Key findings towards optimising adalimumab treatment: the concentration-effect curve

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Abstract

Background and objectives The recommended dose of adalimumab is 40mg every other week for all patients with inflammatory diseases, but variation in pharmacokinetics between patients suggests that individual patients may be under or over-treated. A concentration-effect curve for adalimumab has not been published yet.

Methods In a prospective observational cohort study, 221 consecutive patients with rheumatoid arthritis (RA) were treated with 40mg adalimumab subcutaneously every other week. Adalimumab trough levels were measured using an ELISA, and clinical efficacy was measured based on differences in disease activity between baseline and 28 weeks of follow-up.

Results Adalimumab levels varied between 0 - 20 ug/mL. Clinical efficacy improved with increasing adalimumab concentration and reached a maximum (mean DAS28 improvement of 2) with levels between 5 – 8 mg/mL. Levels exceeding 8 ug/mL were illustrated to have no additional beneficial effect on disease activity. The Receiver Operator Characteristics (ROC) curve showed an Area Under the Curve (AUC) of 0.695 (95% CI 0.626 – 0.764) for European League Against Rheumatism (EULAR) response and adalimumab levels: good responders versus non and moderate responders. A cut-off of 5 ug/mL had sensitivity of 91% and a specificity of 43%. Adalimumab levels are influenced by concomitant methotrexate use: patients on adalimumab monotherapy had a median adalimumab level of 4.1 ug/mL (IQR 1.3 – 7.7), whereas patients concomitantly taking methotrexate had a median level of 7.4 ug/mL (IQR 5.3 – 10.6, p<0.001).

Conclusions Adalimumab trough levels in a range of 5 - 8 ug/mL are sufficient to reach adequate clinical response. These levels are influenced substantially by concomitant methotrexate use.
INTRODUCTION

Tumour necrosis factor (TNF) inhibitors have become an important part of healthcare worldwide for inflammatory diseases such as rheumatoid arthritis (RA), Crohn’s disease, psoriatic arthritis, and ulcerative colitis. Although billions of dollars are being spent on these agents for patients worldwide and ‘raised the bar’ for goals of therapy for RA,1 2 detailed information about pharmacokinetics, and pharmacodynamics is minimal. Studies undertaken to investigate these characteristics for adalimumab,3-8 mainly focused on finding a safe and effective therapeutic ‘one fits all’ dose, even though there is a wide variation in pharmacokinetics between biological-treated patients.9

We previously observed that good responders had significantly higher serum concentrations than non-responders, and moderate responders,9 and that concentrations reach a median steady state concentration within 28 weeks of treatment.10 The adalimumab level reached in serum depends on different factors such as absorption rate after subcutaneous injection, distribution throughout the body and clearance of the drug. These factors are influenced by physical differences between patients such as gender, age and disease state.11 12

Furthermore, pharmacokinetics is profoundly influenced by immunogenicity. The production of anti-drug antibodies (ADA) leads to neutralization of the drug and the formation of drug-ADA immune complexes that alter the clearance rate of the drug. In 94% of the patients who produce ADA, these antibodies can be detected within the first 28 weeks of treatment,10 13 and over 98% of anti-adalimumab antibodies inhibit the binding of adalimumab to TNF.14 Consequently, the administered dose will be partly or completely inactivated. Therefore, patients producing higher amounts of ADA will have lower or no measurable concentrations of functional adalimumab (i.e., adalimumab that can still bind to TNF).14-16 Immunogenicity of adalimumab in RA patients has been described to be associated with lower adalimumab concentration and a lower likelihood of minimal disease activity or clinical remission.17 18

Concomitant use of immunomodulators could diminish the formation of ADA via a suppressive effect on the immune system, and in case of adalimumab, methotrexate was confirmed to have an effect in reducing immunogenicity in a dose-dependent manner,19 and could thereby result in higher adalimumab levels and enhanced therapy.

Although ADA are closely linked with adalimumab concentration, with an antigen binding test they can only routinely be measured adalimumab concentrations are low or not
detectable, since this assay is susceptible for drug interference. Therefore the adalimumab concentration is in our opinion a more reliable parameter to monitor, in addition to clinical response.\textsuperscript{15}

This study is the first to relate serum trough concentrations to clinical effect with a concentration-effect curve, in patients who used adalimumab for 28 weeks, with or without concomitant use of methotrexate.

**METHODS**

**Patients**

For this study, data was obtained from a prospective observational cohort study consisting of 272 consecutive RA patients treated with adalimumab (Abbvie, Illinois, USA) at the Department of Rheumatology, Jan van Breemen Research Institute | Reade, Amsterdam, the Netherlands as described previously.\textsuperscript{10} Some of these patients were also reported in previous papers.\textsuperscript{9,20-22}

Patients were excluded from this study when the dosing scheme was not 40 mg sc every other week, (n=36). An additional 15 patients were excluded due to lacking samples for repeated measurement of adalimumab concentrations with the newly validated protocol, as described below. Therefore, 221 patients remained for analysis. To analyse clinical response in patients up to 28 weeks of treatment, we used last observation carried forward for patients who discontinued treatment prematurely. The study was approved by the medical ethics committees of the Slotervaart Hospital, and the Jan van Breemen Research Institute | Reade, Amsterdam, the Netherlands. All patients gave written informed consent.

**Clinical response**

Disease activity was assessed at baseline and after 28 weeks of therapy using the disease activity score in 28 joints (DAS28).\textsuperscript{23}

Treatment response was defined according to the European League Against Rheumatism (EULAR) response criteria.\textsuperscript{24}
Measurement of adalimumab concentrations

Trying to understand clinical efficacy early on in treatment, and taking into account ADA development, 28 weeks was chosen to collect serum samples for this study, just before next injection.\textsuperscript{10, 15} To measure drug levels accurately, the previous reported enzyme linked immunosorbent assay (ELISA) was automated using a Tecan Freedom EVO platform,\textsuperscript{13} and validated for measurements in serum and heparin plasma according to the Q2 (R1) guideline and Food and Drug Administration (FDA)-guidelines.\textsuperscript{25, 26} This validation showed a Lower Limit of Quantification (LLOQ) of 10 ng/mL. Accuracy (expressed as % of theoretical value) and precision (expressed as % coefficient of variation) were determined using adalimumab spikes of 10 ng/mL, 4 ug/mL and 20 ug/mL, and were found to be 92-109% and <7%, respectively. Accuracy was not influenced by the presence of methotrexate or prednisolone. To confirm assay specificity, non-spiked sera from healthy donors (n=9) and biological-naïve RA patients (n=64, including rheumatoid factor positive samples) were tested, which all rendered signals below the LLOQ of the assay. Furthermore, spikes using certolizumab-pegol (50 ug/mL), infliximab (250 ug/mL), etanercept (2.4 ug/mL) and golimumab (3.1 ug/mL) did not render detectable signals.

Concentration effect curve

To establish a concentration effect curve after 28 weeks of treatment, all 221 patients were sorted from low to high adalimumab concentration with correlating ΔDAS28. These data were then stratified in eleven groups of 20 patients (last group 21 patients) giving a mean trough level and a mean ΔDAS28, which lowers the inter-variability between patients. To investigate the influence of MTX, patients were divided into a group concomitantly using MTX and the group of patients not using MTX. Because groups were smaller, they were stratified by groups of 10 patients instead of 20, with mean trough level and a mean ΔDAS28.

Statistical analysis

For differences in baseline demographic and clinical variables between response groups, independent samples t test, Mann-Whitney U test or chi-square were used as appropriate. The threshold for significance was set at p< 0.05. To analyse clinical response in patients up to 28 weeks of treatment, we used last observation carried forward for patients who discontinued treatment prematurely.
Receiver operator characteristics (ROC) analysis was used to obtain a representative cut-off value for adalimumab trough levels. To obtain this distinct value a trade off was made between sensitivity and specificity, while taking into account health benefits, non-treatment and costs. Patients were divided into a group of non and moderate responders, and a group of good responders according to the EULAR response criteria. Using logistic regression analysis, the predicted probability of adalimumab concentrations and methotrexate dose combined were calculated, which was thereafter entered in the ROC curve to obtain the Area Under the Curve (AUC) for these combined variables.

For differences in adalimumab concentration between patients with and without methotrexate use and low, intermediate and high dosage use, logistic regression analysis was implemented.

The statistical software package (SPSS Inc, Chicago, IL) version 15.0 and Graph Pad Prism 5 for windows were used to perform statistical analysis.

RESULTS

Of the 221 patients enrolled in this study 193 (87.3%) completed follow-up. The median follow-up period was 28 weeks (IQR 28 – 28). Patient characteristics are shown in Table 1. There were statistically significant differences between patients sex (p = 0.04), ESR (p < 0.001), DAS28 (p = 0.007), previous use of biologicals (p < 0.001), concurrent methotrexate use (p < 0.001), and methotrexate dose (p = 0.001) for patients that did or did not achieve good EULAR response.
Table 1. baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>total patient population n=221</th>
<th>EULAR good responders n=87</th>
<th>EULAR non and moderate responders n=134</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>demographics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>age, mean (SD), y</td>
<td>54 (12)</td>
<td>53 (12)</td>
<td>55 (12)</td>
</tr>
<tr>
<td>sex, female n (%)</td>
<td>176 (80)</td>
<td>63 (72)</td>
<td>113 (84)</td>
</tr>
<tr>
<td><strong>disease status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>disease duration, median (IQR) y</td>
<td>8 (3-18)</td>
<td>7 (3-17)</td>
<td>10 (4-18)</td>
</tr>
<tr>
<td>rheumatoid factor positive, n (%)</td>
<td>160 (72)</td>
<td>59 (68)</td>
<td>101 (75)</td>
</tr>
<tr>
<td>anti-ccp positive, n (%)</td>
<td>155 (70)</td>
<td>60 (69)</td>
<td>95 (71)</td>
</tr>
<tr>
<td>erosive disease, n (%)</td>
<td>163 (74)</td>
<td>63 (72)</td>
<td>100 (75)</td>
</tr>
<tr>
<td>ESR, median (IQR), mm/h</td>
<td>24 (12-41)</td>
<td>18 (9-29)</td>
<td>30 (15-49)</td>
</tr>
<tr>
<td>C-reactive protein, median (IQR), mg/L</td>
<td>12 (6-24)</td>
<td>12 (5-22)</td>
<td>11 (6-29)</td>
</tr>
<tr>
<td>DAS28, mean (SD)</td>
<td>5.3 (1.1)</td>
<td>5.0 (0.9)</td>
<td>5.4 (1.2)</td>
</tr>
<tr>
<td><strong>(co) medication</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>prior DMARDs, mean (SD)</td>
<td>3.2 (1.4)</td>
<td>3.0 (1.4)</td>
<td>3.3 (1.4)</td>
</tr>
<tr>
<td>prior biologics, n (%)</td>
<td>54 (24)</td>
<td>9 (10)</td>
<td>45 (34)</td>
</tr>
<tr>
<td>methotrexate use, n (%)</td>
<td>170 (77)</td>
<td>77 (89)</td>
<td>93 (69)</td>
</tr>
<tr>
<td>methotrexate dose, median (IQR), mg/wk</td>
<td>15 (5-25)</td>
<td>25 (12.5-25)</td>
<td>14 (0-25)</td>
</tr>
<tr>
<td>prednisone use, n (%)</td>
<td>71 (32)</td>
<td>31 (36)</td>
<td>40 (30)</td>
</tr>
<tr>
<td>prednisone dose, median (IQR), mg/d</td>
<td>7.5 (5-10)</td>
<td>5 (5-7.5)</td>
<td>7.5 (5-10)</td>
</tr>
</tbody>
</table>

Abbreviations: CCP, cyclic citrullinated peptide; DAS28, disease activity score in 28 joints; DMARD, disease-modifying antirheumatic drug; ESR, erythrocyte sedimentation rate; IQR, interquartile range.

a For differences between groups, we used the independent samples t test, chi square, or Mann-Whitney U (Wilcoxon) statistic, as appropriate.

b There were significant differences between patients sex (p=0.04), ESR (p=<0.001), DAS28 (p=0.007), prior biologics (p=<0.001), methotrexate use (p<0.001), and methotrexate dose (p=0.001).

Concentration effect curve

In Figure 1, the relationship between adalimumab trough levels and clinical response is shown. All 221 patients were sorted from low to high adalimumab concentration, with each dot representing the mean concentration and correlating ΔDAS28 per 20 patients (last dot is 21 patients) with standard deviations showing intervariability between patients. There were 18 patients with no detectable adalimumab concentration of which 89% could be ascribed to ADA formation. To reach a DAS28 improvement of 1.2 or higher, concentrations around 3 ug/mL appear to be already sufficient. Serum levels up to 8 ug/mL show a positive association with ΔDAS28. However, it appears that levels above 8 ug/mL did not give further improvement of clinical efficacy.
Figure 1. Concentration effect curve. Each point represents the mean (with SD) of 20 data points of 221 trough level concentrations measured at 28 weeks of treatment stratified in ascending order with correlating ΔDAS28 mean (with SD).

**ROC curve**

To establish a cut-off value, the patients were divided into a group of good responders, and a group consisting of non and moderate responders, according to the EULAR response criteria. The adalimumab concentrations were studied in a ROC-curve.

Figure 2 shows an AUC of 0.695 (95% CI 0.626 – 0.764, p < 0.0001). At 5.0 ug/mL a sensitivity of 91% and a specificity of 43% was found. The AUC in the ROC curve is significantly different from 0.5, concluding that adalimumab concentration has the ability to distinguish between the group of good responders and the group of moderate and non responders.
Figure 2. ROC-curve analysis: EULAR non and moderate vs. good response. ROC-curve analysis with trough level concentrations of adalimumab. To optimally distinguish between EULAR non + moderate responders versus good responders a cut-off value of 5 ug/mL was found with an AUC of 0.695 (95% CI 0.626 – 0.764, p<0.0001), with a specificity of 43% and sensitivity of 91%.

**Effect of methotrexate on adalimumab through levels**

The relationship between adalimumab concentration and clinical response is similar for patients concomitantly using MTX (Figure 3A) and patients who were not using MTX (Figure 3B). The patient group using MTX consists of 170 patients and patients not using MTX consists of 51 patients. Both curves show the same trend but with a higher mean improvement of DAS28 for patients in the methotrexate group.

These two groups had an overall significantly different median adalimumab concentration. Patients on adalimumab monotherapy had a median adalimumab level of 4.1 ug/mL (IQR 1.3 – 7.7), with 80.4% non- or moderate responder and only 19.6% good responders. By contrast patients concomitantly taking methotrexate had a median level of 7.4 ug/mL (IQR 5.3 – 10.6, p < 0.001), and 45% of this population was good EULAR responder and 55% was non- or moderate responder.
Figure 3. Concentration effect curve. Each point represents the mean (with SD) of 10 data points of 221 trough level concentrations measured at 28 weeks of treatment stratified in ascending order with correlating ∆DAS28 mean (with SD). A) shows the concentration-effect curve for adalimumab patients using concomitant MTX divided in groups of 10 patients each and B) shows the same curve for adalimumab patients not using MTX.

Table 2 shows the median adalimumab concentrations for patients with no concomitant methotrexate use (n = 51), low dose methotrexate (5 – 10 mg/week, n=34), intermediate dose methotrexate (12.5 – 20 mg/week, n = 49) or high dose methotrexate use (≥ 22.5 mg/week, n = 87) as well as the percentages of patients achieving EULAR response per methotrexate dose group. There was a significant difference in adalimumab levels between patients not using MTX and low, intermediate and high dose MTX users (p = 0.034; 0.026; and 0.001, respectively), but no significant difference in levels were seen between MTX user groups.

<table>
<thead>
<tr>
<th>concomitant MTX-dose</th>
<th>n</th>
<th>median adalimumab level (ug/mL)</th>
<th>IQR</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mg/ week</td>
<td>51</td>
<td>4.1</td>
<td>1.3 – 7.7</td>
</tr>
<tr>
<td>5-10 mg/week</td>
<td>34</td>
<td>8.0</td>
<td>4.0 – 10.5</td>
</tr>
<tr>
<td>12.5-20 mg/week</td>
<td>49</td>
<td>6.9</td>
<td>4.8 – 11.1</td>
</tr>
<tr>
<td>≥ 22.5 mg/week</td>
<td>87</td>
<td>7.7</td>
<td>5.5 – 10.5</td>
</tr>
</tbody>
</table>

DISCUSSION

With the identification of this concentration-effect relationship of adalimumab in patients with RA, new opportunities have emerged to optimize treatment and reduce costs.
Although adalimumab concentrations vary widely between patients, with an AUC of 0.695, a drug level of 5 ug/mL has a predictive value of good clinical response according to the EULAR response criteria. In addition, even in patients with low adalimumab concentrations around 3 ug/mL clinical effect was found. However, in patients without detectable drug levels there was no clinical effect of adalimumab. Most importantly, we illustrated no additional improvement of disease activity in patients with adalimumab concentrations exceeding 8 ug/mL.

In 2002 it was described that dose titration in adalimumab is feasible without losing clinical efficacy.\textsuperscript{27} We observed that levels exceeding 8 ug/mL, compared to 5 - 8 ug/mL, had no additional improvement of disease activity, which roughly comes down to one third of the patients. These findings together imply that in the group of patients exceeding these concentrations it might be able to successful lower the dose interval without losing clinical efficacy.

Over-treatment of this expensive drug results in a substantial waste of health care resources. Therefore, rather than avoiding or anticipating on toxic concentrations, therapeutic drug monitoring (TDM) of adalimumab would primarily aim to reduce costs without affecting treatment efficacy. However, it remains to be investigated if patients will indeed retain an optimal clinical response with lower concentrations. A dose decrease might potentially lead to issues with increased immunogenicity, since it is unknown whether lower adalimumab concentrations will result in breaking tolerance.

Vice versa, patients with low drug levels can be identified. For these patients TDM based dose titration might assist in restoring or improving clinical response, although costs of adalimumab may limit the usefulness of this approach in patients with RA. Moreover, this will only be useful in patients with absent or low ADA levels.\textsuperscript{16}

In our patient population, one of the main factors influencing pharmacokinetics was the concomitant use of methotrexate: on average, patients with adalimumab monotherapy had an adalimumab concentration of 4.1 ug/mL, whereas patients concomitantly treated with methotrexate had a median concentration of 7.4 ug/mL. One factor that can explain this effect is influenced by the fact that adalimumab patients concomitantly treated with methotrexate are less prone to develop ADA,\textsuperscript{19} and since ADA bind to the idiotype of adalimumab,\textsuperscript{14} functional drug levels are higher in patients taking concomitant methotrexate. This corresponds to our data in which of the 18 patients having no detectable adalimumab concentration, 61% were on adalimumab monotherapy and 89% had detectable ADA levels.
Furthermore, there might be a synergistic effect between methotrexate and adalimumab. Even in patients failing to respond to methotrexate, there will be some level of suppression of inflammation, resulting in fewer targets for adalimumab to bind to and therefore higher functional drug levels. Therefore, we can conclude that even the use of a low concomitant methotrexate dose aids in optimizing treatment with adalimumab, at least during the first 6 months of treatment, since patients taking methotrexate might need a lower dose of adalimumab to obtain an effective concentration with maximal clinical benefit. Whether this effect of methotrexate will also be accomplished by other immunosuppressive agents needs further investigation.

Whether the current results can be extrapolated to other biologics or inflammatory diseases is debatable. For drugs in which pharmacokinetics is influenced by immunogenicity this could be justified, however half-life, dose, dosing interval and administration route influence pharmacokinetics and differ between drugs. Furthermore, concentrations needed to obtain maximal clinical effect will differ between inflammatory diseases. In addition to variable pharmacokinetics of drugs in different inflammatory diseases, methotrexate co-treatment is not common in all diseases and sometimes even discontinued before the initiation of a biologic.\textsuperscript{18,28} Even though methotrexate has no clinical effectiveness in some inflammatory diseases, i.e. ankylosing spondylitis, it might be worthwhile to consider concomitant methotrexate therapy.

Important prerequisites for the construction of a concentration-effect curve are the availability of a standardized, validated assay and controlled timing of blood sampling.\textsuperscript{29} For the current study we used our newly validated and automated adalimumab concentration ELISA. Furthermore, all patients in our cohort were requested to donate blood just before their next adalimumab injection. Nevertheless, there might have been some variation in this timing between patients adding to the variation in drug levels.

Standard deviations of $\Delta$DAS28 in Figure 1 were large and overlapping between groups. This indicates that pharmacokinetics of a drug is not the only factor defining whether a patient is or will be a responder. Other factors contributing to response to therapy will be patient and disease related and need to be investigated further in order to optimize treatment in these patients. For instance, in some patients the disease may not be driven by
TNF and a TNF-inhibitor may not be the right type of drug. These patients might benefit from biologics with other mechanisms to suppress inflammation.

In conclusion, the concentration-effect curve is a new instrument in the treatment of adalimumab. We identified the therapeutic range of 5 to 8 ug/mL for maximal clinical effect. This range can be used for TDM based treatment adaptations, to titrate the dose of adalimumab toward this range. This will lead to a more optimal use of the expensive drug. Concomitant methotrexate use is an important factor influencing pharmacokinetics of adalimumab and should therefore, if possible, be used to optimize treatment with adalimumab. TDM based individually tailored treatment will result in maximal clinical benefit with the lowest possible dose of the drug.
Reference list


