three month etanercept treatment in 41 RF positive rheumatoid patients. The lines in the boxes indicate median points; the edges of the boxes indicate the lower and upper quartile points.

etanercept, other factors such as the disease duration might play a role in the modulation of anti-CCP production during anti-TNF $\alpha$  treatment.

IgM-RF is also an important predictor of radiographic damage in rheumatoid arthritis.<sup>32</sup> Previous studies have reported that the levels of IgM-RF antibodies could be reduced following infliximab treatment.<sup>27–30</sup> In this trial, the progressive decrease of IgM-RF levels in etanercept treated patients may have some prognostic importance. Acute phase reactants such as ESR and C reactive protein are very sensitive to change in disease activity. The levels of ESR and C reactive protein in the study decreased rapidly, beginning in the first month after the injection, and these reductions appeared to be sustained throughout the etanercept treatment. Similar results were observed with another TNF $\alpha$  blocker, infliximab.<sup>33</sup>

The mechanism of etanercept that blocks the production of these autoantibodies is still unknown. The pivotal role of TNF $\alpha$  in the inflammatory and proliferative processes of rheumatoid arthritis has been established. Except for the inhibition of TNF $\alpha$  activity, etanercept significantly reduces the number of peripheral blood mononuclear cells secreting IL1 $\beta$ , IL6, and interferon  $\gamma$ .<sup>34</sup> High levels of IL13 in rheumatoid sera have also been modulated by etanercept.<sup>35</sup> Etanercept is able to induce cell type specific apoptosis in the synovial monocyte/macrophage population and to decrease the number of these inflammatory cells in rheumatoid joints.<sup>36</sup> These anti-inflammatory effects may account for the reduction in acute phase reactants and autoantibody generation. However, more studies are needed to confirm and elucidate the role of etanercept in reducing these autoantibodies.

In this study, we showed a significant decrease in the levels of anti-CCP and RF in the sera of rheumatoid patients after three months of etanercept treatment. While a reduction in the anti-CCP level after infliximab treatment was not found to be concordant with previous studies, this is the first evidence of the downregulation of this specific antibody following etanercept treatment. The effect of etanercept on anti-CCP and RF production may reflect an important therapeutic action of this agent. However, this study was limited to a three month observation and a relatively small group of patients. A longer follow up in a larger scale trial is necessary to further confirm the influence of etanercept on anti-CCP and RF in patients with rheumatoid arthritis.

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