Immunogenicity does not influence treatment with etanercept in patients with ankylosing spondylitis


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M K de Vries, I E van der Horst-Bruinsma, M T Nurmohamed, et al.

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Immunogenicity does not influence treatment with etanercept in patients with ankylosing spondylitis

M K de Vries,1 E van der Horst-Bruinsma,1 M T Nurmohamed,1,3 L A Aarden,2 S O Stapel,2 M J L Peters,1,3 J C van Denderen,3 B A C Dijkmans,1 G J Wolbink2,3

ABSTRACT

Background: Immunogenicity, specifically the onset of antibodies against tumour necrosis factor (TNF) blocking agents, seems to play an important role in non-response to treatment with these drugs.

Objectives: To assess the relation of clinical response of ankylosing spondylitis (AS) to etanercept with etanercept levels, and the presence of antibodies to etanercept.

Methods: Patients with AS were treated with etanercept 25 mg twice weekly, according to the international Assessment in Ankylosing Spondylitis (ASAS) working group consensus statement. Sera were collected at baseline and after 3 and 6 months of treatment. Clinical response was defined as a 50% improvement or as an absolute improvement of 2 points on a 0–10 scale) Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) score. Functional etanercept levels were measured by a newly developed ELISA, measuring the binding of etanercept to TNF. Antibodies against etanercept were measured with a two-site assay and antigen binding test. Clinical data were used to correlate disease activity with serum etanercept levels.

Results: In all, 53 consecutive patients were included. After 3 months of treatment 40 patients (76%) fulfilled the response criteria. Mean etanercept levels were 2.7 mg/litre and 3.0 mg/litre after 3 and 6 months respectively. Characteristics and etanercept levels of responders and non-responders were similar. No antibodies to etanercept were detected with any of the assays.

Conclusion: Etanercept levels of responders and non-responders were similar and no antibodies to etanercept were detected with any of the assays. This study indicates that etanercept is much less immunogenic compared with the other TNF-blocking agents.

Ankylosing spondylitis (AS) is a chronic inflammatory disease, which can result in invalidating deformities of the joints and spine at an early age. Until recently, treatment was based mainly on non-steroidal anti-inflammatory drugs (NSAIDs) and physical therapy. Most disease-modifying antirheumatic drugs (DMARDs) do not seem to be effective in AS, although properly conducted studies are lacking.1 The introduction of tumour necrosis factor (TNF) blocking agents, ie, infliximab,2 etanercept1 and adalimumab3 have changed the treatment options in AS radically. The majority of patients with AS, who fulfil the Assessment of Ankylosing Spondylitis (ASAS) working group guidelines for anti-TNF treatment, respond very well. Nevertheless, TNF-blocking agents still fail to reach efficacy in approximately 50% of patients with AS. A possible explanation for this failure could be the formation of antibodies, which results in lower or undetectable serum levels of the biological agent.

For etanercept, however, it is unclear whether a relation between clinical response and the formation of antibodies is present in patients with AS. In addition, many questions concerning immunogenicity have not yet been answered and different methods of detection of anti-etanercept antibodies are being used, which makes the results difficult to compare.4,5 In our previous studies, we demonstrated a correlation between clinical response and serum trough infliximab levels, adalimumab levels and the onset of antibodies against these drugs.6,7 In this study, we used the same approach as in our previous studies to investigate the relation between clinical response, functional etanercept levels and the detection of anti-etanercept antibodies in patients with AS. Additionally, in a few patients the etanercept levels were measured daily to investigate their course over time.

PATIENTS AND METHODS

Patients and study protocol

Consecutive patients with AS, attending the outpatient clinics of the Jan van Breenem Institute or of the VU University Medical Center, who were scheduled for treatment with etanercept were included and followed prospectively. All patients with AS fulfilled the modified New York criteria and started using etanercept according to the ASAS consensus statement on the initiation of TNF-blocking agents in AS.7 According to this ASAS consensus, patients must have an insufficient response to non-steroid anti-inflammatory drugs (NSAIDs) and a Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) above 4 (0–10 scale) before starting treatment with etanercept. After tuberculosis was excluded by means of a tuberculin skin test and chest x-rays, subcutaneous injections with etanercept 25 mg were taken twice a week. Concomitant medication remained unaltered for at least 3 months after the start of etanercept treatment. Demographic data collected at baseline were recorded from medical histories and patient medical records. The study was approved by the medical ethical committees of the two institutions, and all patients gave their written informed consent.

Outcome measures

Data were collected at baseline and after 3 and 6 months of treatment. During every visit
questionnaires such as the BASDAI, Patient Global Disease Activity score and Bath Ankylosing Spondylitis Functional Index (BASFI) were obtained, and after 3 months patients were checked as to whether they met the ASAS response criteria.

The primary outcome measure was clinical response after 3 months of treatment with etanercept, according to the International ASAS Consensus Statement for the use of TNF agents in patients with AS, which is equivalent to the Dutch guidelines for continuation of TNF-blocking agents.\(^1\) In this consensus statement, ASAS response was defined as a 50% improvement or as an absolute improvement of 2 points of the BASDAI (0–10 scale) and an expert opinion in favour of continuation of treatment after 3 months. Routine laboratory tests (erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) levels) were performed. A CRP value below 8.0 mg/litre was considered to be normal.

Etanercept levels and antibodies against etanercept were measured in patient sera at baseline and after 3 and 6 months. Furthermore, in five patients serum etanercept levels were measured daily between two consecutive subcutaneous injections.

### Assessment of functional serum etanercept levels

Etanercept levels were measured by means of a newly developed ELISA, based on the principle that etanercept is captured through its ability to bind TNF (Sanquin, Diagnostic Services, Amsterdam, The Netherlands). The sensitivity of detection is 1 ng/ml (= 0.001 mg/litre).

In short, a mouse monoclonal antibody directed against TNF (CLB TNF/5) was coated overnight at room temperature (0.2 \(\mu\)g/well) onto flat-bottomed microtitre plates. Recombinant TNF (5 ng/well) in high-performance ELISA (HPE) buffer (Sanquin) was added and remained in place for 1 h. After washing the plates with phosphate buffered saline/0.02% Tween, patient serum samples were added in different dilutions in HPE buffer and incubated for 1 h at 37°C. Plates were washed with phosphate buffered saline (PBS)/0.04% Tween, and incubated with biotinylated polyclonal rabbit antibodies against etanercept in 100 \(\mu\)l HPE buffer for 1 h at 37°C. Subsequently, after washing the plates, poly(horseradish peroxidase)-conjugated streptavidin was added (30 min at 30°C), followed by incubation with tetramethylbenzidine (TMB). The reaction was stopped with 2 M H\(_2\)SO\(_4\). Absorption at 450 nm was determined, and results were related to a calibration curve as applied in the two-site assay for the interpretation of patient results. Cut-off level for a positive signal was set at 1.07% binding (mean+SSD of the pre-treatment values).

### Antigen binding test

Additionally, testing for antibodies was carried out by using an antigen binding test, which is in principle similar to the tests we routinely use for detection of antibodies against infliximab.\(^12\) Pepsin-treated \(^{125}\)I-radio labelled etanercept was used for the detection of antibodies. The same rabbit-derived calibration curve as applied in the two-site assay was used for the interpretation of patient results. Cut-off level for a positive signal was set at 1.07% binding (mean+SSD of the pre-treatment values).

### Statistical analysis

The last observation was carried forward in patients who had dropped out before 6 months of treatment, due to ASAS non-response, adverse events, or loss to follow-up. Data were expressed as mean (SD) or median (interquartile range) where appropriate. The distribution of variables was tested for normality and transformed if possible. Independent Student t tests were used for variables with a normal distribution and nonparametric tests (Wilcoxon signed-rank test or Mann–Whitney U test) for skewed variables. Pearson \(\chi^2\) tests were conducted for dichotomous variables.

Logistic regression analyses were conducted to examine associations between ASAS response and serum etanercept level, and were corrected for the possible influence of demographic, clinical and laboratory variables. Subsequently, a linear regression model was used to investigate whether serum etanercept levels were associated with any of the demographic, clinical or laboratory variables. The following variables were tested: gender, age, ethnicity, body mass index (BMI) and Human leukocyte antigen (HLA) B27, baseline bodyweight, baseline BMI, decrease of Patient Global Assessment score, absolute decrease of ESR and CRP level.

Calculations were made using SPSS V14.0 software (SPSS Inc., Chicago, Illinois, USA). The threshold for significance was set at \(p<0.05\).

### RESULTS

A total of 53 patients were included in this study during the period July 2004 to March 2006. The demographic data and baseline characteristics are shown in table 1.

Bias due to missing data was excluded, as ASAS response did not differ between patients with available data and those with missing data at 6 months.

Treatment with etanercept resulted in significant clinical improvement after 3 and 6 months, when compared to baseline (table 1). The mean BASDAI, Patient Global Disease Activity score, BASFI and ESR were even lower after 6 months of treatment.

Baseline characteristics did not differ significantly for responders and non-responders (table 2).

After 5 months of treatment, 40 of the patients (76%) met the ASAS response criteria.

The etanercept levels were measured in 48 patients after 3 months of treatment and in 41 patients after 6 months of treatment: 10 of 41 samples turned out to be samples from ASAS non-responders. These patients should have stopped treatment with etanercept after 3 months because of insufficient improvement of the BASDAI. However, they continued their treatment based on the expert opinion of the rheumatologist.

At baseline serum etanercept levels were undetectable, mean serum etanercept levels were 2.7 mg/litre (SD 1.2 mg/litre) and...
3.0 mg/litre (SD 1.0 mg/litre) after 3 and 6 months of treatment respectively. In each patient etanercept levels could be measured and were within the same range (fig 2). The mean etanercept levels of responders and non-responders were similar, which could be explained by the fact that antibodies against etanercept were not detected in these patients with any of the assays (table 2 and fig 2). As well as the lack of association between clinical response and serum etanercept levels, there was also no significant association between gender, age, ethnicity, HLA B27, baseline bodyweight, baseline BMI, decrease of Patient Global Assessment score, absolute decrease of ESR and CRP level and serum etanercept levels.

In addition, in five patients etanercept levels were measured at daily intervals between two subsequent injections with a mean of 3.5 mg/litre, SD 1.2 mg/litre and a variance of 1.5. Furthermore, sera of six non-responders were taken 3 months after the final etanercept injection and, as expected, etanercept was no longer present in these sera and antibodies against etanercept could not even be detected in these patients.

### Adverse events

One patient had an adverse reaction of flushing and dyspnoea after 2 months of treatment with etanercept. Another patient developed urticaria and atopic dermatitis after 27 months of treatment with a pre-filled syringe of etanercept, however, this patient showed none of these symptoms after re-treatment with etanercept in the form of lyophilised powder. Both patients had previously developed an infusion reaction to infliximab after which they had switched to etanercept. No antibodies against etanercept were detected in any of the patients with adverse reactions.

### DISCUSSION

In all, 76% of the patients with AS were classified as responders to etanercept after 3 months of treatment, which is comparable to the response rate observed in clinical trials. No correlation was found between etanercept levels, formation of antibodies against etanercept and clinical response. All patients had detectable serum levels of etanercept and no antibodies against etanercept were found. Interestingly, there seemed to be no difference in mean etanercept levels and the onset of antibodies against etanercept between responders and non-responders. These findings are in contrast with our previous studies with infliximab and adalimumab in rheumatoid arthritis RA and AS, where we found a strong correlation between decreased response and the onset of antibodies against TNF-blocking agents. These antibodies against TNF-blocking agents often resulted in low or absent serum levels of these drugs, which would explain the decrease in efficacy, but apparently this is not the case with etanercept. Therefore, this study seems to confirm the hypothesis that etanercept is less immunogenic than other TNF-blocking agents.

Although etanercept levels have not yet been correlated with clinical response, some studies do report on the detection of antibodies against etanercept. In these, etanercept has been labelled as having antibody formation in less than 6% of the cases and no clear relation to clinical response was detected. These discrepancies regarding anti-therapeutic drug antibodies could be explained by the use of different detection methods. ELISA is known to give more false positive signals than antigen binding assays, and it is therefore difficult to compare other results with our method. The detection method for anti-etanercept antibodies used by our laboratory may be regarded as not very sensitive. However, this does not agree with the fact that functional etanercept levels could be measured in all patients, because the presence of antibodies would have resulted

### Table 1 Demographic data and baseline characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline</th>
<th>3 Months</th>
<th>6 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males (number, %)</td>
<td>40 (76)</td>
<td>3.1 2.9*</td>
<td>2.5 1.7*</td>
</tr>
<tr>
<td>Age (years)</td>
<td>41 (11)</td>
<td>3.2 2.4*</td>
<td>2.5 2.1*</td>
</tr>
<tr>
<td>Caucasian (number, %)</td>
<td>45 (85)</td>
<td>4.1 2.5*</td>
<td>3.5 2.5*</td>
</tr>
<tr>
<td>BMI</td>
<td>25 (4.4)</td>
<td>5 (1–58)*</td>
<td>4 (1–33)*</td>
</tr>
<tr>
<td>HLA B27 + (number, %)</td>
<td>44 (88)</td>
<td>4 (1–44)*</td>
<td>4 (1–74)*</td>
</tr>
<tr>
<td>Peripheral arthritis (number, %)</td>
<td>28 (53)</td>
<td>2.7 1.2</td>
<td>3.0 1.0</td>
</tr>
<tr>
<td>BASDADI</td>
<td>6.4 (1.3)</td>
<td>6.2 (2.1)</td>
<td>6.2 (2.1)</td>
</tr>
<tr>
<td>Patient Global Disease Activity score</td>
<td>7.2 (1.5)</td>
<td>4.2 (2.5)</td>
<td>4.2 (2.5)</td>
</tr>
<tr>
<td>CRP (median, range)</td>
<td>22 (1–114)</td>
<td>5 (1–58)*</td>
<td>4 (1–33)*</td>
</tr>
<tr>
<td>CRP (median, range)</td>
<td>17 (1–92)</td>
<td>4 (1–44)*</td>
<td>4 (1–74)*</td>
</tr>
<tr>
<td>Serum etanercept levels</td>
<td>0</td>
<td>2.7 1.2</td>
<td>3.0 1.0</td>
</tr>
<tr>
<td>Antibodies to etanercept</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Except where indicated otherwise, the values are the mean (SD). Mean serum etanercept and antibodies to etanercept levels are in mg/litre. Normal CRP <8.0 mg/litre; normal ESR <10 mm/h.

Significance level: p <0.001.

BasDAI, Bath Ankylosing Spondylitis Disease Activity Index (0–10); BASFI, Bath Ankylosing Spondylitis Functional Index (0–10); BMI, body mass index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; HLA B27, human leukocyte antigen B27.
in an enhanced clearance and removal of etanercept. Furthermore, similar techniques for measuring functional etanercept levels and detecting of antibodies against etanercept were proven to be sensitive in our previous studies with other TNF-blocking agents.

Several arguments are in favour of the hypothesis that etanercept shows less immunogenicity compared with other TNF-inhibitors. Firstly, etanercept has a less immunogenic structure compared with the other TNF-blocking agents. Etanercept is a dimeric fusion protein consisting of two TNF receptors, linked to the Fc portion of an IgG1. Only the fusion part of the molecule can contain immunogenic epitopes. Infliximab is a chimerical monoclonal IgG1 antibody against TNFα, partly consisting of murine protein. Adalimumab is a fully human monoclonal antibody against TNFα. These monoclonal antibodies have more epitopes within the variable region of the antibody to which an immune response can be directed. Secondly, major fluctuations in serum levels may precipitate an immune response and the development of antibodies against the TNF-blocking agent. This is mainly the case in treatment with infliximab, which is administered once every 6 to 8 weeks. Treatment with etanercept, however, produces stable levels between the two injections and is dosed much more frequently, which is in line with previous findings.16–19 The variance of 1.5 of the etanercept levels measured at daily intervals between two subsequent injections measured in our five patients was very low, compared with the variance of all serum trough infliximab levels measured in our previous study, which was 80. The overall variance of all etanercept levels was 1.3, which is also very low. Thirdly, there may be different mechanisms for non-response. Theoretically, non-responders can be divided into two categories: true non-responders, whose illness is not mainly caused by excess production of TNFα, and non-responders whose illness is caused by inadequate blocking of TNFα. The latter can be caused by enhanced clearance or as a result of inadequate dosing.

A dose–response relation of etanercept in AS has not been investigated previously. There is a possibility that small differences in etanercept levels could not be found because of the random timing of sampling between two injections. The most important argument against this view is probably the observation that etanercept levels, measured at several time intervals between the two subsequent injections in the five patients, were quite stable. Furthermore, other authors have confirmed that etanercept levels are likely to be stable between two injections.16 Additionally, the mean etanercept levels were equal in responders and non-responders and this measurement error would influence both groups in the same way.

In summary, all patients with AS had detectable etanercept levels, regardless of whether they were responders or non-responders. In contrast with previous studies with other TNF-blocking agents, no antibodies against etanercept were detected with any of the assays. This study indicates that immunogenicity does not play an important role in explaining the non-response of patients with AS to treatment with etanercept.

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**Competing interests:** None declared.

**Ethics approval:** The study was approved by the medical ethical committees of the VU University Medical Center and the Jan van Breemen Institute.

**Patient consent:** Informed consent was obtained for the publication of the details in this report.

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**Table 2** Baseline characteristics and baseline ESR, CRP and etanercept levels after 3 months of treatment for ASAS responders and non-responders

<table>
<thead>
<tr>
<th>Variables</th>
<th>Responders (n = 40)</th>
<th>Non-responders (n = 13)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender (number, %)</td>
<td>29 (73)</td>
<td>11 (85)</td>
<td>0.38</td>
</tr>
<tr>
<td>Age (years)</td>
<td>41 (11.2)</td>
<td>41 (8.8)</td>
<td>0.82</td>
</tr>
<tr>
<td>Caucasian (number, %)</td>
<td>36 (90)</td>
<td>9 (69)</td>
<td>0.07</td>
</tr>
<tr>
<td>BMI</td>
<td>25 (4.8)</td>
<td>24 (3.1)</td>
<td>0.53</td>
</tr>
<tr>
<td>HLA B27+ (number, %)</td>
<td>34 (90)</td>
<td>10 (83)</td>
<td>0.57</td>
</tr>
<tr>
<td>Peripheral arthritis (number, %)</td>
<td>21 (53)</td>
<td>7 (54)</td>
<td>0.99</td>
</tr>
<tr>
<td>BASDAI</td>
<td>6.4 (1.3)</td>
<td>6.2 (1.4)</td>
<td>0.57</td>
</tr>
<tr>
<td>Patient Global Disease Activity (VAS)</td>
<td>7.1 (1.5)</td>
<td>7.4 (1.9)</td>
<td>0.31</td>
</tr>
<tr>
<td>BASFI</td>
<td>6.2 (2.1)</td>
<td>6.2 (2.2)</td>
<td>0.86</td>
</tr>
<tr>
<td>ESR (median, range)</td>
<td>22 (1–114)</td>
<td>22 (1–87)</td>
<td>0.82</td>
</tr>
<tr>
<td>CRP (median, range)</td>
<td>18 (1–91)</td>
<td>10 (1–92)</td>
<td>0.45</td>
</tr>
<tr>
<td>Etanercept level (mg/litre)</td>
<td>2.7 (1.3)</td>
<td>2.8 (1.1)</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Except where indicated otherwise, the values are the mean (SD). ASAS, Assessment in Ankylosing Spondylitis; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index (0–10); BASFI, Bath Ankylosing Spondylitis Functional Index (0–10); BMI, body mass index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; HLA B27, human leukocyte antigen B27; VAS, visual analogue scale.

**Figure 2** Etanercept levels (mg/litre) for Assessment of Ankylosing Spondylitis (ASAS) responders and non-responders after 3 months of treatment.
REFERENCES


