Therapeutic drug monitoring to optimize biological treatment: opportunities and challenges

Eva-Linda Kneepkens
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Eva-Linda Kneepkens
promotor: prof. dr. M.T. Nurmohamed
copromotor: dr. G.J. Wolbink
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CHAPTER 1

General introduction
Certain cytokines and other pro-inflammatory mediators play a key role in activation of the inflammatory cascade, causing persistence of clinical symptoms in patients with chronic inflammatory rheumatic diseases. This knowledge regarding disease mechanisms has resulted in the development of biological therapeutics targeting specific molecules of the immune system, like, Tumor Necrosis Factor (TNF), interleukin-6 (IL-6), B- or T-cells.1-3

The availability of these biological therapeutics has improved treatment options and prognosis of patients with chronic rheumatic inflammatory diseases considerably; nonetheless, not all patients obtain sufficient clinical response, and biological therapeutics are associated with a high financial burden.4-7 Thus, factors influencing clinical response and cost-effectiveness of biological therapeutics are currently an important focus of interest for clinicians, scientists and policy makers.

Causes for (partial) clinical non-response or loss of response often remain unknown or are assumed to be multifactorial. However, considering mechanism of action of molecular targeted therapies, the most apparent cause of lack of response is incompletely target blockade or the targeted molecule is not the (only) key factor contributing to persistence of inflammation in the patient. For complete target blockade sufficient drug levels of a biological therapeutic are needed.

Drug levels of biological therapeutics are determined by dosage and several pharmacokinetic factors.8 The wide variability in serum trough levels of biological therapeutics found in patients receiving standard dosing suggests that pharmacokinetic factors vary between patients and, to a lower extent, within patients over time, causing under and over treatment in some patients.8,9 Therefore, a personalized dosing strategy of a biological therapeutic is more rational and cost-effective compared with a standard dose for all. This is supported by evolving evidence that dose reduction and/or dosing interval prolongation of biologicals can be successful in a substantial proportion of patients with sustained low disease activity.10-12

A personalized dosing strategy of a biological therapeutic to optimize treatment and reduce costs can be based on disease activity, parameters of inflammation and/or drug level measurements. This thesis focusses especially on the opportunities of drug level measurements to optimize treatment.

Biological therapeutics in rheumatology

Biological therapeutics include a wide range of medicinal products created by biological instead of chemical processes.13 In rheumatology, prescribed biological therapeutics consist of immunoglobulin G (IgG)-antibodies, or a derivate thereof, directed against specific molecules of the immune system.14

In this thesis several biological therapeutics were investigated: infliximab, adalimumab and golimumab (human IgG-antibodies against TNF); etanercept (a TNF-
in dosage (dose and dosing interval) and route of administration (intravenous (iv) or subcutaneous (sc)).

Pharmacokinetics of biological therapeutics

What the body does with the drug once it is administered is investigated by studying pharmacokinetics. The pharmacokinetic process can be divided into (dosing), absorption (in case of sc administration), distribution and clearance, which can take place simultaneously in the body and is influenced by several factors related to: the drug (e.g. immunogenicity and half-life), treatment (e.g. dosing and co-medication), patient (e.g. gender, age and body weight, compliance) or disease (e.g. level of target molecule).

Immunogenicity will be discussed below in more detail, because it is a pharmacokinetic factor specific for biological therapeutics and an important topic in this thesis.

Immunogenicity

Biological therapeutics are proteins which can be recognized as foreign by the immune system, thereby evoking an immunogenic response. For example, previous studies of adalimumab have shown that a substantial number of patients with rheumatoid arthritis (RA) have detectable anti-drug antibodies (ADAs). Moreover, the vast majority of ADAs against adalimumab, infliximab, golimumab and certolizumab pegol are neutralizing, and thus interfere with the binding capacity of the drug to the target molecule, and thereby affecting clinical outcome.

Despite the presence of detectable ADAs in patients treated with biological therapeutics, controversy regarding the clinical relevance of immunogenicity still exists. This is partly due to heterogeneity of studies regarding factors influencing formation and detection of ADAs, like dosage, route of administration, co-medication, patients characteristics (e.g. severity of disease) and type of assay used (i.e. sensitivity for drug-interference, lack of standardized assays).

However, most importantly, the clinical relevance of immunogenicity is determined by the remaining functional drug available to block the target effectively. Therefore, drug level measurements are the first step, and, if the drug level is very low or undetectable, the next step is to investigate whether immunogenicity is the cause for this.

Therapeutic Drug Monitoring of biological therapeutics

Routine measurement of drug levels is called therapeutic drug monitoring (TDM), meaning dose adjustments based on the measurement of plasma or blood concentrations to optimize treatment on an individual level. TDM is performed in a variety of therapeutic agents, such as agents with a narrow therapeutic index (e.g. some anti-epileptic agents, lithium and digoxin) and some antimicrobial or antiviral agents, or in a particular clinical context, such as an overdose.

Several criteria to assess whether a therapeutic agent is eligible for TDM have been described, including pharmacokinetic variability, narrow therapeutic range, a defined concentration-effect relationship and the availability of a suitable laboratory assay.

Most of these criteria can be applied on biological therapeutics, like a marked drug level variability has been reported in for several biological therapeutics in different studies. Validated assays to measure drug level are available, mostly a drug-specific enzyme-linked immune sorbent assay (ELISA), which is easy to perform and for which costs are low. A concentration-effect relationship has been described for several biological therapeutics in different studies as well. However, biological therapeutics do not have a narrow therapeutic index, although the high costs associated with over treatment of biological therapy can be considered as financial toxicity.

To study the added value of TDM in, for example, a randomized controlled trial (RCT), the optimal therapeutic drug level range should be identified. This can be investigated by determining the concentration-effect curve.

Concentration-effect curve of biological therapeutics

Biological therapeutics are molecular targeting therapies, thus, after binding all target or after saturation of all target receptors the maximum effect is obtained. This can be visualized by a concentration-effect curve: with increasing drug levels, response rates increase until a plateau is obtained; increasing drug levels will no longer contribute to higher response rates. For example, the previously described concentration-effect curve of adalimumab in RA at 26 weeks of treatment shows that a concentration around 3 mg/L is already sufficient to obtain a Disease Activity Score of 28 joints (DAS28) improvement of 1.2 points or more. Serum levels up to 8 mg/L show a positive association with DAS28 improvement, however, levels above 8 mg/L do not contribute to an additional improvement in clinical efficacy. Consequently, patients with levels below 3 mg/L are at risk of under treatment, while patients with levels above 8 mg/L are at risk of over treatment. The optimal therapeutic target range of adalimumab seems to lie within 3-8 mg/L for RA patients with active disease. However, this is based on mean values per group of 20 patients, and the inter-variability in clinical outcome (DAS28 improvement) between patients within one group can be large. In psoriasis a similar optimal range in adalimumab drug level was identified, but, inter-variability in clinical outcome (Psoriasis Area and Severity Index (PASI) improvement) was much smaller compared with RA. For AS assessing a therapeutic window seems more complicated compared with RA and psoriasis. Although, differences in pathophysiology and treatment could contribute to that, differences in objectivity of outcome measurement are a possible explanation too; a hypothesis which will be discussed in this thesis in more detail.

Optimizing biological therapy is an important topic considering the increasing number of diseases for which molecular targeted therapy is registered, and the associated increased costs. Currently, several research projects studied the predictive value of drug level cut-offs (e.g. optimal range, predictive good or poor clinical outcome) of biological therapeutics.
for different chronic inflammatory diseases, like RA (adalimumab and etanercept), psoriasis (adalimumab), Crohn’s disease (infliximab and adalimumab). Moreover, interest in measurements of drug level and immunogenicity of biologics is expanding to neurology (natalizumab in multiple sclerosis (MS)), and even oncology. Nevertheless, no consensus currently exist with regard to the necessity of TDM of biologics due to several limitations. Like, the reported cut-off values vary between the different studies, and, to date, for most diseases and biological therapeutics an optimal range has not been identified. In addition, an efficacious drug level of biological therapeutic for complete target blockade is probably highly individual. Therefore, most available preliminary algorithms for optimizing biological therapeutics in patients with chronic inflammatory diseases are currently based on assessment of immunogenicity. However, as mentioned previously, clinical impact of immunogenicity depends on the remaining functional drug level to block the target pathway, thus, drug level measurement should be the first step.

Scope of this thesis

Biological therapeutics are molecular targeting therapies, therefore, we hypothesize that drug levels sufficient for effective target blockade are enough; higher drug levels are unnecessarily, because all target is already blocked; while a drug level below the threshold for complete target blockade is suboptimal. To obtain and maintain drug levels within the optimal target range a personalized dosing scheme seems more rational compared to a standard dosing for all patients. In addition, TDM might offer opportunities to optimize treatment, but further research is needed to gain more knowledge on variability in drug level per biological therapeutic and disease, insight in relevant pharmacokinetic factors and the concentration-effect relationship. Currently, for some biological therapeutics and/or chronic inflammatory rheumatic diseases this knowledge is not available. This thesis is a part of the puzzle to fill this gap.

The aim of this thesis is to investigate the relationship between serum drug level, clinical outcome and the effect of pharmacokinetic factors on drug level for several biologicals and/or diseases for which limited or no data is currently available. Hence, golimumab and tocilizumab for rheumatoid arthritis (RA), adalimumab and etanercept for ankylosing spondylitis (AS) and adalimumab in psoriatic arthritis (PsA). Moreover, two additional studies were conducted; tapering of tumor necrosis factor (TNF)-inhibitors in spondyloarthritis (SpA) patients and the feasibility of a dried blood spot on material obtained via a finger prick for the measurement of adalimumab drug levels and assessment of immunogenicity.

Chapter 2: describes the concentration-effect relationship of adalimumab in ankylosing spondylitis (AS) and psoriatic arthritis (PsA), and the effect of immunogenicity.

Chapter 3: reports the concentration-effect relationship of etanercept in AS.

Chapter 4: describes the concentration-effect relationship of golimumab in RA, and the effect of immunogenicity.

Chapter 5: reports the concentration-effect relationship of tocilizumab in RA, and the effect of immunogenicity.

Chapter 6: the effect of dose tapering of infliximab, etanercept and adalimumab on disease activity, flares and drug concentration compared to standard regimen in spondyloarthriosis (SpA) patients is studied. In Chapter 7 the possibilities to develop a dried blood spot obtained by finger prick to measure adalimumab drug levels and anti-adalimumab antibodies is investigated. Chapter 8: contains a general discussion and a summary of the results.
REFERENCES


CHAPTER 2a

Immunogenicity, adalimumab levels and clinical response in ankylosing spondylitis patients during 24 weeks of follow-up

Eva L Kneepkens, James Cheng-Chung Wei, Michael T Nurmohamed, Kai-Jieh Yeo, CY Chen, Irene E van der Horst-Bruinsma, Desiree van der Kleij, Theo Rispens, Gertjan Wolbink, Charlotte LM Krieckaert

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ABSTRACT

Background Immunogenicity influences adalimumab levels and therefore clinical response in patients with rheumatic diseases.

Objectives To study the relationship between clinical response, adalimumab levels and anti-drug antibodies (ADA) in ankylosing spondylitis (AS).

Methods Observational cohort study of 115 consecutive AS patients treated with adalimumab in the Netherlands (n = 85) and Taiwan (n = 30), monitored during 24 weeks. Adalimumab levels and ADA titres were determined using an enzyme linked immunosorbent assay (ELISA) and an antigen binding test (ABT), respectively, designed by Sanquin Research, Amsterdam. Response to adalimumab treatment was defined as a Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) response and disease activity was measured using the Ankylosing Spondylitis Disease Activity Score (ASDAS) using C-reactive protein (CRP).

Results At baseline, median BASDAI (IQR) was 6.4 (4.5-7.6) and mean ASDAS (SD) was 3.5 (1.0). After 24 weeks, 49 (42.6%) patients were BASDAI50 responders and mean ASDAS (SD) for responders was 1.5 (1.0) vs 2.6 (1.0) for non-responders (p<0.001). Thirty-one (27.0%) patients had detectable ADA. After 24 weeks adalimumab levels (mg/L) (IQR) were significantly higher in ADA-negative patients than in ADA-positive patients (12.7 (8.2-18.0) vs 1.2 (0.0-2.0), (p<0.001)). A significant association was demonstrated between adalimumab levels and ASDAS (p = 0.02; RC -1.1; 95% CI -2.0 to -0.2). Eleven (9.6%) patients had no detectable adalimumab levels and high detectable ADA titres (>100 AU/mL). In these patients, CRP and erythrocyte sedimentation rate (ESR) remained elevated during treatment.

Conclusions Adalimumab levels are related to clinical response in AS patients measured with ASDAS and are influenced by ADA detectable with an ABT.

INTRODUCTION

Approximately 40% of ankylosing spondylitis (AS) patients do not respond to tumor necrosis factor (TNF)-inhibitors.1 Part of the non-response cannot be explained; however, an important reason for non-response is low drug levels as a result of the development of antidrug antibodies (ADA). Previous studies of adalimumab treatment in AS patients showed percentages of detectable ADA around 30% during 6-12 months of follow-up, leading to low or undetectable adalimumab levels and assessment of spondyloarthritis (ASAS) non-response.2,3 A diminished treatment response associated with ADA development has been described for rheumatoid arthritis (RA) to a larger extent.4,5,6 These studies, from three different medical centers, observed different proportions of patients that developed ADA. In patients with AS, the frequency of ADA might be higher compared with patients with RA, due to the lack of concomitant disease modifying antirheumatic drugs (DMARDs) (particularly methotrexate) in the treatment of AS. In RA, methotrexate has been shown to reduce the percentage of detectable ADA.7,10 Currently, there is no firm evidence to support a significant benefit of methotrexate monotherapy in the treatment of AS.11 Several randomized controlled trials (RCTs) have studied the beneficial effect of methotrexate in addition to infliximab in AS patients; however, a significant difference in disease activity or infliximab levels could not be demonstrated by these trials.12-15

Previous studies showed that serum drug levels may vary widely between patients, but despite these observed variations,2,4,5 pharmacokinetics of TNF-inhibitors is currently not taken into account when treating AS patients. Considering the high costs of TNF-inhibitors, there is a need to optimize TNF-inhibitor treatment by identifying causes for non-response and preventing over treatment in responders. The aim of this study was to investigate the relationship between clinical response, adalimumab levels and ADA in AS in order to explore the utility of drug level and ADA testing for the optimisation of adalimumab treatment in AS patients.

PATIENTS AND METHODS

Study design and patients
This prospective observational cohort study consisted of 126 consecutive adult patients with AS (according to the 1984 modified New York Criteria16) who received adalimumab therapy at the department of Rheumatology of the Jan van Breemen Research Institute | Read, Amsterdam and Chung Shan Medical University Hospital, Taichung. All patients had failed to respond to at least two non-steroid anti-inflammatory drugs (NSAIDs) in
the maximal tolerable dose or had contraindications for the use of NSAIDs before start of TNF-inhibitors according to the ASAS consensus statement of initiation and continuation of TNF-inhibiting therapy in AS.17 Patients were treated either with adalimumab and concomitant NSAID or DMARD therapy or with adalimumab monotherapy. All patients started with adalimumab 40 mg subcutaneously every other week. If mandatory, as judged by the treating rheumatologist, the dosing frequency of adalimumab could be adapted to 40 mg per week or every 3 weeks. The study was approved by the Medical Ethics Committee of both institutes. All patients gave written informed consent.

Clinical response
At the Jan van Breemen Research Institute | Reade, Amsterdam disease activity was assessed at baseline, 4, 12 and 24 weeks. In the Chung Shan Medical University Hospital, Taichung, disease activity was assessed at baseline, 8 and 20 weeks. For analysis weeks 8 and 12 and weeks 20 and 24 were combined. Disease activity was assessed using the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)18 and Ankylosing Spondylitis Disease Activity Score (ASDAS) using c-reactive protein (CRP).19 Active disease was defined as a BASDAI ≥ 420 or an ASDAS ≥ 2.1,21,22 Response to adalimumab treatment was defined accordingly to the international ASAS consensus statement for the use of TNF-inhibitors in AS and was defined as a 50% improvement or an absolute improvement of two points of the BASDAI (0-10 scale), further mentioned here as BASDAI50 response.17

Measurements of adalimumab concentrations
Trough serum samples were taken at each visit and adalimumab concentrations were measured by enzyme linked immunosorbent assay (ELISA), based on the principle that adalimumab is captured via its ability to bind TNF. Adalimumab binding was assessed by incubation with biotinylated rabbit IgG directed to the adalimumab idiotype. Details on this assay can be found in previous publications.7, 23 The lower limit of quantitation of this assay is 0.01mg/L.

Measurements of ADA
ADA titres were determined using an antigen binding test (ABT) as described previously.7,24-25 Patients were defined as positive for ADA if titres were above 12 AU/ml on at least one occasion, in combination with serum adalimumab levels below 5.0 mg/L, as previously described by Bartelds et al.6

Statistical analysis
For statistical analysis, statistical package for the social sciences (SPSS) version 17.0 was used. For differences between groups, we used independent sample t test, Mann Whitney U test or X² test, as appropriate. The threshold for significance was set at p < 0.05. The generalized estimating equation (GEE) approach was used to investigate the association between adalimumab levels and disease activity or response over time. The influence of confounders on this association was investigated. Variables considered as potential confounders were chosen from all available baseline variables if unrelated to ASDAS (hence, not in analysis: CRP, erythrocyte sedimentation rate (ESR) and BASDAI) or BASDAI (hence, not in analysis: ASDAS and Visual Analogue Scale general disease activity). Variables were included in the regression model as confounders if the β changed 10% or more after inclusion of the variable. To analyze clinical response at 24 weeks of treatment, we used last observation carried forward for patients who stopped treatment due to non-response, adverse events, loss to follow-up or missing data.

RESULTS
Patient characteristics
Of 126 suitable patients, 115 (91.3%) were included in this study and 11 (8.7%) were excluded, because only baseline samples were available. The demographic data and the baseline characteristics are shown in table 1. For an additional 11 patients the assessment of response status was not possible due to missing baseline BASDAI (n = 8) or missing follow-up BASDAI (n = 3). Forty-nine patients were BASDAI50 responder after 24 weeks, and 55 patients were BASDAI50 non-responders. Baseline characteristics did not differ significantly for responders and non-responders except for Bath Ankylosing Spondylitis Functional Index (BASFI) (p = 0.02). Patients from Taiwan had a more severe and long-standing disease as shown in table 2.

Discontinuation of treatment
Nine patients (7.8%) dropped out before 24 weeks, five patients due to treatment failure in the opinion of the patient or physician, three patients due to adverse events (palmoplantar pustular psoriasis, increased liver enzymes and multiple myeloma) and one patient due to lost to follow-up. Of the patients dropping out due to failure, one patient had detectable ADA.

Clinical response
After 24 weeks, 106 (92.2%) patients were still on adalimumab treatment. In one patient, dosing frequency was increased to adalimumab once a week and in four patients adalimumab dosing frequency was decreased to once per 3 weeks. Mean ASDAS for BASDAI50 responders was 1.5 (SD 1.0) vs 2.6 (SD 1.0) for non-responders (p<0.001). There was no significant difference in BASDAI response between patients from the Netherlands and Taiwan (47.3% vs 46.7%, respectively; p = 0.95). Six patients did not have 24 weeks of follow-up yet, and response data at 24 weeks of 20 (17.4%) patients were missing. To exclude bias due to missing data a sensitivity analysis was performed for patients who completed 6 months of adalimumab treatment; this did not alter the results. More information on the method we used for the sensitivity analysis can be found in the supplementary file.
Adalimumab concentration and antibodies against adalimumab

Thirteen (11.3%) patients had detectable ADA at week 12 after start of treatment and 31 (27.0%) at week 24. There was no significant difference in the proportion of patients with ADA for BASDAI50 responders and non-responders 14 (28.6%) and 14 (25.5%) respectively; p = 0.56. Of the 5 patients who used concomitant methotrexate, none had ADA, and of the 34 patients using concomitant sulfasalazine at baseline 10 had ADA.

Table 1 Demographics and clinical characteristics at baseline for BASDAI50 responders (last observation carried forward) and non-responders.

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Total population n = 115</th>
<th>BASDAI50 responder n = 49</th>
<th>BASDAI50 non-responder n = 55</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, mean ± SD</td>
<td>42 ± 11</td>
<td>40 ± 10</td>
<td>44 ± 12</td>
</tr>
<tr>
<td>Male, no. (%)</td>
<td>78 (68)</td>
<td>34 (69)</td>
<td>35 (64)</td>
</tr>
<tr>
<td>BMI, median (IQR)</td>
<td>24.9 (22.8 -27.8)</td>
<td>24.1 (22.7-26.7)</td>
<td>25.4 (22.7-26.9)</td>
</tr>
</tbody>
</table>

Disease status

| Disease duration, years, median (IQR) | 8 (3 - 15) | 8 (3 - 16) | 7 (2 - 15) |
| HLA-B27 positive, no. (%) | 95 (83) | 42 (86) | 45 (82) |
| CRP mg/L, median (IQR) | 7 (3 - 17) | 8 (3 - 18) | 6.5 (3 - 17) |
| ESR mm/h, median (IQR) | 25 (9 - 40) | 28 (14 - 40) | 14 (7 - 40) |
| ASDAS CRP, median (SD) | 3.5 ± 1.0 | 3.4 ± 1.0 | 3.5 ± 1.0 |
| BASDAI, median (IQR) | 6.4 (4.5 - 7.6) | 6.3 (4.2 - 7.5) | 6.6 (5.2 - 7.5) |
| GDA VAS, median (IQR) | 7 (5 - 8) | 6 (5 - 8) | 7 (5 - 8) |
| BASFI, median (SD) | 5.2 ± 2.5 | 4.5 ± 2.6** | 5.8 ± 2.6** |

DMARD therapy

| Prior biologicals, no. (%) | 21 (18.3) | 6 (12.2) | 9 (16.4) |
| Methotrexate use, no. (%) | 5 (4.3) | 2 (4.1) | 3 (3.6) |
| Sulfasalazine use, no. (%) | 34 (29.6) | 16 (32.7) | 16 (29.1) |
| NSAID use, no. (%) | 75 (65.2) | 33 (67.3) | 36 (65.5) |

Table 2 Demographics and clinical characteristics at baseline between patients from the Netherlands and Taiwan.

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Total population n = 115</th>
<th>Netherlands n = 85</th>
<th>Taiwan n = 30</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, mean ± SD</td>
<td>42 ± 11</td>
<td>43 ± 12</td>
<td>37 ± 12</td>
<td>0.01</td>
</tr>
<tr>
<td>Male, no. (%)</td>
<td>78 (68)</td>
<td>52 (61)</td>
<td>26 (87)</td>
<td>0.01</td>
</tr>
<tr>
<td>BMI, median (IQR)</td>
<td>24.9 (22.8 -27.8)</td>
<td>24.8 (22.9-27.8)</td>
<td>25.0 (22.7-27.3)</td>
<td>0.82</td>
</tr>
<tr>
<td>Disease status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease duration, years, median (IQR)</td>
<td>8 (2.5-15)</td>
<td>7 (2-14)</td>
<td>12 (5.7-16.3)</td>
<td>0.04</td>
</tr>
<tr>
<td>HLA-B27 positive, no. (%)</td>
<td>95 (82.6)</td>
<td>65 (76.5)</td>
<td>30 (100)</td>
<td>0.003</td>
</tr>
<tr>
<td>CRP mg/L, median (IQR)</td>
<td>7.0 (3-17)</td>
<td>5.0 (2-11.5)</td>
<td>15.6 (12-28)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ESR mm/h, median (IQR)</td>
<td>25 (8.5-39.5)</td>
<td>15 (7-31)</td>
<td>38 (30.5-54.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ASDAS CRP, median (IQR)</td>
<td>3.5 (1)</td>
<td>3.1 (2.4-3.8)</td>
<td>4.3 (3.7-4.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BASDAI, median (IQR)</td>
<td>6.4 (4.5-7.6)</td>
<td>6.1 (4.3-7.3)</td>
<td>7.4 (6.5-8)</td>
<td>0.001</td>
</tr>
<tr>
<td>GDA VAS, median (IQR)</td>
<td>7 (5-8)</td>
<td>6 (5-8)</td>
<td>7 (5-8)</td>
<td>0.15</td>
</tr>
<tr>
<td>BASFI, median (IQR)</td>
<td>5.2 ± 2.5</td>
<td>4.7 ± 2.5</td>
<td>6.5 ± 2.1</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

DMARD therapy

| Prior biologicals, no. (%) | 21 (18.3) | 21 (24.7) | 0 (0) | 0.002 |
| Methotrexate use, no. (%) | 5 (4.3) | 5 (5.9) | 0 (0) | 0.32 |
| Sulfasalazine use, no. (%) | 34 (29.6) | 8 (7) | 26 (86.7) | <0.001 |
| NSAID use, no. (%) | 75 (65.2) | 45 (52.9) | 30 (100) | <0.001 |

BMI = Body Mass Index; HLA-B27 = Human Leukocyte Antigen B27; CRP = C-reactive protein; ESR = Erythrocyte sedimentation rate; ASDAS-CRP = Ankylosing Spondylitis Disease Activity Score using CRP; BASDAI = Bath Ankylosing Spondylitis Disease Activity Index; GDA VAS = General Disease Activity on a Visual Analogue Scale (0-10); BASFI = Bath Ankylosing Spondylitis Functional Index; NSAID = Non-steroid anti-inflammatory Drugs.

There was a significant difference between patients with and without BASDAI50 response for **BASFI (p = 0.01).

After 24 weeks, median adalimumab level was 9.7 mg/L (3.9-15.7) and varied from undetectable to 56.7 mg/L in patients with adalimumab in a dose of 40 mg every other week. Adalimumab levels (mg/L) were significantly different for patients without and with ADA 12,7 (IQR 8.2-18.0) and 1.2 (IQR 0.0-2.0), respectively; p<0.001 (figure 1). At 24 weeks of follow-up median adalimumab levels were significantly higher for Dutch patients (12.6 (5.9-18.5)) vs Taiwanese patients( 6.1 (1.1-11.4) mg/L(p = 0.001); this was a result of the higher percentage of ADA-positives among patients from Taiwanese patients (12 (40.0%)) vs Dutch patients (19 (22.4%)) (p = 0.06).
Patients could be divided into four groups according to the height of the ADA titre and adalimumab level: 77 (67%) patients with high adalimumab levels (median 12.7 mg/L; IQR 7.1-16.5) and no ADA, 20 (17.4%) patients with adalimumab levels <5 mg/L (median 2.0 mg/L; IQR 1.6-3.6) and intermediate ADA (12-100 AU/mL) titres (median 35 AU/mL; IQR 21 - 57), 11 (9.6%) patients with no detectable adalimumab levels (0.0 mg/L; IQR 0.0-0.0) and high ADA (>100 AU/mL) titres (median 670 AU/mL; IQR 319 - 13600) and 7 (6.1%) patients with transient ADA titres (fluctuating over time and >12 AU/mL on at least one visit) (median 14 AU/mL; IQR 0-32) but adalimumab levels (median 7.4 mg/L; IQR 5.1-18.6) remained at >5 mg/L (figure 1).

Although, a lower percentage of Dutch patients tested positive for ADA as compared with Taiwanese patients, among the 11 patients with high ADA levels and no detectable adalimumab most patients were Dutch (n = 8).

Clinical response and adalimumab concentrations

There was no statistically significant difference in adalimumab levels between BASDAI50 responders and non-responders (12.0 (3.2-16.0) and 7.4 (4.1-15.8), respectively; p = 0.3). As shown in table 3, GEE analysis demonstrated a significant association between adalimumab and disease activity, measured with ASDAS and BASDAI. There were no confounders for the association between adalimumab and disease activity, measured with ASDAS and BASDAI. BASFI was the only confounder for the association between adalimumab and BASDAI, and after correction significance (p = 0.05) decreased but a trend in favor of BASDAI remained. Also, there was a statistically significant association of adalimumab with ESR in GEE (table 3).

CRP, ESR and BASDAI of most patients decreased during follow-up, but in 11 patients with high ADA titres and no detectable adalimumab levels CRP and ESR remained high during follow-up, although BASDAI decreased over time for these patients (figure 1). For patients with intermediate detectable ADA titres and low adalimumab levels (<5 mg/L), ESR remained elevated over time.

ESR at baseline were significantly higher in the patients with ADA compared with patients without ADA (35.5(13.0-52.5) vs 19.0 (7.0-34.0), respectively; p = 0.008). CRP at baseline were not significant different between both groups (9.1 (3.7-24.5) vs 7.0 (3.0-16.5), respectively; p = 0.4). The patient number was to small to compare between patients with no, intermediate and high ADA titres.

DISCUSSION

Our results show that adalimumab levels vary widely among patients and ADA were detected in 27% of AS patients. Adalimumab levels were significantly lower for patients with ADA compared with patients without ADA. A significant association between adalimumab and ASDAS, BASDAI and ESR was established with GEE analysis. In

![Figure 1](image)

**Figure 1** Median adalimumab trough levels (mg/L), BASDAI score (0-10), ESR (mm/Hr) and CRP (mg/L) for AS patients divided in four groups: no detectable ADA titres with normal adalimumab levels (> 5 mg/L); intermediate detectable ADA titres with low adalimumab levels (<5 mg/L); intermediate detectable ADA titres with low adalimumab levels (<5 mg/L) and patients with transient ADA during 24 weeks of follow-up.

**Table 3** Clinical measurements in association with adalimumab levels over time, analyzed using GEE analysis.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>P-value</th>
<th>RC</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASDAS CRP</td>
<td>0.02</td>
<td>-1.1</td>
<td>-2.0 to -0.2</td>
</tr>
<tr>
<td>BASDAI</td>
<td>0.02</td>
<td>-0.5</td>
<td>-0.9 to 0.1</td>
</tr>
<tr>
<td>CRP</td>
<td>0.15</td>
<td>-0.01</td>
<td>-0.02 to 2.1</td>
</tr>
<tr>
<td>ESR</td>
<td>&lt;0.001</td>
<td>-0.1</td>
<td>-0.15 to -0.06</td>
</tr>
</tbody>
</table>

ASDAS-CRP = Ankylosing Spondylitis Disease Activity Score using CRP; BASDAI = Bath Ankylosing Spondylitis Disease Activity Index; CRP = C-reactive protein; ESR = Erythrocyte sedimentation rate.
particularly in patients with high ADA titres, the adalimumab levels were absent and in these patients CRP and ESR remained elevated throughout 24 weeks of follow-up.

In this study, we combined two populations in which baseline characteristics were different between both cohorts (table 2). We found a higher ADA percentage in patients from Taiwan, who had a more severe and longstanding disease with more physical limitations at baseline, compared with patients from the Netherlands. Probably patients with high levels of inflammation and a more severe and longstanding disease are more prone to extensively develop ADA as is shown in RA.7

Although CRP levels at baseline are elevated only in a proportion of AS patients, it might be helpful in assessing disease activity and response in AS because suppression of inflammation is the mechanism of action of TNF-inhibition therapy.

First, CRP and ESR at baseline have been described as a predictor for BASDAI50 response.26 In our study, CRP and ESR were higher for BASDAI50 responders compared with non-responders, although this difference was not significant.

Second, at 24 weeks of treatment, ESR was significantly higher in ADA-positive patients. Additionally, in patients with high ADA titres and no adalimumab level, CRP and ESR remained elevated throughout 24 weeks of treatment, although the number of patients with high ADA titres and no adalimumab levels was too small to analyze separately. To study the association between adalimumab and disease activity, clear measurements for disease activity and response are needed. BASDAI is a validated measurement of disease activity and the most used, but this is a patient-reported questionnaire only. ASDAS using CRP or ESR as an alternative has been introduced as an alternative measurement for assessing disease activity in AS19,21 and might be a more objective measurement because it includes inflammatory parameters.

Data on adalimumab level and immunogenicity are limited. A previous study in 60 AS patients of whom 20 patients were treated with adalimumab showed that adalimumab levels were negatively correlated with BASDAI and ESR after 6 months, with CRP after 12 months and with ASDAS CRP after 3 months (p<0.05). Median adalimumab levels at 6 months of treatment were 6.8 µg/mL (5.9–11.4) and 1.6 µg/mL (0.0–2.4) for patients without and with ADA, respectively. ADA was detected in 30% of patients at 1 year of treatment.7 de Vries et al80 found an ADA percentage of 31 in AS patients treated with adalimumab for 6 months, in correspondence with diminished or undetectable adalimumab levels. In our study, ADA detectable with an ABT were found in 27% of patients. These results show that the percentage of patients developing ADA within 6 months of adalimumab treatment is higher for AS patients compared with RA patients at 6 months, which might be due to the differences in pathophysiology or rather due to the differences in cofactors such as concomitant DMARD use or differences in ADA testing.27

In our study, seven patients had transient ADA titres (fluctuating ADA titres over time and > 12 AU/mL at least one visit); possibly these patients develop tolerance for adalimumab, which was described earlier.28

Several factors can influence drug levels of TNF-inhibitors, of which ADA development, dose and concomitant methotrexate use are the most important. The number of patients with concomitant methotrexate or sulfasalazine use in our study was too small to detect a significant relationship between the development of ADA and the use of concomitant DMARDs, as well as detect a significant difference in drug levels between patients with adalimumab monotherapy or in combination therapy. Previous studies have shown that RA patients with ADA significantly less often used concomitant methotrexate,7,9 which has appeared to be an important factor in reducing immunogenicity in a dose-dependent manner.10 Also, a better clinical response and higher drug levels of biologics have been described for TNF-inhibitors with concomitant methotrexate compared with TNF-inhibitor monotherapy in RA.28 Currently, there is not enough evidence to support any benefit of methotrexate in the treatment of AS. Therefore, it is also not used as concomitant therapy with TNF-inhibitor treatment.11,30 Several RCTs studied the beneficial effects of methotrexate (varying from 10 to 15 mg/week) in addition to infliximab but no significant difference in disease activity or infliximab levels was found.12–15 In these studies, response was defined according to ASAS20 or BASDAI50 response criteria; patient numbers varied between 26 and 76, and follow-up was relatively short.12–15 Possibly these factors influenced the results of these studies.

Previous studies described lower infliximab levels for RA as reported for AS.30–33 Possibly beneficial effect of additional methotrexate is only seen when TNF-inhibitor drug levels reach a critical level. This is an adalimumab level that is low enough for the immune system to overcome by the production of ADA and therefore cause loss of response. If TNF-inhibitor dosage can be lowered when methotrexate is added in the treatment of AS patients, this might save costs. It would be interesting to study the effect of methotrexate on the immunogenicity and drug levels of TNF-inhibitors in AS patients prospectively.

There are some limitations to this study. First a limitation of this study was the missing data, although a sensitivity analysis did not alter the results. This was mostly due to the fact that patient questionnaires were not completed. Second, a small amount of the samples might not have been exactly trough level since adalimumab is an at-home administered drug.

In conclusion, adalimumab levels varied widely among patients; however, some patients improved, based on clinical measurements such as BASDAI or ASDAS CRP despite low adalimumab levels. Currently, the variation in pharmacokinetics of TNF-inhibitors is not taken into account in the treatment of AS. TNF-inhibitor treatment is expensive and due to the large observed variations in drug levels a personalized treatment strategy is necessary to identify under treatment and over treatment. This is especially important in AS, where the treatment options are limited and clear clinical measurements for disease activity are lacking.
SUPPLEMENTARY MATERIAL CHAPTER 2a

Sensitivity analysis

For the sensitivity analysis we compared the baseline characteristics of patients with missing BASDAI50 response data at 24 weeks (n = 20) to patients without missing BASDAI50 response data at 24 weeks (n = 65). For differences between groups we used independent sample T test, Mann-Whitney U or Chi square, as appropriate. The threshold for significance was set at p < 0.05. The first group consisted only of patients who did not have BASDAI response data at 24 weeks of treatment although, this data should have been available. Patients who dropped out before 24 weeks of treatment due to treatment failure or side effects and patients who did not have 24 weeks of follow-up at the time of data analysis, were not included in the first group but in the latter. Baseline characteristics did not differ significantly between both groups.

Only the Dutch cohort had missing data as defined above, therefore, and because of significant differences in baseline characteristics between patients from the Netherlands and Taiwan, we performed the sensitivity analysis for Dutch patients separately.

REFERENCES

Adalimumab treatment in ankylosing spondylitis


Anti-adalimumab antibodies and adalimumab drug concentrations in psoriatic arthritis; an association with disease activity at 28 and 52 weeks of follow-up

Erik H Vogelzang, Eva L Kneepkens, Michael T Nurmohamed, Arno WR van Kuijk, Theo Rispens, Gertjan Wolbink, Charlotte LM Krieckaert

Ann Rheum Dis. 2014 Dec;73(12):2178-82
ABSTRACT

Objectives To investigate the relationship between antidrug antibodies (ADA), adalimumab concentrations and clinical response in patients with psoriatic arthritis (PsA) during 52 weeks of follow-up.

Methods This prospective cohort study included 103 consecutive patients with PsA. Disease Activity Score of 28 joints (DAS28), Erythrocyte Sedimentation Rate, C-reactive protein and Psoriasis Area and Severity Index were assessed. Adalimumab concentrations and ADA were measured in serum trough samples, using an ELISA and a radio immunoassay, respectively.

Results Adalimumab concentrations were significantly lower at 28 and 52 weeks in patients with detectable ADA compared with patients without detectable ADA (at week 28: 1.3 mg/L (IQR 0.0–3.2) versus 8.7 mg/L (IQR 5.7–11.5), p<0.001; at week 52: 0.9 mg/L (IQR 0.0–2.9) vs 9.4 mg/L (IQR 5.7–12.1), p = 0.0001). DAS28 at 28 weeks (2.16 vs 2.95, p = 0.023) and 52 weeks (2.19 vs 2.95, p = 0.024) showed a significant difference; patients with detectable ADA had a poorer clinical outcome than patients without.

Conclusions Patients with detectable ADA had lower adalimumab concentrations and a significantly poorer clinical outcome compared with patients in whom ADA were not detected.

INTRODUCTION

A large registration trial in psoriatic arthritis (PsA) patients demonstrated an improved clinical outcome for adalimumab compared with placebo. However, some patients failed to respond to treatment. Studies in patients with rheumatoid arthritis (RA), ankylosing spondylitis (AS) or Crohn’s disease (CD) have shown that absent or diminished response, in some patients, is associated with lower drug concentrations of tumor necrosis factor (TNF) inhibitors due to the formation of anti-drug antibodies (ADA). A small study in 22 PsA patients reported a relationship between the development of ADA (n = 4, 18%), lower adalimumab concentrations and diminished clinical response during 12 months of treatment.

The current study describes adalimumab concentrations, clinical response and the formation of ADA in a cohort of 103 PsA patients treated with adalimumab.

METHODS

Study design and patients
One hundred and three PsA patients with mainly peripheral joint involvement treated with adalimumab were consecutively included in this prospective observational cohort study at the Jan van Breemen Research Institute | Reade, Amsterdam, The Netherlands. Patients included in this cohort failed to respond adequately to at least one disease modifying antirheumatic drug (DMARD), according to the European League Against Rheumatism (EULAR) recommendations for the management of PsA. All patients were treated with adalimumab 40 mg injected subcutaneously (sc) every other week. Patients were treated with adalimumab monotherapy or adalimumab with concomitant DMARDs with or without additional prednisone. Patients were included in the analysis if there was a baseline visit and at least one subsequent visit where serum samples and clinical data were obtained. Approval of this study was obtained from the medical ethics committee. All patients gave their written informed consent.

Clinical response
Disease activity was assessed at baseline and every subsequent visit thereafter at 4, 16, 28, 40 and 52 weeks of treatment. The primary outcome measure for clinical response was DAS28. Secondary outcome measures were: Erythrocyte Sedimentation Rate (ESR), C reactive protein (CRP) and Psoriasis Area Severity Index (PASI).
Adalimumab treatment in psoriatic arthritis

Measurement of adalimumab concentration and antidrug antibodies against adalimumab

The measurement of adalimumab concentrations has been described previously. In short, an ELISA, designed by Sanquin, Amsterdam, The Netherlands, was used to measure trough serum adalimumab concentrations. Adalimumab was captured via its ability to bind TNF. As previously described for the measurement of ADA, IgG, a radio immunoassay (RIA) was used. Patients were defined as being positive if ADA titres were above 12 AU/mL in combination with serum adalimumab concentrations below 5 mg/L on at least one occasion, as previously described.

Statistical analysis

For differences between groups, we used the independent sample t test, \( \chi^2 \) or Mann–Whitney U test, as appropriate. The independent sample t test or Mann–Whitney U was used to investigate the relationship between the drug concentrations and the detection of ADA or the use of concomitant methotrexate. A linear regression model was used to investigate the relationship between the primary or secondary outcome measures and the development of ADA. All statistical tests were two-sided, with a threshold for significance set at \( p<0.05 \). The following variables were considered potential confounders: methotrexate use and dose and prednisone use and dose. Variables were included in the regression model if \( \beta \) changed 10% or more. The last observation was carried forward for patients with incomplete data. All statistical analyses were performed using SPSS V.19.0.

RESULTS

Patients

Table 1 shows the baseline characteristics of patients included in this study. There was a significant difference in Body Mass Index (BMI), DMARD use, methotrexate use, prednisone use and dose between patients with and without detectable ADA. Patients with detectable ADA had a higher BMI, were less frequently treated with a DMARD, methotrexate and prednisone. Also prednisone dosage was lower in patients with detectable ADA.

During 52 weeks of follow-up, 32 (31%) patients dropped out of the study. Of these patients, 15 (47%) stopped treatment due to failure, 9 (28%) were lost to follow-up and 8 (25%) dropped out due to side effects.

Detection of ADA

During follow-up for 52 weeks, 23 (22%) patients developed detectable ADA. In 3 patients, ADA were intermittently detectable. The ADA titre in these patients ranged from 13 to 186 AU/mL and their adalimumab concentrations ranged from 0.8 to 5.9 mg/L.

Adalimumab concentrations

Adalimumab was not detectable in any of the baseline samples. The adalimumab concentration over time for patients with or without detectable ADA is shown in figure 1A. At week 28, patients who had detectable ADA had significant lower median
Adalimumab treatment in psoriatic arthritis

### Figure 1

**A** Shown are the median adalimumab concentrations (and IQR) for patients who had detectable antidrug antibodies (ADA) compared with patients with no detectable ADA. Patients with detectable ADA had significantly higher median adalimumab concentrations at weeks 28 and 52, both P<0.001. **B** Median adalimumab concentrations (and IQR) are shown of patients using concomitant methotrexate (no other disease modifying antirheumatic drug) or monotherapy. Patients using concomitant methotrexate had significantly lower concentrations at weeks 28 and 52, P = 0.0001. **C** Concentration-effect curve, with each point representing the mean adalimumab concentration of 10 patients (except the last point, n = 13) of which through adalimumab concentrations were measured and stratified in ascending order with their corresponding ΔDAS28.

### Table 2

**Clinical outcome at 28 and 52 weeks.**

<table>
<thead>
<tr>
<th>Week 28</th>
<th>Patients with detectable ADA (n = 23)</th>
<th>Patients without detectable ADA (n = 80)</th>
<th>P-value *</th>
<th>Adjusted p-value †</th>
<th>Adjusted RC (95%CI) †</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAS28, mean (SD)</td>
<td>2.95 (1.44)</td>
<td>2.16 (1.14)</td>
<td>0.007</td>
<td>0.8 (0.2-1.4)</td>
<td>0.023</td>
</tr>
<tr>
<td>ESR mm/h, median (IQR)</td>
<td>5 (2-11)</td>
<td>5 (2-10)</td>
<td>0.048</td>
<td>4.8 (0.0-9.7)</td>
<td>0.085</td>
</tr>
<tr>
<td>CRP mg/l, median (IQR)</td>
<td>2 (1-3)</td>
<td>1 (1-3)</td>
<td>0.03</td>
<td>2.7 (0.3-5.1)</td>
<td>0.016</td>
</tr>
<tr>
<td>PASI, median (IQR)</td>
<td>0 (0-0.8)</td>
<td>0 (0-0.6)</td>
<td>0.169</td>
<td>0.5 (0.0-1.1)</td>
<td>0.392</td>
</tr>
</tbody>
</table>

**Week 52**

<table>
<thead>
<tr>
<th>Patients with detectable ADA (n = 23)</th>
<th>Patients without detectable ADA (n = 80)</th>
<th>P-value *</th>
<th>Adjusted p-value †</th>
<th>Adjusted RC (95%CI) †</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAS28, mean (SD)</td>
<td>2.95 (1.41)</td>
<td>2.19 (1.20)</td>
<td>0.011</td>
<td>0.8 (0.2-1.3)</td>
</tr>
<tr>
<td>ESR mm/h, median (IQR)</td>
<td>5 (2-11)</td>
<td>5 (2-10)</td>
<td>0.110</td>
<td>4.0 (-0.9-8.9)</td>
</tr>
<tr>
<td>CRP mg/l, median (IQR)</td>
<td>2 (1-3)</td>
<td>1 (1-3)</td>
<td>0.029</td>
<td>2.3 (0.2-4.3)</td>
</tr>
<tr>
<td>PASI, median (IQR)</td>
<td>0 (0-0.8)</td>
<td>0 (0-0.4)</td>
<td>0.182</td>
<td>1.0 (0.4-1.6)</td>
</tr>
</tbody>
</table>

*P-value for patients without antidrug antibodies (ADA) vs patients with ADA, linear regression model. † At weeks 28 and 52 for DAS28 and ESR confounders are methotrexate use and dosage, for CRP the confounder is methotrexate dose and for PASI methotrexate use was a confounder. Linear regression model. ‡ Linear regression model was used without transformation of the data since the residuals were normally distributed. CRP, C-reactive protein; DAS28, 28-Joint Disease Activity Score; ESR, Erythrocyte Sedimentation Rate; PASI, Psoriasis Area Severity Index; RC, regression coefficient.
Adalimumab concentration of 1.8 mg/L, IQR 0.0–3.5 compared with 8.8 mg/L, IQR 4.9–11.9 in patients treated with adalimumab and concomitant methotrexate. This difference was also statistically significant, p<0.001.

**Clinical response and ADA**

There was a significant difference at 28 and 52 weeks of treatment between patients with and without ADA for DAS28 and CRP, and for ESR only at 52 weeks, as shown in table 2. All clinical outcome variables showed a consistent pattern: patients with detectable ADA had a poorer clinical outcome at weeks 28 and 52 than patients without detectable ADA.

**Concentration effect curve**

Figure 1C shows a concentration-effect curve. All patients were sorted from low to high adalimumab concentration. Each dot represents 10 patients (the last dot represents 13 patients) and their mean adalimumab concentration at week 28 and mean DAS28 improvement compared with baseline. Concentrations of approximately 1.0 mg/L already show reasonable efficacy. Adalimumab concentrations between 5–8 mg/L appear optimal. In 48 (47%) patients, adalimumab concentrations exceeded 8 mg/L. Furthermore, 36 (35%) patients had adalimumab concentrations below 5 mg/L. Of these patients 21 (58%) had detectable ADA.

**DISCUSSION**

This study shows that detectable ADA was associated with lower adalimumab concentrations in patients with PsA, which is associated with a poorer clinical outcome at 28 and 52 weeks of treatment.

Adalimumab concentrations reflect the amount of unbound drug available in the serum, which can bind to the target. If none or insufficient concentrations of free drug are available, the inflammation cannot be suppressed sufficiently. Therefore, measuring drug concentrations in patients who do not respond adequately could give more insight to the reason why there is an inadequate response. As is shown, one important factor of inadequate drug concentrations is the formation of ADA. However, in several studies, a relationship is found also between high CRP concentrations, which may be considered as an indirect marker of TNF, and higher infliximab clearance. Thus indicating that other factors also, such as the severity of the inflammation, influence drug concentrations.

This study identified that the optimal concentration range of adalimumab in PsA patients in this cohort, might be 5–8 mg/L. This is in concordance with the range that has been found in patients with RA. With this range, a proportion of the patients receive under treatment or over treatment. The adalimumab dose can probably be tapered in a proportion of patients with low disease activity in combination with high adalimumab drug concentrations, without an increase in disease activity.

For RA, it has been shown that some factors can influence ADA development, like concomitant methotrexate, however, for PsA, data are limited. In a study of PsA patients, an association between ADA development and concomitant methotrexate use was not found, and the study cautions clinicians in extrapolating the practice of administering combination therapy with methotrexate. However, a study in patients with RA concluded that methotrexate use reduced the development of ADA in a dose-dependent manner. Patients treated with adalimumab and concomitant methotrexate had higher adalimumab concentrations than patients with adalimumab monotherapy indicating that concomitant methotrexate can influence adalimumab concentrations and therefore, might be of clinical importance. Some patients used other concomitant DMARDs than methotrexate. It would be interesting to see whether these DMARDs are also able to reduce ADA formation like methotrexate.

DAS28 was chosen as a primary outcome measure. This comprises the risk that not all relevant joints are encompassed, because commonly affected joints in PsA, such as distal interphalangeal joints, ankles and toes, are not included in the DAS28. Nevertheless, DAS28 has been shown to discriminate effectively between placebo and infliximab or etanercept in patients with polyarticular PsA. Also, in this study, significant differences between groups could be demonstrated using DAS28, which further supports its use. Since there is no standard clinical outcome measure for PsA, other variables were chosen as a secondary clinical outcome measure.

In conclusion, this study demonstrates that ADA results in lower adalimumab concentrations and poorer clinical outcome. This is a major concern for PsA patients treated with adalimumab. Further studies regarding measuring drug concentrations would be relevant, since this could give more insight on the cause of inadequate response, especially since treatment options in PsA are limited. Obviously, information on drug concentrations could lead to a more tailored, evidence-based therapy for patients that is potentially cost saving.
REFERENCES


Lower etanercept levels are associated with high disease activity in ankylosing spondylitis patients at 24 weeks of follow-up

Eva L Kneepkens, Charlotte LM Krieckaert, Desiree van der Kleij, Mike T Nurmohamed, Irene E van der Horst-Bruinsma, Theo Rispens, Gertjan Wolbink

ABSTRACT

Background Previous data has shown that etanercept levels are associated with clinical response in rheumatoid arthritis. However, for ankylosing spondylitis (AS) data regarding this topic is inconclusive.

Objectives To investigate the relationship between etanercept levels and clinical response in AS patients.

Methods Observational prospective cohort study of 162 AS patients treated with etanercept, monitored during 24 weeks of treatment. Etanercept trough levels were determined, retrospectively, using an ELISA. Disease activity was measured using AS Disease Activity Score, including C-reactive protein (CRP) (ASDAS) and Bath Ankylosing Spondylitis Disease Activity index (BASDAI). Active disease was defined as ASDAS ≥ 2.1. Since etanercept is an at home administered drug there might have been some variation in trough level sampling.

Results At 24 weeks etanercept levels were significantly higher in patients with ASDAS < 2.1, (3.8 mg/L; (IQR 2.5-5.2)) compared to patients with ASDAS ≥ 2.1 (2.3 mg/L (IQR 1.2-3.4); (p = <0.001). Generalized estimating equation (GEE) analysis demonstrated a statistically significant association between etanercept levels and ASDAS, BASDAI, CRP and erythrocyte sedimentation rate (ESR) (all p<0.001). When patients were categorized into quartiles according to the height of etanercept levels, the lowest quartile (etanercept < 1.80 mg/L) comprised 35% of all patients with ASDAS ≥ 2.1 while the highest quartile comprised only 14%.

Conclusions Disease activity and inflammation are associated with etanercept levels in AS patients at 24 weeks of treatment. Measuring etanercept levels might help in identifying overtreatment and under treatment and optimize etanercept therapy in AS.

INTRODUCTION

The efficacy and safety of tumor necrosis factor (TNF)-inhibitor treatment has been demonstrated for ankylosing spondylitis (AS), although approximately 40% do not respond to TNF-inhibitor treatment. All TNF-inhibitors are prescribed in a fixed dose without taking differences in pharmacokinetics into account, however, a substantial proportion of patients remain in a state of low disease activity after dose reduction. In addition, dose registration trials of etanercept show that a proportion of rheumatoid arthritis (RA) patients achieve acceptable response rates with a lower dose than standard. A previous study of 108 AS patients treated with etanercept 50 mg subcutaneously (SC) weekly showed that administration of etanercept 50 mg twice weekly in AS non-responders did not result in significantly higher response rates (76% vs 71%) in comparison to 50 mg SC once weekly. Possibly, non-responders with low drug levels could profit from a dose increase but drug levels were not taken into account in this study.

The above indicates that a proportion of patients is under treated or over treated and that there are opportunities to adapt etanercept dose to individual needs. This potentially saves costs, which is important considering the high financial burden of anti-TNF treatment. Currently, due to lacking dose tapering guidelines, adaptations of TNF-inhibitor dosing regimens are based solely on the clinical opinion of the rheumatologist. Previous studies have shown an association between etanercept levels and response in RA patients. For AS the available data on this subject is limited. Since therapeutic options in AS are still limited, it is crucial to identify parameters that would aid in optimized use of TNF-inhibitors. In a study of 53 AS patients, etanercept levels were similar for responders and non-responders. Another study, in which 20 AS patients were treated with etanercept, demonstrated a significant negative correlation between etanercept level and C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) but not with Bath Ankylosing Spondylitis Disease Activity index (BASDAI).

The aim of this study was to investigate the relationship between clinical response and etanercept levels in a large group of AS patients, in order to explore the utility of drug level testing for the optimisation of etanercept treatment in AS.

PATIENTS AND METHODS

Study design and patients

This prospective observational cohort consisted of 162 consecutive included adult patients with AS (according to the 1984 modified New York Criteria) who received
etanercept at the department of Rheumatology of the Jan van Breemen Research Institute | Reade, Amsterdam. All patients had failed to respond to at least two non-steroid anti-inflammatory drugs (NSAIDs) in the maximal tolerable dose, or had contraindications for the use of NSAIDs before start of TNF-inhibition treatment according to the ASAS consensus statement of initiation and continuation of TNF-inhibition therapy in AS.10 Patients were treated either with etanercept and concomitant NSAID and/or disease modifying anti-rheumatic drug (DMARD) therapy or with etanercept monotherapy. All patients started with etanercept 50 mg SC every week. If mandatory, as judged by the rheumatologist, the dosing frequency of etanercept could be adapted to 50 mg twice weekly or every 2 weeks. The study was approved by the Medical Ethics Committee of the Slotervaart Hospital and Jan van Breemen Research Institute | Reade. All patients gave written informed consent.

Clinical response

Disease activity was assessed at baseline and after 4, 12 and 24 weeks of therapy and measured with the Ankylosing Spondylitis Disease Activity Score, including CRP (ASDAS),13 and the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI).14 Active disease was defined as ASDAS ≥ 2.1.15,16

Measurements of etanercept concentrations

Trough serum samples were taken at each visit and etanercept concentrations were measured, retrospectively, by EUSA, designed by Sanquin (Amsterdam), based on the principle that etanercept is captured via its ability to bind TNF. Assays were performed using a fully automated ELISA platform. Briefly, microtitre plates were coated overnight with a mouse monoclonal anti-TNF antibody. After washing 5 times with phosphate buffered saline containing 0.02% Tween (PBS-T), recombinant TNF in high-performance ELISA (HPE) buffer was added. After one hour plates were washed with PBS-T. Subsequently, patient serum samples in different dilutions was added and incubated for 1 hour at 37°C. After washing the plates with PBS-T, plates were incubated with biotinylated polyclonal rabbit antibodies against etanercept in 100 µl HPE buffer for 1 hour at 37°C. Plates were washed, and poly(horseradish peroxidase)-conjugated streptavidin was added for 30 min at 30°C, followed by incubation with tetramethylbenzidine (TMB). Afterwards reaction was stopped and absorption at 450 nm was assessed. Test results were compared to a titration curve of etanercept, which was present in each plate. The lower limit of quantification of this assay is 0.1mg/L.

Statistical analysis

For statistical analysis statistical package for the social sciences (SPSS) V.17.0 was used. For differences between groups we used independent sample t test, Mann-Whitney U test or χ² test, as appropriate. The threshold for significance was set at p < 0.05. The generalized estimating equation (GEE) approach (using an exchangeable correlation matrix) was used to investigate the association between etanercept levels and disease activity or inflammation over time. The influence of confounders on this association was investigated. Variables considered as potential confounders were chosen from all available baseline variables if unrelated to ASDAS (hence, not in analysis: BASDAI, CRP and ESR) or BASDAI (hence, not in analysis: ASDAS and visual analogue score (VAS) general) or CRP (not in analysis ASDAS and ESR) or ESR (not in analysis ASDAS and CRP). Variables were included in the regression model as confounders if the b changed 10% or more after inclusion of the variable. To analyze clinical response in patients at 24 weeks of treatment we used last observation carried forward for patients who discontinued etanercept treatment prematurely.

RESULTS

Patient characteristics

The baseline characteristics of 162 patients are shown in table 1. For 11 (6.8%) patients the assessment of response status was not possible due to missing ASDAS data at 24 weeks of treatment. To exclude missing data bias a sensitivity analysis was performed for patients who completed 6 months of etanercept treatment; this did not alter the results (data not shown). Eighty-four patients (52%) had an ASDAS < 2.1 and 67 (41%) patients had an ASDAS ≥ 2.1 at 24 weeks of follow-up. At baseline patients with ASDAS ≥ 2.1 had a higher Body Mass Index (BMI) (p = 0.001), BASDAI (p = 0.03) and Bath Ankylosing Spondylitis Functional Index (BASFI) (p<0.001) and a lower number of patients was HLA-B27 positive (p = 0.02).

Discontinuation of treatment

In total 14 (8.6%) patients dropped out of the study before 24 weeks of treatment: 12 patients stopped due to treatment failure and two due to adverse events (injection site reaction, recurrent infections). Of the 14 patients who dropped out, 13 patients had an ASDAS ≥ 2.1 at the time of drop out.

Clinical response and etanercept concentrations

After 24 weeks 148 (91.4%) patients were still on etanercept treatment. In one patient etanercept dosing frequency was decreased to once per 2 weeks and none had a dose increase. At week 24 median etanercept level was 3.0 mg/L (IQR 1.8 - 5.0) and varied from undetectable to 9.7 mg/L. Etanercept levels at 24 weeks of treatment were significantly higher in patients with ASDAS < 2.1 (3.8 mg/L; IQR 2.5-5.2) compared to patients with ASDAS ≥ 2.1 (2.3 mg/L; IQR 1.2-3.4) (p <0.001) (figure 1).

GEE analysis demonstrated a significant association between etanercept concentration and disease activity. ASDAS: regression coefficient (RC) -1.02, 95% coincidence interval (95%CI) -1.17 to -0.87, p = 0.001, no confounding variables were found; BASDAI: RC...
Etanercept treatment in ankylosing spondylitis comprised only 14%. The highest quartile (etanercept level ≥ 4.6 mg/L) comprised 36% of all patients with ASDAS ≥ 2.1 while the highest quartile

Table 1 Baseline demographics and clinical characteristics for the total population and for ASDAS inactive to moderate disease activity (< 2.1) and high disease activity (≥ 2.1) at 24 weeks of follow-up (last observation carried forward).

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Total patient population*</th>
<th>Inactive to moderate disease activity</th>
<th>High disease activity</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 162</td>
<td>n = 84</td>
<td>n = 67</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years, mean ± SD</td>
<td>43 ± 11</td>
<td>40 ± 11</td>
<td>44 ± 11</td>
<td>NS</td>
</tr>
<tr>
<td>Male, no. (%)</td>
<td>115 (71)</td>
<td>64 (76.2)</td>
<td>42 (64.2)</td>
<td>NS</td>
</tr>
<tr>
<td>BMI, median (IQR)</td>
<td>25.7 (23.4-29.7)</td>
<td>24.7 (23.1-27.8)</td>
<td>27.7 (24.4-31)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Disease status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease duration, years, median (IQR)</td>
<td>8 (3-15)</td>
<td>9 (4-17)</td>
<td>6.5 (1-15)</td>
<td>NS</td>
</tr>
<tr>
<td>HLA-B27 positive, no. (%)</td>
<td>118 (72.8)</td>
<td>67 (79.8)</td>
<td>44 (65.7)</td>
<td>0.02</td>
</tr>
<tr>
<td>CRP mg/L, median (IQR)</td>
<td>12 (3-27)</td>
<td>13 (3-27)</td>
<td>11 (3-21)</td>
<td>NS</td>
</tr>
<tr>
<td>ESR mm/h, median (IQR)</td>
<td>22 (6-38)</td>
<td>21 (5-37)</td>
<td>22 (6-40)</td>
<td>NS</td>
</tr>
<tr>
<td>ASDAS CRP, mean ± SD</td>
<td>3.6 ± 0.9</td>
<td>3.5 ± 0.9</td>
<td>3.8 ± 0.9</td>
<td>NS</td>
</tr>
<tr>
<td>BASDAI, median (IQR)</td>
<td>6.1 (5.1-7.1)</td>
<td>5.7 (4.6-6.8)</td>
<td>6.6 (5.5-7.6)</td>
<td>0.03</td>
</tr>
<tr>
<td>GDA VAS, median (IQR)</td>
<td>7 (4-8)</td>
<td>6 (3-8)</td>
<td>7 (4-8)</td>
<td>NS</td>
</tr>
<tr>
<td>BASFI, mean ± SD</td>
<td>5.9 ± 2.3</td>
<td>5.2 ± 2.4</td>
<td>6.6 ± 1.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>DMARD therapy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prior biologics, no. (%)</td>
<td>10 (6.2)</td>
<td>3 (3.6)</td>
<td>7 (10.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Methotrexate use, no. (%)</td>
<td>4 (2.5)</td>
<td>4 (4.8)</td>
<td>0 (0)</td>
<td>NS</td>
</tr>
<tr>
<td>Sulfasalazine, use no. (%)</td>
<td>16 (9.9)</td>
<td>11 (13.1)</td>
<td>5 (7.5)</td>
<td>NS</td>
</tr>
<tr>
<td>NSAID use, no. (%)</td>
<td>114 (70.4)</td>
<td>65 (77.4)</td>
<td>45 (67.2)</td>
<td>NS</td>
</tr>
</tbody>
</table>

BMI = Body Mass Index; HLA-B27 = Human Leukocyte Antigen B27; CRP = C-reactive protein; ESR = Erythrocyte sedimentation rate; ASDAS-CRP = Ankylosing Spondylitis Disease Activity Score using CRP; BASDAI = Bath Ankylosing Spondylitis Disease Activity Index; GDA VAS = General Disease Activity on a Visual Analogue Scale (0-10); BASFI = Bath Ankylosing Spondylitis Functional Index; DMARD = Disease Modifying Antirheumatic Drug; NSAID = Non-steroid anti-inflammatory Drugs.

* Of 11 patients ASDAS at 24 weeks of treatment could not be calculated because baseline ASDAS was missing (n = 8) or only baseline ASDAS was available (n = 3).
11 patients, only the last dot represents 12 patients. The cut-off for clinically important improvement based on ASDAS is set at a ΔASDAS ≥ 1.1 points. Most dots are above this cut-off value, although a large variability is seen among patients which is represented by the standard deviation. A clear therapeutic window could not be identified at 24 weeks in our cohort of AS patients (figure 3).

DISCUSSION

Our data, of 162 AS patients, showed an association between etanercept level and ASDAS, BASDAI, CRP en ESR with a GEE analysis. Thirty-five percent of patients with an ASDAS ≥ 2.1 at 24 weeks of treatment had low etanercept levels (< 1.80 mg/L) and it needs to be studied further whether these patients will benefit from a dose increase. A previous study of 108 AS patients treated with etanercept 50 mg SC weekly showed that administration of etanercept 50 mg twice weekly in AS non-responders did not result in significantly higher response rates (76% vs 71%) compared to 50 mg SC once weekly, although drug levels were not taken into account.7 Possibly, patients with high disease activity who will benefit from a dose increase can be identified by measuring etanercept level. This strategy can only be cost-effective if etanercept dose is also lowered in a selective group of AS patients with low disease activity. Our data shows that 36% of patients with high etanercept levels (> 4.6 mg/L) had an ASDAS < 2.1. Possibly, dosing interval of etanercept can be prolonged in these patients without an increase of disease activity. This is currently a hot topic, since the costs of biologic treatment increased enormously during the last decade. At the moment, limited data is available on success rates of etanercept interval prolongation and discontinuation in AS patients with low disease activity.13 Our concentration-effect curve does not show clear cut-offs to identify the therapeutic window of etanercept in our AS cohort. For optimising etanercept treatment in AS patients it is important to identify and validate the therapeutic window of etanercept, specified for AS, in a larger group of patients because it provides information, additional to clinical parameters, regarding under treatment and over treatment.

Data on etanercept level in association with response and disease activity in AS is limited and inconclusive. One study, in which 20 AS patients were treated with etanercept, showed data comparable to our results. They found a significant negative correlation between etanercept level and CRP and ESR. However, unlike our data, their data did not show this correlation with BASDAI.10 Another study of 53 AS patients could not detect a difference in etanercept levels between responders and non-responders (based on BASDAI50 response criteria).9 Probably, patient numbers in these studies were too small to detect a significant difference for BASDAI and BASDAI50 response.

In this study ASDAS was used to assess disease activity, because it also includes parameters of inflammation (ESR or CRP). This is important since suppression of inflammation is the mechanism of action in TNF-inhibition therapy. Our study shows that BMI, BASDAI, BASFI and HLA-B27 at baseline were statistically significantly different between both groups. ASDAS is developed recently and therefore limited data on predictors of response are currently available. Predictors for other response criteria are available but cannot directly be extrapolated. Increased levels of CRP or ESR have been described as a predictor for ASDAS major improvement (ΔASDAS > 2.0) previously, however, further research on predictors of ASDAS response is needed.

An important cause of non-response to adalimumab or infliximab treatment is subclinical drugs levels due to anti-drug antibody (ADA) formation in AS patients. Etanercept is probably not or only marginally immunogenic, since we previously did not detect ADA against etanercept with an antigen binding test (ABT).8 Other studies have detected ADA against etanercept, however, these ADA had no apparent effect on clinical outcome. Anti-etanercept antibodies were not studied in the current study, since, the relationship between etanercept drug level and clinical outcome was the primary focus. Although immunogenicity is not an important factor in etanercept treatment, non-response is an issue in etanercept treatment too. Other factors influencing pharmacokinetics of etanercept, like concomitant DMARD use and patient related factors, might be possible explanations for non-response to etanercept.22 In our study, patients with low etanercept levels at 24 weeks of treatment had a statistically significant higher BMI and BASDAI score at baseline compared to patients with high etanercept levels. BMI has been described as a factor influencing etanercept levels in RA8 and AS patients, but data on this topic is inconclusive. Possible explanations might be the inflammatory effect of adipose tissue influencing target load or a difference in body distribution influencing the volume of distribution. The influence of concomitant DMARDs on etanercept level and response could not be studied here due to small numbers of patients using sulfasalazine or methotrexate.

There are some limitations to this study. First, a limitation of this study was missing BASDAI questionnaires or CRP measurement. The sensitivity analysis did not alter the results. Second, since etanercept is an at home administered drug there might have been some variation in trough level sampling.

In conclusion, our data shows a clear association between disease activity, inflammatory markers and etanercept levels in AS patients at 24 weeks of treatment. This indicates that therapeutic drug monitoring can be beneficial in addition to clinical measurements alone, in characterizing etanercept non-responders (low vs normal to high etanercept levels) and identifying responders in whom etanercept interval can be prolonged. However further research on the possibilities of etanercept interval prolongation in AS patients with low disease activity is needed.
REFERENCES


Golimumab trough levels, anti-drug antibodies and clinical response in patients with rheumatoid arthritis treated in daily clinical practice

Eva L Kneepkens, Chamaida Plasencia, Charlotte LM Krieckaert, Dora Pascual-Salcedo, Desiree van der Kleij, Michael T Nurmohamed, M Teresa López-Casla, Roeland Wieringa, Theo Rispens, Gertjan Wolbink

**TNF-inhibitors (TNFi)** are effective in the majority of patients with rheumatoid arthritis (RA), however, an important reason for non-response is low drug level due to immunogenicity. To our knowledge no data, collected during a prospective observational study, is currently available regarding the relationship between golimumab level, immunogenicity and response in RA.

This prospective observational cohort consisted of 37 consecutive adult patients with RA, according to the American College of Rheumatology 1987 revised criteria, in whom golimumab 50 mg subcutaneously once monthly was initiated according to the judgment of the rheumatologist and who were recruited from two departments (Spain and the Netherlands). The study was approved by both Medical Ethics Committees. Clinical response was defined as Disease Activity Score using 28 joint count (DAS28) <3.2, calculated with erythrocyte sedimentation rate (ESR) (mm/ hour). Patients were eligible for inclusion when clinical data and sera of baseline with ≥ one follow-up visit were available.

Clinical measurements and trough level sera were collected at baseline and 4, 16, 28 and 52 weeks (Netherlands), or half yearly (Spain), thereafter. Golimumab levels were measured analogously to adalimumab using TNF for capture and rabbit anti-golimumab for detection (LLOQ 5 ng/mL, accuracy 103%, precision 12%). Anti-drug antibodies (ADA) were measured, using an ADA radio-immune assay, described previously. Cut-off (mean+3 SD) was based on a serum panel of 80 healthy donors and 15 sera containing anti-CCP, ANA, and/or RF. All baseline samples were ADA against golimumab negative.

For statistical analysis SPSS V.17.0 and Graph Pad Prism 5 for windows were used. Threshold for significance was set at p<0.05. To analyse the association between golimumab level and response at one year, last observation carried forward was used for patients who discontinued golimumab treatment prematurely.
Table 1 Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Total patient population</th>
<th>DAS28 &lt; 3.2</th>
<th>DAS28 ≥ 3.2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td>n = 37</td>
<td>n = 15</td>
</tr>
<tr>
<td>Age, years, mean ± SD</td>
<td>51.7 ± 13.9</td>
<td>50.4 ± 15.5</td>
<td>52.6 ± 12.9</td>
</tr>
<tr>
<td>Female, no. (%)</td>
<td>31 (83.8)</td>
<td>10 (66.7)</td>
<td>21 (95.5)*</td>
</tr>
<tr>
<td>BMI, median (IQR)</td>
<td>25.5 (22.7-27.3)</td>
<td>23.5 (20.7-26.8)</td>
<td>26 (23.2-29)</td>
</tr>
</tbody>
</table>

**Disease status**

<table>
<thead>
<tr>
<th></th>
<th>Total patient population</th>
<th>DAS28 &lt; 3.2</th>
<th>DAS28 ≥ 3.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease duration, years, mean ± SD</td>
<td>12.3 ± 8.7</td>
<td>11.6 ± 10.3</td>
<td>12.8 ± 7.7</td>
</tr>
<tr>
<td>Rheumatoid factor positive, no(%)</td>
<td>25 (67.6)</td>
<td>9 (60)</td>
<td>16 (72.7)</td>
</tr>
<tr>
<td>Anti-CCP positive, no. (%)</td>
<td>22 (59.5)</td>
<td>8 (53.3)</td>
<td>14 (63.6)</td>
</tr>
<tr>
<td>Erosive disease, no. (%)</td>
<td>18 (48.6)</td>
<td>7 (46.7)</td>
<td>11 (50)</td>
</tr>
<tr>
<td>CRP mg/L, median (IQR)</td>
<td>6.4 (2-18)</td>
<td>2 (1-19)</td>
<td>9 (2-18)</td>
</tr>
<tr>
<td>ESR mm/h, median (IQR)</td>
<td>29 (7.5-42)</td>
<td>7 (4-38)</td>
<td>34 (21.8-48)**</td>
</tr>
<tr>
<td>DAS28, mean ± SD</td>
<td>4.4 ± 1.3</td>
<td>3.1 ± 1</td>
<td>5.1 ± 1.1***</td>
</tr>
</tbody>
</table>

**DMARD therapy**

<table>
<thead>
<tr>
<th></th>
<th>Total patient population</th>
<th>DAS28 &lt; 3.2</th>
<th>DAS28 ≥ 3.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior biologicals, no. (%)</td>
<td>24 (64.9)</td>
<td>8 (53.3)</td>
<td>16 (72.7)</td>
</tr>
<tr>
<td>Methotrexate use, no. (%)</td>
<td>24 (64.9)</td>
<td>11 (73.3)</td>
<td>13 (59.1)</td>
</tr>
<tr>
<td>Methotrexate dose (mg/ wk), median (IQR)</td>
<td>20 (10.6-25)</td>
<td>25 (15-25)</td>
<td>12.5 (8.8-25)</td>
</tr>
<tr>
<td>Prednisone use, no. (%)</td>
<td>16 (43.2)</td>
<td>6 (40)</td>
<td>10 (45.5)</td>
</tr>
<tr>
<td>Prednisone dose (mg/ day), median (IQR)</td>
<td>5 (5-10)</td>
<td>6.3 (4.3-10)</td>
<td>5 (5-10)</td>
</tr>
</tbody>
</table>

Normally distributed continuous variables are represented by mean values ± standard deviation (SD) and non-normally distributed continuous variables are represented by median values (interquartile range (IQR)); dichotomous variables are represented by numbers (percentages of total).

For differences between groups at baseline, the independent sample t test was used for normally distributed continuous variables, Mann-Whitney U was used for non-normally distributed continuous variables and the chi² test was used for dichotomous variables.

*p = 0.02; **p = 0.02; ***p < 0.001

DAS28 = Disease Activity Score using 28 joint count; BMI = Body Mass Index; CCP = cyclic citrullinated peptide; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; DMARD = disease modifying anti-rheumatic drugs.

Figure 1a Median golimumab trough level (mg/L) (with interquartile range) was higher in patients with a DAS28 <3.2 vs ≥3.2 at week 52 of treatment (p = 0.023).

Figure 1b The association between golimumab trough level and disease activity over time during one year of follow-up, analyzed with a generalized estimating equation (GEE).

Figure 1c Percentage of patients with DAS28 <3.2 and ≥3.2 stratified according to the golimumab level at 52 weeks of treatment. Each group contains 9 patients (25% of all patient) and the last quartile 10.
For baseline characteristics see table 1.

At week 52, 15 patients (40.5%) were responder and 22 (59.5%) non-responder. Nineteen patients (51.4%) discontinued golimumab treatment prematurely due to inefficacy (11), side effects (7) or other reasons (1); with a median drug survival of 16 weeks. Median golimumab level (mg/L) at week 52 was 0.55 (0.27-1.48) and was significantly higher, analyzed with a χ² test, in responders, 1.36 (0.5-1.82), compared with non-responders, 0.43 (0.23-0.84) (p = 0.023) (Figure 1a). Generalized estimating equation analysis demonstrated, after adjustment for baseline values, a statistically significant inverse association between golimumab level and C-reactive protein (CRP) (mg/L)/ESR. After correction, a trend remained visible for DAS28 (figure 1b).

All patients were stratified according to the golimumab level at week 52 and divided into quartiles (figure 1c). The lowest quartile (golimumab <0.25 mg/L) comprised 32% of all non-responders, while, the highest (golimumab >1.4 mg/L) comprised 47% of all responders.

During 52 weeks, 3 patients were ADA positive (ADA >12 AU/mL one ≥1 occasion in combination with golimumab levels <0.1 mg/L). All 3 patients discontinued golimumab prematurely due to inefficacy. One patient used concomitant methotrexate. However, the assay used to detect ADA can be influenced by drug interference, resulting in an underestimation of ADA. The percentages of patients with ADA against golimumab found in prior studies varied between 2.1% to 13%. However, head-to-head comparison of ADA percentages is complicated, since, several factors can influence immunogenicity and clinical non-response is multifactorial.

In conclusion, responders had a significantly higher golimumab trough level at one year of treatment. ESR and CRP were statistically significantly inversely associated with golimumab level over time. Three patients had high ADA titres resulting in undetectable golimumab levels and thus in a poor clinical outcome. These results can be used to further optimize golimumab treatment in RA.

There are some limitations to this study: limited patient number, the majority of patients used prior TNFi and golimumab discontinuation rate was relatively high.

REFERENCES

Serum tocilizumab trough concentration can be used to monitor systemic IL-6 receptor blockade in patients with rheumatoid arthritis: a prospective observational cohort study

Eva L Kneepkens, Inge AM van den Oever, Chaimaida H Plasencia, Dora Pascual-Salcedo, Annick de Vries, Margreet Hart, Michael T Nurmohamed, Alejandro Balsa, Theo Rispens, Gertjan Wolbink

ABSTRACT

Objectives To investigate pharmacokinetics (PK) and –dynamics of tocilizumab (TCZ) in daily practice.

Method An observational study of 66 consecutive RA patients treated with TCZ 8 mg/kg once every 4 weeks intravenously, monitored during 24 weeks. Spearman rank test was used to investigate the correlation between TCZ concentration and C-reactive protein (CRP). Clinical improvement was assessed at week 24 using Disease activity Score of 28 joints (DAS28) compared to baseline; And its relationship with TCZ concentration was investigated using linear regression analyses. TCZ trough concentrations and anti-drug antibodies were measured using an ELISA and Antigen Binding Test, respectively.

Results At baseline, 26 patients (39.4%) had a CRP above 10 mg/L with a median of 37.7 (21.9-49.7). TCZ concentration above 1 mg/L was sufficient to normalize CRP levels. The spearman rank test showed a correlation coefficient of -0.460, p<0.0001. TCZ concentration varied widely, with concentrations <1 mg/L in 17-31% of patients, depending on time point of measurement. Anti-TCZ antibodies were detected in one sample. Linear regression analysis showed a coefficient of 0.080 with a 95% confidence interval of 0.039 to 0.113, p<0.001, for the association between TCZ concentration and ∆DAS28. No confounders were identified.

Conclusions TCZ standard regimen results in a wide variety of serum TCZ trough concentrations; which is mostly due to target-binding, and to a lesser extent to immunogenicity. The majority of patients obtained TCZ concentrations >1 mg/L, which is sufficient for CRP normalisation. Therefore, dose taper strategies might be possible in a substantial proportion of patients.

INTRODUCTION

The pathogenesis of rheumatoid arthritis (RA) involves the release of pro-inflammatory mediators like Interleukin 6 (IL-6). IL-6 is a multifunctional cytokine and is associated with inflammation, chronic synovitis, bone destruction of joints and pathogenesis of RA. Moreover, IL-6 is the most important cytokine stimulating hepatocytes to produce C-reactive protein (CRP). IL-6 can activate target cells, like hepatocytes, via the IL-6 receptor (IL-6R) which occurs in the body in the form of a membrane bound IL-6R (mIL-6R) and a soluble IL-6R (sIL-6R), called classic and trans-signalling pathway, respectively. The sIL-6R/IL-6 complex can only activate cells which express cell surface glycoprotein-130.

Tocilizumab (TCZ) is a humanized antibody which competitively inhibits both sIL-6R and mIL-6R and is an effective treatment for RA. Currently, it can be administered intravenously (iv) and subcutaneously, with or without concomitant methotrexate. Randomised controlled trials (RCTs) show that TCZ treatment substantially reduced biomarkers of inflammation, like CRP, and influence serum levels of IL-6 and sIL-6R. With regard to CRP, Nishimoto et. al. shows that a TCZ concentration above 1 mg/L is sufficient for CRP normalisation. Although, clinical response rates are promising not all patients seem to respond sufficiently to TCZ treatment, which is reported for anti-Tumor Necrosis Factor (TNF) treatment too.

Clinical inefficacy to biological treatment is multifactorial, but in anti-TNF treatment immunogenicity can be a major factor influencing pharmacokinetics (PK). The presence of high titres of anti-drug antibodies (ADA) reduce the amount of free drug available to bind the target resulting in a reduced clinical response in the majority of patients with detectable ADA. Evolving evidence shows that PK of TCZ is influenced by target-binding (amount of IL-6R) and to a lesser extent by immunogenicity.

Identifying factors which can predict clinical response to a biological agent is important, since, this knowledge can be used to optimize treatment in individual patients. Currently, all data on serum TCZ concentrations, immunogenicity and clinical response are obtained from RCTs. The aim of this study is to investigate variation in serum TCZ trough concentrations and the relationship with clinical measurements in RA during 24 weeks of follow-up.
Patient population and study design

This prospective observational cohort consisted of 66 consecutively included adult RA patients, diagnosed according to the American College of Rheumatology 1987 revised criteria.14 All patients started TCZ between April 2009 and June 2014. Two cohorts were combined (The Netherlands, n = 34, Spain, n = 32). All patients had active disease, meaning a Disease Activity Score of 28 joint count (DAS28), using erythrocyte sedimentation rate (ESR), of > 3.2, despite prior treatment with disease modifying anti-rheumatic drugs (DMARDs) and/or biologics. All patients started with TCZ standard regimen (8 mg/kg per 4 weeks iv) and concomitant DMARDs with or without prednisone, only with concomitant prednisone or TCZ as monotherapy. Adaptations in TCZ regimen could be made, based on the expert opinion of the rheumatologist, in case of: clinical inefficacy, adverse events, sustained low disease activity or remission. Patients were eligible for inclusion in the final analyses, if a serum sample (trough concentration) of ≥ 1 follow-up visit from week 12 onwards was available, taken after TCZ standard regimen and in combination with availability of corresponding measurements of DAS28 and/or CRP. The study was approved by the Medical Ethics Committee of the Slotervaart hospital and the Jan van Breemen research institute | Reade, Amsterdam (The Netherlands); and by the Medical Ethics Committee of La Paz hospital, Madrid (Spain). All patients gave written informed consent in accordance with the Helsinki Declaration.

Outcome measures

Disease Activity was measured with DAS28-ESR.15 In the Dutch cohort DAS28, parameters of inflammation (CRP and ESR) and serum trough samples were collected at baseline and 4, 12 and 24 weeks thereafter. In the Spanish cohort serum samples and parameters of inflammation were collected before every TCZ infusion. However, DAS28 and its separate components were measured at baseline and at 24 weeks. The duration of follow-up in this study was 24 weeks.

To investigate the relationship between serum TCZ trough concentration (further mentioned as TCZ concentration) and clinical response at week 24, defined as an improvement compared to baseline in DAS28 (ΔDAS28) and swollen joint count of 28 joints (ΔSJC28). To obtain the concentration-effect curve at week 24, last observation carried forward was used for patients in whom follow-up data of week 12 was available but not yet of week 24. This seemed appropriate since steady-state of TCZ standard regimen is seen, on average, from week 8 onward.20 The relationship between TCZ concentration and serum CRP (further mentioned as CRP) was investigated separately, since serum CRP can be used as a surrogate marker for systemic IL-6R blockade.1,6,7

Tocilizumab concentration measurement

To measure TCZ concentrations an immunoassay was developed using rabbit anti-TCZ antibodies to capture TCZ, and rabbit anti-TCZ F(ab′)2, fragments for detection. Maxisorp enzyme-linked immune sorbent assay (ELISA) plates were coated overnight at room temperature with 0.125 µg/ml rabbit anti-TCZ in phosphate buffered saline (PBS). The specific rabbit anti-idiotype antibodies were produced analogously as described for natalizumab.21 Plates were washed five times with PBS/ 0.02% Tween. Next, plates were washed and incubated for 1 hour with patient serum which was serially diluted in High performance ELISA buffer (HPE). After washing 5 times with PBS-Tween (PT), plates were incubated for 1 hour with biotinylated anti-TCZ F(ab′)2 fragments (125 ng/ml in HPE). After washing, streptavidin-poly-horseradish peroxidase (HRP) (Sanquin) (1:10,000, in HPE) was added for 1 hour at 37 °C. After washing, the ELISA was developed with 100 µg/ml tetramethylbenzidine in 0.1 M sodium acetate (pH 5.5) containing 0.003% (v/v) H2O2. The reaction was stopped with 2 M H2SO4. Absorption was measured at 450 nm related to a titration curve of TCZ in each plate. The Lower Limit of Quantification (LLOQ) in serum was 200 ng/ml; overall precision and accuracy were 8% and 93%, respectively. Serum samples were collected at trough concentration, meaning just before the next infusion.

Anti-tocilizumab antibody measurement

Measurement of anti-TCZ antibodies was essentially carried out as described before.22 One microliter of serum diluted in buffer containing IVIg F(ab′)2, to prevent anti-hinge reactivity23 was incubated overnight with 1 mg Protein A Sepharose (GE healthcare, Chalfont St. Giles, UK) and 2.5 ng biotinylated F(ab′)2, TCZ in a final volume of 800 µl. Subsequently, samples were washed with 0.005% PT and ca. 1 ng 125I-labeled streptavidin was added in 800 ul final volume of PBS albumin tween (PBS-AT) (PBS / 0.01M Ethylenediaminetetraacetic acid (EDTA) / 0.3% Bovine Serum Albumin / 0.004% Tween-20 / 0.05% NaNO3) and incubated overnight. Unbound label was removed by washing, and Sepharose-bound radioactivity was measured. Antibody levels were compared to a standard serum of an immunized rabbit containing ADA and expressed in arbitrary units (AU). A lower limit of detection was based on mean + 3 standard deviation (SD) measured in a panel of 50 sera from healthy donors and 15 sera containing anti-cyclical citrullinated peptide (CCP), antinuclear antibody (ANA), and/or Rheumatoid Factor (RF).

Statistical analyses

For statistical analyses SPSS V.21.0 and Graph Pad Prism 6.0 for windows were used. Results are displayed as number and percentage or mean ± SD, when normally distributed, or as median and interquartile range (IQR), when not-normally distributed. For differences in baseline characteristics between patients of the Spanish cohort versus the Dutch, an independent sample t test, Mann–Whitney U test or χ2 test was used as appropriate. The threshold for significance was set at p<0.05. To investigate the correlation between TCZ concentration and CRP, the Spearman rank test was used. To investigate the relationship between TCZ concentration and ΔDAS28 at week 24, a linear regression analysis was used. Potential confounders of this relationship...
were investigated using the baseline characteristics (see table 1 of the result section), excluding the separate components of the DAS28, since baseline DAS28 itself was already included. A variable was considered to be a confounder, if it changed the regression coefficient by ≥10%. For this analysis, last observation carried forward was used as explained in the method section. Sensitivity analyses showed no significant difference in baseline characteristics or outcome data (ΔDAS28 and TCZ concentration) at week 12 between patients with and without outcome data at week 24.

**RESULTS**

The baseline characteristics of the 66 included RA patients are shown in table 1. In addition, patients in the Spanish cohort had a significantly lower median ESR (mm/hr) level of 23.5 (14.3-35.8) compared to 44 (22.5-63.0); p = 0.009, for Dutch patients; Dutch patients more often used ≥1 prior biological(s) (34 (100%)) compared with Spanish patients (19 (59.4%)); p<0.001.

**Discontinuation and follow-up**

A total of 8 patients (12.1%) discontinued TCZ treatment between week 12 and 24 due to inefficacy (n = 5) or adverse events (n = 3). For 3 patients clinical data and serum of week 12 was available, however, they did not have 24 weeks of follow-up yet at the time of data extraction.

**Serum tocilizumab trough concentration and anti-tocilizumab antibodies**

The available samples and median TCZ concentrations per time point are shown in table 2, with the lowest and highest concentration and the number of patients with TCZ concentrations below 1 mg/L.

In total, 9 patients had TCZ concentrations below 1 mg/L at ≥2 subsequent visits, of which 3 patients at every time point. This means that some patients had TCZ concentrations below 1 mg/L repeatedly, despite receiving TCZ standard regimen. However, this could not be established in all patients due to missing samples, therefore, this number might be an underestimation. Although, TCZ concentrations below 1 mg/L were found (repeatedly) in several patients, an anti-TCZ antibody signal was seen in only 2 patients with the assay used. In one of these patients, a weak anti-TCZ antibody signal was consistently detected, including in pre-treatment samples, independently of TCZ concentrations. This patient was therefore not considered anti-TCZ antibody positive.

**Table 1** Baseline demographics and clinical characteristics.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Baseline demographics and clinical characteristics.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total patient population</strong> n = 66</td>
<td></td>
</tr>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
</tr>
<tr>
<td>Age (years), mean ± SD</td>
<td>56.0 ± 12.9</td>
</tr>
<tr>
<td>Female, number (%)</td>
<td>54 (81.8)</td>
</tr>
<tr>
<td>BMI, mean ± SD</td>
<td>26.4 ± 5.4</td>
</tr>
<tr>
<td>Spanish, number (%)</td>
<td>32 (48.5)</td>
</tr>
<tr>
<td><strong>Disease status</strong></td>
<td></td>
</tr>
<tr>
<td>Disease duration (years), median (IQR)</td>
<td>11.0 (5-17)</td>
</tr>
<tr>
<td>Rheumatoid factor positive, number (%)</td>
<td>47 (71.2)</td>
</tr>
<tr>
<td>Anti-CCP positive, number (%)</td>
<td>47 (71.2)</td>
</tr>
<tr>
<td>CRP (mg/L), median (IQR)</td>
<td>6.8 (2.1-31.9)</td>
</tr>
<tr>
<td>ESR (mm/hr), median (IQR)</td>
<td>34 (16.5-47.5)</td>
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<tr>
<td>DAS28, mean ± SD</td>
<td>5.4 ± 1.4</td>
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<tr>
<td>Tender joint count, median (IQR)</td>
<td>9.5 (4-14.5)</td>
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<tr>
<td>Swollen joint count, median (IQR)</td>
<td>6 (2.8-10.3)</td>
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<tr>
<td>VAS GDA patient (0-100 mm), mean ± SD</td>
<td>60.0 ± 25.8</td>
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<tr>
<td><strong>DMARD therapy</strong></td>
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<tr>
<td>Prior biologics, number (%)</td>
<td>53 (80.3)</td>
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<tr>
<td>Methotrexate use at baseline, number (%)</td>
<td>42 (63.6)</td>
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<tr>
<td>Methotrexate dose (mg/week), mean ± SD</td>
<td>15.5 ± 7.3</td>
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<tr>
<td>Prednisone use at baseline, number (%)</td>
<td>46 (69.7)</td>
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<tr>
<td>Prednisone dose (mg/day), mean ± SD</td>
<td>5.8 ± 4.4</td>
</tr>
<tr>
<td>Other DMARD use (with or without methotrexate), number (%)</td>
<td>29 (43.9)</td>
</tr>
</tbody>
</table>

**Table 2** Median serum TCZ trough concentration (mg/L) during 24 weeks of follow-up.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Median serum TCZ trough concentration (mg/L) during 24 weeks of follow-up.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Week 4</strong></td>
<td><strong>Week 12</strong></td>
</tr>
<tr>
<td>Number of patients on drug (%)</td>
<td>66 (100)</td>
</tr>
<tr>
<td>Number of available samples (%)</td>
<td>49 (74.2)*</td>
</tr>
<tr>
<td>Median TCZ (mg/L)</td>
<td>3.4</td>
</tr>
<tr>
<td>Min. TCZ (mg/L)</td>
<td>0</td>
</tr>
<tr>
<td>Max. TCZ (mg/L)</td>
<td>18.2</td>
</tr>
<tr>
<td>Number of patients with TCZ &lt;1 mg/L (%)</td>
<td>15 (30.6)**</td>
</tr>
</tbody>
</table>

* Percentage is based on the number of patients on drug.
** Percentage is based on the number of available samples per time point.
To provide an insight into the course of CRP normalisation over time with the corresponding TCZ concentration, figure was also divided per time point (i.e. week 4, 12 and 24), see supplementary material. This figure shows that only one patient had an elevated CRP level at the time point of drop-out (week 12) and in almost all other patients CRP normalised over time. None of the patients had increased CRP levels during follow-up in combination with TCZ >1 mg/L at any time point.

A similar course of improvement was seen for ESR, nevertheless, the cut off of TCZ of 1 mg/L was less marked compared to CRP. With TCZ concentrations above 6 mg/L, no increased ESR levels (>20 mm/hr) were found (data not shown).

Concentration-effect curve of tocilizumab at week 24

In Figure 2, the relationship between TCZ concentration and ΔDAS28 (figure 3a) and ΔSJC28 (figure 3b) for the individual patients at week 24 is presented. Twelve patients (18%) did not achieve a ΔDAS28 of ≥1.2, which is considered to be a clinically significant change. Eight of these 12 patients had a TCZ concentration <1 mg/L. In addition, 3 of these 8 patients had a CRP level above 10 mg/L. Linear regression analysis showed a regression coefficient of 0.080 with a 95% confidence interval of 0.039 to 0.113, p<0.001, for the association between TCZ concentration and ΔDAS28 at week 24 of treatment. No confounders were identified.

Eight patients had more swollen joints at week 24 of treatment compared to baseline of whom 5 had a TCZ concentration below 1 mg/L. Two of these 5 patients had increased CRP levels, 2 patients did not but had increased CRP levels at previous time points and one had normal CRP levels. Roughly, in patients with TCZ concentrations of approximately 4 mg/L SJC had improved or stabilised in the majority of patients.

DISCUSSION

The aim of this study is to investigate variation in serum TCZ trough concentrations and the relationship with clinical measurements in RA during 24 weeks of follow-up; in a prospective observational cohort. This study shows that serum TCZ trough concentrations vary widely between patients with TCZ standard regimen, as was seen previously in anti-TNF treatment. Moreover, the majority of patients obtained TCZ concentrations which were sufficient to normalize serum CRP. A statically significant ΔDAS28 improvement with increasing TCZ concentrations was seen, but, for clinical implications this change was small.

For anti-TNF treatment it has been shown that immunogenicity can have a profound effect on PK. However, PK of TCZ appears to be different, since, anti-TCZ antibodies were detected only in one patient, although, TCZ concentrations below 1 mg/L were found (repeatedly) in several patients. Moreover, these low concentrations were especially evident during early treatment phase which is not in accordance with

Figure 1. The relationship between tocilizumab (TCZ) concentration (mg/L) and C-reactive protein (CRP) levels (mg/L), all samples from week 4 onward were stratified from low to high according to TCZ concentration with correlating CRP levels, thus, one dot represents one sample and not one patient. Based on this figure, a TCZ concentration above 1 mg/L (marked with vertical dashed line) is sufficient to normalize serum CRP levels (≤10 mg/L) (marked with horizontal dashed line). The spearman rank correlation coefficient was showed a significant but moderately strong negative correlation coefficient of -0.460, p<0.0001.
reported PK variations due to immunogenicity in anti-TNF treatment. The influence of immunogenicity might have been underestimated in this study, since, the assay used to detect anti-TCZ antibodies is drug sensitive, meaning only ADA exceeding TCZ concentration will be detected.\textsuperscript{24,25} Previously reported data of RCTs suggests that immunogenicity is not a major factor in PK of TCZ,\textsuperscript{10,11,13} but, these trials included a different patient population as compared to daily clinical practice. Moreover, comparing ADA results is difficult, since, ADA production and detection can be influenced by several factors like: time point of sampling, assay format, concomitant immunomodulation therapy (e.g. methotrexate) and dosing.\textsuperscript{26} Thus, it remains an interesting question, whether TCZ has a less immunogenic structure, or detectability is more complex due to drug interference or immunological tolerance is induced by high dosing.\textsuperscript{27,28} Therefore, it would be interesting to investigate immunogenicity of TCZ with a drug tolerant assay as was done for adalimumab previously.\textsuperscript{29,30} 

Another explanation for the variation in TCZ concentration between patients is target binding. In patients with more target, clearance of TCZ is increased, thus, lower serum TCZ trough concentrations will be detected by the assay. Moreover, a TCZ concentration above 1 mg/L was sufficient to normalise CRP levels, and the spearman rank test showed a statistically significant moderately strong negative correlation between TCZ and CRP. CRP is mainly produced by hepatocytes via IL-6 activation and can therefore be used as a surrogate marker for systemic IL-6R binding.\textsuperscript{1,6,7} Target-binding influencing PK of TCZ has been suggested previously in several studies. Inhibition assays showed that the binding between IL-6 and sIL-6R was suppressed, in a dose dependent manner, by adding TCZ at concentrations between 0.002 and 4 mg/L.\textsuperscript{31} Nishimoto et al. shows that TCZ concentrations above 1 mg/L resulted in >95% binding of sIL-6R in a sIL-6R/TCZ immune complex with subsequent inhibition of CRP production.\textsuperscript{12} Another clinical trial showed that these TCZ concentrations were obtained in the majority of patients from week 8 onward.\textsuperscript{11} In addition, the TCZ concentration at which 50% of its maximal effect was observed was lower in patients with high IL-6 levels at baseline, which may be the result of IL-6 overproduction or lower expression of mIL-6R, thus, slower clearance.\textsuperscript{14} Different amounts of target, in normal or inflammatory state, might be explained by sIL6R polymorphisms;\textsuperscript{32,33} but was, to our knowledge, not studied in combination with serum TCZ concentrations. 

Although, CRP can be used as a surrogate marker for systemic IL-6R binding, clinical response is more complex. Clinical response is multifactorial and clinical outcome measurements frequently used in RA (like, DAS28, Clinical Disease Activity Index (CDAI),\textsuperscript{34} Simplified Disease Activity Index (SDAI))\textsuperscript{35} are composite measurements which reflect total disease activity, but do not discriminate between the role of a particular cytokine versus other factors (e.g. other cytokines, established bone damage, psychological and social factors). Linear regression analysis showed a statistically significant ΔDAS28 improvement with increasing TCZ concentrations; but, for clinical implications this change was small. Moreover, normalised CRP did not result in good clinical outcome, measured with DAS28 or SJC28, in all patients. In addition, predictive value of IL-6, sIL-6R or CRP on clinical outcome is contradictory.\textsuperscript{12,13,17,36,37} Nevertheless, TCZ, like all biologics given in RA, is a molecular targeting therapy, thus, the highest obtainable result is complete target blockade; although, this does not necessarily translate to an appropriate clinical response in all patients. The assay used for TCZ concentrations measures the surplus of unbound TCZ, a detectable serum trough concentration means all systemic, and potentially all local,\textsuperscript{38,39} IL-6R is blocked. Therefore, TCZ concentration measurements can be used as a surrogate marker for systemic target blockade. Considering, the amount of non-responders to biological treatment and the high costs associated with these therapeutics, a dose based on target levels seems more rational. To apply Therapeutic Drug Monitoring (TDM) of biologicals in daily clinical practise for treatment optimisation, an optimal therapeutic concentration range for effective target blockade must be identified.\textsuperscript{40,41} Due to the direct relationship of IL-6 and CRP, TCZ is an interesting biological to investigate the optimal therapeutic concentration.
range for complete target blockade vs. for clinical response. To investigate the additional value of TDM, a prospective TCZ dose taper trial is necessarily, including: clinical measurements, TCZ concentrations and other potential markers, for target blockade (IL6, sIL-6R, CRP and calprotectin);12,13,17,36,42,43,44 as well as, biomarkers for progression of bone damage, since, IL-6 plays an important role in bone metabolism.3,45

Some limitations of the current study need to be addressed. Two cohorts were combined to increase patient numbers resulting in slight differences in measurements per time point. Differences in patient characteristics were limited and both cohorts consisted of mainly Caucasians. On the other hand, possible bias due to non-compliance, change in TCZ dose or interval or wrong timing of sampling was excluded since TCZ was given iv.

In conclusion, TCZ standard regimen results in a wide variety of serum TCZ trough concentrations between patients; and target-binding seems to explain this variation more compared to immunogenicity. Moreover, the majority of patients obtained TCZ concentrations above 1 mg/L, which is sufficient to normalise serum CRP. Therefore, TCZ standard regimen is an over treatment with regard to systemic IL-6R blockade in the majority of RA patients; however, clinical response is multifactorial and might require more than only sufficient blockade of a single cytokine pathway.

SUPPLEMENTARY MATERIAL CHAPTER 5

For this figure the same procedure was followed as for figure 1 (main text), but, this figure represents the relationship between tocilizumab (TCZ) concentrations (mg/L) with correlating C-reactive protein (CRP) levels (mg/L) separated per time point (week 4 (A), week 12 (B) and week 24 (C)). Thus, one dot represents one patient.
REFERENCES


Comparing tapering strategy to standard dosing regimen of Tumor Necrosis Factor inhibitors in patients with spondyloarthritis in low disease activity

Chamaida Plasencia, Eva L Kneepkens, Gertjan Wolbink, Charlotte LM Krieckaert, Samina Turk, Victoria Navarro-Compán, Merel L’Ami, Mike T Nurmohamed, Irene van der Horst-Bruinsma, Teresa Jurado, Cristina Diego, Gema Bonilla, Alejandro Villalba, Diana Peiteado, Laura Nuño, Desiree van der Kleij, Theo Rispen, Emilio Martín-Mola, Alejandro Balsa, Dora Pascual-Salcedo

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ABSTRACT

**Objectives** To compare clinical outcomes, incidence of flares, and administered drug reduction between patients with spondyloarthritis (SpA) under TNF inhibitor (TNFi) tapering strategy with patients receiving a standard regimen.

**Methods** In this retrospective study, 74 patients with SpA from Spain on tapering strategy (tapering group; TG) were compared with 43 patients from the Netherlands receiving a standard regimen (control group; CG). The Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) was measured at visit 0 (prior to starting the TNFi), visit 1 (prior to starting tapering strategy in TG and at least 6 months with BASDAI < 4 after starting the TNFi in the TG and CG), visit 2 (6 months after visit 1), visit 3 (1 year after visit 1), and visit 4 (the last visit available after visit 1).

**Results** An overall reduction of the administered drug was seen at visit 4 in the TG [dose reduction of 22% for infliximab and an interval elongation of 28.7% for infliximab, 45.2% for adalimumab, and 51.5% for etanercept] without significant differences in the BASDAI between the groups at visit 4 (2.15 ± 1.55 in TG vs 2.11 ± 1.31 in CG, p = 0.883). The number of patients with flares was similar in both groups [22/74 (30%) in the TG vs 8/43 (19%) in the CG, p = 0.184].

**Conclusion** The tapering strategy in SpA results in an important reduction of the drug administered, and the disease control remains similar to that of the patients with SpA receiving the standard regimen.

INTRODUCTION

There is a growing interest in optimizing biological therapy in patients with spondyloarthritis (SpA), because the costs of this treatment are high, the longterm risks are unknown, and the treatment options are limited.\(^1\)\(^-\)\(^9\) The therapeutic strategy once low disease activity (LDA) is achieved has not been clearly identified and little evidence is available regarding the predictors of maintaining LDA after lowering Tumor Necrosis Factor inhibitor (TNFi) dose in patients with SpA.\(^10\) Considering that TNFis are the only biologicals available in patients with SpA, changes in the therapy regimen should be made with caution. In a study of patients with ankylosing spondylitis (AS) taking infliximab, a stable clinical course was observed despite decreased doses and extended intervals of administration during the 1-year study period.\(^6\) Another study in patients with AS taking etanercept showed that remission was maintained in a high percentage of patients after halving the dose.\(^1\)

Several studies have demonstrated an association between the serum drug levels and the clinical response.\(^1\)\(^-\)\(^4\) In a study of patients with rheumatoid arthritis (RA) who were treated with adalimumab, the optimal drug levels for maintaining a good clinical course were defined;\(^15\) nevertheless, the optimal drug levels required to maintain stability in LDA or remission are unknown. The effect of biological therapies depends on the concentration and the immunogenic properties of these drugs.\(^16\) It is beneficial to consider the pharmacokinetics of TNFi in the care of patients with SpA to optimize treatment and to reduce the risk of under- or overtreatment.

The introduction of TNFi into the management of AS and psoriatic arthritis has increased treatment costs.\(^1\)\(^7\)\(^-\)\(^9\) A number of economic evaluations have been performed. A comparison of different TNFi found less favorable cost-effectiveness results for infliximab;\(^1\)\(^7\)\(^,\)\(^8\)\(^,\)\(^10\) however, these findings should be interpreted cautiously because of the variability in the dose regimen and drug pricing. Actual clinical data on TNFi for longterm use have not been published. The use of the tapering strategy in SpA patients with LDA might lead to cost reductions.

In recent years there has been a tendency at La Paz University Hospital, Madrid, Spain, to use a tapering strategy, with drug and anti-drug antibody (ADA) level monitoring in patients with SpA who have sustained LDA. Conversely, in the Netherlands, the label dose is maintained even when a good clinical response has been registered in patients with SpA. Our main objectives were to compare the longterm clinical disease activity, incidence of flares, and incidence of ADA at the end of the study between patients with SpA under a tapering strategy versus patients with SpA taking a standard dose. Our secondary targets were analyzed only in the SpA tapering group: the change in serum drug levels (infliximab, adalimumab or etanercept) during the study, and predictors associated with good response to tapering.
METHODS

Patients, clinical assessment and therapy regimen

In this retrospective observational study, 2 SpA cohorts taking TNFi were analyzed: a cohort from Spain under a tapering strategy (tapering group: TG) and a cohort from the Netherlands taking a standard therapy regimen (control group: CG). First, 528 patients with SpA (282 from Spain and 246 from the Netherlands) under TNFi (infliximab, adalimumab and etanercept) were recruited, but after the selection period only 117 patients with SpA fulfilled the inclusion criteria (74 patients from Spain and 43 patients from the Netherlands). The number of patients from the Netherlands was lower because no control group was available for infliximab and during the matching process of both cohorts, several patients with SpA were excluded (see the supplementary file).

All the selected patients with SpA (87 patients with AS, 11 patients with nonradiographic SpA, 8 with SpA associated with inflammatory bowel disease, and 11 psoriatic patients) had axial involvement and 49% (58) of them had also some peripheral manifestations (arthritis, enthesitis, dactylitis). The patients with AS fulfilled the revised New York criteria, and the remaining patients with SpA with nonradiographic axial SpA fulfilled the Assessment of Spondyloarthritis Society classification and diagnostic criteria. All the selected patients with SpA had a sustained LDA of at least 6 months, defined by Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) < 4, and also fulfilled 1 of these conditions: normal C-reactive protein (CRP) or ΔBASDAI > 50%.

Disease activity was measured by BASDAI at the different timepoints: visit 0 (prior to starting TNFi), visit 1 (before starting the tapering strategy in TG and after at least 6 months with LDA in the TG and CG), visit 2 (6 months after visit 1), visit 3 (1 year after visit 1), visit 4 (the last visit available after visit 1), and visit flare (visit with the worst flare between visit 1 and visit 4). Clinical activity was monitored every 6 months during the study, and the same periods of clinical evaluations were considered in the control group to avoid overestimating flares.

The tapering strategy was done as follows: in infliximab-treated patients, the tapering strategy included a gradual dose reduction (5 mg/kg to 4 mg/kg to 3 mg/kg) and/or interval administration per weeks (8 weeks to 9 weeks to 10 weeks, to a maximum of 15 weeks), adalimumab administration was prolonged 1 week until a maximum of 6 weeks and etanercept was delayed 3 days for a maximum of 3 weeks as long as the physician decided that the interval of administration could be modified based on clinical and serological markers. The CG continued the standard therapy regimen throughout the study. The patients gave written informed consent prior to the start of the biological therapy for the use of their clinical data and serum for research.

Flares were recorded during the followup after visit 1 and were defined as BASDAI ≥ 4 and a ΔBASDAI ≥ 2 in comparison with the BASDAI at pre-tapering (visit 1). In the TG, in a flare episode, the TNFi dose could be increased or the interval could be shortened to regain LDA. When a flare was registered in the CG, an intense regimen of nonsteroidal antiinflammatory drugs (NSAID) and/or nonbiologic disease-modifying antirheumatic drugs (DMARD) was used to control the disease activity.

In the selection period, the first step was to select a SpA cohort from Spain under tapering strategy who fulfilled the inclusion criteria. Later, both cohorts were matched according to several demographic, serological, and clinical characteristics to ensure that both groups were similar (age, sex, disease duration, HLA-B27 positivity, the disease activity (BASDAI) at baseline and at visit 1 (before starting the tapering strategy), duration of inactive disease prior to visit 1, and the time of followup between visit 1 and visit 4). All included patients were white. Patients with SpA who did not fulfill these requirements were excluded from the study to avoid misinterpretations using heterogeneous cohorts (see the supplementary file).

Serum samples and assays to measure drug and antidrug antibodies

Blood samples were collected a maximum of 24 h before drug administration for subcutaneous TNFi or immediately before intravenous infusions of infliximab. The serum drug concentrations (infliximab, adalimumab and etanercept) were determined by ELISA, as described previously. A radioimmunoaassay was performed to detect ADA in the patients with SpA, as previously described.

Statistical analysis

First, descriptive analyses were performed for the demographic and clinical variables. The results are shown as means and SD for continuous variables and relative frequencies for categorical variables. The frequency data were compared using the Pearson chi-squared and Fisher exact tests. The continuous data were compared between groups using the Mann-Whitney U and Wilcoxon nonparametric tests. Later, the associations between the independent variables and the outcomes were investigated using a univariate logistic regression model. Estimates for these associations are shown as standardized linear coefficient. SPSS 20.0 software was used for the analyses, and p values < 0.05 were considered statistically significant.
RESULTS

Patient characteristics

In Table 1 the demographic characteristics are shown comparing the TG versus CG. Most patients were receiving monotherapy in the CG. The time in LDA prior to visit 1 was higher in the TG although not statistically different. Patients taking infliximab had more time in LDA prior to the tapering strategy, but in patients taking etanercept and adalimumab this time was very similar (infliximab: 1.5 ± 1.3 years in TG; adalimumab: 0.7 ± 0.2 years in TG vs 0.8 ± 0.5 years in CG, p = 0.6; etanercept: 0.8 ± 0.1 years in TG vs 0.9 ± 0.4 years in CG, p = 0.5).

Table 1 Demographic characteristics of 117 patients with SpA.

<table>
<thead>
<tr>
<th>SpA patients, n = 117</th>
<th>TG, n = 74</th>
<th>CG, n = 43</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)</td>
<td>54 (73)</td>
<td>31 (72)</td>
<td>0.9</td>
</tr>
<tr>
<td>Age, yrs, mean ± SD</td>
<td>50.3 ± 12.5</td>
<td>47 ± 9.3</td>
<td>0.2</td>
</tr>
<tr>
<td>Disease duration, yrs, mean ± SD</td>
<td>15.2 ± 9.3</td>
<td>14.4 ± 7.6</td>
<td>0.9</td>
</tr>
<tr>
<td>HLA-B27, n (%)</td>
<td>54/59 (91)</td>
<td>47/43 (95)</td>
<td>0.45</td>
</tr>
<tr>
<td>Baseline BASDAI, mean ± SD</td>
<td>5.8 ± 1.6</td>
<td>5.8 ± 1.3</td>
<td>0.96</td>
</tr>
<tr>
<td>Baseline CRP, mg/L, mean ± SD</td>
<td>14.4 ± 23.7</td>
<td>15 ± 15.7</td>
<td>0.2</td>
</tr>
<tr>
<td>Subtypes of SpA</td>
<td></td>
<td></td>
<td>0.183</td>
</tr>
<tr>
<td>Ankylosing spondylitis</td>
<td>51 (70)</td>
<td>36 (84)</td>
<td></td>
</tr>
<tr>
<td>Non-radiographic SpA</td>
<td>8 (10)</td>
<td>3 (7)</td>
<td></td>
</tr>
<tr>
<td>SpA associated with IBD</td>
<td>5 (7)</td>
<td>3 (7)</td>
<td></td>
</tr>
<tr>
<td>Psoriatic SpA</td>
<td>10 (13)</td>
<td>1 (2)</td>
<td></td>
</tr>
<tr>
<td>Prior biological use, n (%)</td>
<td>10 (14)</td>
<td>5 (12)</td>
<td>0.7</td>
</tr>
<tr>
<td>Duration of LDA prior to visit 1, yrs, mean ± SD</td>
<td>1.2 ± 1.1</td>
<td>0.7 ± 0.2</td>
<td>0.24</td>
</tr>
<tr>
<td>Duration of follow-up between visit 1 and 4, yrs, mean ± SD</td>
<td>2.3 ± 1.1</td>
<td>2.4 ± 1</td>
<td>0.6</td>
</tr>
<tr>
<td>Baseline co-therapy, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methotrexate only (MTX)</td>
<td>11 (15)</td>
<td>3 (7)</td>
<td>0.2</td>
</tr>
<tr>
<td>Other DMARD only (OD)</td>
<td>17 (23)</td>
<td>6 (14)</td>
<td>0.2</td>
</tr>
<tr>
<td>MTX + OD</td>
<td>8 (11)</td>
<td>1 (2)</td>
<td>0.1</td>
</tr>
<tr>
<td>TNFi monotherapy</td>
<td>38 (51)</td>
<td>33 (77)</td>
<td>0.007</td>
</tr>
</tbody>
</table>

SpA: spondyloarthritis; TG: tapering group; CG: Control group; BASDAI: Bath Ankylosing Spondylitis Disease Index; CRP: C-reactive protein; IBD: inflammatory Bowel Disease; DMARD: disease-modifying antirheumatic drugs; TNFi: tumor necrosis factor inhibitor.

Clinical response during the study

The clinical course measured by BASDAI was similar in the 2 groups during the study (Figure 1). In a subgroup analysis to compare the clinical activity between the various TNFi, no significant differences were observed (Figure 2). The patients in the TG taking etanercept had higher clinical activity at visit 2; however, this difference was not significant (Figure 2).

The majority of patients with SpA had LDA at the end of the study [63/74 (85.1%) in the TG vs 39/43 (90.7%) in the CG at visit 4, p = 0.386], even after a subanalysis comparing the 2 groups per TNFi [infleximab: 30/35 (85.1%) in the TG at visit 4; adalimumab: 15/17 (88.2%) in the TG vs 19/21 (90.5%) in the CG at visit 4, p = 0.823; etanercept: 18/22 (81.8%) in the TG vs 20/22 (90.9%) in the CG at visit 4, p = 0.380].

Flares during the study

Thirty patients with SpA (26%) experienced a flare during our study [22/74 (30%) in the TG vs 8/43 (19%) in the CG, p = 0.184]. No differences were observed in the number of flares between groups (1.4 ± 0.7 in the TG vs 1.5 ± 0.5 in the CG, p = 0.486) or in the time to the first flare after visit 1 (1.3 ± 0.8 yrs in the TG vs 1.3 ± 1.2 yrs in the CG, p = 0.841). Table 2 shows the proportion of patients with flares, the number of flares.
and the time to the first flare for patients of the TG and CG divided by TNFi. Most patients, after having a flare, reached the LDA at the end of the study (19 patients, 63%). Three out of 22 patients in the TG dropped out the therapy due to inefficacy and no patients in the CG with flare needed to discontinue the therapy (only 1 patient discontinued in the CG but it was due to an adverse event).

In the 22 patients under tapering strategy, more patients with flare were in the infliximab group [infliximab: 14/35 (40%); adalimumab: 2/17 (12%); etanercept: 6/22 (27%); p = 0.108]. In the TG, the nonbiologic DMARD were intensified in 1 patient and the NSAID were used at flare in 13 patients. Most patients in the TG with a flare who were treated with infliximab or adalimumab needed to increase the dose or shorten the interval of administration to regain control over the disease activity [infliximab: 13/14 (93%); adalimumab: 2/2 (100%); etanercept 2/6 (33.3%)]. The clinical activity at the worst registered flare in the TG was lower in the etanercept patients (inflliximab: 5.9 ± 1.2; adalimumab: 6.3 ± 0.4; etanercept: 4.7 ± 0.5; p = 0.028). In the CG, 7 patients with flares intensified NSAID and 1 patient started nonbiologic DMARD.

The incidence of antidrug antibodies appearance at the end of the study
Only 2 patients treated with infliximab in the TG were positive for ADA at pre-tapering (visit 1). Sixteen patients (14%) had detectable ADA at the end of our study, and the majority of these patients were in the TG [14/73 (19.2%) ADA-positive in the TG (11 with infliximab and 3 with adalimumab) vs 2/43 (4.7%) in the CG (all with infliximab), p = 0.028]. No ADA-positive patients could be detected in the group of patients with SpA treated with etanercept. ADA were detected in 6 out of the 30 patients (20%) with a flare (5 patients taking infliximab in TG and 1 patient taking adalimumab in CG), but only 2 patients under infliximab in the TG needed to drop the therapy because of secondary inefficacy. At the end of our study, no differences were observed in clinical activity (BASDAI) in patients who developed or not ADA at visit 4 in both groups (TG: 2.2 ± 1.6 in ADA-negative vs 2.0 ± 1.6 in ADA–positive, p = 0.659; CG: 2.1 ± 1.3 in ADA-negative vs 2.3 ± 0.2 in ADA–positive, p = 0.603).

The influence of the tapering on serum drug levels
A significant reduction in the drug levels was observed between visit 1 (pre-tapering) and visit 4 (at the end of our study) in the TG (Figure 3). Only 2 patients taking adalimumab and 7 patients taking etanercept did not have the drug levels available at visit 1.

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**Figure 2** Comparison of clinical activity (BASDAI) between in tapering and control groups in each TNFi. The clinical activity was measured by BASDAI (mean ± SD, represented in X-axis) in each TNFi at different time points during the study: visit 0 (prior starting TNFi), visit 1 (pre-tapering), visit 2 (6 months after visit 1), visit 3 (1 year after visit 1) and visit 4 (last visit available after visit 1).

**Figure 3** The influence of the tapering on serum drug levels. A significant reduction in the drug levels was observed between visit 1 (pre-tapering) and visit 4 (at the end of our study) in the TG (Figure 3). Only 2 patients taking adalimumab and 7 patients taking etanercept did not have the drug levels available at visit 1.

**Table 2** Comparison of flares between tapering and control group. The proportion of SpA patients with flares, number of flares between visit 1 and 4, and time to first flare in each TNFi are shown.

<table>
<thead>
<tr>
<th>SpA patients, n = 117</th>
<th>IFX</th>
<th>ADL</th>
<th>ADL</th>
<th>ETN</th>
<th>ETN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flares, n = 30 patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. patients with flares, n/N (%)</td>
<td>14/35 (40)</td>
<td>3/21 (14)</td>
<td>2/17 (12)</td>
<td>0.431</td>
<td>5/22 (23)</td>
</tr>
<tr>
<td>No. flares, mean ± SD</td>
<td>1.5 ± 0.7</td>
<td>1.4 ± 0.6</td>
<td>2 ± 1.4</td>
<td>0.519</td>
<td>1.2 ± 0.5</td>
</tr>
<tr>
<td>Time to appearance of 1st flare, yrs, mean ± SD</td>
<td>1.2 ± 0.5</td>
<td>0.9 ± 0.6</td>
<td>1 ± 0.1</td>
<td>1.000</td>
<td>1.6 ± 1.4</td>
</tr>
</tbody>
</table>

SpA: spondyloarthritis; TG: Tapering group; CG: Control group; IFX: infliximab; ADL: adalimumab; ETN: etanercept.
Predictors of a good clinical outcome to tapering strategy

In the tapering group, several demographic, clinical and serological factors were studied at baseline and at pre-tapering to predict which patients are more likely to present a flare during the tapering strategy (see Table 3). The male gender (OR: 3.5; 95% IC: 1.18-10.4) was the only predictive factor that demonstrated to be protective for having a flare (data shown in Table 3).

Reduction of the administered drug in the tapering group during the study

At the end of the study (visit 4), the SpA patients in the TG received a substantially lower amount of drug compared with the patients in the CG (infliximab dose was 4.40 ± 0.81 mg/kg; interval of administration for infliximab is 11.22 ± 1.80 weeks, for adalimumab is 3.74 ± 1.21 weeks and for etanercept is 2.09 ± 0.59 weeks).

Table 3 Predictive clinical baseline and pre-tapering factors predicting a flare during tapering strategy. Demographic, clinical, and serological characteristics were analyzed to predict a flare in SpA patients under tapering strategy by means of univariate logistic regression analysis at baseline and pre-tapering.

<table>
<thead>
<tr>
<th>Predictive Factor</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>At baseline</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male sex</td>
<td>3.50</td>
<td>1.18-10.4</td>
</tr>
<tr>
<td>Age</td>
<td>1.03</td>
<td>0.99-1.07</td>
</tr>
<tr>
<td>Disease duration</td>
<td>0.98</td>
<td>0.92-1.04</td>
</tr>
<tr>
<td>Naïve to biologicals</td>
<td>0.99</td>
<td>0.23-4.26</td>
</tr>
<tr>
<td>HLA-B27</td>
<td>0.31</td>
<td>0.05-2.1</td>
</tr>
<tr>
<td>Monotherapy</td>
<td>0.55</td>
<td>0.20-1.51</td>
</tr>
<tr>
<td><strong>At pre-tapering</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time in inactive disease</td>
<td>1.22</td>
<td>0.75-1.98</td>
</tr>
<tr>
<td>BASDAI</td>
<td>1.51</td>
<td>0.91-2.52</td>
</tr>
<tr>
<td>CRP levels</td>
<td>1.03</td>
<td>0.89-1.20</td>
</tr>
</tbody>
</table>

Overall, the reduction of the administered drug at visit 4 in the TG was 22% for infliximab, and the interval was extended to 28.7%. The dose reduction was 45.2% for adalimumab and 51.5% for etanercept. The majority of the patients in the tapering group continued with the tapering strategy at visit 4 [34/35 (97.1%) on infliximab; 16/17 (94.1%) on adalimumab; 19/22 (86.4%) on etanercept].

Figure 3 The decrease of serum trough drug levels in SpA patients under tapering strategy along the study. The drug levels (Mdn, IQR ng/ml) of the different TNFi were measured during the study at different time points in the tapering group: visit 1 (pre-tapering) and visit 4 (the last visit available after visit 1). Not all patients had the serum drug levels at visit-1 in adalimumab and etanercept.

### Table 3

<table>
<thead>
<tr>
<th>Predictive Factor</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>At baseline</strong></td>
<td></td>
<td></td>
</tr>
<tr>
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</tr>
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</tr>
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</tr>
<tr>
<td>Naïve to biologicals</td>
<td>0.99</td>
<td>0.23-4.26</td>
</tr>
<tr>
<td>HLA-B27</td>
<td>0.31</td>
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</tr>
<tr>
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<td>0.20-1.51</td>
</tr>
<tr>
<td><strong>At pre-tapering</strong></td>
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<td></td>
</tr>
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</tr>
<tr>
<td>CRP levels</td>
<td>1.03</td>
<td>0.89-1.20</td>
</tr>
</tbody>
</table>

BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; CRP: C-reactive protein; SpA: spondyloarthritis.
DISCUSSION

To our knowledge, this work is the first retrospective observational longitudinal followup study comparing the clinical and serological outcomes between patients with SpA using a tapering strategy versus a standard regimen in daily clinical practice. Although an important reduction in the administered drug was achieved in the TG (infliximab dose 22% and interval 28.7%; adalimumab interval 45.2%; etanercept interval 51.5%), the percentage of patients who maintained a BASDAI < 4 at the end of our study was similar in both groups. The development of flares was low during the study but the frequency was a little higher in patients under the tapering strategy, mainly in patients receiving infliximab.

The evidence regarding the discontinuation and dose titration of TNFi in patients with SpA is sparse and varied.1,2,4-6 Most of the studies that focused on TNFi discontinuation failed to demonstrate that this strategy resulted in good control of disease activity. These studies included heterogeneous populations, different outcome measurements, and variable followup periods, making it difficult to extrapolate the results to other patient populations.6,7 The evidence for dose-titration in patients with SpA is inconclusive.1,4 In patients with AS who were treated with etanercept, Cantini, et al observed that remission was possible in at least 50% of the patients and remission was maintained in the majority of patients after halving the dose.1 Similar results were seen in patients with AS treated with infliximab in cases in which the clinical improvement was sustained during the course of the study despite a reduced dose and longer infusion intervals.6 In our study, we showed that the clinical course in the TG was similar to that in the CG. These findings suggest that the tapering strategy is superior to discontinuation of the TNFi in patients with SpA who have LDA.

In considering a tapering strategy for patients with SpA with sustained LDA, one of the most important concerns of rheumatologists is the increased risk of flares and the inefficacy of TNFi after a flare. However, most publications regarding withdrawal of biological therapies in patients with SpA have shown that re-starting is safe and effective in most patients.1,4,5 Our data demonstrate that 26% of the patients developed a flare during the study. The number of patients with a flare was slightly higher in the TG, without significant differences. An important issue is that more than 60% of these patients had inactive disease at the end of the study; the dropout rate due to inefficacy was very low. No patients taking etanercept in our study dropped out after flaring; a probable explanation is that the median of clinical activity in flares was lower in these patients when comparing with infliximab or adalimumab. The data about therapeutic changes on biological and classic DMARD after flaring were collected. However, it was not possible to obtain proper data on the use of NSAID during flares because of the retrospective design of our study. Globally, these data reflect that even in a selected SpA cohort in LDA, flares are present during the followup in patients under tapering or standard therapy regimen, and tight clinical monitoring is needed to make therapeutic decisions as soon as possible to avoid undesirable outcomes.

In general, ADA detection was low in patients in our study (14%), but it should be noted that it was more frequent in patients on tapering strategy who were treated with infliximab. It is widely known that ADA detection is more frequent in patients with low drug levels.23 These results should be studied in a larger population to investigate whether dose tapering of TNFi results in more inefficacy (hence, more dropouts) because of ADA development. A study showed that patients with SpA who develop ADA–positivity to the first TNFi have a good clinical response after switching to a second TNFi.24 However, patients who developed ADA to the first TNFi were more prone to present with ADA to the second TNFi.25 Prior to our present report, there had been no evidence about what happens with drug levels and ADA appearance when a tapering strategy is carried out in patients with SpA in LDA. Our data show that patients under tapering strategy had a progressive decrease of drug levels after tapering and also presented more frequency of ADA, although the data are sparse. But these findings in our cohort are not associated with a higher incidence of flares or dropouts, indicating that, in some patients, the disease may be completely inactive and the drug may not be the main factor that influences this status. Currently, there are some doubts about whether drug and ADA measurements are useful in patients on a TNFi dose-tapering strategy.

Several studies of randomized clinical trials and registries have attempted to identify predictors of the responses to TNFi in patients with AS.27-31 Data from registries have shown that elevated inflammatory markers, a lower Bath Ankylosing Spondylitis Functional Index, and younger age at baseline were associated with better clinical responses. In a prospective observational cohort study in patients with AS treated with TNFi, these factors were observed to be independent baseline predictors of responses and/or continuation of TNFi:22 higher Ankylosing Spondylitis Disease Activity Score, higher ESR or CRP levels, the presence of peripheral arthritis, younger age, male sex, a lower modified Schöber test, and lower BASDAI. Predictive markers of having a flare after tapering strategy in patients with SpA have not been previously described. In our cohort, we found that male sex was a predictive factor to protect from flare when dose titration was made in patients with SpA in low disease activity.

Biological treatment is expensive; therefore, tapering strategies have important economic implications. In a study of patients with RA in which infliximab was down-titrated or discontinued, a mean cost reduction of 3474 euros (US $ 3883) per patient was observed during 1 year.33 From the results of our study, it is not possible to calculate the exact financial savings because of the differences in tapering strategies. However, an important reduction in the administered TNFi was reached, without relevant clinical changes, after tapering in patients with SpA. There were cost reductions, the patients were not overtreated, and they were less likely to develop potential adverse events or infections.

Our study had some limitations: the patients were from different countries, there was no control group for the patients treated with infliximab, the design was retrospective, and the number of patients was small. Although included patients were from different
countries, both cohorts were matched to ensure they were as homogeneous as possible. Because of the strict criteria in the selection period, many patients were excluded. One inconvenience of the study was not finding a control group for patients treated with infliximab, but this drug is not used much in the patients with SpA from the Netherlands, and after matching the few Dutch patients, it was impossible to find a homogeneous group for comparison. On the other hand, it was very useful to show what happened to patients taking infliximab when a tapering strategy was done, even if a control group was absent. Although the design was retrospective, these data reflect the type of patients that we usually find in daily clinical practice.

The tapering strategy in patients with SpA with low disease activity appears to be feasible, resulting in an important reduction of the administered drug; disease control remains similar to that of patients with SpA on the standard dosing regimen. The incidence of flares and ADA detection was low in both cohorts during our study, but a little higher in patients under the tapering strategy, indicating that a tight clinical and serological monitoring should be done in these patients to avoid unexpected clinical outcomes.

SUPPLEMENTARY MATERIAL CHAPTER 6

Flowchart describing patient selection and inclusion. BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; CRP: C-reactive protein.
REFERENCES


CHAPTER 8

DISCUSSION
AND SUMMARY
The benefits of biological therapeutics for patients with chronic inflammatory rheumatic diseases are evident, nevertheless, a substantial proportion of patients does not respond sufficiently to biological treatment, or loses its initial clinical response; in addition, the increasing number of patients receiving biological treatment has resulted in a huge financial burden for society. Therefore, optimizing biological treatment is currently an important and broadly studied topic in rheumatology and beyond, e.g. dermatology, gastroenterology, neurology and oncology.

An approach to explore opportunities to optimize biological treatment is studying pharmacokinetics and pharmacodynamics. In general, these studies showed that a standard dose results in a wide variety of serum trough levels, which are often associated with clinical response. Therefore, differences in pharmacokinetic factors between patients and within a patient are clinically relevant, suggesting a personalized dosing scheme is more rational as compared to a standard dose for all patients. This personalized dosing scheme should include a dose-to-target strategy, because, biological therapeutics are molecular targeted therapies.

We hypothesized that drug levels within the therapeutic window for effective target blockade are optimal; higher levels are unnecessarily and lower drug levels are suboptimal. Based on this hypothesis, therapeutic drug monitoring (TDM) can help to identify the cause of insufficient clinical response and over treatment. First, patients with insufficient clinical response with undetectable serum trough levels probably have incomplete target blockade, while patients with detectable serum trough levels might benefit more of a biological with another mode of action. Obtaining detectable serum trough levels depends on dosing, compliance and pharmacokinetic factors like immunogenicity. Second, serum trough levels above the threshold needed for effective target blockade will only contribute to unnecessarily high costs and in those patients the dose can be tapered, probably independent of disease activity.

The aim of this thesis was to investigate the relationship between serum drug level, clinical outcome and the effect of pharmacokinetic factors on drug level for several biologicals and/or diseases for which limited or no data was available at start of this thesis. Hence, golimumab and tocilizumab for rheumatoid arthritis (RA), adalimumab and etanercept for ankylosing spondylitis (AS) and adalimumab in psoriatic arthritis (PsA). Moreover, two additional studies were conducted; tapering of tumor necrosis factor (TNF)-inhibitors in spondyloarthritis (SpA) patients and the feasibility of a dried blood spot on material obtained via a finger prick for the measurement of adalimumab drug levels and assessment of immunogenicity.

In Chapter 2 the relationship between serum trough levels of adalimumab and clinical outcome was studied in patients with AS and PsA. Previous studies have shown that a lower number of patients with detectable anti-drug antibodies (ADA) against adalimumab or infliximab were detected in RA patients using concomitant...
Concomitant methotrexate is used less frequently in PsA and rarely in AS.10,18,19 Therefore, we expected to find a higher amount of patients with detectable ADA in the AS cohort.

The number of patients with ADA against adalimumab detectable with an antigen-binding test (ABT) was 27% at week 24 (AS), 22% at week 52 (PsA) and approximately 21% at week 52 of treatment (RA).6 Concomitant methotrexate use at baseline in the AS, PsA and RA cohort was, respectively, 4%, 78% and 74%. Therefore, it seems likely that some AS patients could benefit from concomitant methotrexate to optimize biological treatment. Nevertheless, the effect of other factors influencing formation and detectability of immunogenicity cannot be excluded,22,23 because this was not a head-to-head comparison.

Preliminary results suggest that other immunosuppressant therapeutics (e.g. sulfasalazine) are also associated with a lower number of patients with detectable ADA against adalimumab or infliximab, and detection of higher adalimumab serum trough levels, although, the impact may be less than for concomitant methotrexate use.24,25 The potential beneficial effect of other immunosuppressant medication has been described for other chronic inflammatory diseases, like Crohn’s Disease (CD), too.26-28 This provides opportunities to optimize biological treatment in AS patients and patients who do not tolerate methotrexate.

The mechanism thereof has not been identified yet. Possibly, concomitant immunosuppressant therapeutics have a synergic effect on inflammation, hereby reducing TNF which results in higher detectable functional serum trough levels of the therapeutic antibody. However, higher drug levels might also interfere with ADA detection resulting in a higher risk of false negative results and this can complicate interpretation of the data29,30 Another explanation is that these co-medications have a direct effect on formation of ADA resulting in higher functional drug levels of the therapeutic antibody.1 For methotrexate it has been suggested that suppression of early T-cell and B-cell expansion might be responsible for the modulation of the immune response, thereby the formation of ADA is reduced.31

Reducing the immunogenic risk is important, especially, if options in biological treatment are limited and/or consequences of suboptimal treatment are severe (e.g. as in IBD, multiple sclerosis (MS)). In addition, if the use of concomitant immunosuppressant agents increases serum levels of biologics, this will enable a greater dose reduction of the biological and a dose reduction in a larger proportion of patients, which will evidently contribute to higher savings.

In Chapter 3 the relationship between serum trough level of etanercept on clinical outcome was studied in patients with AS. Low serum etanercept trough levels are associated with poorer clinical outcome, however, etanercept is only marginally immunogenic.7,34,35 Nevertheless, under treatment and over treatment are also found in patients treated with etanercept. Gender, concomitant methotrexate use, body weight and glomerular filtration rate (GFR) were identified as possible pharmacokinetic factors.7 Other potential important pharmacokinetic factors for biological treatment should be studied.36 In addition, compliance to therapy and variations in time point of sampling might have been of influence too, because etanercept (and most other biologicals) is an at-home administered drug.

Patients with low serum etanercept trough levels and insufficient clinical response might benefit of a dose increase; however, in rheumatology this is not recommended due to the high costs associated with a dose increase,37 but for other diseases this is optional (e.g. inflammatory bowel disease (IBD) and psoriasis).46,47 Dose adaptions can be made according to algorithms in which treatment decisions are often based on immunogenicity and, in case the therapeutic window is known, on drug levels.38-47 Nevertheless, TDM and assessing immunogenicity during biological treatment have a limited position in current clinical guidelines due to a lack of randomized controlled trials (RCTs) and meta-analyses.37,48,49

In Chapter 4 the relationship between serum golimumab trough level on clinical outcome was studied in patients with RA. Golimumab is dosed 50 mg subcutaneously once a month and adalimumab is dosed 40 mg once every two weeks, therefore, we expected that ADA might be detected more frequently in golimumab treated patients. This assumption was based on the previous research which has shown that higher dosing is associated with less detection of immunogenicity.50 This knowledge is used in hemophilia to induce immune tolerance via a high dosing strategy of factor VIII treatment, but unfortunately results are poor.51-54 Nevertheless, the number of patients with detectable ADA against golimumab was limited; possibly, golimumab is less immunogenic due to differences in characteristics and production process.23 In addition, the influence of other factors cannot be excluded,22,23 like a difference in sensitivity for drug interference between both assays,6,29,30 because this was not a head-to-head comparison and the number of patients included in the golimumab study was limited.

The relationship between variations in dosing, risk of an immunogenic response and immune tolerance is an interesting topic. Patients with a transient immunogenic response were reported in chapter 2, in a study of RA patients treated with adalimumab55,56 and is supported by data of natalizumab and infliximab.57-61 The mechanism behind the development of immune tolerance is currently unknown, but it seems to be a state of immune unresponsiveness specific to a particular antigen induced by previous exposure to that antigen. For therapeutic antibodies it has been shown that ADA originated from different naïve B-cells and underwent extensive hypermutation; resulting in ADA (IgG) with high avidity for the idiotype of the biological therapeutic62,63 For this maturation process, activation of T- and B-cells is required.64,65 In contrast, if higher dose is associated with reduced risk of immunogenicity, a dose reduction and/or interval prolongation might be associated with a higher risk of evoking an immunogenic response. This is an important topic, because dose reduction of a biologicals is currently broadly studied and this topic will be further discussed during the summary of chapter 6.64,65

In Chapter 5 the relationship between serum tocilizumab trough level and clinical outcome was studied in patients with RA. We expected to identify immunogenicity
as main pharmacokinetic factor of clinical relevance, like was found for TNF-inhibitors. 
Interestingly, undetectable serum tocilizumab trough levels could not be explained by immunogenicity. This suggest that another pharmacokinetic factor plays a more important role, which is probably target-binding and this is discussed below.

A serum tocilizumab trough level above 1 mg/L is sufficient for systemic blockade of the IL-6 pathway. Firstly, because it is sufficient to normalize C-reactive protein (CRP), which is produced by the liver and can be considered as a surrogate marker for membrane bound IL-6 receptor saturation. Secondly, this drug level is sufficient to bind more than 95% of systemically present soluble IL-6 receptor. Nonetheless, it might be that bioavailability of tocilizumab is slightly different in target tissue as compared to the systemic compartment but, to our knowledge, data regarding this issue is lacking. In IBD, one preliminary study has investigated the correlation between serum and tissue TNF-inhibitor drug level and endoscopic remission to identify the ‘therapeutic tissue drug level’. Overall correlation between serum drug level and endoscopic remission was good, but some patients with active IBD had a higher ‘serum to tissue anti-TNF mismatch’ suggesting serum drug levels are not always a good predictor for local bioavailability. This could be explained by increased local target load, or possibly, delay in saturation of target cells as was seen in our tocilizumab study. Nevertheless, these data cannot be extrapolated to chronic inflammatory rheumatic diseases, because in active IBD there will be an increased protein clearance, including losses in the stool.  

Our study shows that some patients have undetectable serum tocilizumab trough levels despite receiving standard dose, which suggests that they have more target load (i.e. IL-6 receptors) than others, and therefore, might benefit from a higher tocilizumab dose at start of treatment to saturate all IL-6 receptors. In addition, most of these patients obtain sufficient levels over time, suggesting that some process of receptor down-regulation occurs. Nevertheless, little is known about variations in target between patients (e.g. polymorphism of the IL-6 receptor) or up and down regulation of IL-6 receptors during disease course and treatment.

Due to the direct relationship between the IL-6 pathway and CRP, tocilizumab is ideally suited to investigate the therapeutic window for effective target pathway blockade. Currently, optimal ranges are known for adalimumab (RA, PsA and psoriasis) and infliximab (IBD), but these are often based on conventional measurements of disease activity. Only for IBD preliminary results show that higher serum anti-TNF levels are associated with endoscopic remissions, but the therapeutic window for endoscopic remission has not been studied.

Conventional measurements to assess disease activity in chronic inflammatory rheumatic diseases might not always be a good representation of amount of target blockade. These measurements (e.g. disease activity score (DAS) or Bath AS Disease Activity Index (BASDAI)) represent several domains, like active inflammation, clinical symptoms and patient perception on well-being and quality of life. These composite measurements are suitable to assess the overall treatment goal, but not to discriminate between the role of the target molecule versus other factors, like the influence of other inflammatory mediators, the contribution of non-inflammatory domains or potential misdiagnosis. To optimize biological treatment in a rational, personalized and cost-effective manner it will be important to approach this kind of treatment as molecular targeted therapy, thus, dosing guided by amount of target inhibition. If, target is not measurable in a valid manner, a surrogate marker could be used, like CRP as surrogate marker for systemic IL-6 pathway inhibition or CD86 for monitoring belatacept treatment in adult kidney transplant recipients. In the absence of suitable surrogate markers, TDM can add value to clinical measurements alone to optimize treatment, because detectability of functional drug levels provide additional information with regard to inhibition of the target pathway, not only in case of under treatment but also with regard to over treatment.

Currently, only a few tapering studies of biologicals included drug level measurements and assessment of immunogenicity, and for IBD even a RCT of TDM guided tapering of infliximab is available. In chapter 6 we studied the effect of dose tapering of TNF-inhibitors. As expected dose reduction and/or interval prolongation results in a significant reduction of serum trough levels; which is sufficient in most patients to maintain a state of low or minimal disease activity. In case of a flare, reintroduction of the prior dose is often sufficient to gain control over disease activity again. However, in rare cases clinical inefficacy is accompanied by detectable ADA titres in patients in whom ADA were not detected prior to the dose adaptation. However, contradictory results for RA regarding the risk of immunogenicity have been reported. Assessing immunogenicity is influenced by drug interference and, thus, factors like, dose reduction or discontinuation or not sampling at trough level will influence the results, therefore, conclusions should be made with caution.

In chapter 7 a new method to obtain material for drug level measurements and assessment of immunogenicity was investigated. Currently, all data on TDM and immunogenicity of biological treatment is obtained by venipuncture, which requires a visit to the hospital. Development of a dried blood spot (DBS) obtained via an at home performed finger prick will enable self-sampling, with the results ready for immediate decision-making at consultation of the rheumatologist. Moreover, self-sampling is easy and minimally invasive, only a small volume is required, and is convenient for storage and transportation. Therefore, development of a self-sampling method for TDM of biologicals will be an important step for gaining more pharmacokinetic knowledge and for implementation of TDM of biologicals.

Our study shows promising results, with a good correlation between adalimumab and ADA levels measured in serum obtained by venipuncture compared with the measurements obtained by finger prick. Precision and accuracy are within acceptable limits as described by Food and Drug Administration (FDA) and European Medicines Agency (EMEA) guidelines. For further development it will be necessarily to investigate, if material collected by the patient at home will be of sufficient quality to
measure adalimumab and ADA levels with the DBS method. Moreover, the possibility of using a DBS obtained by finger prick to measure drug levels of other biological therapeutics should be studied as well, for example, for adalimumab a correction factor was needed because the proportion of IgG was lower in the material obtained by finger prick compared to serum.

Concluding remarks and future research

Most of the data used in this thesis was obtained from prospective observational cohort studies investigating long-term efficacy and safety of biological treatment in patients with chronic inflammatory rheumatic diseases. Prior to start of this thesis some of the data of these cohorts regarding drug level measurements and immunogenicity has been published, especially for patients with RA. This thesis is a continuation of that work, and represents a dynamic process characterized by an evolving insight and emerging of new research questions. In short, focus of interest has shifted from immunogenicity as a possible explanation for insufficient clinical response, to drug level in relationship with clinical outcome, to biologicals as molecular targeting therapies which should monitored accordingly.

Although, the clinical impact of immunogenicity is mostly determined by the remaining functional drug level, it remains important to characterize the immunogenic response. Several initiatives are currently being conducted, like the Anti-Biopharmaceutical Immunization: prediction and analysis of clinical relevance to minimize the RISK (ABIRISK)\textsuperscript{90} and the Impact of immunogenicity on anti-TNF response after switch (INTENT) study (EudraCT Number: 2015-002284-42).\textsuperscript{87}

With the development of more accurate drug tolerant assays\textsuperscript{55,56,92} it will be interesting to investigate the characteristics in patients who develop a transient immunogenic response versus patients who develop an extensive immunogenic response. For the latter, some risk factors have been identified,\textsuperscript{1,6,35} but, especially the genetic susceptibility remains largely unkown.\textsuperscript{93-96}

Before TDM of a biologic therapeutic can be prospectively studied, the therapeutic window should be identified, as was done for adalimumab (RA, PsA and psoriasis) and infliximab. Nevertheless, in the absence of reliable methods to measure target levels, or in the absence of surrogate markers thereof, these optimal ranges can only be based on conventional measurements of disease activity. The balance between dosing of a biologic, clinically relevant pharmacokinetic factors, therapeutic window, target molecule inhibition and regulation of receptors of the target molecule is a very interesting and unknown area to explore. A better insight in that process can help to understand the mechanism of action of biologicals, and thereby contribute to a more rational and cost-effective treatment strategy of biologicals with regard to starting, switching and initiating dose adaptations of biologicals. At the moment, an assay to measure TNF complexes is in development as a part of the activities of the MOlecular Diagnostics in Rheumatoid Arthritis (MODIRA) consortium.\textsuperscript{97}

The tapering study conducted in this thesis was not TDM guided, but the data of the adalimumab concentration-effect curves as discussed in the introduction and the tocilizumab data show that the number of patients eligible for dose reduction of a biological based on TDM versus clinical outcome measurements might be slightly different. Currently, a RCT TDM guided dose reduction of adalimumab in RA study is being conducted (NTR3509).\textsuperscript{98} Patients with high serum adalimumab trough levels (>8 mg/L), independently of DAS28, will be randomly assigned to continuation of adalimumab every other week or prolongation of the dosage interval to once every 3 weeks. At the end of the study the ΔDAS28 between both groups will be compared and cost-effectiveness of the TDM guided dose reduction will be assessed.

The above mentioned RCT is based on the therapeutic window of adalimumab as assessed in the concentration effect curve.\textsuperscript{91,80,81} However, the efficacious dose of a biological therapeutic is probably highly individual; for example, serum adalimumab range of 5-8 mg/L applies to RA patients with active disease in general. For patients with sustained low or minimal disease activity or remission the overall range is probably lower. However, in individual cases the optimal range may be higher or lower depending on the amount of TNF and TNF-receptors present in the system. Therefore, tapering possibilities do not depend on drug level itself, but on excess of anti-TNF compared to target load and its pathway. If, both could be measured reliably, this might be an opportunity to predict the chance of successful dose tapering in patients treated with biological therapy. To date, such a predictor has not been identified.

The therapeutic window of etanercept has not been identified yet, therefore, a TDM guided dose reduction is not possible. Currently, a RCT is being conducted in adult patients with chronic inflammatory rheumatic diseases and juvenile idiopathic arthritis (JIA), during which serum samples are collected at all time points (NTR3903/NTR4634).\textsuperscript{98} Possibly, this study will provide more insight in pharmacokinetics of etanercept, therapeutic window and TDM guided taper possibilities of etanercept.

A tapering study of tocilizumab will be interesting to study the hypothesis that TDM guided tapering based on target blockade is a more rational and cost-effective strategy compared to clinical measurements alone, ideally, this should be a double blind RCT. This study should include assessment of local bioavailability, several mediators of inflammation and potential surrogate markers as well as polymorphisms of IL-6 receptor and markers for bone destruction; however, large patients number will be needed for which multi-center collaboration is required.
Conclusion

Considering the differences in clinically relevant pharmacokinetic factors, between patients and within a patient, a personalized dosing scheme of biological therapeutics is more rational compared with a standard dose for all. Biologicals are unique therapeutics, molecular targeting therapies, which require a new and innovative approach of dosing and monitoring based on target load. In the absence of opportunities to measure target itself or availability of good surrogate marker, TDM can provide important additional information regarding target pathway blockade compared with clinical measurements alone. A better understanding of the dynamics between dosing, pharmacokinetic and pharmacodynamics factors, and especially, drug level relative to target amount will contribute to develop a more rational and cost-effective treatment strategy of biologicals to start, switch and initiate biologicals or to taper the dose of a biological.

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Reumatoïde artritis, artritis psoriatica en ankyloserende spondylitis (ziekte van Bechterew) zijn chronische inflammatoire ziekten die gekenmerkt worden door een abnormale reactie van het afweersysteem. Dit kan resulteren in een scala aan symptomen, afhankelijk van het type ziektebeeld, waaronder zwelling en pijn aan de gewrichten, omringende pezen en de wervelkolom, en afwijkingen aan de huid. De ontstekingen kunnen op termijn ook het botweefsel onherstelbaar beschadigen.

De abnormale reactie van het afweersysteem vormt het aangrijpingspunt van de behandeling via ontstekingsremmende medicijnen, die eventueel in combinatie met pijnstillende medicijnen gegeven worden. Inmiddels zijn er verschillende groepen van ontstekingsremmende medicijnen beschikbaar en dit proefschrift focust op de groep van biologicals. De patiënten die biologicals krijgen voorgeschreven hebben vaak eerder onvoldoende gereageerd op andere ontstekingsremmende medicijnen of konden deze door bijwerkingen niet verdragen.

Het afweersysteem bestaat uit verschillende moleculen, cellen en weefsels met een remmende of stimulerende werking op de afweerreactie. Er zijn verschillende typen biologicals beschikbaar die zich onder andere onderscheiden doordat ze een andere doelcel (target) hebben of op een andere manier deze blokkeren. De meeste ervaring is inmiddels opgedaan met biologicals die de werking van tumor necrosis factor (TNF) blokkeren. Bij onvoldoende effect van deze zogeheten TNF-blokkers kan geswitchd worden naar een biological met een ander target.

Biologicals verminderen de klachten aanzienlijk of geheel in een groot deel van de patiënten, maar bij ongeveer 40% is het effect onvoldoende en blijft de chronische ontsteking (deels) bestaan, met alle negatieve gevolgen van dien. Daarnaast is het chronisch gebruik van biologicals ook kostbaar: ongeveer 14.000 euro per patiënt per jaar (www.medicijnkosten.nl). Het kosteneffectief optimaliseren van de behandeling met biologicals (reduceren van onder- en overbehandeling) is daarmee een belangrijk onderwerp in de klinische praktijk en in de wetenschap, zowel binnen als buiten de reumatologie.

Biologicals worden voorgeschreven in een standaarddosering, dat wil zeggen, iedere volwassen patiënt krijgt dezelfde dosis ongeacht potentiële farmacokinetische verschillen (met uitzondering van het gewicht van de patiënt bij per infuus toegediende biologicals). Eerder onderzoek heeft aangetoond dat met deze standaarddosering de medicijnspiegel in het bloed, gemeten net voordat de volgende injectie wordt toegediend (dalspiegel), sterk varieert tussen mensen. Dit suggereert dat een individuele dosering rationeler is en dat dit onder- of overbehandeling kan reduceren. Het identificeren van de optimale dosis per patiënt gebeurt momenteel aan de hand van klinische uitkomstmaten, wat redelijk goed gaat, maar het meten van medicijnspiegels zou van toegevoegde waarde kunnen zijn.
De doelstelling van dit proefschrift is om de relatie te onderzoeken tussen de medicijンspiegel van enkele biologicals, de farmacokinetiek en het klinische effect in verschillende patiëntengroepen, waarvoor bij start van het onderzoek weinig tot geen literatuur beschikbaar was (hoofdstuk 2: adalimumab en artritis psoriatica en de ziekte van Bechterew; hoofdstuk 3: etanercept en de ziekte van Bechterew; hoofdstukken 4 en 5: golimumab en tocilizumab en reumatoïde artritis). Daarnaast werden nog twee andere onderzoeken beschreven: een afbouwstudie van TNF-blokkers (hoofdstuk 6) en een vingerprikeltest om de adalimumab-medicijンspiegel te meten (hoofdstuk 7).

In hoofdstuk 2 werd de relatie onderzocht tussen de medicijンspiegel van adalimumab (een TNF-blokker) en het effect op de ziekteactiviteit in patiënten met de ziekte van Bechterew of artritis psoriatica, met in het bijzonder aandacht voor de ontwikkeling van antistoffen tegen adalimumab. Biologicals zijn eiwitstructuren die als lichaamsvreemd herkend kunnen worden door het afweersysteem waardoor antistoffen aangemaakt worden tegen het medicijn, wat een effectieve werking van het medicijn belemmert. Dit fenomeen wordt immunogeniciteit genoemd.

In 27% en 22% van de patiënten met respectievelijk de ziekte van Bechterew en artritis psoriatica waren antistoffen tegen adalimumab in het serum aanwezig. Dit percentage was vooraf voor patiënten met de ziekte van Bechterew hoger dan eerder gevonden in een groep patiënten met reumatoïde artritis, waarschijnlijk omdat patiënten met de ziekte van Bechterew minder vaak behandeld worden met andere afweerverlaginge medicijnen zoals methotrexaat. Over methotrexaat en enkele andere afweerverlaginge medicijnen is bekend dat ze het risico op immunogeniciteit verlagen en de medicijンspiegel van de biologicals kunnen verhogen. Het gebruik van biologicals in combinatie met andere afweerverlaginge medicijnen is daarmee een manier om de behandeling met biologicals te optimaliseren.

In hoofdstuk 3 werd de relatie onderzocht tussen de medicijンspiegel van etanercept (een TNF-blokker) en het effect op de ziekteactiviteit in patiënten met de ziekte van Bechterew. In deze studie werden gevonden dat patiënten met een lagere etanercept medicijンspiegel vaker een hogere ziekteactiviteit behielden na start van de behandeling. Door de structuur van etanercept is immunogeniciteit een onwaarschijnlijke verklaring voor de gevonden variatie in medicijンspiegel. Welke andere factoren het verschil in medicijンspiegel kunnen verklaren is grotendeels onbekend.

De verschillen in medicijンspiegel tonen aan dat een individuele dosis waarschijnlijk tot een optimale behandeling met etanercept leidt.

In hoofdstuk 4 werd de relatie onderzocht tussen de medicijンspiegel van golimumab (een TNF-blokker) en het effect op de ziekteactiviteit in patiënten met reumatoïde artritis. Tijdens de behandeling met deze TNF-blokker werd eveneens tussen patiënten een grote variatie in medicijンspiegel gevonden, maar het aantal patiënten met detecteerbare antistoffen tegen golimumab was beperkt. Dit kan komen door de gebruikte testmethode (assay), want de meeste assays kunnen alleen antistoffen tegen een biological detecteren in afwezigheid van medicijn in het bloed (drug interference). Andere verklaringen kunnen zijn: de beperkte onderzoekspopulatie of een minder immunogenestructuur van golimumab. Ook een verschil in dosis kan effect hebben op de immunogeniciteit. Bestuderen van factoren die de immunogeniciteit beïnvloeden kan belangrijke aanknopingspunten bieden om de behandeling op individueel niveau te optimaliseren.

In hoofdstuk 5 werd de relatie onderzocht tussen de medicijンspiegel van tocilizumab en het effect op de ziekteactiviteit in patiënten met reumatoïde artritis. Tocilizumab is een interleukine-6 (IL-6) receptor (IL-6R) blokker. Net als bij de TNF-blokkers werd ook bij tocilizumab een grote variatie in medicijンspiegels gevonden, maar dit kon niet verklaard worden door immunogeniciteit, zoals bij de meeste TNF-blokkers. De medicijンspiegel van tocilizumab lijkt veel meer beïnvloed te worden door target-binding (de IL-6R): hoe meer target er in het lichaam aanwezig is, hoe minder medicijn in het bloed gedetecteerd kan worden. Tocilizumab is een interessante biological vanwege de directe relatie tussen IL-6 en c-reactive protein (CRP). De resultaten van dit onderzoek gaven nieuwe inzichten ten aanzien van de balans tussen dosis, farmacokinetiek, target en target doelcellen (de receptor).

Als de medicijンspiegel door verschillende farmacokinetische factoren in het lichaam, zoals immunogeniciteit en/of target-binding, te laag wordt om alle doelcellen te neutraliseren dan, wordt de ontstekingscascade onvoldoende geremd en blijven ontstekingsymptomen bestaan (overbehandelde). Daarom is de optimale dosis van een biological de dosering waarmee in de individuele patiënt een medicijンspiegel wordt behaald die hoog genoeg is om al het target te binden. Een dosering die boven die medicijンspiegeldrempel uitstijgt, levert waarschijnlijk geen extra klinische verbetering meer op (overbehandeling). Tussen onder- en overbehandelen ligt het optimale terapeutische medicijンspiegeldrempel (range).

De hoeveelheid target in het lichaam van de individuele patiënt is nu nog niet (betrouwbaar) te meten. Daarom kan de terapeutische medicijンspiegeldrempel alleen bepaald en gemonitord worden door middel van bestaande meetinstrumenten die de ziekteactiviteit objectiveren (zoals de disease activity score, DAS, of bath ankylosing spondylitis disease activity index, BASDAI). Deze conventionele meetinstrumenten bestaan uit meerdere componenten die de verschillende domeinen van de ziekte vertegenwoordigen(rond andere ontsteking, pijn, algemeen welbevinden) en gezamenlijk één maat geven voor de gehele ziekteactiviteit. Daarmee zijn deze meetinstrumenten ook zeer geschikt om de totale behandeling te monitoren, maar een ontlegeling van het afweersysteem kan door veel meer moleculen, cellen of weefscellen veroorzaakt worden dan alleen het target van de biological die toegediend wordt. Daarnaast wordt de ervaren ziekteactiviteit ook door meer factoren bepaald dan alleen de ontsteking. Met andere woorden, de hoeveelheid target in de individuele patiënt laat zich waarschijnlijk niet altijd één-op-één vertalen naar een verandering in ziekteactiviteit zoals gemeten met conventionele meetinstrumenten. Daarom zijn alternatieve methoden nodig om het effect van een biological op moleculair niveau te monitoren en om de behandeling op een rationele en kosteneffectieve wijze te optimaliseren. In de afwezigheid van meetbare target, of
een surrogaatmarker daarvan, is een medicijnspiegel een geschikt alternatief omdat het
additionele informatie geeft over effectieve versus ineffectieve targetblokkade. Een ruim
meetbare medicijnspiegel geeft aan dat na binding van al het target er nog vrij medi-
cijn in het bloed over is. Als er desondanks persisteerende ontstekingssymptomen zijn,
dan zijn hiervoor waarschijnlijk één of meerdere andere onderdelen van het afweersys-
team verantwoordelijk. Anderzijds, als er geen medicijnspiegel meetbaar is in combi-
natie met persisteerende ontstekingssymptomen, dan kan het zijn dat de dosering voor
deze patiënt onvoldoende is om al het target effectief te binden, bijvoorbeeld doordat er
sprake is van immunogeniciteit of zeer hoge targetlevels. Het meten van de medicijn-
spiegel kan daarom in bepaalde gevallen bijdragen aan het optimaliseren van de biolo-
gicalbehandeling.

In hoofdstuk 6 werd onderzocht of een lagere dosering dan de standaarddosering
van een TNF-blokker mogelijk is in sommige patiënten, met behoud van en lage ziek-
teactiviteit. Minder frequent toedienen van het medicijn is voor de patiënt prettiger en
bespaart ook veel geld, maar of het ook tot lagere langetermijnrisico’s leidt is moment-
teel onbekend. Ons onderzoek laat zien dat in een aanzienlijk aantal patiënten met
langdurige lage ziekteactiviteit de dosering van de TNF-blokker verlaagd kan worden.
In het geval er toch een substantiële toename van ziekteactiviteit (flare) optreedt na
dosisreductie, dan is terugkeren naar de voorgaande dosis meestal voldoende. Echter,
het is onbekend of een dosisreductie van sommige biologicals het risico op immuno-
geticiteit verhoogt. Een immunogene reactie kan de werking van de biological vermin-
deren en daarom is het belangrijk dit fenomeen te bestuderen in afbouwstudies. Onze
studie laat zien dat het optreden van detecteerbare antistoffen na dosisreductie zeld-
zaam is.

In hoofdstuk 7 werd een nieuwe methode onderzocht om de medicijnspiegel van
adalimumab en de spiegel van antistoffen tegen adalimumab te meten. Momenteel is
het noodzakelijk dat patiënten voor bloedafname naar het ziekenhuis komen, bij voor-
keur op dalspiegelmoment (vanwege drug interference). Dit vormt in de dagelijkse klini-
sche praktijk een obstakel om medicijnspiegels te bepalen, daarnaast belemmert het
ook de mogelijkheid om mid-intervalspiegels te meten om meer kennis over de farmaco-
kinetiek te verzamelen. In hoofdstuk 7 wordt een methode beschreven om bloed te verz-
melen via een vingerprik. De studie laat zien dat de medicijnspiegel van adalimumab en
van antistoffen tegen adalimumab in het bloed goed meetbaar zijn via de vingerprik in
vergelijking met de conventionele methode van veneuze bloedafname. Deze methode is
patiëntvriendelijker en biedt een oplossing voor bestaande belemmeringen bij het meten
van medicijnspiegels van biologicals in de dagelijkse praktijk.

Concluderende opmerkingen en aanbevelingen voor toekomstig onderzoek

Voor het optimaliseren van de behandeling met biologicals is het van belang om
immunogeniciteit beter te begrijpen, vooral indien van immune tolerance, dit is
het fenomeen dat het immuunsysteem niet meer op de biological reageert alsof
het een lichaamsverweer is. Het identificeren van factoren die het risico op
immunogeniciteit verhogen of verlagen (onder andere genetische variaties). Verschillende
initiatieven hiervoor zijn al gestart, zoals het ontwikkelen van drugtolerante assays
(methode om antistoffen tegen een biological te meten in de aanwezigheid van medicijn
in het bloed) en studies zoals Anti-Biopharmaceutical Immunization: prediction and
analysis of clinical relevance to minimize the RISK (ABIRISK) (www.abirisk.eu) en Impact
of immunogenicity on anti-TNF response after switch (INTENT) (EudraCT Number: 2015-002284-42).

Daarnaast is het voor het optimaliseren van de behandeling met biologicals van
belang om het therapeutische medicijnspiegelbereik per biological, per ziektebeeld en
per ziektefase te identificeren. Bij voorkeur met behulp van het target zelf of een
geschikte surrogaatmarker daarvan, maar in afwezigheid van beide zal dit gedaan
moeten worden op basis van de conventionele meetinstrumenten die de ziekteactiv-
itieit objectiveren. Het verder exploreren van de balans tussen dossieres, farmacokine-
tiek, therapeutische medicijnspiegelbereik, targetblokkade en de interactie tussen
target en receptor is een zeer interessant. De kennis over deze balans zal bijdragen
aan een beter begrip over de werking van dit soort medicijnen en een meer rationele
en kosteneffectieve manier van behandelen ondersteunen. Momenteel is er een assay
in ontwikkeling om TNF in complex te meten, als onderdeel van het MOlecular Diagnos-
tics in Rheumatoid Arthritis (MODIRA) consortium.

Het identificeren van het therapeutische medicijnspiegelbereik is een belangrijke
stap voor het opstellen en uitvoeren van prospectieve, TDM-gestuurde randomized
controle trials (RCT’s), zoals momenteel voor adalimumab gedaan wordt (NTR3509).
Het identificeren van de range is noodzakelijk voor het (routinematig) meten van
medicijnspiegels om dosisaanpassingen te sturen in de dagelijkse praktijk. Maar
uiteindelijk is zo’n range een gemiddelde en wordt de individuele range bepaald door
de hoeveelheid target dat op een bepaald moment in het lichaam van de patiënt aan-
wezig is. De kans op succesvolle dosisreductie lijkt daarom vooral afhankelijk te zijn
van de verhouding tussen medicijnspiegel en hoeveelheid target in het lichaam van de
individuele patiënt.

In conclusie, gezien de klinisch relevante verschillen in farmacokinetiek tussen
patiënten, kan geconcludeerd worden dat een individuele dosering rationeler is dan
een standaarddosering. Biologicals behoren tot een bijzondere medicijngroep die een
unieke binding aangaan met een specifiek target en op die manier de werking ervan
blokkeren. Dit vraagt om een nieuwe en innovatieve manier van doseren en monito-
ren, namelijk een manier die gebaseerd op de verhouding tussen de medicijnspiegel

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Nederlandse samenvatting
DANKWOORD

Vele mensen hebben direct of indirect bijgedragen aan de totstandkoming van dit proefschrift en hiervoor wil ik al deze mensen bedanken! Ondanks dat ik mij realiseer dat ik iemand vergeet, wil ik een aantal mensen in het bijzonder noemen.

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**LIST OF PUBLICATIONS**

Serum tocilizumab trough concentration can be used to monitor systemic IL-6 receptor blockade in patients with rheumatoid arthritis: a prospective observational cohort study.

**Kneepkens EL**, van den Oever I, Plasencia CH, Pascual-Salcedo D, de Vries A, Hart M, Nurmohamed MT, Balsa A, Rispen T, Wolbink G.


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J Rheumatol. 2015 Sep;42(9):1638-46.
Adalimumab trough concentrations in patients with rheumatoid arthritis and psoriatic arthritis treated with concomitant disease-modifying antirheumatic drugs.

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Lower etanercept levels are associated with high disease activity in ankylosing spondylitis patients at 24 weeks of follow-up.

Kneepkens EL, Krieckaert CL, van der Kleij D, Nurmohamed MT, van der Horst-Bruinsma IE, Rispens T, Wolbink GJ.

Immunogenicity, adalimumab levels and clinical response in ankylosing spondylitis patients during 24 weeks of follow-up.

Kneepkens EL, Wei JC, Nurmohamed MT, Yeo KJ, Chen CY, van der Horst-Bruinsma IE, van der Kleij D, Rispens T, Wolbink G, Krieckaert CL.

CURRICULUM VITAE

The availability of biologicals has improved the prognosis of chronic rheumatic inflammatory diseases considerably; nonetheless, not all patients obtain sufficient clinical response and these therapeutics are expensive. Therefore, to identify factors associated with clinical response and cost-effectiveness of biological therapeutics remains an important focus of interest.

Currently, all biologicals are registered in a standard dose for all patients without taking differences in pharmacokinetics into account. However, this standard dose results in a wide variability in serum trough levels, thus, standard dosing results in an under or over treatment in a proportion of patients. This is supported by promising results of dose reduction and interval prolongation studies.

Biologicals are molecular targeting therapies, therefore, we hypothesize that drug levels sufficient for effective target blockade are enough; higher drug levels are unnecessarily, because all target is already blocked; while a drug level below the threshold for complete target blockade is suboptimal.

To obtain and maintain drug levels within the optimal target range a personalized dosing scheme seems more rational and cost-effective compared to a standard dosing for all patients. Currently, dose adaptations are based on clinical measurements, but what are the possibilities of therapeutic drug monitoring to optimize biological treatment?

This thesis describes the relationship between serum drug level, pharmacokinetic factors and clinical outcome for several biologicals and diseases. Moreover, it contains a TNF-inhibitor tapering study in spondyloarthritis and a study regarding the feasibility of a dried blood spot obtained by finger prick to measure adalimumab and anti-adalimumab levels.