Anti-TNFα treatment in rheumatoid arthritis

Een wetenschappelijke proeve op het gebied van de Medische Wetenschappen

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**Abbreviations:**

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<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>ACE</td>
<td>Angiotensin Converting Enzyme</td>
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<tr>
<td>ACR</td>
<td>American College of Rheumatology</td>
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<tr>
<td>AE</td>
<td>Adverse Event</td>
</tr>
<tr>
<td>ANA</td>
<td>Anti Nuclear Antibodies</td>
</tr>
<tr>
<td>ASA</td>
<td>Acetyl Salicylic Acid</td>
</tr>
<tr>
<td>AUC</td>
<td>Area Under the Curve</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Intervals</td>
</tr>
<tr>
<td>COMP</td>
<td>Cartilage Oligomeric Matrix Protein</td>
</tr>
<tr>
<td>COPD</td>
<td>Chronic Obstructive Pulmonary Disease</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive Protein</td>
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<tr>
<td>CTC</td>
<td>Common Toxicity Criteria</td>
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<tr>
<td>DAS</td>
<td>Disease Activity Score</td>
</tr>
<tr>
<td>DMARD</td>
<td>Disease Modifying Anti Rheumatic Drug</td>
</tr>
<tr>
<td>DCART</td>
<td>Disease Controlling Anti Rheumatic Drug</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiograph</td>
</tr>
<tr>
<td>ESR</td>
<td>Erythrocyte Sedimentation Rate</td>
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<tr>
<td>EULAR</td>
<td>European League Against Rheumatism</td>
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<tr>
<td>GCP</td>
<td>Good Clinical Practice</td>
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<tr>
<td>HAQ</td>
<td>Health Assessment Questionnaire</td>
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<tr>
<td>HPA-axis</td>
<td>Hypothalamus-Pituitary-Adrenal axis</td>
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<tr>
<td>HC gp-39</td>
<td>Human Cartilage glycoprotein 39</td>
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<tr>
<td>ICAM</td>
<td>Intra Cellular Adhesion Molecule</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>JSN</td>
<td>Joint Space Narrowing</td>
</tr>
<tr>
<td>MED</td>
<td>Minimal Erythema Dose</td>
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<tr>
<td>MMP</td>
<td>Matrix Metalloproteinase</td>
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<tr>
<td>MTX</td>
<td>Methotrexate</td>
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<tr>
<td>MoAb</td>
<td>Monoclonal Antibody</td>
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<tr>
<td>NSAID</td>
<td>Non-Steroidal Anti Inflammatory Drug</td>
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<tr>
<td>OR</td>
<td>Odds Ratio</td>
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<tr>
<td>OTC</td>
<td>Over The Counter</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate Buffered Saline</td>
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<tr>
<td>PK</td>
<td>Pharmacokinetic</td>
</tr>
<tr>
<td>PMA</td>
<td>Phorbol Myristate Acetate</td>
</tr>
<tr>
<td>PMN</td>
<td>Polymorphonuclear cells</td>
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<tr>
<td>RA</td>
<td>Rheumatoid Arthritis</td>
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<tr>
<td>RACT</td>
<td>Rheumatoid Arthritis Clinical Trials</td>
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<tr>
<td>RAI</td>
<td>Ritchie Articular Index</td>
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<tr>
<td>RF</td>
<td>Rheumatoid Factor</td>
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<tr>
<td>RIA</td>
<td>Radio Immuno Assay</td>
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<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
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<tr>
<td>RR</td>
<td>Relative Risk</td>
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<tr>
<td>SAE</td>
<td>Serious Adverse Event</td>
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<tr>
<td>SASP</td>
<td>Sulfasalazin</td>
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<tr>
<td>SD</td>
<td>Standard Deviation</td>
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<tr>
<td>SDD</td>
<td>Smallest Detectable Difference</td>
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<tr>
<td>SJC</td>
<td>Swollen Joint Count</td>
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<tr>
<td>STZ</td>
<td>Serum-Treated Zymosan</td>
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<tr>
<td>TJC</td>
<td>Tender Joint Count</td>
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<tr>
<td>TNF</td>
<td>Tumour Necrosis Factor $\alpha$</td>
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<tr>
<td>UVB</td>
<td>Ultraviolet type B</td>
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<tr>
<td>VAS</td>
<td>Visual Analogue Scale</td>
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<td>WBC</td>
<td>White Blood Cell</td>
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Chapter I

Introduction

Alfons A den Broeder
**General introduction**

Rheumatoid Arthritis (RA) is a common disease, the most prominent feature of which is a symmetric chronic polyarthritis. This leads to pain, stiffness and functional limitations and can also lead to structural damage of the cartilage and the underlying bone in the joint. Treatment of RA has traditionally consisted of Disease Modifying Anti-Rheumatic Drugs (DMARDs), a diverse group of drugs, the disease modifying properties of which have often been discovered by chance. These agents are however not effective in all patients and in addition their usefulness is limited by side effects (1).

In the last decade, the knowledge about the pathogenesis of RA has increased. Proinflammatory cytokines like Tumour Necrosis Factor α (TNFα) and interleukin β (IL-1β) are known to play a pivotal role in the inflammation in RA and other chronic inflammatory diseases (2-4). Based on these insights, new - so-called biological - agents have been developed. These agents have specifically been designed to block the action of immune cells or to antagonise cytokines and other mediators produced by these cells (5). Among these new therapies, strategies to neutralise the function of TNFα seem the most promising. Blockade of the actions of TNFα can be accomplished with monoclonal antibodies directed against TNFα or with TNF receptor fusion proteins that bind with TNFα, preventing its binding with signalling receptors. Both modalities have been shown to be effective in the treatment of RA (6,7).

One monoclonal antibody that recently has been approved for treatment of RA (infliximab) has been constructed using both human and murine protein sequences. The use of such chimeric antibodies is potentially limited by the formation of human anti-chimeric antibodies (HACA), which may lead to a shortened half-life time or adverse reactions (8). Adalimumab (D2E7, Knoll) is a fully human monoclonal antibody against TNFα, developed using phage display techniques (9) undergoing phase III studies.

The main subject of this thesis is the evaluation of several aspects of treatment of patients with RA with the human anti-TNFα MoAb adalimumab. These aspects include efficacy, mode of action, safety and use in daily clinical practice. Chapter 6 and 7 also include data derived from patients treated with a TNF p55 receptor fusion protein (Ro 45-2081, lenercept, Roche) (10,11).

To study the effects of any treatment intervention in patients with RA, the impact on disease activity has to be measured. As no single variable can be considered the “golden standard” for disease assessment in RA, a wide variety of variables have traditionally been used in rheumatoid arthritis clinical trials. In the last decade much progress has been made in the assessment of disease activity in RA. Consensus has been reached on a minimum set of variables that should be measured in clinical trials and powerful combined disease activity indices have been developed (12,13).

In chapter two, the current state-of-the-art in disease assessment in RA is reviewed. In addition, the level of compliance in recently published clinical studies towards the
guidelines that have been developed was assessed. To this aim, all clinical studies comparing two or more interventions that have been published in 1999 were reviewed.

Aspects of efficacy of treatment with anti-TNFα are discussed in chapter three and four. In chapter three the short-term efficacy, toxicity and pharmacokinetics of i.v. treatment with increasing doses of adalimumab were studied in a randomised, placebo-controlled study in 120 patients. These patients with a longstanding disease had previously been treated with a large number of DMARDs. The patients were randomly assigned to one of five dose groups or placebo. The American College of Rheumatology (ACR) improvement criteria and the European League Against Rheumatism (EULAR) response criteria were used to judge individual response percentages and the toxicity was assessed using the Common Toxicity Criteria. The goals of treatment of RA are not only ameliorating the signs and symptoms of the disease, but also preventing radiologic damage and development of functional disability. The first reports on the effect of TNFα blocking strategies on this important outcome variable show that these therapies retard radiologic progression (6,7,14). Since radiologic progression is a slow process, the effect of a treatment intervention on radiologic damage can only be judged after a substantial period of time. This way an undesired delay will occur. Therefore, the search for markers that can predict radiologic outcome at an early timepoint has been intensified in recent years (15). In chapter four the effect of long-term treatment with monotherapy with anti-TNFα on radiological outcome is studied in 47 patients. Radiological outcome is compared between patients that have been treated with anti-TNFα for two years and patients that dropped out earlier because of toxicity or lack of efficacy and were treated with other DMARDs. In addition, a number of biological markers were tested at baseline to assess their predictive value for radiologic progression. These markers were chosen since they reflect bone and cartilage turnover (HC gp-39, COMP and the metalloproteinases 1 and 3) (16-18) or because they are involved in endothelial activation (sE-selectin and sICAM-1), which plays an important role in the inflammation that characterises RA (19).

The mode of action of TNFα blockade in RA has not been unravelled yet. This subject is targeted in chapter five to seven. Several studies with another IgG1 anti-TNFα MoAb (infliximab) have shown that blocking TNFα reduces the acute phase reaction and decreases the local and systemic levels of adhesion molecules in RA patients (20-22). In vitro studies have also shown that neutralisation of TNFα reduces the production of IL-1β in synovial cultures (23). Whether such therapies also downregulate the synovial expression of IL-1 and TNFα in RA has not been fully elucidated yet, although some data exist supporting this theory (24,25). In chapter five the short-term effects of the first dose of adalimumab or placebo on the homeostasis of the two main pro-inflammatory cytokines IL-1β and TNFα at the systemic and the synovial level are investigated.
The HPA axis could also play a role in the mechanism of action. Although acute TNFα or IL-1 administration is known to stimulate the hypothalamus-pituitary-adrenal axis (HPA-axis) (26,27), the HPA axis in patients with RA does not seem to be activated and has even been reported to be impaired (28). Glucocorticoids, both endogenous and exogenous, are strong inhibitors of TNFα production (29). Therefore, it can be hypothesised that TNFα production in RA is enhanced by relative deficit of glucosteroid activity. Alternatively, it could be that TNFα has a suppressive effect on the HPA axis in this chronic inflammatory state, in contrast to the effects of TNFα in acute models. In this scenario, blockade of TNFα could lead to increased activity of the HPA axis. The latter would be concordant with the clinical and laboratory effects of anti-TNFα treatment that occur very rapidly and mimic treatment with corticosteroids. In chapter six the effect of treatment with anti-TNFα on the HPA axis is assessed. Eighteen patients receiving adalimumab, lenercept or placebo were studied. Several variables that reflect the activity of the HPA axis were measured.

RA can be accompanied by pathology occurring outside the joints. One of the common extra-articular manifestations of RA is vasculitis. Vasculitis in RA usually affects small vessels and commonly involves the skin, causing nail-fold infarcts, and in more severe cases digital gangrene and leg ulcers (30). Vasculitis can also affect larger vessels leading to organ damage. The effect of TNFα-blockade on RA related vasculitis is unknown. Chapter seven describes the positive effect of treatment with lenercept on skin vasculitis in one patient with RA.

Neutrophilic granulocytes (neutrophils) play an important role in the inflammation that characterises RA. These cells are abundantly present in RA synovial fluid and increased production of reactive oxygen species (ROS) by these cells can induce cartilage damage (31,32). Modulation of neutrophil functions could therefore be beneficial in RA. TNFα is involved in the in-vivo priming of neutrophils and in the induction of ROS production and chemotaxis (33-35). It has been suggested that reduction of neutrophil priming and ROS production may be implicated in the mechanism of action of several antirheumatic drugs, including methotrexate and leflunomide (36-39). Whether this also holds true for TNFα blocking agents is still unknown.

On the other side, alteration of neutrophil function by TNFα blocking agents could yield adverse effects. Data derived from experimental animal models suggest increased susceptibility for infections (40-42) and impaired clearance of (pre) malignant cells (43-45) after TNFα neutralisation. These adverse effects are partially mediated through an impaired function of phagocytic cells, including neutrophils. In chapter eight the effect of treatment with anti-TNFα on neutrophil function is assessed. Neutrophil ROS production and chemotactic capacity were measured ex-vivo. Moreover, two in vivo models were used to study neutrophil migration. Migration into the synovial joints was measured using scintigraphy with radiolabelled...
autologous leukocytes. Neutrophil migration to a non-target organ (the skin) was assessed using an ultraviolet type B skin inflammation model. Except for local injection site reactions (46) and infusion reactions (47,48), no clearly related specific adverse effects of anti-TNF\(\alpha\) treatment have been published, although it is suggested that blockade of TNF\(\alpha\) could possibly lead to reactivation of prior tuberculosis infections. In chapter nine a patient is described that develops a nephrotic syndrome during treatment with anti-TNF\(\alpha\). The possible pathophysiological pathways are discussed.

Two anti-TNF\(\alpha\) agents have recently reached clinical practice, but their exact place in the treatment of RA has yet to be established (49). Anti-TNF\(\alpha\) strategies share some unique characteristics compared to standard DMARD therapies. The onset of action is very rapid, especially after intravenous administration. Also, based on the blocking characteristics of these agents – blocking TNF with a fixed ratio - (50) and the huge differences in cytokine levels in patients with RA (51), it is conceivable that the inter-individual variation in effective dose of anti-TNF\(\alpha\) is large. If present, this large inter-patient variation could result in gross over- or undertreatment when standard dosing schedules are applied. In addition, treatment with TNF\(\alpha\) blocking strategies is characterised by very high costs. For prevention of possible adverse effects resulting from overtreatment and to diminish costs, individual dosing is warranted.

To this aim, we developed a treatment protocol that can be used in daily clinical practice (chapter ten). This protocol was tested in patients that had been treated with anti-TNF\(\alpha\) for two years and had stable disease activity. The disease activity was monitored using the Disease Activity Score (DAS28) (52), a combined disease activity index, ranging from 0-10. The dose of anti-TNF\(\alpha\) was lowered in steps and adjusted for each individual patient based on the DAS28.

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Chapter II

Assessment of disease activity in Rheumatoid Arthritis Clinical Trials: past accomplishments and future aims

Alfons A den Broeder, Anke M van Gestel, Piet LCM van Riel
Abstract
For many years the clinical evaluation of patients with rheumatoid arthritis (RA) has been hampered by the lack of consensus about variables that should be used to follow the course of the disease. We summarise the progress that has been made in the last decade in assessment of rheumatoid arthritis; the core set variables, the Disease Activity Score (DAS), and the EULAR and ACR improvement criteria. Furthermore the compliance towards the use of the core set criteria and improvement criteria was reviewed in clinical studies published in 1999. Although consensus has been reached on the core set variables and powerful improvement criteria have been developed, our results show that standardisation of the instruments to measure these variables has not taken place. Prudent implementation of these tools in clinical studies is not yet common practice. This observation warrants further attention.

Introduction
For many years the clinical evaluation of patients with rheumatoid arthritis (RA) has been hampered by the lack of consensus about variables that should be used to follow the course of the disease. This has led to the use of a broad spectrum of variables to measure disease activity. These include clinical variables like joint scores, grip strength, walking time, morning stiffness, questionnaires to assess functional capacity, and patient’s and observer’s judgement of pain and disease activity using Visual Analogue Scales (VAS). Laboratory variables often employed are acute phase reactants or viscosity. Also different outcome measures are being used including radiographic damage, and recently also magnetic resonance imaging, ultrasound and bone density.

The validity and test characteristics of many of these variables, however, are poor or unknown. Also for both practical and economical reasons the variables that are being employed in a clinical study should not yield redundant information, e.g. should not give information already provided by another measure. Furthermore, it is difficult to compare the results of clinical studies or to perform meta-analyses when different measures are used. The same holds true for comparison of baseline patient characteristics.

In the last decade much effort has been directed towards refining and validating disease assessment to overcome the aforementioned problems. In this report, first an overview is given of the current existing guidelines for measuring disease activity in RA. In addition, the level of compliance towards these recommendations is assessed by reviewing clinical studies concerning RA published in 1999 in seven journals.
Overview of existing guidelines

Both the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) have created core sets based on a minimal set of validated variables that needs to be measured in Rheumatoid Arthritis Clinical Trials (RACT) (1,2). The variables included in the core set are the following: number of tender joints, number of swollen joints, patient’s pain score, patient’s global assessment of disease activity, observer’s global assessment of disease activity, an acute phase reactant, a physical function score, and a radiologic evaluation when the study duration is equal to or exceeds one year. Consensus regarding these core sets has been reached based on the validity and the sensitivity to change for the variables used in clinical trials. Those measures that do not show good test characteristics and those that give redundant information have been excluded. It has still to be determined whether these core sets have to be extended in the future with other variables such as socio-economic status and psychological variables.

To assess individual respondership in RACT, composite improvement criteria based on these core set variables were developed. The ACR has proposed the preliminary revised criteria for response (table 1), dichotomous criteria for response that include all the core set variables excluding radiologic outcome (3). The EULAR has adopted the Disease Activity Score (DAS), a continuous score of disease activity ranging from 0 to 10 that is calculated using three or four core set variables. Using the DAS, the EULAR respondership criteria (table 1) have been created, defining response in three groups: no, moderate and good response (4). Both improvement criteria, although designed using a different approach, demonstrate good validity, are sensitive to change and can discriminate between placebo and effective treatment (5).

Table 1. Improvement/respondership criteria developed by the ACR and the EULAR

<table>
<thead>
<tr>
<th>ACR criteria*</th>
<th>EULAR criteria**</th>
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</thead>
<tbody>
<tr>
<td>20% improvement swollen and tender joint counts</td>
<td>DAS28 = 0.56VT28 + 0.28S28 + 0.7lnESR + 0.014GH</td>
</tr>
<tr>
<td>20% improvement in 3 out of 5 variables</td>
<td>Good response : DAS28 below 3.2 and improvement with 1.2</td>
</tr>
<tr>
<td>Acute phase reactant</td>
<td>Moderate response = DAS28 below 5.1 and improvement with 0.6-1.2 or DAS28 above 5.1</td>
</tr>
<tr>
<td>Patient’s assessment of pain</td>
<td>and improvement&gt;1.2</td>
</tr>
<tr>
<td>Patient’s assessment of global disease activity</td>
<td></td>
</tr>
<tr>
<td>Observer’s assessment of global disease activity</td>
<td></td>
</tr>
<tr>
<td>Patient’s assessment of physical function</td>
<td></td>
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</tbody>
</table>

* ACR 50% and 70% should read 50% or 70% respectively instead of 20%.
** The equation shown is the calculation of the Disease Activity Score from the components: tender joint count (T28), swollen joint count (S28), the Erythrocyte Sedimentation Rate (ESR) and the 100 mm VAS for the patients global disease activity (GH).

The original DAS score is calculated with another formula including the Ritchie Articular Index and 44 swollen joint count instead of 28 tender and swollen joint counts.
Although it is clear now which variables to assess in RACT, the methods used to measure these variables still lack standardisation. Several joint counts for example have been and are still being used, ranging from 68/66 joint counts to 28 joint counts. Because the latter have been demonstrated on several occasions to give as much information as the more comprehensive joint counts, it makes sense to use these simple counts (6,7,8). Consensus is leaning towards the use of these simple joint counts, and these have been included now in the EULAR core set recommendations (9).

For other core set variables, however, standardisation is further away. Both different semi-quantitative scores (e.g. Likert) and different VAS (horizontal/vertical, different sizes, with or without marks) are being used to determine patient’s and observer’s global assessment of disease activity and pain. Also for assessment of radiologic damage different scores (e.g. Larsen, Sharp, modified Sharp/van der Heijde, Kellgren) exist. Functional status is often assessed by the Health Assessment Questionnaire (HAQ), of which several translations and versions (for example modified HAQ) have been developed, but also other questionnaires are being used. Even for laboratory variables standardisation has not reached an optimum. The ESR is usually measured according to the Westergren method (although this is often not mentioned) but CRP levels are still not interchangeable between different laboratories. All the different instruments that are being used to measure core set variables yield different results that are not interchangeable in the majority of cases. To prevent the “Babylonian confusion of languages” in the comparison of results of RACT, further standardisation of the instruments for measuring these core set variables should be reached.

Compliance towards existing guidelines

Methods

To investigate whether the aforementioned guidelines are being followed, all the clinical studies concerning therapeutic interventions in RA patients published in 1999 in the following seven Journals were reviewed: Lancet, New England journal of Medicine, Arthritis and Rheumatism, Annals of Rheumatic Diseases, Rheumatology, Journal of Rheumatology and the Scandinavian journal of Rheumatology. Inclusion criteria were comparison of two or more treatment modalities for RA (including placebo) and a study duration > 3 months. Studies that compared treatment with analgesics or NSAIDs were excluded. The primary study measure on which the conclusions were based (individual response criteria), the variables assessed in the study, and the method of assessment and references were determined. Deviations from the current recommendations for disease assessments that were found were classified as either major or minor deviations. Major deviations are those that do not allow valid conclusions to be made regarding the number of patients that show individual response. This includes the absence of individual response criteria or the use of not validated response criteria. The absence of one or more core set
variables is also regarded as major deviation because this severely hampers the possibility of comparison with other studies. The second group, labelled minor deviations, includes the use of not recommended instruments to measure a particular variable with, missing references regarding the instruments that were used (e.g. HAQ version or translation). Differences in radiologic score were not taken into account, as radiologic evaluation is only recommended in clinical studies with a follow-up equal to or exceeding one year.

Results
Twelve studies fulfilled the inclusion criteria (appendix A). Results are depicted in table 2. For each study, the primary outcome measure is shown. Furthermore, the core set variables that are assessed are listed with the method used for evaluation of that particular variable.

Major deviations were found in seven studies. These consisted of the lack of an individual response measure (study 1, 4 and 6) and the use of non-validated or adjusted response criteria (study 2, 9 and 11). In five studies (study 1, 2, 4, 11 and 12) not all core set variables were assessed although study 12 provides correct individual response percentages using the DAS and the EULAR response criteria.

Minor deviations from the guidelines are seen in all studies. These included for example the use of the modified HAQ instead of the original HAQ and missing references or description of the methods used. The studies printed in bold (n=5) are complying with the existing guidelines.

Discussion
The last decade the methods available to follow the course of RA have been vastly improved. Consensus has been reached on a core set of variables to measure and powerful improvement criteria have been developed and validated. The results of the review of clinical studies, however, show that the guidelines that have been developed for measuring disease activity in RA are not being followed in the majority of studies in 1999. Only five of twelve studies are compliant with the current recommendations and even these studies differ in the instruments that are used to measure the core set variables with.

Regarding the use of composite response criteria, things seem clear. For individual improvement or response status, both the EULAR criteria using the DAS and the ACR criteria have been extensively validated and at least one of these response criteria should therefore be used. The use of alternative response criteria, for example ACR improvement criteria with 20% improvement in 2 out 4 variables instead of 3 out 5 variables is thus lacking rationale.

The same holds true for the core set of variables that should at least be included in RACT. The assessment of extra variables does not influence the primary endpoints for efficacy of a clinical study, but missing variables could hamper comparability with other clinical studies.
Table 2. RA clinical studies published in 1999 (n=12): response criteria and core set variables.

<table>
<thead>
<tr>
<th>Study no</th>
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<tr>
<td>DAS28(4)</td>
<td>ACR20</td>
<td><strong>DAS(3)+</strong></td>
<td>-</td>
<td>ACR20</td>
<td>-</td>
<td>ACR20</td>
<td>ACR20</td>
<td>Paulus *</td>
<td>ACR20</td>
<td>ACR20</td>
<td>DAS(3)+</td>
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</tr>
<tr>
<td>(2 of 3 variables)</td>
<td>EULAR</td>
<td>ACR20</td>
<td>ACR50</td>
<td>ACR50/70</td>
<td>(2 of 4 variables)</td>
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<td></td>
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<td>Core set variables</td>
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<td>T40</td>
<td>RAI</td>
<td>T66</td>
<td>T68</td>
<td>T48, RAI</td>
<td>T28</td>
<td>T68</td>
<td>T66</td>
<td>T68</td>
<td>T69 G</td>
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<tr>
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<td>S66</td>
<td>S64</td>
<td>S66</td>
<td>S66 G</td>
</tr>
<tr>
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<td>-</td>
<td>V 100mm</td>
<td>V 100mm</td>
<td>0-10</td>
<td>V 100mm</td>
<td>V 100mm</td>
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<td>-</td>
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<tr>
<td>Pt's global †</td>
<td>?</td>
<td>0-10</td>
<td>L 1-5</td>
<td>-</td>
<td>0-10</td>
<td>1-4</td>
<td>V 100mm</td>
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<td>1-5 **</td>
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<td>Observer's global †</td>
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<td>L 1-5</td>
<td>-</td>
<td>0-10</td>
<td>1-4</td>
<td>V 100mm</td>
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<td>V 100mm</td>
<td>V 100mm</td>
<td>V 100mm</td>
<td>1-5 **</td>
</tr>
<tr>
<td>ESR/CRP</td>
<td>esr/crp</td>
<td>Crp</td>
<td>esr/crp</td>
<td>esr/crp</td>
<td>crp</td>
<td>esr/crp</td>
<td>esr</td>
<td>esr</td>
<td>crp/esr</td>
<td>crp</td>
<td>crp/esr</td>
<td>Crp/Esr</td>
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<td>HAQ</td>
<td>HAQ</td>
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<td>HAQ</td>
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<td>MHAQ</td>
<td>HAQ</td>
<td>MHAQ</td>
<td>HAQ</td>
<td>LEE</td>
</tr>
</tbody>
</table>

* Paulus criteria need a change of 2 scales on a 5-point scale instead of a VAS
** 1 to 5, 1 being no improvement and 5 being excellent improvement
# RAI= Ritchie Articular Index, G = graded (0-3)
† L= Likert scale, V=VAS
§ (M)HAQ = (modified) Health Assessment Questionnaire
- not done
? the variable is assessed but the instrument is not mentioned
Consensus has, however, not been reached yet on the instruments to measure the core set variables with. The recommendations leave room for several different instruments to measure for example functional capacity and patients’ and observers’ opinion on disease activity and pain. Although it is possible that these instruments turn out to be interchangeable, the influences of the use of these different instruments on the individual response criteria are still unknown. The ACR improvement criteria for example have been developed using the original HAQ to measure functional capacity. Recently the modified HAQ is often used instead. The latter makes use of transition questions (is a function better, the same or worse compared to before) and it is not clear how these questions can be used to calculate 20% improvement. This does not imply that the modified HAQ can never be used as part of the ACR improvement criteria. Rather, validation of this method has to take place prior to this substitution.

The question raises why support for the proposed core sets and response criteria is lacking. It can be argued that this is caused by the short time between publication of the core-sets (1992) and response criteria (1995) and the design of the studies included in our survey. Although the design of some of these clinical studies may date from before 1995 (publication of response criteria), the core set variables published in 1992 should however at least have been included.

In conclusion we would like to stress the importance of unambiguous and careful use and description of methods used for clinical assessment. There is a lot to gain if optimisation is reached at all levels: a smaller sample size needed to find significant differences, less work and costs due to fewer variables that need to be assessed and finally comparability between patient populations and responder percentages. To meet the goals mentioned above also the compliance towards the use of the proposed core sets and improvement criteria in RACT should be carefully monitored in the future, as there is still room for improvement.

REFERENCES


Appendix A


Chapter III

A single-dose, placebo controlled study of the fully human anti-TNFα antibody adalimumab (D2E7) in patients with established rheumatoid arthritis

Alfons A den Broeder, Leo BA van de Putte, Rolf Rau, Manfred Schattenkirchner, Piet LCM van Riel, Oliver Sander, Christina Binder, Helmut Fenner, Yvonne Bankmann, Raja Velagapudi, Joachim Kempeni, and Hartmut Kupper
Abstract

Objectives This dose-ranging study assessed the pharmacokinetics, the safety profile, and efficacy of the fully human anti-TNFα monoclonal antibody adalimumab (D2E7) in patients with long-standing active rheumatoid arthritis.

Methods This was a randomised, double blind, placebo-controlled study of single intravenous injections of ascending doses (0.5 to 10 mg/kg) of adalimumab in 5 cohorts of 24 patients each (18 adalimumab, 6 placebo). The clinical response was measured by changes in composite scores as defined by the criteria of the European League Against Rheumatism and the American College of Rheumatology.

Results Single doses of adalimumab showed a rapid onset of clinical effect (24 hours to 1 week) with peak efficacy at 1 to 2 weeks which was sustained for at least 4 weeks and as long as 3 months in some patients. EULAR response was seen at least once during the 4 week period after study drug injection in 29% of patients in the placebo group as well as in 41%, 78%, 72%, 89%, and 100% in the 0.5, 1.0, 3.0, 5.0, and 10.0 mg/kg group, respectively. No dose-related increases in adverse events were observed in the adalimumab-treated patients when compared to that seen in the placebo group. Adalimumab systemic drug exposure (AUC0-inf) increased linearly with an increase in dose. The mean total serum clearance was 0.011 to 0.017 L/h, and the steady state volume of distribution ranged from 4.7 to 5.5 L. The estimated mean terminal half-life ranged from 10.0 to 13.6 days for the five cohorts with an overall mean half-life of 12 days.

Conclusion Treatment with the fully human monoclonal antibody adalimumab is safe and well tolerated when administered as a single intravenous injection at doses up to 10 mg/kg and is associated with a clinically significant improvement in the signs and symptoms of active rheumatoid arthritis.

Introduction

Current treatments for rheumatoid arthritis (RA) have little impact on the long-term course of the disease (1). Recently the focus of novel therapies for RA has shifted towards attempts to target the cellular inflammatory mechanisms involved in long-term tissue destruction in order to slow or prevent disease progression. The pro-inflammatory cytokines, interleukin (IL)-1 and tumour necrosis factor (TNF)-α, are known to play an important role in the induction and maintenance of inflammatory synovitis and articular matrix degradation and therefore modulation of their synovial activity provides the potential for novel therapeutic interventions in RA (2,3). Biological agents such as anti-cytokine antibodies and soluble receptors that bind and neutralise these mediators have been developed as potential therapies and several are currently being evaluated. These new biological treatment modalities include chimeric and humanised monoclonal antibodies (4-6), as well as fusion proteins of soluble TNF receptor proteins with human immunoglobulin Fc regions (7). Whilst therapy with these modalities has shown promising results in terms of tolerability and duration of clinical benefit, long-term use of non-human constructs
may be limited by immune responses mounted against their foreign components thereby reducing their half-life and thus efficacy (8). In addition, there may be an increased risk of adverse events due to immune complex formation. Given these limitations, the development of a fully human (i.e., 100% human peptide sequences in a natural configuration) therapeutic antibody has considerable potential to provide improved safety and efficacy in long-term use (8).

Adalimumab is a fully human anti-TNFα monoclonal antibody (MoAb) consisting of 100% human sequences and has been developed using phage display technology (9). The adalimumab antibody has been extensively tested in animal models of disease and in standard in vitro and in vivo safety and toxicity assays. Adalimumab has been shown to be both safe and highly effective in these experimental systems. The purpose of this dose-ranging study reported here was to assess the clinical pharmacology profile and evaluate the safety, tolerability and efficacy of adalimumab over a range of doses in patients with active rheumatoid arthritis. The study results have been previously reported in abstract form (10).

Patients and methods
This was a multicenter, double blind, randomised, placebo-controlled, ascending single-dose study in cohorts of patients with active RA carried out in one centre in the Netherlands and two in Germany. The study was conducted under GCP guidelines in accordance with the principles of the Declaration of Helsinki and followed Ethics Committee approval. All patients gave written informed consent to participate in the study.

Patients and study design
The study population included males and females ≥ 18 years of age (women of childbearing potential required a negative pregnancy test and had to use a reliable method of contraception to be included in the study) with a diagnosis of RA and evidence of active inflammatory synovitis as defined by a disease activity score (DAS) of ≥ 3.2 at study entry (11). Body weight of patients was limited to ≤ 100 kg. Important exclusion criteria included intra-articular or intra-muscular corticosteroid treatment within 4 weeks prior to study screen, joint surgery within 2 months prior to study screen (if those joints were to be included in the study assessment), and treatment with any investigational drugs of a chemical or biological nature 2 months or 12 months (respectively) prior to pre-study screen.

After a 3-week washout period for DMARDs, a single intravenous injection lasting 3-5 minutes of study drug was administered to qualified patients hospitalised for 24 hours. The study had five ascending cohorts with 24 patients each. Within each cohort, 18 patients received adalimumab and 6 received matching placebo. Doses of adalimumab were 0.5, 1, 3, 5 and 10 mg/kg, respectively. The cohort with the next higher dose was started only if no dose-limiting adverse events were observed at the previous dose level.
Concomitant treatment with anti-rheumatic/anti-inflammatory drugs was not allowed during the study with the exception of stable doses of NSAIDs and/or corticosteroids with a maximum daily dose equivalent to 10 mg of prednisolone. Analgesics including OTC preparations, propoxyphene, or codeine alone or in combinations were not allowed with the exception of infrequent acetylsalicylic acid (ASA: maximum daily dose of 500 mg) or equivalent treatments for mild pain. Regular intake of low-dose ASA for prophylaxis of myocardial infarction was allowed.

Patients were followed with weekly examinations for at least 4 weeks. If the study drug showed efficacy in individual patients beyond this 4-week period, they continued under observation until their disease status deteriorated in order to assess the duration of efficacy of single-dose administration up to month 3. After completion of this phase of the study, patients were offered the option to enter a continuation phase either at the end of the 4-week follow-up period (day 29) or upon clinical deterioration. The results of the continuation phase will be reported separately.

Pharmacokinetic assessments
Pharmacokinetic (PK) analysis was performed using a non-compartmental approach for intravenous (iv) bolus delivery. The PK analyses were performed using commercially available PK software (KINETICA TM 2000, Version 3.0, InnaPhase, France). The PK variables assessed were the model-dependent parameters for serum concentrations of drug: area under the serum concentration-time curve from time zero to infinity (AUC 0-inf); serum clearance (CL); volume of distribution at steady-state (Vss); and terminal log-linear phase half-life (t½).

Blood samples were collected pre-dose, after end of injection (5 min) and 20 minutes, 1, 4, 8, 12, and 24 hours post-dose. Additional samples were taken weekly for up to six weeks post-dose and bi-weekly thereafter up to a maximum of 12 weeks post-dose. Samples were assayed for adalimumab content using a validated double-antigen, sandwich ELISA method. The assay is sensitive (limit of quantitation = 0.72 μg/mL), precise (CV < 12%; interassay) and accurate (deviation from nominal < ± 20%; interassay).

Safety assessments
Safety and tolerability were evaluated based on reported adverse events, standard hematology and biochemistry parameters and urinalysis, vital signs and ECG. In addition, total immunoglobulins M, G, A, and E, as well as coagulation factors (aPTT, PT and fibrinogen) and complement activity (C1q, C3, C4) were assessed using standard methods. Pulse rate, blood pressure, body weight, and temperature were measured pre-dose and at various times up to day 29. A twelve lead ECG was recorded pre-dose and 4-6 hours post-dose as well as on day 29.

Efficacy assessments
Efficacy analyses up to day 29 were based on the intent-to-treat population within this period. Efficacy variables were determined at each patient visit. Tender joint
count (TJC) and Ritchie Articular Index were assessed in 53 joints or regions by pressure and joint manipulation on physical examination. Standard pain grading was used for calculation of the Ritchie Index (12). For swollen joint count (SJC), 44 joints were rated by physical examination as either swollen or not swollen. Patient’s global assessment of disease activity, pain and general health and physician’s assessment of disease activity were recorded using standard 100-mm visual analogue scales (VAS). Erythrocyte Sedimentation Rate/1hr (ESR) was assessed as a measure of the acute phase reaction.

From the above assessments, a Disease Activity Score (DAS) was calculated according to the formula: 0.53938 x \sqrt{\text{Ritchie Index}} + 0.06465 x \text{swollen joint count} + 0.33 x \ln(\text{ESR} \text{ mm/1st hour}) + 0.00722 x \text{patient’s general health assessment} (11). The functional capacity was assessed using either the Dutch HAQ (Health Assessment Questionnaire) or the German HAQ depending on the center (13, 14). EULAR responses were compared to those defined by the American College of Rheumatology (ACR) at the 20% and 50% improvement levels (15). Responses based on EULAR criteria were defined as good response (DAS decrease from baseline > 1.20 and current DAS ≤ 2.40), no response (DAS decrease from baseline ≤ 0.6 or patients with an improvement of >0.6 but ≤ 1.20 and DAS attaining >3.7). The improvement of the remaining patients is classified as moderate response (11).

For the ACR 20 and 50 a positive response was considered to be 20 or 50% respectively for improvements in Ritchie Index, swollen joint count and at least three of five other variables (patient’s assessment of pain, patient’s or physician’s assessment of disease activity, HAQ score and ESR).

Statistical methodology
Descriptive statistics and simple linear regression analyses were performed to indicate variability in PK data and to establish dose-proportionality. Analyses of efficacy data were descriptive. Accordingly, 95% confidence intervals (CI) were calculated for treatment group means or percentages, for differences between dose groups of adalimumab treatment versus placebo and actual or percent differences from pre-dose data. Safety data were also analysed using descriptive statistics.

Results
Patients
A total of 120 patients were randomised to receive study medication. No significant differences between study groups were detected in pre-treatment characteristics or baseline disease activity (Table 1). The mean age of patients in the study ranges from 53 to 59 years, and most were female (64%). Overall, the mean duration of RA was 11.5 years. All randomised patients had received therapies for RA prior to study entry. These included both drug treatment and surgery.
Table 1. Demographic and baseline disease characteristics

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Adalimumab (mg/kg)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>No. Patients</td>
<td>31</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td>Male</td>
<td>9 (29)</td>
<td>7 (41)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>22 (71)</td>
<td>10 (59)</td>
</tr>
<tr>
<td>Age in yr. ± SD (Mean (range))</td>
<td>55±11</td>
<td>54±15</td>
<td>58±8</td>
</tr>
<tr>
<td></td>
<td>Duration of RA in yr. (mean)</td>
<td>10.0</td>
<td>11.0</td>
</tr>
<tr>
<td>Measures of arthritis activity (mean values)</td>
<td>DAS</td>
<td>5.16</td>
<td>5.14</td>
</tr>
<tr>
<td></td>
<td>TJC [0-53] (no.)</td>
<td>23.5</td>
<td>23.8</td>
</tr>
<tr>
<td></td>
<td>SJC[0-44] (no.)</td>
<td>18.4</td>
<td>18.4</td>
</tr>
<tr>
<td></td>
<td>ESR (mm/hr)</td>
<td>39.0</td>
<td>36.3</td>
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<tr>
<td></td>
<td>CRP (mg/L)</td>
<td>41.4</td>
<td>37.5</td>
</tr>
<tr>
<td></td>
<td>Physician’s global assessment (mm on VAS)</td>
<td>62.1</td>
<td>59.1</td>
</tr>
<tr>
<td></td>
<td>Patient’s global assessment (mm on VAS)</td>
<td>60.5</td>
<td>66.0</td>
</tr>
<tr>
<td></td>
<td>Patient’s assessment of pain (mm on VAS)</td>
<td>58.6</td>
<td>63.2</td>
</tr>
<tr>
<td></td>
<td>Morning stiffness (min)</td>
<td>132.9</td>
<td>106.8</td>
</tr>
<tr>
<td></td>
<td>HAQ</td>
<td>1.57</td>
<td>1.57</td>
</tr>
</tbody>
</table>

DAS = Disease Activity Score; TJC = Tender Joint Count; SJC = Swollen Joint Count; ESR = Erythrocyte Sedimentation Rate; CRP = C-reactive protein; VAS = Visual Analogue Scale; HAQ = Health Assessment Questionnaire.

The most frequent drug treatments were with disease modifying anti-rheumatic drugs (DMARDs), glucocorticoids and non-steroidal anti-inflammatory drugs (NSAIDs). The mean number of previous DMARDs per patient was 3.6. The most frequently given DMARDs were methotrexate (88%), gold preparations (78%), sulfasalazine (71%), chloroquine/hydroxychloroquine (43%), azathioprine (40%), penicillamin (29%), cyclosporin A (10%) and cyclophosphamide (8%). All randomised patients completed the double-blind phase of the study except for one patient in the 0.5 mg/kg group.
who received study drug but withdrew from the study after 3 weeks due to a serious adverse event (necrotising pancreatitis).

**Pharmacokinetic**

Pharmacokinetic parameters derived using non-compartmental analyses are summarised in Table 2.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Cmax (µg/mL)</th>
<th>AUC(_{0\text{–inf}}) (µg*h/mL)</th>
<th>t(_{1/2}) (h)</th>
<th>CL (L/h)</th>
<th>CL (mL/h/kg)</th>
<th>Vss (L)</th>
<th>Vss (L/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>25 (22)</td>
<td>2729 (707)</td>
<td>284 (119)</td>
<td>0.015</td>
<td>0.068</td>
<td>5.28</td>
<td>0.068</td>
</tr>
<tr>
<td>1</td>
<td>68 (76)</td>
<td>4363 (1807)</td>
<td>242 (170)</td>
<td>0.017</td>
<td>0.089</td>
<td>4.97</td>
<td>0.075</td>
</tr>
<tr>
<td>3</td>
<td>78 (28)</td>
<td>14229 (3703)</td>
<td>267 (90)</td>
<td>0.015</td>
<td>0.061</td>
<td>5.46</td>
<td>0.082</td>
</tr>
<tr>
<td>5</td>
<td>144 (46)</td>
<td>32963 (11556)</td>
<td>326 (129)</td>
<td>0.012</td>
<td>0.065</td>
<td>4.73</td>
<td>0.070</td>
</tr>
<tr>
<td>10</td>
<td>294 (74)</td>
<td>67115 (17385)</td>
<td>321 (117)</td>
<td>0.011</td>
<td>0.038</td>
<td>4.70</td>
<td>0.065</td>
</tr>
</tbody>
</table>

Cmax = peak serum adalimumab concentration immediately after end of injection; AUC\(_{0\text{–inf}}\) = total area under the serum concentration-time curve; t\(_{1/2}\) = half-life; CL = serum clearance; Vss = volume of distribution at steady-state.

Mean serum concentration-time profiles (log-linear) following single IV bolus doses of 0.5 to 10 mg/kg adalimumab are shown in Figure 1. Serum adalimumab concentrations appeared to have declined bi-exponentially. Mean serum adalimumab clearance ranged from 0.011 to 0.017 L/h (0.183 to 0.283 ml/min). The estimated mean steady-state volume of distribution ranged from 4.7 to 5.5 L (0.070 to 0.086 L/kg), indicating that the drug mainly resides in the vascular compartment. The mean terminal half-life ranged from 242 to 326 h (10.0 to 13.6 days). The overall mean half-life was 12 days for all dose groups. Systemic exposure (AUC\(_{0\text{–inf}}\)) increased linearly with an increase in dose following single intravenous bolus doses ranging from 0.5 to 10.0 mg/kg (Figure 2). The clearance, terminal half-life, and steady-state volume of distribution appeared to be dose-independent, indicating linear kinetics.
Figure 1. Mean serum profile of adalimumab following a single IV injection (semi-log plot)

Figure 2. Relationship between area under the serum concentration-time curve and dose following single IV injections of adalimumab

\[ y = 6947.9x - 2840.4 \]

\[ R^2 = 0.8632 \]
Safety and tolerability
The injections of adalimumab and placebo were well tolerated. All patients in both adalimumab- and placebo-treated groups experienced at least one adverse event (AE). In general, overall AE frequencies did not differ between the placebo and the adalimumab groups and no increased rate of AEs was seen with increasing doses of adalimumab (Table 3). This was also the case for individual AEs. The most frequently reported clinical AEs were fever (body temperature ≥ 37°C), headache, and hypertension (Table 4). Pruritis and rash occurred in six and five patients, respectively, treated with adalimumab and none on placebo. No injection site reactions were reported. Two patients experienced serious adverse events (SAEs) following treatment with adalimumab. One had a necrotising pancreatitis with abdominal pain after 1 injection of 0.5 mg/kg adalimumab. Retrospectively, it was discovered that this patient had a strongly elevated serum lipase at baseline and had hidden a history of alcohol abuse. The second from the 1 mg/kg group had a known long history of epilepsy and had an epileptic seizure 5 days after injection of adalimumab. The plasma concentration of the concomitant medication phenytoin has been analysed retrospectively and proved to be much too low at the time of the event. The dosage of the anti-epileptic medication was adjusted and the patient was treated repeatedly with adalimumab in the continuation study without further adverse events.

Changes from baseline in clinical laboratory values, vital signs, and ECG were small in all treatment groups. There were only a few cases of clinical laboratory values meeting grade 3 or 4 of the common toxicity criteria (CTC). Most of these were low lymphocytes (29% placebo, 28% adalimumab), which was also a common condition at study entry (22%).

Table 3. Overview of numbers (%) of patients with treatment emergent adverse events

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Adalimumab (mg/kg)</th>
<th>Any Adalimumab</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Patients</td>
<td>31</td>
<td>17 18 18 18 18 18 18</td>
<td>89</td>
</tr>
<tr>
<td>Clinical AE</td>
<td>27 (87)</td>
<td>15 (88) 15 (83) 18 (100) 11 (61) 16 (89)</td>
<td>75 (84)</td>
</tr>
<tr>
<td>Laboratory AE</td>
<td>31 (100)</td>
<td>16 (94) 16 (89) 16 (89) 18 (100) 16 (89)</td>
<td>82 (92)</td>
</tr>
<tr>
<td>Possibly drug-related AE</td>
<td>18 (58)</td>
<td>12 (71) 12 (67) 11 (61) 8 (44) 9 (50)</td>
<td>52 (58)</td>
</tr>
<tr>
<td>Severe/life threatening AE</td>
<td>7 (23) 5 (29) 4 (22) 4 (22) 3 (17) 2 (11)</td>
<td>18 (20)</td>
<td></td>
</tr>
<tr>
<td>Serious AE</td>
<td>1 (0)</td>
<td>1 (6) 1 (6) 0 (0) 0 (0) 0 (0)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>AE leading to withdrawal</td>
<td>0 (0)</td>
<td>1 (6) 0 (0) 0 (0) 0 (0) 0 (0)</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>

AE=adverse event
### Table 4. Most frequent (≥2 patients) treatment emergent clinical adverse events

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Placebo n=31</th>
<th>0.5</th>
<th>1.0</th>
<th>3.0</th>
<th>5.0</th>
<th>10.0</th>
<th>Any Adalimumab n=89</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever (no. [%])</td>
<td>9 (29)</td>
<td>5 (29)</td>
<td>7 (39)</td>
<td>4 (22)</td>
<td>3 (17)</td>
<td>4 (22)</td>
<td>23 (26)</td>
</tr>
<tr>
<td>Headache</td>
<td>2 (6)</td>
<td>2 (12)</td>
<td>5 (28)</td>
<td>5 (28)</td>
<td>2 (11)</td>
<td>3 (17)</td>
<td>17 (19)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>3 (10)</td>
<td>7 (41)</td>
<td>2 (11)</td>
<td>2 (11)</td>
<td>1 (6)</td>
<td>2 (11)</td>
<td>14 (16)</td>
</tr>
<tr>
<td>Asthenia</td>
<td>3 (10)</td>
<td>2 (12)</td>
<td>3 (17)</td>
<td>0</td>
<td>0</td>
<td>1 (6)</td>
<td>6 (7)</td>
</tr>
<tr>
<td>Rhinitis</td>
<td>2 (6)</td>
<td>1 (6)</td>
<td>2 (11)</td>
<td>2 (11)</td>
<td>1 (6)</td>
<td>0</td>
<td>6 (7)</td>
</tr>
<tr>
<td>Dizziness</td>
<td>2 (6)</td>
<td>0</td>
<td>3 (17)</td>
<td>1 (6)</td>
<td>0</td>
<td>1 (6)</td>
<td>5 (6)</td>
</tr>
<tr>
<td>Flu syndrome</td>
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<td>1 (6)</td>
<td>0</td>
<td>2 (11)</td>
<td>5 (6)</td>
</tr>
<tr>
<td>Pruritis</td>
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<td>0</td>
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<td>1 (6)</td>
<td>2 (11)</td>
<td>6 (7)</td>
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<tr>
<td>Rash</td>
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<td>2 (12)</td>
<td>0</td>
<td>0</td>
<td>2 (11)</td>
<td>1 (6)</td>
<td>5 (6)</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>1 (3)</td>
<td>1 (6)</td>
<td>0</td>
<td>1 (6)</td>
<td>0</td>
<td>2 (11)</td>
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<td>0</td>
<td>1 (6)</td>
<td>1 (6)</td>
<td>1 (6)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Joint disorder</td>
<td>2 (6)</td>
<td>0</td>
<td>1 (6)</td>
<td>1 (6)</td>
<td>2 (11)</td>
<td>0</td>
<td>4 (4)</td>
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<tr>
<td>GI pain</td>
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<td>0</td>
<td>0</td>
<td>2 (11)</td>
<td>0</td>
<td>0 (0)</td>
<td>2 (2)</td>
</tr>
</tbody>
</table>

**Efficacy: primary efficacy variables**

In all the adalimumab dose groups, reductions in DAS compared with baseline scores were observed. In the placebo group, by contrast, only slight mean changes in DAS from baseline were found at all evaluated time points. The improvement in DAS was smallest in the 0.5 mg/kg group. In the higher adalimumab dosage groups 1.0 to 10.0 mg/kg the improvement was much larger, but of similar magnitude between the groups (Table 5).

For the adalimumab dose groups, an initial decline in DAS was seen as early as 24 hours after injection, further decreasing up to day 8, and returning toward baseline by days 22 and 29. For all doses other than 0.5 mg/kg, DAS remained below the pretreatment level up to day 29.

Relative to placebo, treatment with adalimumab 0.5 mg/kg resulted in statistically significantly greater decreases from baseline in DAS at days 8 and 15. For the adalimumab 1.0 mg/kg group, the change from baseline in DAS versus placebo was statistically significant at all evaluated time points up to day 29. This was also the case for the adalimumab 3.0 mg/kg, 5 mg/kg and 10.0 mg/kg groups.

Response rates based on EULAR and ACR 20 criteria (using ESR as the acute phase reactant variable) are shown in Figure 3. In the higher adalimumab dose groups, 60 to more than 80% of patients achieved EULAR and ACR 20 response status between 24 hours to 29 days of treatment.
Table 5. Disease Activity Scores (DAS) over time. Mean (95% CI) baseline measurements and differences from baseline

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Adalimumab (mg/kg)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Patients</td>
<td>31</td>
<td>17</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Baseline</td>
<td>5.16</td>
<td>5.14</td>
<td>5.81</td>
<td>5.35</td>
<td>4.93</td>
<td>5.63</td>
</tr>
<tr>
<td>(4.73, 5.59)</td>
<td>(4.72, 5.55)</td>
<td>(5.32, 6.31)</td>
<td>(4.93, 5.77)</td>
<td>(4.53, 5.32)</td>
<td>(5.09, 6.17)</td>
<td></td>
</tr>
<tr>
<td>Difference from baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 hours</td>
<td>-0.16</td>
<td>-0.25</td>
<td>-0.81</td>
<td>-0.49</td>
<td>-0.33</td>
<td>-0.72</td>
</tr>
<tr>
<td>(-0.34, 0.02)</td>
<td>(-0.49, -0.01)</td>
<td>(-1.10, -0.53)</td>
<td>(-0.76, -0.22)</td>
<td>(-0.64, -0.02)</td>
<td>(-1.00, -0.43)</td>
<td></td>
</tr>
<tr>
<td>Day 8</td>
<td>-0.09</td>
<td>-0.76</td>
<td>-1.28</td>
<td>-1.50</td>
<td>-1.21</td>
<td>-1.29</td>
</tr>
<tr>
<td>(-0.38, 0.20)</td>
<td>(-1.12, -0.39)</td>
<td>(-1.58, -0.97)</td>
<td>(-1.88, -1.13)</td>
<td>(-1.69, -0.74)</td>
<td>(-1.85, -0.73)</td>
<td></td>
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<tr>
<td>Day 15</td>
<td>-0.03</td>
<td>-0.82</td>
<td>-1.37</td>
<td>-1.45</td>
<td>-1.19</td>
<td>-1.50</td>
</tr>
<tr>
<td>(-0.34, 0.27)</td>
<td>(-1.31, -0.33)</td>
<td>(-1.77, -0.97)</td>
<td>(-1.87, -1.04)</td>
<td>(-1.69, -0.68)</td>
<td>(-1.88, -1.13)</td>
<td></td>
</tr>
<tr>
<td>Day 22</td>
<td>0.24</td>
<td>-0.35</td>
<td>-1.14</td>
<td>-1.27</td>
<td>-1.56</td>
<td>-1.53</td>
</tr>
<tr>
<td>(0.00, 0.48)</td>
<td>(-0.88, 0.18)</td>
<td>(-1.58, -0.71)</td>
<td>(-1.73, -0.81)</td>
<td>(-2.13, -0.99)</td>
<td>(-1.99, -1.08)</td>
<td></td>
</tr>
<tr>
<td>Day 29</td>
<td>0.26</td>
<td>0.16</td>
<td>-0.81</td>
<td>-1.05</td>
<td>-1.52</td>
<td>-1.08</td>
</tr>
<tr>
<td>(0.02, 0.50)</td>
<td>(-0.32, 0.64)</td>
<td>(-1.31, -0.31)</td>
<td>(-1.54, -0.55)</td>
<td>(-2.08, -0.96)</td>
<td>(-1.54, -0.62)</td>
<td></td>
</tr>
</tbody>
</table>

Therapeutic effects became evident within 24 hours to 1 week after adalimumab administration and peaked after 1-2 weeks, with dose response seeming to reach a plateau at 1 mg/kg adalimumab. In contrast, only 29% of patients on placebo achieved EULAR response status. The ACR 50 responder rates at any time up to day 29 were 18%, 17%, 28%, 28% and 17% in the 0.5, 1, 3, 5, 10 mg/kg adalimumab groups, respectively.

Figure 3. Percentage of patients with EULAR/ACR 20 response at any time.
The proportion of all patients with EULAR response to a single dose of adalimumab over time was also analysed. The adalimumab dose groups had consistently longer periods of response (defined as time from injection until non-responsiveness) compared to placebo. Duration of response was longer in the 1.0, 3.0, 5.0, and 10.0 mg/kg groups compared with the 0.5 mg/kg group and the placebo group. Only 6% of the placebo and 0.5 mg/kg treated patients had a EULAR response lasting until day 29, whereas 33%, 39%, 61% and 61% of the patients in the 1.0, 3.0, 5.0, and 10.0 mg/kg group had responses lasting until day 29 or longer. In some patients the response lasted up to 3 months. There was no apparent difference in response-duration in the dose groups above 0.5 mg/kg.

Secondary efficacy variables
Further confirmation of the beneficial effects of adalimumab treatment on inflammatory synovitis during the trial was demonstrated by the reduction from baseline in the individual components of the EULAR and the ACR response criteria (Table 6). Following therapy with adalimumab, there were improvements in all these parameters with the least pronounced improvements being in the 0.5 mg/kg group. Improvements in the higher dose groups (1-10 mg/kg) were of similar magnitude.

Table 6. Maximum median reduction (%) from baseline in individual components of EULAR or ACR response criteria at any time up to day 29 following single injection of adalimumab or placebo

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Adalimumab (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>No. Patients</td>
<td>31</td>
<td>17</td>
</tr>
<tr>
<td>TJC</td>
<td>5.6</td>
<td>29.4</td>
</tr>
<tr>
<td>SJC</td>
<td>0</td>
<td>31.3</td>
</tr>
<tr>
<td>ESR</td>
<td>0</td>
<td>27.5</td>
</tr>
<tr>
<td>CRP</td>
<td>4.8</td>
<td>49.5</td>
</tr>
<tr>
<td>HAQ</td>
<td>0</td>
<td>15.7</td>
</tr>
</tbody>
</table>

TJC = Tender Joint Count (Ritchie Index); SJC = Swollen Joint Count; ESR = Erythrocyte sedimentation rate; CRP = C reactive protein; HAQ = Health Assessment Questionnaire

The improvements were sustained with values for each parameter remaining below baseline at day 29, except the Ritchie articular index in 0.5 mg/kg group. Patient’s and physician’s global assessments of disease activity and patient’s assessment of pain and general health measured using a 100-mm visual analogue scale largely paralleled the above findings. Placebo effects were minimal, and the effects of the 0.5 mg/kg dose were less marked than those resulting from treatment with the higher adalimumab doses. As with the other measures of efficacy, the effects with the higher adalimumab doses (1 mg/kg and higher) persisted at least up
to the day 29 observation. Acute phase reactants CRP and ESR have shown an impressive drop within one week after injection of adalimumab for all dose groups.

**Discussion**

This is the first report of a clinical study of the fully human anti-TNFα monoclonal antibody adalimumab in patients with active RA. Analysis of pharmacokinetic data indicated that systemic drug exposure (AUC) increased proportionally with an increase in dose. The drug mainly resides in the vascular compartment (Vss = 4.7 to 5.5 L) and is cleared very slowly from serum (CL = 0.011 to 0.017 L/h). The estimated mean terminal half-life ranged from 10.0 to 13.6 days for all the five cohorts with an overall half-life of 12 days for all doses.

The results of this double blind, placebo-controlled trial demonstrated that single intravenous doses of adalimumab from 0.5 to 10 mg/kg were safe and well tolerated. No dose-related increases in adverse events were observed, and the two reported SAEs did not appear to be treatment related. The relatively high rates of abnormal clinical laboratory findings in the study were to be expected given the patient population and there were no differences between placebo and adalimumab groups. Compared with placebo, treatment with adalimumab produced a statistically significant reduction in disease activity as assessed by standard clinical endpoints. Adalimumab produced rapid and sustained reductions in disease activity with the maximum clinical response seen within 1 to 2 weeks. The treatment effect became evident as early as 24 hours following adalimumab administration. Approximately 60-80% of the patients in the higher adalimumab dose groups achieved a significant therapeutic response (EULAR and ACR20) with a single dose, which was sustained in some patients for up to 3 months. Considering the longstanding, therapeutically refractory nature of the disease in the RA patients studied and the relatively stringent primary efficacy parameters used (EULAR and ACR 20 and 50), it is noteworthy that such positive and sustained responses could be achieved following single doses of adalimumab. In this single dose study, the dose-response relationship was flat at doses between 1 and 10 mg/kg/day, whereas the lowest dose of adalimumab (0.5 mg/kg) was less effective than the higher doses for all parameters evaluated.

In conclusion, single intravenous injections between 0.5 to 10 mg/kg of the fully human anti-TNFα monoclonal antibody adalimumab exhibited systemic drug exposure linearly related to dose and were well tolerated in patients with active RA. The results provide the first clinical evidence that the fully human antibody adalimumab can be safely and effectively used to treat RA. Patients from this study continue to be treated in long-term studies that will provide data on chronic safety and efficacy.
Acknowledgements
We would like to thank M Wibberg (Datamap GmbH, Freiburg, Germany) and Dr D Compagnone (Knoll AG) for data management and statistical support, Dr P Noertershäuser for pharmacokinetic analyses.

REFERENCES

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Chapter IV

Long-term anti-TNFα monotherapy in rheumatoid arthritis. Effect on radiologic course and prognostic value of markers of cartilage turnover and endothelial activation.

Alfons A den Broeder, Leo AB Joosten, Tore Saxne, Dick Heinegård, Helmut Fenner, Andre MM Miltenburg, W LH Frasa, Wim A Buurman, Piet LCM van Riel, Leo BA van de Putte, Pilar Barrera
Abstract

Aims To investigate the effect of prolonged neutralisation of tumour necrosis factor alpha (TNFα) on the radiologic course in rheumatoid arthritis (RA). To assess whether the radiologic course can be predicted by clinical variables or biological markers of cartilage and synovium turnover and of endothelial activation.

Patients and methods Forty-seven patients with active RA enrolled at our centre in monotherapy trials with adalimumab (D2E7), a fully human anti-TNFα monoclonal antibody, were studied for two years. X-rays of hands and feet obtained at baseline and after 1 and 2 years were scored in chronological order by a single, blinded observer using the modified Sharp method. Radiologic course was classified as stable or progressive using the smallest detectable difference as cut-off. The relationship between radiologic course and serum markers of cartilage and synovium turnover (metalloproteinases MMPs 1 and 3), cartilage oligomeric matrix protein (COMP), human cartilage glycoprotein-39 (HC gp-39)), endothelial activation (soluble E-selectin and ICAM-1) and integrated measures of disease activity were assessed using univariate and multivariate analysis.

Results At study completion, 42% of the patients presented no radiological progression. In this group, more patients were still on anti-TNFα therapy (87% versus 51%; \( P = 0.03 \)) and baseline COMP and sICAM-1 levels were lower (\( P = 0.01 \) and 0.04, respectively) compared to the group with progressive disease. In a logistic regression model these three factors in combination were predictive for radiologic outcome (\( P = 0.03 \)). CRP and DAS area under the curve were significantly correlated with changes in radiologic scores after two years (\( r=0.40 \) and 0.37, \( P < 0.05 \)). Long-term TNFα neutralisation decreased the levels of COMP, sICAM, MMPs and HC gp-39. sE-selectin was not decreased after two years.

Conclusion Our results suggest that long-term monotherapy with anti-TNFα has a positive effect on radiologic outcome and modulates cartilage and synovium turnover as measured by biological markers. Baseline serum sICAM-1 levels and moreover baseline COMP levels may be helpful to identify patients with progressive or non-progressive radiologic outcome.

Introduction

Rheumatoid Arthritis (RA) is characterised by a chronic polyarthritis leading to irreversible cartilage damage. In the last decade therapeutic options for the treatment of RA have been broadened by the development of specifically targeted biological agents. Among these, strategies aimed at specific neutralisation of pro-inflammatory cytokines, such as tumour necrosis factor alpha (TNFα) and interleukin-1 (IL-1) seem the most promising. Studies with monoclonal antibodies (MoAb) against TNFα and with TNF
receptor fusion proteins have proven the clinical efficacy of TNFα neutralisation in RA (1,2) and these treatment modalities have been introduced in clinical practice.

Adalimumab (D2E7) is a fully human IgG1 anti-TNFα MoAb developed using phage display techniques (3). Studies in more than 1200 patients have demonstrated the efficacy and safety of prolonged administration of this compound either alone (4-7) or in combination with methotrexate (8).

Adalimumab and other TNFα blocking agents have a very short lag-time to clinical response and induce a rapid decrease in non-specific acute phase reactants such as C-reactive protein (CRP) and ESR (9,10). Based on the short-term effects of TNFα neutralisation on several other biological markers, it has been proposed that this therapy reduces endothelial activation (11,12) and angiogenesis (13) and might be chondroprotective (14,15). Whether these effects are maintained during long-term anti-TNFα therapy and whether they correlate with outcome variables has not been elucidated yet.

The first reports with one-year follow-up indeed suggest that TNFα neutralisation retards radiological progression (16-18). Tissue derived molecular markers have been suggested as possible prognostic factors for future joint damage (19). If this can be verified, such markers could be attractive tools for treatment decisions and patient selection and also for monitoring of therapy aimed at slowing joint damage progression.

In the present study, the influence of long-term treatment with anti-TNFα therapy with adalimumab on radiological course was assessed. Furthermore early and long-term effects of this treatment on biological markers of cartilage and synovium turnover (cartilage oligomeric matrix protein (COMP), metalloproteinases 1 and 3 (MMPs) and human cartilage glycoprotein-39 (HC gp-39, also known as YKL-40)) and on markers of endothelial activation (sE-selectin and intercellular adhesion molecule-1 (sICAM-1)) were examined. The interrelationships and potential prognostic value of these markers on the radiological outcome after 2 years were also investigated.

**Patients and Methods**

**Patients**

All patients with RA included in phase I clinical studies with adalimumab monotherapy at our center were studied. Patients fulfilled the 1987 ACR criteria (20), had an active disease, defined by a disease activity score (DAS (21)) > 3.2 at baseline and underwent a washout period for DMARDs of at least 3 weeks prior to study initiation. Stable dosages of NSAIDs and steroids (dose <10mg/day prednisone equivalent) were allowed during the study. The 2-year follow-up encompassed a 6 to 8 weeks placebo-controlled, randomised, dose ranging (0.5 – 10 mg/kg) trial followed by an ongoing open label phase. In the latter, all patients, including those initially randomised to placebo, received adalimumab, at dosages of 3 mg/kg i.v. biweekly (5,6) or 1 mg/kg s.c. weekly (4). In the
intravenous clinical studies with adalimumab, a plateau in response was seen at the level of 1.0 mg/kg biweekly and beyond. The pharmacokinetics of subcutaneously administered adalimumab are the same three to four weeks after therapy start (4-7). Therefore, it was decided to pool data derived from these two treatment modalities. Anti-TNFα therapy was withheld in case of adverse events or lack of efficacy. The latter was defined as a DAS decrease of less than 1.2 in the placebo-controlled phase and according to the judgement of the clinical rheumatologist in the subsequent open phase. In case of withdrawal, patients were treated using the best available choice of DMARDs taking previous therapies into account.

Methods

Radiological and clinical assessments

Standard X-rays of hands and feet were obtained at baseline and after 1 and 2 years. Erosions, joint space narrowing (JSN) and total scores (erosion plus narrowing) were evaluated in sequential order by a single observer (AdB) blinded for therapy and clinical response using the Sharp/van der Heijde method (22). Readings were performed in chronological order since this method has been shown to be more sensitive to change than random readings (23). Moreover, instead of using an arbitrary cut-off point, the radiologic course of each patient was classified as stable or progressive using the smallest detectable difference (SDD) calculated according to Lassere et al (24,25). The SDD is the smallest change a measuring instrument can detect, taking the measurement error (in our case the intra-observer variation) into account. The SDD was calculated using a set of 75 radiographs from 25 patients assessed twice by the same observer within an interval of 9 months.

The area under the curve (AUC) for DAS and CRP were calculated using measurements obtained at baseline and every twelve weeks during the 2-year follow-up period. For patients who dropped out before study completion, data was collected during an additional post study follow up period of 12 months. If patients dropped out before the end of year one, data collected at the last clinical observation were carried forward. Radiograph data were then separately obtained at year 2.

Markers of cartilage and synovium turnover and endothelial activation.

Serum samples had been collected during the study and stored at –80°C until assay. For all markers, short-term changes after the first administration of anti-TNFα MoAb or placebo were analysed in samples collected at baseline, day 1 and day 14. Long-term changes were assessed using samples collected after a 2-year follow-up for all markers with exception of the MMPs. The latter were assessed at month 6 due to technical reasons. All samples from the same patient were analysed simultaneously to minimise inter-assay variations.
Systemic levels of COMP were measured using a previously described ELISA with an intra-assay variation of < 5% (26,27). The concentrations of collagenase (MMP-1) and stromelysin-1 (MMP-3) were measured by ELISA according to the manufacturer’s instructions (Binding Site, Birmingham; intra-assay variation <13%) (28). The adhesion molecules sE-selectin and intercellular adhesion molecule-1 (sICAM-1) were measured by ELISA as previously described (29,30). Intra-assay variation for both essays is < 10%. HC gp-39 levels were assessed as follows. Polystyrene 96-well microplates (Nunc Maxisorb C12) were coated overnight at 4 °C with 150 µL/well of 1.0 µg/ml mouse anti-HC gp-39 capture antibodies (HC gp-39 8B) in coat buffer (CB). The supernatant was discarded and the wells were incubated for one hour at room temperature with 200 µL PBS, 1.0 % BSA to block excess free binding sites. Subsequently, plates were washed three times with 500 µL PBST and incubated for one hour at room temperature with 100 µL sample, 100 µL recombinant HC gp-39 standard in assay buffer (PBS, 10 % FCS), 100 µL REF in assay buffer (ten times the highest STD) and 100 µL recombinant HC gp-39 control samples (at 5.0, 25.0 and 75.0 ng/ml) in assay buffer. Plasma samples were diluted 1:10 in assay buffer (10% FCS, 0.1% Triton-X, 0.2% Kathon CG in PBS) After this incubation the plates were washed three times with 500 µL PBST and incubated for one hour at room temperature with horseradish peroxidase labeled with mouse anti-HC gp-39 detection antibodies (HC gp-39 10B) at a dilution of 1:10.000 in assay buffer. Then, the plates were washed three times with 500 µL PBST and incubated for five minutes with 100 µL tetramethylbenzidine/ureum peroxide substrate solution (Organon Teknika). The enzyme reaction was stopped by addition of 50 µL 4 mol/L sulfuric acid to each well. The absorbencies were measured with a microplate reader. Calibration curves (0.15-24 ng/ml) for recombinant HC gp-39 were constructed using 4-parameter-logistic regression, and HC gp-39 concentrations in study samples and quality control samples were calculated with the data reduction package PhIRSt (Phoenix International Reporting System, version 2.1). The LOQ was determined at 0.15 ng/ml. The assay has been validated and the accuracy of the quality control samples was higher than 94.7% and the CV% was lower than 9.7%.

Statistical analysis:
Within-groups comparisons were made using paired Student’s t-test and Wilcoxon signed rank test. For comparisons between groups Mann Whitney U tests and chi-square test were used as appropriate. Correlations were tested with Pearson or Spearman correlation tests. The use of parametric or non-parametric test was dependent on the data distribution. P-values are reported without adjustments for multiple comparisons.
Multiple logistic regression analysis was performed to elucidate whether markers of cartilage and synovium turnover and endothelial activation had prognostic value using the radiologic progression from 0 to 2 years and from 1 to 2 years as dependent
variable. Additional independent variables considered were treatment status at study completion (0 = on therapy, 1 = withdrawal), IgM rheumatoid factor (0 = negative, 1 = positive) and the DAS and Sharp scores at baseline. Potential interactions between independent variables were tested prior to inclusion in the model. Analysis was performed using the Astute Base Module version 1.50 Package and SAS version 6.12.

Results

Clinical course

A total of 47 patients were studied whose characteristics are shown in Table 1. At inclusion, patients had active disease, long disease duration and had been refractory to a large number of DMARDs prior to anti-TNFα therapy. No significant differences between patients randomised to adalimumab or placebo were observed in the blinded study. The course of the follow-up is depicted in Figure 1.

<table>
<thead>
<tr>
<th>Table 1. Patient characteristics at baseline; patients with and without radiologic progression. Data expressed as mean ± SD unless otherwise stated.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Radiologic course</strong></td>
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<tr>
<td>Age (yr)</td>
</tr>
<tr>
<td>Female sex (%)</td>
</tr>
<tr>
<td>Disease duration (yr)</td>
</tr>
<tr>
<td>RF positive (%)</td>
</tr>
<tr>
<td>Previous DMARDs (nr)</td>
</tr>
<tr>
<td>Concomitant steroids (%)</td>
</tr>
<tr>
<td>DAS</td>
</tr>
<tr>
<td>Ritchie Articular Index</td>
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<tr>
<td>Swollen Joints (nr)</td>
</tr>
<tr>
<td>ESR (mm)</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
</tr>
<tr>
<td>Sharp score, median (25-75 perc)</td>
</tr>
</tbody>
</table>

As shown, a total of 41 (87%), 33 (70%) and 26 (55%) patients were still on therapy and maintained responder status after 6 months, 1 and 2 year follow-up respectively. Clinical improvement occurred rapidly after initiation of therapy. Two weeks from baseline 16 out
of 35 patients (46%) treated with adalimumab but none in the placebo group (0/12) had achieved responses according to the EULAR definitions. The course of the DAS and CRP in the whole patient group is shown in Figure 2.

![Figure 2](image.png)

**Figure 2.** Course of the median DAS and CRP during two-year follow-up in this study. Intention-to-treat analysis.

**Radiologic Course**
As expected from the long disease course, most patients had high radiologic scores at baseline (Table 1). In the whole patient group (radiographic data present at all timepoints in 36 patients), the median changes in radiologic scores after 1 and 2 years were 5 and 9 for erosions and 3 and 5 for narrowing respectively. For this study, the smallest detectable difference (SDD) in total Sharp/van der Heijde scores were 8 and 13 after 1 and 2 years, respectively. Using these cut-off points, a total of 53% (n=19/36) and 42% (n=21/36) of the patients presented no signs of radiological progression after 1 and 2 years follow-up respectively.
Comparison between the groups with stable radiologic course and those with radiological progression showed that the former group encompassed more patients still on therapy at study completion than the latter (87% [n=13/15] versus 51% [n=11/21] respectively; \( P = 0.03 \)). The Sharp scores at baseline tended to be lower and the
percentage of females higher among patients with stable radiological course but this did not reach statistical significance (Table 1).

Characteristics of patients who still received anti-TNFα treatment at study completion were similar to those who had switched to other therapies (data not shown) with the exception of the radiological course, which was stable in 54% (n=31/24) of the former but in only 17% (2/12) of the latter (P = 0.03).

**Relationship between radiologic course, disease activity and biological markers**

The serum levels of the biological markers at baseline were compared between patients with stable and progressive radiologic course. These groups only differed in baseline COMP and sICAM-1 levels, which were higher in the progressive group (P = 0.01 and P = 0.04 respectively; figure 3.). After two years, COMP and sICAM-1 had decreased in patients with X-ray progression (P = 0.0003 and P = 0.03 compared to baseline for COMP and sICAM-1 respectively) and remained low and unchanged in patients with stable radiologic course.

![Figure 3](image-url)

**Figure 3.** Concentrations of COMP [A] and sICAM-1 [B] in patients with and without radiologic progression. The boxes illustrate the mean (horizontal bar), 25 and 75 percentile (box) and 5 and 95 percentile (bars). Significance is shown as p<0.05, tested between groups (‡).
The primary reason for withdrawal is mentioned: patients that did not reach respondership after three administrations of adalimumab were withdrawn according to the study protocol.
12 months

adalimumab, n=33

n=7

Adverse events, n=3
Lack of efficacy, n=2
Remission, n=1
Death, n=1

2 years

adalimumab, n=26

Other Treatments, n=19

Methotrexate 6
Sulfasalazine 1
Cyclophosphamide 3
Steroids 2
Leflunomide 2
Gold salts 1
Azathioprine 2
DMARDs comb. 1
No therapy 1
Concomitant steroids 9
Correlations between baseline radiological and clinical variables and biological markers are outlined in Table 2. As shown, the classical acute phase reactant CRP correlated with both MMPs, HC gp-39 and, to a lesser extent, also with sICAM-1 but importantly not with COMP levels. COMP and HC gp-39, were significantly correlated with age and this was not due to a confounding effect of disease duration (data not shown).

We performed univariate analysis to assess which variables could be prognostic for changes in radiologic scores over time. The changes in total Sharp/van der Heijde scores between 0 and 2 years showed modest correlations with the AUC for CRP and DAS ($r = 0.40$ and $0.37$ respectively; $P < 0.05$) and with baseline COMP and sICAM-1 levels ($r = 0.30$ and $0.37$; $P = 0.06$ and $0.04$ respectively). None of the biological markers or clinical parameters at baseline showed a significant correlation with the radiologic changes between 1 and 2 years follow-up (data not shown). All analyses were also performed for both patient groups (adalimumab treated patients and patient that dropped out) separately. This approach did not yield different results (data not shown).

| Table 2. Correlations between radiologic score, acute phase and markers at baseline |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                | Duration        | SE-select       | sICAM-1         | MMP-1           | MMP-3           | HC gp-39        | COMP            |
| Sharp score                    | 0.49 †          | ns              | ns              | 0.50 †          | ns              | 0.39 *          | ns              |
| Age                            | 0.48 ‡          | ns              | ns              | 0.33 *          | ns              | 0.56 †          | 0.57 ‡          |
| CRP                            | 0.33 *          | 0.46 †          | 0.65 ‡          | 0.53 ‡          | 0.56 †          | ns              |
| sE-selectin                    | 0.35 *          | 0.36 *          | ns              | ns              | ns              |
| sICAM-1                        | 0.35 *          | ns              | ns              | ns              |
| MMP-1                          | 0.45 †          | 0.39 *          | ns              |
| MMP-3                          | 0.37 *          | ns              |
| HC gp-39                       | ns              |

* $R$ is shown

$P < 0.05$ (*); $P < 0.01$ (†); $P < 0.001$ (‡); not significant (ns).

Multiple Logistic Regression analysis results.

Two models, using the radiological changes between 0 and 2 years and between 1 and 2 years respectively as dependent variable were initially considered. The latter was abandoned after the negative results of the univariate analysis. The final logistic regression model using presence/absence of radiologic progression from 0 to 2 years as dependent variable included the following independent variables: treatment status (on therapy or dropout; 0 or 1), baseline sICAM-1 and baseline COMP. Rheumatoid factor (RF) was not included as explanatory variables, since all but one patient were RF
positive. The DAS and CRP at baseline were also left out of the model since they did not differ between patients with stable and progressive course (Table 1). Although baseline Sharp scores were not associated with progression using univariate tests ($r = 0.28$, $P = 0.09$), this variable was tested in the model, because differences - although not significant - were found at baseline between patients with and without progression (Table 1).

The logistic regression model is shown in Table 3. Patients remaining on anti-TNF$_\alpha$ therapy had a four-fold higher chance to have stable radiologic course. Higher COMP levels at baseline were associated with worse radiologic outcome independently from the effect from the treatment. The association of baseline sICAM levels with radiologic outcome largely disappeared. As shown, none of these three individual variables reached significance, but the complete model had a significant explanatory effect on the radiologic progression as indicated by the $P$ value for the chi-square for covariates (0.03). The disappearance of significance for the individual independent variables between the two treatment groups in the logistic regression model is caused by listwise deletion of missing values at baseline (1 COMP level and 1 sICAM level). Inclusion of baseline Sharp in the model scores did not change these results (data not shown).

| Table 3. Multiple Logistic Regression results of predictors for radiologic progression |
|-----------------------------------------------|-----------------|-----------------|---|
| Dependent variable                           | Progression 0 – 2 year |
| Chi-square for covariates                    | $\beta$ | OR          | CI          | $P$  |
| Independent variables                         |          |              |              |     |
| Treatment at two years (0=on therapy, 1=drop-out) | 3.99   | 0.60 ; 26.30 | 0.15        |
| Baseline sICAM-1                              | 0.09   | -0.15 ; 0.33 | 0.43        |
| Baseline COMP                                 | 0.41   | -0.02 ; 0.84 | 0.06        |
| Intercept                                    | -5.64  |              |             |     |

Parameter estimate ($\beta$), standard error (SE), confidence interval (CI), independent variables $(x_1...x_k)$, dependent variable is $1$

$$1 + \exp [- (\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3)]$$

Markers of endothelial activation and cartilage turnover

Short-term and long-term changes in the marker levels during anti-TNF$_\alpha$ therapy are outlined in Figure 4. Short-term measurements within 2 weeks after the first dose, allowed comparison between anti-TNF$_\alpha$ and administration of placebo. As observed,
none of the markers showed significant changes after placebo. In contrast, the levels of MMP-1, HC gp-39 and the adhesion molecules sE-selectin and sICAM-1 decreased significantly ($P < 0.05$) and MMP-3 also tended to drop (NS) within two weeks after starting anti-TNFα therapy. This was not the case for COMP levels that remained stable during the first two weeks.

Long-term measurements allowed comparison between patients still on anti-TNFα treatment and those who dropped-out and were treated with DMARDs. HC gp-39 and COMP levels were significantly decreased after 2 years ($P < 0.0001$ and $< 0.001$ respectively) irrespective of the therapy that patients received at this time point (Figure 4 lower panel). The levels of sICAM-1 values showed a modest decrease only in patients who were still on anti-TNFα therapy ($P < 0.05$) but not in those patients who had switched to other treatments. In contrast, the final concentrations of sE-selectin did not differ from baseline values (Figure 4 mid panel). Further reductions in MMP-1 and 3 concentrations ($P < 0.05$) were found after 6 months of therapy in the whole patient group (Figure 4 upper panel). At this time point, the number of patients who had switched to other therapies was too small to allow for comparisons with those still on anti-TNFα.

**Discussion**

Radiologic assessment remains the cornerstone of outcome assessment in RA, but has important drawbacks such as the extensive lag-time to change after therapeutic interventions. This has resulted in an intensified search for predictors of outcome in RA in recent years. The need for better monitoring of clinical decisions and aspects of cost-effectiveness is especially high for novel and expensive biological agents such as those directed against TNFα. Potential predictors for outcome could be used a priori for adequate patient selection or for adjustment of therapy.

This is, to our knowledge, the first long-term follow-up study on both the effect of anti-TNFα monotherapy on radiologic course and on prognostic value of biological markers of cartilage and synovium turnover and endothelial activation.

Concerning the radiological assessments, our findings support the notion that sustained TNFα neutralisation can modulate the outcome in advanced RA. First, a stable radiologic course was observed in 54% of the patients who were still receiving anti-TNFα therapy after two years compared to only 17% of the patients who had to switch from anti-TNFα to the best available choice of antirheumatic drugs during the same observation period. Although dropout patients had been treated for variable amounts of time with adalimumab, this fact should most likely have made differences between the two groups only smaller. The same holds true for the possible positive effect of treatment with DMARDs in patients that had dropped out. Switching was mostly due to other reasons than lack of response (Figure 1), which suggests a positive effect of anti-
TNFα in modulating the radiologic course. Also, our results show that blocking TNFα resulted in a rapid and sustained reduction of CRP and DAS and that radiologic progression is associated with time-integrated measures of these variables. Such a relationship has been previously described in patients with RA receiving conventional DMARD therapy (31) but not in patients with longstanding disease treated with anti-TNFα.

Recently published reports on random X-ray readings after one-year treatment with this (18) and other anti-TNFα strategies in patients with advanced disease (17) and early RA (16) have suggested that blocking TNFα can arrest radiological progression in RA. Our findings show that this may be the case in most patients who remain on therapy for prolonged periods. It should be stressed that the follow-up in our study was longer than in previous reports and that X-rays were scored in chronological order as originally described and recently recommended (32). In contrast to random readings, this method assumes that radiological damage is irreversible and thus detects more progression but is also more sensitive to change (23). Moreover, we addressed recent OMERACT recommendations (33,34) by taking intraobserver reproducibility into account.

Regarding the effect of anti-TNFα therapy on biological markers, we observed a rapid and sustained down-modulation of non-specific acute phase reactants such as CRP. With the notable exception of COMP, all markers studied correlated positively with CRP levels and most of them dropped within two weeks of therapy initiation. This is in line with previous data on the kinetics (35,36) and on the effect of anti-TNFα on these markers in patients with RA (11,14,15). COMP levels in contrast, did not change over the short term and our results confirmed the acute-phase-independent nature of this marker (37,38).

Our study was directed to identify potential predictors of radiologic outcome. Analysis of the relationship between biological markers and radiologic course yielded some interesting findings. Among the biological markers studied, univariate analysis showed that only baseline COMP and sICAM-1 had moderate correlations with radiologic course over the 2-years follow-up. This was corroborated by the fact that patients with X-ray progression had significantly higher levels of COMP and sICAM-1 compared to those with stable radiologic course ($P = 0.01$ and $0.04$ respectively). These findings are of interest since previous studies have found that high COMP levels were prognostic for large joint destruction in RA (37), Månsson thesis, Lund 1999. Our data suggest that, in contrast to data published concerning early RA (38), COMP levels might also predict small joint damage in RA. sICAM-1 is known to be upregulated in synovial tissue and its soluble form is found in high concentrations in patients with RA (39). Down-regulation of the expression of this and other adhesion molecules has also been implicated in the mechanism of action of anti-TNFα (11,12) and other antirheumatic drugs (40,41).
Figure 3. Short and long-term changes in markers of endothelial activation (sE-selectin [C], sICAM-1 [D]) and cartilage and synovium turnover (metalloproteinases MMP-1 [A] and MMP-3 [B], cartilage oligomeric matrix protein or COMP [E] and human cartilage glycoprotein-39 or HC gp-39 [F]). The boxes illustrate the mean (horizontal bar), 25 and 75 percentile (box) and 5 and 95 percentile (bars). Short-time changes: changes observed within two weeks after the first dose of anti-TNFα (continuous line) or placebo (dashed line) in the double-blind phase. Long-term changes: changes occurring between baseline and the two years follow up, patients still on therapy (gray boxes) or dropout (white boxes).

MMP-1, MMP-3, sE-selectin and sICAM-1 levels are displayed in μg/l, COMP and HC gp-39 levels are shown in mg/l. Significance is shown as p<0.05, tested within groups (†) and between groups (‡).
In addition, antibodies that block ICAM-1 have been tested with favourable results in RA patients (42). The relation of baseline sICAM-1 levels with radiological course has however not been previously described, although higher sICAM-1 levels have been found in patients with radiologic damage in a cross-sectional study (43).

Circulating MMPs were associated with acute phase parameters but not found to be prognostic for radiologic course up to two years or between one and two years in our study. This relationship has been previously suggested in patients with early RA (44,45). The long median disease duration of our study population is most likely the reason for this difference, as also another study including patients with longstanding RA yielded a negative result (46). Whether differences in therapies used in our and above-mentioned studies also contribute to this apparent contrast is not clear.

Besides the higher baseline levels of COMP and s-ICAM-1 (Figure 4) and the higher drop-out rate observed among patients with progressive radiologic course, this group tended to have higher baseline X-ray scores than those showing no progression (Table 2). This difference was, however, not significant and neither disease activity nor radiologic damage at baseline were prognostic for radiologic course. Inclusion of the latter in the regression model did not change the results of the model.

Logistic regression using baseline COMP, sICAM-1 and therapy status at completion demonstrated that these independent variables together had significant prognostic value for the radiologic outcome after two years, although each individual variable failed to reach significance in this statistical model. This could be due to the size of the sample and confirmation of these data is therefore needed in a larger cohort. The model, however, shows that both the treatment with anti-TNFα and baseline COMP levels are independently associated with radiologic outcome.

Although this was not the main goal of our study, the levels of biological markers at study completion were compared with those at baseline. Interestingly, decreases in COMP levels were observed irrespective of the therapy at study completion whereas sICAM-1 levels were lower than baseline only in patients still on anti-TNFα therapy (Figure 3, panel D for sICAM-1 and E for COMP). This association with therapy at study end might explain the disappearance of the prognostic value of sICAM-1 in the multivariate analyses. In patients with a stable radiologic course COMP and sICAM-1 levels remained low and unchanged. Of note, both MMPs and HC gp-39 levels but not E-selectin showed further decreases on the long term. Earlier studies showed that sICAM-1, but not sE-selectin levels were elevated in patients with RA (39). This shows that the endothelial markers sICAM-1 and sE-selectin are differentially regulated and do not possess the same properties as disease markers.

In conclusion, our study indicates that sustained anti-TNFα monotherapy with adalimumab has a positive effect on radiologic outcome. This intervention has rapid effects on biological markers of cartilage and synovial turnover and endothelial
activation, which are associated with the acute phase reaction but not on COMP levels, which strengthens the importance of serum COMP as a selective cartilage marker. Besides sustained anti-TNFα therapy during study period, only baseline COMP and to a lesser extent sICAM-1 levels were prognostic of the radiological course. These markers could potentially be used to identify patients at risk for development of radiological damage.

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Chapter V

Effects of therapy with a fully human anti-tumour necrosis factor $\alpha$ monoclonal antibody on the local and systemic homeostasis of interleukin-1$\beta$ and tumour necrosis factor $\alpha$ in patients with rheumatoid arthritis

Pilar Barrera, Leo AB Joosten, Alfons A den Broeder, Leo BA van de Putte, Piet LCM van Riel, Wim B van den Berg
Abstract

Objectives To study the short-term effects of a single dose of adalimumab, a fully human anti tumour necrosis factor (TNFα monoclonal antibody (MoAb), on the local and systemic homeostasis of interleukin-1β (IL-1β) and TNFα in patients with rheumatoid arthritis (RA).

Methods All patients with RA enrolled in a phase I, single-dose, placebo-controlled study with adalimumab in our centre were studied. Systemic cytokine levels, acute phase reactants and leukocyte counts were studied at day 0, 1 and 14 after the first administration of anti-TNFα MoAb (n = 39) or placebo (n = 11). The cellularity and the expression of IL-1β and TNFα in synovial tissue were studied in knee biopsies obtained at baseline and at day 14 in 25 consenting patients.

Results A single dose of anti-TNFα MoAb induced a rapid clinical improvement, a decrease in acute phase reaction and increased lymphocyte counts in patients with active RA. The protein levels of IL-1β in the circulation were low and remained unchanged but the systemic levels of IL-1β mRNA, \( P = 0.002 \) and the concentrations of IL-1β receptor antagonist (IL-1ra) and IL-6 \( P = 0.0001 \) dropped already within 24 hours and this persisted up to day 14. Systemic levels of TNFα mRNA were low and remained unchanged though total TNFα (free and bound) in the circulation increased after adalimumab, reflecting most likely the presence of TNF:anti-TNFα complexes \( P < 0.005 \) at day 1 and 14. Both TNF receptors dropped below baseline levels at day 14 \( P < 0.005 \).

Despite clinical improvement of arthritis, no consistent immunohistological changes were observed two weeks after anti-TNFα administration. Endothelial staining for IL-1β tended to decrease in treated patients \( P = 0.06 \) but not in responders. The staining for IL-1β and TNFα in sublining layers and vessels were mutually correlated \( r = 0.47 \) and 0.58 respectively, \( P < 0.0005 \) and the microscopic scores for inflammation correlated with sublining TNFα and IL-1β scores \( r = 0.65 \) and 0.54 respectively, \( P < 0.0001 \) though none of these showed significant changes during the study.

Conclusions Blocking TNFα in RA results in downregulation of IL-1β mRNA at the systemic level and in reduction of the endogenous antagonists for IL-1β and TNFα and of other cytokines related to the acute phase response, such as IL-6, within days. At the synovial level, anti-TNFα therapy does not modulate IL-1β and TNFα on the short term. The synovial expression of these cytokines does not reflect clinical response to TNFα neutralisation.
Introduction
In recent years, several approaches aimed at specific neutralisation of proinflammatory cytokines such as interleukin-1β (IL-1β) and tumour necrosis factor α (TNFα) have proven successful in chronic inflammatory diseases, such as rheumatoid arthritis (RA) (1-4) and Crohn’s disease (5,6).

In patients with RA, TNFα has been targeted using monoclonal antibodies (MoAb), either chimeric (2,3) or humanised (4), and TNF receptor-fusion proteins (7,8). Some of these TNFα antagonists have shown their efficacy in multicenter, placebo-controlled trials and have been approved by the FDA. A potential drawback of some TNFα blocking agents is the development of human anti-chimeric antibodies (HACA), which may hamper or shorten the therapeutic effects in the long-term. This complication could be overcome using MoAb devoid of murine regions.

Adalimumab (D2E7, Knoll-BASF Germany) is a fully human IgG1 MoAb anti-TNFα with high specificity for recombinant and natural TNFα and it is generated with phage display techniques (9). Studies in more than 1200 patients show that repeated intravenous or subcutaneous administration of adalimumab is safe and result in rapid clinical improvement in patients with active RA (10-13).

Several studies with another IgG1 anti-TNFα MoAb have clearly shown that blocking TNFα reduces the acute phase reaction and decreases the local and systemic levels of adhesion molecule in RA patients (14-17). In vitro studies have also shown that neutralisation of TNFα reduces the production of IL-1β in synovial cultures (18). Whether such therapies also downregulate the synovial expression of IL-1 and TNFα in RA has not been fully elucidated yet. Observations in small numbers of patients suggest that this might be the case (14,19,20). In the present study we investigated the short-term effects of the first dose of adalimumab or placebo on the homeostasis of the two main pro-inflammatory cytokines IL-1β and TNFα at the systemic and the synovial level.

Patients and Methods
Patients
Patients with RA according to the ACR criteria (21) and with active disease, defined by a disease activity score (DAS (22) > 3.2, enrolled in a double-blind multicenter clinical trial with adalimumab at our centre were studied. DMARD therapy was withheld and NSAIDs and/or low-dose oral steroids (< 10 mg/day) were kept constant in a 3-week washout period prior to and during the study. Patients were randomly assigned to receive 0.5, 1, 3, 5 or 10 mg/kg of adalimumab and each dose group included 2 patients treated with placebo, who received active drug at 6 weeks if they still fulfilled the entry criteria (11,13).

Adalimumab or placebo was administered as a slow i.v. infusion over 3 to 5 minutes. The preparation consists of a non-pyrogenic solution of 25 mg/ml adalimumab MoAb in 1.2% mannitol, 0.12% citric acid, 0.02% sodium citrate. The placebo consists of the same ingredients, except for the exclusion of adalimumab.
Results of the multicenter study including 120 patients in three centres show that clinical response is already apparent within 24 hours and maximal between 1 to 2 weeks after adalimumab administration (11,13). The clinical effect is maximal at a dose of 1mg/kg and shows a plateau in the dose response curve thereafter (11,13). Therefore, for the aims of this study, we focused on the short-time effects observed the first 2 weeks after infusion of adalimumab/placebo and subdivided the patients in three dose groups consisting of placebo (n = 11), 0.5 mg/kg (n = 8) and 1-10 mg/kg adalimumab (n = 31).

Concentrations and gene expression of cytokines in peripheral blood
Blood samples were drawn at 8-9 a.m. on day 0, 1 and 14 after infusion in endotoxin-free Vacutainer tubes with 15% EDTA-K3 (Becton&Dickinson, Rutherford, NJ). Samples were directly centrifuged (2250 g, 10 min and 15000 g, 5min) to assess circulating cytokine concentrations in platelet-free plasma. Aliquots were stored at -20°C until assay.

IL-1β was measured using a high sensitivity ELISA (sensitivity 0.1 pg/ml; Quantikine HS, R&D, Minneapolis, USA) according to the manufacturer’s instructions. IL-1ra was measured by radioimmunoassays (RIA) (sensitivity 40 pg/ml; inter- and intra-assay variation < 10% respectively). IL-6 and both soluble TNF receptors (sTNFR) were measured by ELISA (sensitivity 8 pg/ml and 0.1 ng/ml respectively) as previously described (23,24). Total TNFα was measured by ELISA (sensitivity 10 pg/ml). The latter had been validated in spiking experiments showing adequate TNFα recovery and no hampering by therapeutic concentrations of adalimumab (range 25 to 250 mg/l) (11).

Aliquots of 500 µL blood aimed for RNA isolation were mixed with guanidiumisothiocyanate (GITC) at 1:1 ratio and stored at -70°C. Isolation of whole-blood mRNA and reverse transcriptase polymerase chain reaction (RT-PCR) analysis were performed as previously described by Netea et al (25). Briefly, aliquots of 0.5 µg total RNA were diluted in 20 µl RT buffer (50mM Tris-HCL pH 8.3, 75 mM KCl, 3 mM MgCl₂) containing 10 mM dithiothreitol, 5 µM random hexamers, 250 µM dNTPs, 20 U RNASin and 200 M-MLV RT. RT reaction was performed for 10 minutes at 20°C, 45 minutes at 42°C and 10 minutes at 95°C in a Mastercycler 5330 (Eppendorf, Hamburg, Germany). PCR reaction mixtures consisted of 3 µl cDNA in 50 µl PCR buffer (20mM Tris-HCL pH 8.4, 50 mM KCl, 1.5 mM MgCl₂, 0.001% gelatine) containing 100 µM dNTPs, 1.25 U Taq polymerase and 0.3 µM of each primer (25). A total of 29 PCR cycles (denaturation 30 sec at 92°C, annealing 30 sec at 55°C and extension 90 sec at 72°C) were performed for IL-1β mRNA and TNFα mRNA and 24 cycles were performed for β2-microglobulin. PCR products underwent electrophoresis on 2% agarose gel and were stained with ethidium bromide. Gels were scanned on a densitometer and analysed using the Molecular Analyst™ software. Results are expressed as ratio IL-1β or TNFα mRNA to the housekeeping gene.
Synovial biopsies and immunohistochemistry

Percutaneous synovial biopsies of the knee were obtained with a Parker Pearson needle at baseline and 14 days after the first dose of adalimumab/placebo in all consenting patients. Biopsy was preceded by knee joint examination for tenderness (0 = none, 1 = response on questioning, 2 = spontaneous response, 3 = withdrawal) and swelling (0 = none, 1 = thickening without loss of bony contours; bulge sign, 2 = loss of bony contours; palpable but not tightly distending effusions, 3 = bulging synovial proliferation; tightly distending effusions). Macroscopic knee joint scores were calculated by adding up the tenderness and swelling scores. An average of 30 biopsies was obtained at each occasion and immediately fixed in 10% formalin and embedded in paraffin. Serial 7 μM microtome sections were mounted on superfrost slides and either stained with haematoxylin and eosin (HE) or used for histochemical staining as previously described (26,27). For each marker, all sections were stained in the same run to minimise interassay variations. Slides were incubated with anti-TNFα (IgG1, Monosan, Uden, The Netherlands) or anti-IL-1β MoAbs (IgM, 12E9, Oncogene Science, Manhasset, NY, USA). This primary step was followed by incubation with normal horse serum and with biotinylated horse anti-murine IgG. Slices were stained with avidin peroxidase (Elite kit, Vector, Burlingame, CA), developed with diaminobenzidin (DAB) and counterstained with haematoxylin for 3 minutes. Controls consisted of a) irrelevant primary isotype-specific IgG1 and IgM antibodies obtained from normal horse serum and b) by omitting the secondary antibodies.

All areas of each section were randomly analysed by two blinded observers using semiquantitative five point scales. HE sections were scored for the presence of lymphocytes, plasma cells and polymorphonuclear cells (PMN) with a scale ranging from 0 (no or minimal infiltration) to 4 (abundant inflammatory infiltrate), the lining hyperplasia was also scored (0 : 1-2, 1 : 3-4, 2 : 5-6 and 3 : > 6 layers). An “inflammation score” was calculated by adding these four components (range 0-15) as previously described by Tak et al (14). The staining for IL-1β and TNFα in lining, sublining and vessels was also scored semiquantitatively on a five-point scale (0 to 4). Differences in readings of 1 point were taken as the average, differences exceeding 1 point were resolved by mutual agreement.

Statistical Analyses

Analysis was performed using the SAS statistical package (SAS 6.04 PC version). Data are expressed as mean ± SD or as median (p25-p75) if appropriate. Within groups comparisons were analysed by paired Student’s t or Mann Whitney rank sum test. Baseline comparisons between groups were performed using one-way ANOVA or Kruskal Wallis tests. Correlations are expressed using the Spearman’s rank correlation coefficient.
Results

Clinical results, acute phase reaction and leukocyte subsets

The baseline characteristics of the patients included in the present study are summarised in Table 1. Patients were subdivided in placebo, 0.5 mg/kg and 1-10 mg/kg since, as previously mentioned, the dose-response curve in the multicenter study (n = 120) showed a plateau at doses of 1 mg/kg adalimumab (11, 13). Administration of adalimumab, but not placebo, resulted in a rapid reduction of disease activity as measured by the DAS, a composite disease activity score (22) (Figure 1 upper panel), and its individual components including swollen and tender joint counts, patient wellbeing (data not shown) and ESR (Figure 1 mid panel). In the group treated with 1-10 mg/kg adalimumab, the decrease in DAS was already significant within 24 hours post-infusion (mean ± SD 4.87 ± 0.79 versus 5.25 ± 0.95; \( P < 0.002 \)) and reached a nadir at week 2 (mean ± SD 4.06 ± 0.85, \( P < 0.0001 \)). The 0.5 mg/kg group showed a drop in DAS values significant at week 2 (\( P < 0.01 \)). At this time point, 65% and 37.5% of the patients treated with 1 - 10 and 0.5 mg/kg adalimumab respectively fulfilled the EULAR criteria for clinical response (28) in contrast to only one patient (9%) receiving placebo. Sixteen percent of the patients in the 1-10 mg/kg group showed clinical response already 24 hours after infusion.

Baseline acute phase reaction parameters such as the ESR, C-reactive protein (CRP) and platelet counts tended to be higher in the 1-10 mg/kg group (Table 1) and were also significantly decreased 2 weeks after administration of anti-TNFα MoAb but unchanged or increased in the placebo group (Figure 1 mid and lower panel). The 0.5 mg/kg group which included fewer patients (n = 8) also showed a clear decrease in ESR and CRP after 2 weeks.

Baseline white blood cell (WBC) and leukocyte subset counts were similar in all groups (Table 1). Total WBC counts did not change during the study (data not shown) but a rise in day 1 lymphocyte counts was observed in all groups, being more marked in patients receiving 1- of 10 mg/kg adalimumab (median 14.5% pre-infusion versus 22% at day 1; \( P < 0.01 \)). Only in this group, the lymphocyte counts were still higher than baseline at day 14. Conversely, there was a drop in day 1 polymorphonuclear cells (PMN) counts in all groups which only reached significance in the 1-10 mg/kg group at day 1 (median 77% at baseline and 66.5% at day 1; \( P = 0.02 \)).
Table 1. Baseline characteristics and response percentages according to the dose group.

<table>
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<th>Placebo n = 11</th>
<th>0.5 mg/kg n = 8</th>
<th>1 to 10 mg/kg n = 31</th>
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<td>53 ± 18</td>
<td>57 ± 14</td>
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<td>Female (%)</td>
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<td>RF positivity (%)</td>
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<td>97</td>
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<td>Disease duration, median (p25-p75) (years)</td>
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<td>8 (4 – 15)</td>
<td>10 (5 – 19)</td>
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<td>Previous DMARDs (n)</td>
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<td>4.5 ± 2.6</td>
<td>5 ± 1.7</td>
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<td>Prednisone (%)</td>
<td>72</td>
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<tr>
<td>DAS, mean ± SD</td>
<td>4.8 ± 1.1</td>
<td>5.2 ± 1.3</td>
<td>5.2 ± 1</td>
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<tr>
<td>Ritchie Articular Index, mean ± SD</td>
<td>22 ± 13</td>
<td>25 ± 11</td>
<td>26 ± 11</td>
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<tr>
<td>Swollen joint count, mean ± SD</td>
<td>18 ± 5</td>
<td>18 ± 5</td>
<td>18 ± 6</td>
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<tr>
<td>ESR, median (p25-p75) (mm/h)</td>
<td>24 (7 - 39)</td>
<td>15 (9 - 38)</td>
<td>36 (23 - 55)</td>
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<tr>
<td>CRP, median (p25-p75) (mg/l)</td>
<td>23 (6 - 90)</td>
<td>22 (12 - 42)</td>
<td>63 (33 - 115)</td>
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<tr>
<td>Haemoglobin (mmol/l)</td>
<td>7.6 ± 0.9</td>
<td>7.4 ± 1.2</td>
<td>7.2 ± 1.0</td>
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</tr>
<tr>
<td>Leukocytes, median (p25-p75) (x10^9/l)</td>
<td>8.1 (6.3-10.5)</td>
<td>7.5 (6.5-8.9)</td>
<td>7.2 (5.9-10.4)</td>
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<td>Polymorphonuclears</td>
<td>5.77 (4.76-8.12)</td>
<td>5.07 (4.55-6.51)</td>
<td>4.78 (4.05-8.16)</td>
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<td>Lymphocytes</td>
<td>1.16 (0.85-1.60)</td>
<td>1.37 (1.08-2.26)</td>
<td>1.14 (0.85-1.31)</td>
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<tr>
<td>Monocytes</td>
<td>0.52 (0.40-0.58)</td>
<td>0.42 (0.38-0.49)</td>
<td>0.46 (0.38-0.63)</td>
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<tr>
<td>Platelets mean ± SD (x10^9/l)</td>
<td>329 ± 48</td>
<td>306 ± 65</td>
<td>377 ± 130</td>
<td></td>
</tr>
<tr>
<td>Responders † at (%)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2 weeks</td>
<td>9</td>
<td>37.5</td>
<td>65 ‡</td>
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<tr>
<td>12 weeks</td>
<td>-</td>
<td>50</td>
<td>76.6</td>
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<tr>
<td>24 weeks</td>
<td>-</td>
<td>87.5</td>
<td>70</td>
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† Response according to the EULAR criteria for moderate and good response.
‡ 16 % of the patients showed clinical response already 24 hours after 1-10 mg/kg adalimumab administration.
Figure 1. Disease activity score (DAS; upper panel), ESR (mid panel) and CRP (lower panel) in patients receiving a single dose of placebo or anti-TNFα MoAb (0.5 mg/kg or 1-10 mg/kg doses; bottom). Boxes represent percentiles P25, P50 and P75; vertical lines indicate the P5 and P95 percentiles. Measurements on day 0, 1 and 14 after infusion (x-axis). Comparisons versus baseline shown as *, **, *** representing P < 0.05, < 0.005 and < 0.0005 respectively.
Systemic cytokine measurements
Baseline IL-1β and TNFα levels were below or around the detection limit of the RIAs currently used in our lab and similar to those found in healthy individuals (data not shown). Using a high sensitivity ELISA (Quantikine HS, R&D, Minneapolis, USA), IL-1β protein levels were very low and did not show significant changes after the first dose of adalimumab or placebo (median levels at baseline, day 1 and day 14 were 0.55, 0.4 and 0.5 pg/ml after 1 to 10 mg/kg adalimumab and remained 0.6 pg/ml before and after placebo administration).

Mean baseline TNFα levels (free and bound) measured by ELISA did not change after infusion of placebo but increased after adalimumab administration (41 pg/ml at baseline versus 76 and 63 pg/ml at day 1 (P<0.0001) and day 14 (P<0.01) respectively) which may reflect the formation of circulating TNF-anti-TNF complexes.

To complement the data at the protein level, systemic IL-1β and TNFα mRNA levels in whole blood were measured in the placebo and 1-10 mg/kg adalimumab group using RT-PCR. Baseline IL-1β/β2m ratios in patients with RA were elevated in relation to healthy controls (median [range] 1.45 [0.57-2.95] versus 0.35 [<0.10-0.69]). As shown in Figure 2, IL-1β mRNA expression decreased within 24 hours after adalimumab administration (P = 0.002) and remained lower than baseline at day 14 (P = 0.007) whereas no significant changes occurred after placebo. Systemic TNFα mRNA levels were lower than those of IL-1β overlapped largely with those found in healthy controls (median [range] 0.13 [<0.10 – 0.85] in RA versus <0.10 [<0.10 - 0.30] in controls) and remained unchanged during the study. Systemic levels of IL-1β and TNFα mRNA were not correlated (r [95%CI] = 0.13 [-0.18; 0.43])

The concentrations of IL-1ra and IL-6 decreased sharply after adalimumab administration (P<0.0001 and <0.005 at day 1 and 14 respectively in the 1-10 mg/kg group). Both sTNFR types decreased also after therapy. In patients receiving 1-10 mg/kg adalimumab, the p75 levels dropped from median baseline values of 5 ng/ml to 4.3 (P < 0.005) and 3.7 ng/ml (P = 0.0001) after 1 and 14 days respectively and the p55 sTNFR levels fell from median baseline values of 3.1 ng/ml to 2.5 ng/ml at day 14 (P < 0.002) (Figure 2).

There were positive correlations between acute phase reactants and the endogenous antagonists of IL-1β and TNFα. CRP measurements correlated with the levels of IL-1ra (r = 0.47; P < 0.001), p55 sTNFR (r = 0.66; P < 0.0001) and IL-6 (r = 0.65; P < 0.0001) while ESR levels correlated with p75 sTNFR (r = 0.51; P < 0.002). In contrast, neither TNFα nor IL-1β at protein or mRNA level was related to acute phase parameters.
Figure 2. Circulating concentrations of IL-1ra, IL-6 (upper panel), p55 and p75 sTNFR (central panel). IL-1β and TNFα mRNA in whole blood (lower panel) normalised for the presence of β2 microglobulin. Boxes represent P25, P50 and P75 percentiles; vertical lines indicate the P5 and P95 percentiles. Measurements on day 0, 1 and 14 after infusion (x-axis). Comparisons versus baseline shown as *, **, *** representing P < 0.5, < 0.005 and < 0.0005 respectively.
Immunohistological findings

A total of 25 patients underwent synovial biopsies at baseline and 14 days post-infusion of placebo (n = 8) or 1-10 mg/kg adalimumab (n = 17). At this time in the treated group, the DAS had dropped from 5.0 ± 1.0 to 4.0 ± 1.1 (P < 0.0005) and 11 patients fulfilled the EULAR criteria for clinical response (28). Conversely, DAS scores remained unchanged or increased (4.8 ± 1.3 at baseline versus 5.1 ± 1.6) in the placebo group where only one patient fulfilled the EULAR response criteria (28) (Figure 3, upper panel). The macroscopic joint scores for swelling and pain at the biopsied joint were unchanged 2 weeks after placebo but decreased in treated patients (P = 0.02; Figure 3, mid panel). The microscopic scores for inflammation (14) showed a more scattered pattern and no significant changes in either group (Figure 3, lower panel). Baseline microscopic inflammation scores were weakly related with the macroscopic scores for pain and swelling (r = 0.3; P <0.05).

TNFα and IL-1β staining were observed in all layers of most biopsies. The scores for both cytokines in the lining, sublining and especially in vessels were mutually correlated (r = 0.43; P = 0.002 for the lining and r = 0.47 and 0.58; P < 0.0005 for the sublining and vessels respectively). Nevertheless some differences were found in the staining patterns of both cytokines: TNFα showed a more dense and intense staining in sublining layers, lymphocyte aggregates and vessels whereas IL-1β tended to be more diffuse (Figure 4). The microscopic scores for inflammation were positively correlated with the scores for TNFα and IL-1β in the sublining (r = 0.65 and 0.54 respectively; P < 0.0001) and to a lesser extent with the TNFα staining in the lining (r = 0.38; P = 0.005).

The individual synovial scores for IL-1β and TNFα after administration of anti-TNFα MoAb or placebo are shown in Figure 5. As shown, there was a marked inter- and intra-individual variation in the synovial staining for these cytokines. Decreases larger than 1 point in the scores for IL-1β or TNFα were rare among placebo treated patients. On the other side, both increased and decreased scores for IL-1β and TNFα were detected in the lining and sublining of patients treated with anti-TNFα. With the exception of a trend to decrease in the IL-1β staining in vessels among the treated patients (P < 0.06), no significant changes were observed. Additionally, analysis was performed according to the clinical response achieved at day 14 after infusion. At this time point, a total of 12 patients (all but one in the group treated with anti-TNFα) were responders according to the EULAR criteria (28) (DAS decreased from 4.9 ± 1.1 to 3.4 ± 0.6; P < 0.0001) whereas 13 patients, 7 among them after receiving placebo, were non-responders (mean ± SD DAS 5.1 ± 1.2 versus 5.2 ± 1.2). Macroscopic knee joint score for swelling and pain dropped from 2 ± 2.3 to 0.8 ± 1.1 (P < 0.05) among responders but remained unchanged (2.2 ± 2.0 versus 2.2 ± 2) in the non-responders.
Figure 3. DAS, pain and swelling scores and histologic scores for inflammation in 25 patients undergoing serial biopsies. Time in weeks and placebo (n = 8) or anti-TNFα therapy (n = 17) shown in the x-axis. Scores for n > 1 patient (x exact number) shown in bold lines.
Figure 5. Semiquantitative scores for IL-1β (left columns) and TNFα (right columns). Staining in lining, sublining and vessels in biopsies taken at baseline and at day 14 after administration of placebo (n = 8) or anti-TNFα therapy (n = 17). Scores for n > 1 patient (x exact number) shown in bold lines.
In contrast with the clinical improvement observed in responders, the microscopic inflammation scores (HE) and the staining for IL-1β or TNFα in lining, sublining and vessels were not significant changed from baseline as compared to day 14 (data not shown). Subgroup analysis considering the individual dose of adalimumab administered yielded the same results.

Discussion
Our study demonstrates that in patients with active RA, a single dose of human anti-TNFα antibody in clinically effective and results in rapid downregulation of IL-1β mRNA at the systemic level. Such effect was observed in unstimulated conditions, using a sensitive PCR, normalised for the presence of β2 microglobulin, which corrects for fluctuations in peripheral blood mononuclear cells (PBMNC) counts. The inhibition of IL-1β by anti-TNFα MoAb therapy occurred at the transcriptional level and seemed rather specific. TNFα neutralisation did not alter the levels of TNFα mRNA in the circulation and no change in IL-1β or TNFα mRNA expression was observed in the placebo group.

The production of both IL-1β and TNFα is transcriptionally regulated (29,30) and both cytokines share many pro-inflammatory effects. Therefore, a decrease in IL-1β message might be involved in at least a part of the downstream changes observed after therapy with this and other anti-TNFα MoAb. These include inhibition of IL-6 and acute phase reaction, chemokines and nitric oxide production and decrease in angiogenesis, endothelial activation and cartilage breakdown markers (16,17,31). In our study, baseline mRNA levels of IL-1β in whole blood were elevated in RA patients compared with normal controls. Elevated levels of messenger RNA for IL-1β and other pro-inflammatory cytokines such as IL-8 have also been measured in isolated PBMNC of patients with RA (32, 33) and these may reflect an increased activation status of PBMNC in the circulation. Systemic TNFα mRNA levels were lower, and not correlated with those of IL-1mRNA, which is in line with the earlier reported differential regulation of IL-1β and TNFα in PBMNC (30,32) and in whole blood (29).

Inhibition of the synthesis of IL-1β after blocking TNFα has been shown to occur in rheumatoid synovial cultures (18,34). Direct evidence for such effect in patients with RA has been hard to find since, as we and others have shown (17), protein levels of IL-1β in the circulation are mostly low or undetectable.

Indirect evidence for an effect of blocking TNFα on the homeostasis of IL-1β can be sought in the effects of such therapy on the concentrations of its natural antagonist IL-1ra. This and other studies (35) have shown that the levels of IL-1ra are elevated in patients with RA and positively correlated with acute phase reaction parameters and with IL-6 levels. It has been proposed that in RA, the elevated levels of IL-1ra may reflect an increased production and/or activity of IL-1β (35). Therapy with adalimumab diminished the levels of IL-1ra and IL-6 with similar kinetics and such
effects have also been reported after the administration of cA2, a chimeric anti-TNFα monoclonal antibody, in patients with RA (15,36). The fully human anti-TNFα antibody used in this study is of the same isotype and clinically as efficacious and has other systemic effects common with the chimeric IgG1 anti-TNFα MoAb infliximab. One of those is the reduction of circulating levels of both soluble TNFα receptors after therapy. This has led to the hypothesis that anti-TNFα MoAbs act mainly through neutralisation of TNFα since this cytokine is a major regulator of TNFR release (17). However, decreases in circulating sTNFR levels are not specific for anti-TNFα MoAb therapy and probably mirror the acute phase reaction (24). Another common effect of both anti-TNFα antibodies was a transient rise in lymphocyte counts and a reciprocal drop in granulocyte counts (17) which has not been reported after blocking TNFα with TNFR:Fc fusion proteins. Whether this raise in lymphocytes is due to a deactivation of vascular endothelium, as proposed (37), or is a non-specific phenomenon as suggested by the similar trends seen in placebo group remains to be elucidated.

At the target organ level, significant reductions of pain and swelling at the biopsied joint occurred concomitantly with the systemic improvement observed 2 weeks after adalimumab but not after placebo. The improvement in the macroscopic inflammation scores was also evident when analysed according to the clinical response. The microscopic inflammation scores (HE) and the staining for IL-1β and TNFα in lining, sublining and vessels showed large intra-individual variations. Except for a trend to decrease in the endothelial staining for IL-1β among treated patients we observed no consistent changes two weeks after initiation of anti-TNFα therapy.

Previously Tak et al. reported a reduction in T cells and in the expression of adhesion molecules in RA synovial tissue after TNFα neutralisation (14, 20). It has also been suggested that the synovial expression of IL-1β and TNFα decreases four weeks after an initial dose of anti-TNFα using other TNFα blocking strategies. This was either reported in a very limited number of patients (14) and/or in abstract form (19, 20), which precludes firm conclusions.

Our results are in line with recent observations in experimental arthritis also show that anti-TNFα therapy does significantly reduce joint swelling (38) but leaves the production of IL-1β in synovium unaltered (39). That the local production and expression of IL-1β during arthritis is not driven by TNFα has been elegantly demonstrated in TNFα knockout mice (39). After induction of arthritis in the latter, there is a lack of joint swelling and a marked reduction in the late synovial infiltrate but an ongoing local production of IL-1β. Interestingly, the amelioration in swelling and infiltrate in these mice are abolished if the membrane-bound TNFα is reintroduced in the model (40).

Taken together, our results confirm that TNFα neutralisation with anti-TNFα MoAb in RA is very effective in controlling clinical inflammation and decreasing the acute phase reaction in patients with active RA. Blocking TNFα inhibits IL-1β at the transcriptional level in the circulation and this may be implicated in its mechanism of action. Our study further confirms that the effect of anti-TNFα MoAb is not due to
upregulation of the endogenous antagonists for IL-1β and TNFα since these decrease during therapy mirroring the acute phase reaction. TNFα neutralisation has a very rapid clinical effect and our goal was to analyse short-term systemic and local changes in the homeostasis of IL-1β and TNFα. In the short term, blocking TNFα did not modify the local expression of IL-1β or TNFα or the histological inflammation scores in the joint. Local changes therefore do not precede, or occur concomitantly, with systemic improvement. Whether such changes occur in a delayed fashion is now subjected to further studies.

Our results corroborate the discordance in macro- and microscopic signs of synovial inflammation. Moreover, they suggest that the synovial expression of TNFα and IL-1β, does not relate to concomitant signs of synovitis. Whether the former is predictive for future joint damage has not been elucidated yet. Nonetheless, in view of the independent regulation of these cytokines, future therapeutic efforts that combine neutralisation of IL-1β and TNFα are warranted in patients with RA.

Acknowledgements
We are indebted to Joachim Kempeni and Hartmut Kupper (Knoll, BASF, Germany) for their support. Pr. Dr. Helmut Fenner, Gelterkinden, Germany kindly provided the measurements on TNFα in the circulation. We like to thank Timothy Radstake and Anneke Hijmans for their collaboration in the collection of biopsies and RT-PCR.

REFERENCES


Figure 4 Immunohistochemical staining in the same patient at baseline (TNFα [A]; IL-1β [B]) and 14 days (TNFα [C]; IL-1β [D]) after the first dose of anti-TNFα. Note the unchanged expression of cytokines in synovial lining (s, arrows), sublining and blood vessels (bv). No IL-1β or TNFα staining was observed in controls with an irrelevant primary antibody (E) and omitting the secondary antibody (F). Original magnification 200x.
Anti-TNFα treatment does not affect hypothalamic-pituitary-adrenal axis activity in patients with rheumatoid arthritis
Abstract

Objective Treatment with TNFα neutralising agents yields rapid improvement of disease activity in patients with RA, and it has been suggested that this may be a cortisol-mediated effect. In this study we examined the effect of anti-TNF treatment on the activity of the HPA-axis in these patients.

Methods Eighteen patients with active RA were studied before and after an i.v. injection with a TNFα neutralising agent or placebo. Five patients were treated with the TNFR:FC receptor fusion protein Ro 45-2081 in a study by F. Hoffmann-La Roche, and eight were treated with the human monoclonal antibody adalimumab in a study by Knoll AG. Five patients were studied before and after an injection with placebo in the latter study.

Before the first injection with anti-TNFα, and on the day after injection, plasma ACTH, plasma cortisol and ESR were determined. Before and after injection 24 hrs urinary cortisol excretion was measured, circadian cortisol rhythm was measured in salivary samples, and a composite disease activity score (DAS) was determined.

Results DAS improved in the group of patients treated with anti-TNFα (mean decrease of DAS ± SD: 0.64 ± 0.46, P=0.0007), but not in the placebo group (0.09 ± 0.35). No changes were observed in the treatment group in plasma ACTH and plasma cortisol before and after injection. Neither were there significant changes in 24 hrs urinary cortisol excretion or in the circadian cortisol rhythm.

Conclusion Treatment with TNFα neutralising agents does not influence the activity of the HPA-axis and the achieved improvement is not due to changes in cortisol levels.

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterised by chronic inflammation, predominantly affecting the joints. Although its aetiology still remains to be elucidated, the pathophysiological mechanisms probably consist of a combination of genetical and environmental factors. Activation of the immune system, involving Th1 lymphocytes and several monocyte derived cytokines, has been shown to play an important role in RA. Bi-directional interactions between the immune system and the hypothalamic-pituitary-adrenal axis (HPA-axis) have been identified (1,2). In animal studies it has been shown that the proinflammatory cytokines interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumour necrosis factor α (TNFα) are able to increase the activity of the HPA-axis (3,4). Conversely, cortisol influences cytokine production and inhibits several cytokines, including TNFα.

TNFα is a proinflammatory cytokine, and in the joints of RA patients high levels of this cytokine have been detected, suggesting a crucial role for TNFα in the inflammatory process in RA. Inhibition of TNFα has clearly been shown to reduce disease activity in RA, and recent development of anti-TNFα treatment has been a major break-through in the treatment of RA (5-8). This treatment causes a rapid objective and subjective clinical improvement, which already occur on the first day after injection. The only other treatment of RA by which such a rapid improvement of
disease activity can be achieved is by treatment with corticosteroids. Considering the close interaction between the HPA-axis and cytokines, the question arose, whether the rapid effect of anti-TNFα treatment could be mediated through the HPA-axis. Relatively low plasma levels of cortisol have been reported in patients with RA, which are considered low regarding their level of active inflammation, suggesting a decreased activity of the HPA-axis in RA (9-11). If TNFα is involved in maintaining these reduced plasma cortisol levels, anti-TNFα treatment might restore these levels, resulting in clinical improvement. However, in animal studies i.v. administration of TNFα has been shown to activate the HPA-axis resulting in raised glucocorticoid levels (3,4). Blockade of TNFα could therefore be expected to lower cortisol levels and the rapid improvement in response to this treatment must be due to other factors than raising plasma cortisol levels. The aim of our study was to examine whether anti-TNFα treatment affects the activity of the HPA-axis in patients with RA.

Patients and Methods

Patients

Eighteen patients with RA, who participated in two different studies with a TNF-neutralising agent, were studied before and after an intravenous (i.v.) injection with the TNF-neutralising agent or placebo. The protocols were approved by the hospital’s ethical committee, and written consent was obtained from all patients. All patients fulfilled the American College of Rheumatology criteria for RA, and had active disease, as defined by a Disease Activity Score (DAS) > 3.2 (12). The use of a stable low dose of prednisone with a maximum of 2 mg daily was permitted, but patients were excluded if they had used oral corticosteroids in a dose above 2 mg daily in the previous year, or if they had received an intramuscular injection with corticosteroids in the previous 3 months. Patients using oral contraceptives or other drugs that are known to influence the HPA-axis were also excluded. The use of non-steroidal anti-inflammatory drugs (NSAIDs) was allowed.

Study design and measurements

Five patients were treated with the TNFR:FC receptor fusion protein Ro 45-2081 (F. Hoffmann-La Roche, Basel, Switzerland), and received an intravenous injection with 20 mg Ro 45-2081 before 12:00 a.m. Eight patients were treated with the human monoclonal antibody adalimumab (Knoll AG, Ludwigshafen, Germany) in a dose of 1.0 mg/kg (n=4), 3.0 mg/kg (n=3) or 5.0 mg/kg (n=1). This was administered before 12:00 a.m. by a slow intravenous injection. Five patients received an injection with placebo in the latter study.

In the week before the injection, blood was drawn at 9:00 a.m. for the determination of plasma ACTH, plasma cortisol and ESR, and a joint examination was performed in order to determine DAS. Blood for measurement of ACTH was immediately placed on ice and transported to the laboratory. Urine was collected for the determination of 24 hours cortisol excretion. Salivary samples were collected every 4 hours during 24 hrs
for the determination of the circadian cortisol rhythm, starting at 8:00 a.m. On the first day after the injection, all of these measurements were repeated.

**Laboratory assays**

Plasma cortisol levels were measured by radioimmunoassay (RIA) using an antiserum raised in rabbit against a cortisol-21-hemisuccinate-bovine serum albumin conjugate (13). The sensitivity of the assay was 0.02 μmol/l. The within- and between-assay coefficients of variation were 6.9% and 7.3% at 0.208 μmol/l and 4.2% and 5.6% at 1.03 μmol/l. Plasma ACTH was measured by an immunoradiometric assay (IRMA) based on two polyclonal antibodies (EuroDiagnostics, Arnhem, The Netherlands). Salivary cortisol as well as 24-hour urinary cortisol (i.e. extractable by organic solvents) were measured by RIA after previous extraction and paper chromatography (14).

**Statistical analysis.** Results are expressed as mean ± standard deviation (SD). Statistical comparisons were made within the RA group with paired Student's t-test, using a significance level of p < 0.05. The area under the curve (AUC) was calculated for the cortisol measurements in salivary samples during 24 hours. The small number of patients in the placebo group did not allow statistical analysis, but their results are described in the text and shown in the tables.

**Results**

The characteristics of the two treatment groups are shown in table 1. Thirteen patients were treated with a TNF-neutralising agent and 5 with placebo. All patients had active disease, with a DAS of 5.09 ± 1.18 (mean ± SD) in the treatment group and 4.87 ± 0.74 in the placebo group. ESR was 34 ± 24 mm/hr. in the treatment group and 31 ± 13 mm/hr. in the placebo group.

<table>
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<td>-----------------</td>
</tr>
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<tr>
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<td>Disease duration (yrs)</td>
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<tr>
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<td>Use of prednisone 2 mg daily</td>
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All values are expressed as mean ± SD or number of patients
In the treatment group, a significant improvement of disease activity was seen on the day after the injection with anti-TNFα, as shown by a significant decline of the disease activity score which was 5.09 ± 1.18 before and 4.48 ± 1.21 after injection (p < 0.05) (table 2). In the placebo group these figures were 4.87 ± 0.74 and 4.80 ± 0.57 respectively, showing no improvement. No effect was seen on ESR in both groups.

<table>
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</tr>
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<td>cortisol AUC</td>
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<td>cortisol 8:00 (nmol/l)</td>
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All values are expressed as mean ± SD

* P < 0.05 compared to before treatment

Figure 1. Circadian cortisol levels measured in saliva (nmol/l) before and after treatment with anti-TNFα; mean values ± SD.
Baseline values of plasma ACTH, plasma cortisol and cortisol in 24 hours urine collection, were all in the normal range, and cortisol in salivary samples revealed a normal circadian rhythm with peak values at 8:00 am. (Figure 1). We did not find a significant change in any of these parameters before and one day after an injection with anti-TNF\(\alpha\) treatment. Area under the curve calculated from the salivary cortisol rhythm also showed no significant difference before and after treatment. In the patients who received an injection with placebo, no change in these parameters before and after injection was observed either.

**Discussion**

At baseline patients with active RA had levels of ACTH and cortisol that were in the normal range. As mentioned earlier decreased levels of cortisol have been reported in RA, but also normal levels have been found in several studies. These normal levels however are considered inappropriately low for the degree of inflammation (15). Seven of the 18 patients were men, which is due to the fact that patients using oral contraceptives were excluded, leaving relatively more men than women to be selected for this study.

A significant improvement of disease activity was seen in the treatment group on the day after the injection with the TNF-neutralising agent, and no improvement in the placebo group. Despite this improvement no change was seen in plasma ACTH or cortisol levels after injection, showing that blockade of TNF\(\alpha\) does not affect the activity of the HPA-axis in patients with RA. As interactions between the HPA-axis and the immune system are complex, and probably involve several cytokines, which have been shown to stimulate the activity of the HPA-axis, blockade of one of them, TNF\(\alpha\), may be compensated by other cytokines, like IL-1\(\beta\) and IL-6, resulting in unchanged HPA-axis activity.

Our findings show that the rapid and significant clinical improvement as observed on the first day after anti-TNF\(\alpha\) treatment is not cortisol mediated. Probably the improvement is due to inhibition of local action of TNF\(\alpha\) in the joints, where the highest TNF\(\alpha\) levels are found. However, besides a clear decrease of pain and stiffness of the joints, patients also experience a general feeling of wellbeing and decrease of tiredness that cannot be explained by a local effect in the joints alone. This general improvement suggests that a systemic effect in response to anti-TNF\(\alpha\) treatment takes place as well. Patients who are treated with corticosteroids experience a similar effect, and one could speculate, knowing that corticosteroids inhibit cytokines, including TNF\(\alpha\), that inhibition of cytokines might play a role in the subjective improvement induced by corticosteroid treatment. However, corticosteroids have a wide range of action, and this might only be one aspect of the mechanism by which corticosteroids exert their effect.
In conclusion our study shows that TNFα blockade does not affect the activity of the HPA-axis in patients with RA, and that the rapid improvement in response to anti-TNFα treatment is not a cortisol mediated effect.

REFERENCES

Chapter VII

Isolated digital vasculitis in a patient with rheumatoid arthritis: good response to TNFα blocking treatment

Alfons A den Broeder, Frank HJ van den Hoogen, Leo BA van de Putte
Abstract
TNFα blocking agents are among the most promising new treatments for rheumatoid arthritis (RA). However, no data are present about the effect of these agents on extra-articular manifestations of RA. We describe a patient with small vessel vasculitis that repeatedly responded well to treatment with the soluble p55 TNFα receptor fusion protein Ro 45-2081 (lenercept).

Introduction
Symmetrical polyarthritis is the hallmark of rheumatoid arthritis (RA). In addition, pathology outside the joints can also be found. One of these so-called extra articular manifestations of RA is vasculitis. Vasculitis in RA usually affects small vessels and commonly involves the skin, causing nail-fold infarcts, and in more severe cases digital gangrene and leg ulcers (1). Less frequently, vasculitis of small and medium sized arteries complicates RA and causes damage to peripheral nerves and internal organs. Prevalence of vasculitis in RA varies from 1-5% but post-mortem studies show higher percentages up to 25% (2). Treatment of rheumatoid vasculitis depends on the size of the vessels affected and the impending organ damage. High doses of corticosteroids may be necessary, often in combination with immunosuppressive agents including azathioprine and cyclophosphamide to allow for long-term disease control and reduction of the corticosteroid dose. Treatment with these drugs is associated with considerable side effects.

In the last decade new agents have been developed for the treatment of autoimmune diseases. Among the most promising are TNFα blocking agents. Several agents that block TNFα have been developed and studied in patients with RA (3,4,5). TNFα blockade is achieved either with monoclonal antibodies against TNF1 or with fusion proteins containing human TNF receptors bound to a Fc component of a human IgG antibody. Although excellent efficacy in treatment of RA has been reported for several of these agents, no reports have thus far addressed the effect of TNFα scavenging on extra-articular manifestations. We describe a chance observation in a patient with RA and nailfold lesions responding repeatedly to treatment with the anti-TNFα receptor fusion protein lenercept.

Case report
A 46 year old woman was diagnosed with rheumatoid factor positive, erosive RA in 1982. From the beginning of her disease, ANA could be detected. Testing for disease specific autoantibodies was negative on several occasions. Her medical history was unremarkable. In the following years she was treated unsuccessfully with hydroxychloroquine, intramuscular gold salts, D-penicillamin, azathioprine, methotrexate, and a combination of sulfasalazine and methotrexate. Because of progressive joint destruction she received shoulder prostheses on both sides, an elbow endoprosthesis left, total knee joint replacement on both sides and a
spondylodesis of the first and second cervical vertebra was performed. No extra-articular symptoms were present except for sicca complaints. Due to the uncontrollable disease she was included in 1994 in a study with Ro 45-2081, a fusion protein combining two p55 TNF receptors with the Fc component of an IgG human antibody (lenercept) (6). After a three months placebo controlled phase she was treated with 50mg lenercept intravenously every four weeks. Clinical response was impressive with swollen joint counts decreasing from 32 to 5 and C-reactive protein CRP levels declining from 95 at baseline to 20 after the first injection. Low disease activity was sustained for the following years. Besides lenercept, her medication consisted of oral prednisone 5 mg a day and occasionally paracetamol 500 mg.

In the spring of 1999 she first noticed nailfold lesion on the fingers of both hands. These lesions disappeared after every injection of lenercept and reappeared three weeks thereafter when the effect of lenercept was decreasing (figure 1A and 1B). Clinical response indicated by a drop in joint counts and CRP was present two weeks after each anti-TNFα injection.

Discussion
We describe a patient with RA and small vessel vasculitis manifested by nailfold lesions responding to treatment with lenercept. The response has been well documented during two cycles of anti-TNFα administration. Although the role of TNFα in RA and systemic vasculitides, such as Kawasaki disease (7) and Wegener’s granulomatosis (8), is well established, its role in vasculitis complicating RA is less clear. In one study TNFα levels were found to be increased in patients with small vessel cutaneous vasculitis without RA (9). Nailfold lesions in RA usually have a favourable outcome and generally do not require additional treatment. Thus far, little attention has been paid to extra-articular manifestations in studies examining the efficacy of anti-TNFα treatment in RA. The prompt disappearance of nailfold lesions after lenercept administration observed in our patient could be an indication that blocking of TNFα might be effective in more severe forms of vasculitis and possibly other extra-articular manifestations of RA, some of which are life threatening and currently treated with high doses of corticosteroids and immunosuppressive drugs.

REFERENCES

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Figure 1.
Before (A) and two weeks after (B) injection with lenercept (Ro 45-2081, a sTNFR:Fc p55 fusion protein). Small petechia and surrounding erythema are seen along the nailfold (A). After injection, the lesions are no longer present and the erythema has dramatically improved (B).
Chapter VIII

Neutrophil migration and production of reactive oxygen species (ROS) during treatment with a fully human anti-TNFα monoclonal antibody in RA patients.

Alfons A den Broeder, Geert JA Wanten, Milan Tjioe, Wim JG Oyen, Ton Naber, Piet LCM van Riel, Pilar Barrera
Abstract

Objective To evaluate the effects of therapy with a fully human, anti-tumour necrosis factor monoclonal antibody (anti-TNFα MoAb), on the production of superoxide and other reactive oxygen species (ROS) and on the migration capacity of neutrophils in patients with rheumatoid arthritis (RA).

Patients and methods A total of 29 patients with active RA and 25 healthy controls participated in these studies. Assessments were performed at baseline and two weeks after the first administration of anti-TNFα. The production of ROS was studied both in unstimulated conditions and after stimulation of receptor-dependent (serum treated zymosan, STZ) and receptor independent (phorbol mystrate acetate, PMA) pathways by luminol enhanced chemiluminescence. Additionally, the PMA-induced burst production of superoxide was measured using the cytochrome-c reduction assay. The ex-vivo migration capacity of neutrophils was studied using a modified Boyden chamber model. Potential changes in neutrophil migration to joints and to a non-target organ were assessed by scintigraphy with autologous leukocytes and by histological examination of the skin exposed to ultraviolet B (UVB) irradiation respectively.

Results The baseline production of ROS (both spontaneously and after STZ stimulation) and superoxide and the ex-vivo chemotaxis were similar in RA patients (n=25) and healthy controls (n=25) and remained unchanged after administration of anti-TNFα. The production of ROS after PMA stimulation was slightly higher in RA than in controls (P = 0.04) and this difference disappeared two weeks after the first dose of anti-TNFα (P < 0.05). The scintigraphic study showed that, a single dose of anti-TNFα, but not placebo, markedly decreased the influx of leukocytes to inflamed joints. In contrast, leukocyte infiltration in UVB irradiated skin was not affected by anti-TNFα therapy.

Conclusion In patients with RA, anti-TNFα therapy rapidly decreases the influx of neutrophils into inflamed joints but does not impair either neutrophil migration to non-target organs or their chemotaxis and production of ROS.

Introduction

The efficacy of tumour necrosis factor alpha (TNFα) neutralising approaches in rheumatoid arthritis is nowadays well recognised (1,2). Despite the excellent efficacy/toxicity ratio of these agents, there are some still some concerns on the risk for infections (3-8) carcinogenesis (9-12) and autoimmune disorders (13,14) during TNF neutralisation. Some of these potential adverse effects could, at least partly, be related to an impaired function of neutrophils and other phagocytic cells, during TNFα neutralisation.

On the other side, modulation of the function of neutrophils and phagocytic cells could be beneficial in RA and other chronic inflammatory disorders. TNFα is involved in the priming, chemotaxis and production of reactive oxygen species (ROS) by neutrophils (15-17). These cells are abundant in RA synovial fluid and their
production of ROS and other inflammatory mediators may induce cartilage damage (18,19). Reduction in neutrophil priming, ROS production and chemotaxis have been implicated in the mechanism of action of other rapidly acting antirheumatic drugs such as methotrexate, leflunomide (20-23) and steroids (24). Whether this also holds true for TNFα blocking agents is still unknown. In patients with RA, administration of infliximab, a chimeric anti-TNFα monoclonal antibody, has been reported to reduce the influx of neutrophils into joints and to increase their number in the circulation (25). It has been hypothesized that this could be due to a decreased expression of adhesion molecules (26,27). Interestingly, no increase in circulating neutrophils has been reported during therapy with etanercept, a TNFR-Fc fusion protein. Whether blocking TNFα alters the physiological migration of neutrophils to inflammatory foci in "non-target" organs has not been assessed yet. Still, such effect could be relevant for the risk of infections during TNFα neutralisation especially since this strategy is often combined with agents that alter neutrophil chemotaxis such as methotrexate and steroids (24,28).

Adalimumab (D2E7, Knoll-BASF, Germany) is a fully human, IgG1 monoclonal anti-TNFα MoAb developed using phage display techniques (29) which is now undergoing phase III studies in RA. Its efficacy has been demonstrated in thousands of patients (30-33) and can be measured within days from therapy initiation. In this study, we evaluated the impact of a single dose of adalimumab on neutrophil function in patients with active RA. To this aim, the production of ROS and the chemotactic capacity of neutrophils were assessed using ex-vivo assays. Furthermore, the influx of neutrophils into inflammatory foci, including the joints and a non-target organ such as the skin, were analysed in vivo.

Patients and methods

Patients

Consenting patients with RA, enrolled in double blind, placebo-controlled studies with adalimumab monotherapy at our center were studied. All patients fulfilled the ARA criteria (34), had active disease, defined as a disease activity score (DAS) (35) > 3.2 and had undergone a 4-week washout period for disease modifying antirheumatic drugs (DMARD). NSAIDs and steroids, up to 10mg daily, were kept stable four weeks prior to and during the study. Patients were randomised to initiate treatment with adalimumab subcutaneously (20 to 80mg dose) or placebo. Ex-vivo and in-vivo neutrophil function test and white blood cell (WBC) counts were assessed at baseline and at week 2. At this point, clinical response according to the EULAR criteria (36) was also assessed. All ex-vivo neutrophil function tests were also performed in age- and sex-matched healthy controls. The study was approved by the ethical committee of the University Medical Center Nijmegen, the Netherlands.
Methods

Ex-vivo neutrophil function tests

All reagents used were from Sigma Chemicals (St Louis, MO, USA), unless otherwise stated. Neutrophils were isolated from heparinised blood (Vacutainer, Becton & Dickinson, Rutherford, NJ) as previously described (37). Briefly, whole blood diluted 1:1 in 0.4% trisodium citrate in PBS, was subjected to Percoll gradient centrifugation ($\delta = 1.076$ g/ml, Pharmacia, Uppsala, Sweden, 700xg, 18 min, 25°C). The neutrophil containing cell-pellet was resuspended in 50 ml ice-cold isotonic lysis solution (155 mM NH$_4$Cl, 10 mM KHCO$_3$ and 0.1 mM EDTA), centrifuged (5 min, 400xg, 4°C) and remaining erythrocytes were lysed (lysis solution, 5 min). Cytospin and trypan blue staining showed > 97% neutrophils and > 99% viable cells in all procedures. Neutrophils (final concentration 2 x 10$^6$ cells/ml) were kept at room temperature until assay.

The "long-term" (120 min) spontaneous and stimulated respiratory burst were measured as the production of reactive oxygen species (ROS) that excite luminol (luminol-enhanced chemiluminescence or LECL). To this aim, 200 $\mu$l neutrophils suspension (2 x 10$^6$ cell/ml) and 20 $\mu$l Luminol (5 x 10$^{-4}$ M) per well, were incubated in 96-well microplates at 37°C for 120 min in the absence or presence of a neutrophil stimulus. The latter consisted of serum treated zymosan (STZ), which induces ROS production through complement C3b receptor activation (39) or phorbol myristate acetate (PMA) which yields receptor-independent activation of protein kinase C. STZ and PMA were used at final concentrations of 1 mg/ml and 100 ng/ml respectively. Chemiluminescence was monitored every 30 seconds for 120 minutes using an automated LB96V Microlumat Plus plater luminometer (EG&G Berthold, Bad Wildberg, Germany). The integral area under the curve (AUC) over this period is expressed in relative light units (RLUs). The peak luminescence, expressed in per RLU/second, was calculated using Wingo software (EG&G Berthold, Bad Wildberg, Germany).

The "short-term" burst production of superoxide (O$_2^-$) was measured after stimulation with phorbol myristate acetate (PMA) for 5 minutes using the cytochrome-c reduction assay. The latter measures the superoxide dismutase inhibitable reduction of ferricytochrome c (38), which is expressed as the maximum rate of cytochrome c reduction (in nmol/minute/10$^6$ neutrophils at 550 nm) using 21.1 mM$^{-1}$cm$^{-1}$ as extinction coefficient (39). The assay was performed at 37°C on a thermostatted spectrometer (Perkin-Elmer Lambda 12, Perkin-Elmer Corp., Norwalk, CT, USA). Neutrophil chemotaxis was evaluated using a modification of the Boyden chamber assay (40). Briefly, neutrophils (1 x 10$^6$ /ml, 400 $\mu$l per well) and N-formyl-methionin-leucin-phenylalanin (fMLP, 10$^{-8}$ M, 600 $\mu$l per well) were loaded in the upper and bottom chamber of MilliCell-PC culture plates separated by isopore polycarbonate membranes (12 mm diameter, 10 $\mu$m thickness, 3 $\mu$m pore size) and incubated at 37°C for 1 hour. The number of neutrophils that reached the bottom chamber was counted using a haemocytometer (Coulter counter, Coulter electronics, Mijdrecht).
All ex-vivo neutrophil tests were simultaneously assessed in RA patients and age- and sex-matched healthy controls to correct for inter-assay variation.

**Neutrophil function in-vivo**

*Scintigraphy with technetium-99 labelled autologous white blood cells (Tc-99m-WBC)*

Aliquots of 50 ml venous blood were drawn, mixed with 10 ml methylcellulose containing 0.33% acid citrate dextrose (ACD). Red blood cells were allowed to sediment for one hour. The WBC containing supernatant was centrifuged (10 min, 150 g) and the resulting cell pellet was washed, centrifuged (10 min 150 g) and resuspended in 1.5 ml PBS with 1% human serum albumin (HSA). Thereafter, WBC were labelled with 1 GBq Tc-99m-hexamethylpropyleneamine oxide (HMPAO) at room temperature for 30 minutes. After centrifugation (10 min, 150 g), the WBC pellet was resuspended in 5% glucose and microscopically checked for cell integrity. Labelling efficiency (cell associated activity/total activity) was always > 75%.

A dose of 200 MBq Tc-99m-WBC was administered intravenously. Whole body scintigraphy was obtained 1 and 4 hours after injection, using a single head gamma camera equipped with a parallel hole low energy collimator (Siemens Orbiter, Siemens Inc., Hoffman Estate, IL). Joint uptake was scored semiquantitatively using a 0-2 scale (0 = no, 1 = equivocal, 2 = clearly increased uptake) by a blinded observer (41). The scored joints were the following: sternoclavicular, acromioclavicular and mandibular joints, shoulder, elbow, wrist, metacarpophalangeal joints, proximal interphalangeal joints, hip, knee, ankle joint (scored combined with subtalar joint), forefoot and metatarsophalangeal joints. For each patient, a total joint score was calculated by adding the values of all joint regions at baseline and this was set as 100%. Changes in scintigraphic scores at week 2 are expressed as percentage change from baseline.

**UVB skin model**

Physiological neutrophil migration to inflammatory foci in a non-target organ, was assessed using an ultraviolet light B (UVB) skin model. Briefly, a low dose UVB irradiation was administered to a rectangular skin area (2 x 2 cm) at the lower back, immediately before and two weeks after administration of the first dose of anti-TNFα MoAb. For each individual patient, the UVB dose was adjusted to encompass twice the so-called minimal erythema dose (2 MED). Punch biopsies of the skin were obtained before irradiation (control biopsy) and 24 hours after the first and second UVB irradiation. Biopsies were embedded in Tissue Tek OCT compound (Miles Scientific, Naperville, USA) and snap frozen.

Serial 7 μM cryostat sections were fixed in 100% acetone, air dried, washed in PBS and stained with the following monoclonal antibodies (Dakopatts, Copenhagen, Denmark): anti-elastase (neutrophils), WT-14 (monocyte-macrophage), T-11 (T-lymphocytes) and T-6 (Langerhans cells). This primary step was followed by incubation with normal horse serum and with biotinylated anti-murine IgG (Vecastain ABC kit, Vector Laboratories Inc, USA) for 30 min. Slides were then stained with an
avidin biotin complex solution, developed with 3-amino-9-ethylcarbazole (AEC, Calbiochem, USA), counterstained with haematoxylin and mounted in glycerol-gelatin. Incubation omitting the secondary antibodies was used as negative control. All areas of each section were randomly analysed by a blinded observer using a five point (0-4) semiquantitative scale (0 = no staining, 4 = marked staining).

**Statistical analysis**
Data are expressed as mean or median according to their distribution. Statistical analysis for paired observations was performed using Student’s t-tests and Wilcoxon signed rank tests as appropriate. Between-group comparisons were tested using the Mann-Whitney-U test. Correlation was tested using the Spearman rank correlation test. Analyses were performed using the Astute Base Module version 1.50 Package.

**Results**

**Patients**
A total of 29 patients enrolled in studies with adalimumab at our center, gave informed consent for different studies on the ex-vivo and in-vivo neutrophil function tests. After unblinding, 21 and 8 patients had been randomised to receive active treatment and placebo respectively. Baseline patient characteristics in these two groups were similar (table 1). Already two weeks after the first administration of anti-TNF MoAb 58% of the treated patients, but none of those receiving placebo, fulfilled the EULAR criteria for clinical response.

**Ex vivo neutrophil tests**
Ex vivo neutrophil function tests were performed in 25 patients with RA (18 receiving anti-TNFα and 7 placebo respectively) and 25 age- and sex-matched healthy controls. Total leukocyte counts and WBC subset counts in peripheral blood showed no significant increase in neutrophil counts 2 weeks after the first administration of anti-TNF MoAb (median neutrophil counts 5.86 x 10⁹/L and 6.13 x 10⁹/L at baseline and 2 weeks respectively P = 0.64).
Data on the capacity for ROS production and chemotaxis of neutrophils are shown in table 2. As shown, the production of ROS, including superoxide (O₂), and the ex-vivo chemotactic capacity of neutrophils were largely similar in RA patients and healthy controls (Table 2). At baseline, only the peak production of ROS after PMA stimulation was slightly higher in RA patients than in controls (8.6 ± 3.2 versus 6.8 ± 2.5 RLU/sec, P < 0.05; patient/control ratio 1.46 ± 0.28). No such difference was observed 2 weeks after anti-TNFα administration. The chemotactic activity and the burst production of O₂ after PMA stimulation did not change during the study (Table 2).
Table 1. Baseline patient characteristics

<table>
<thead>
<tr>
<th>Percentage, mean ± SD or median (p25-p75)</th>
<th>Adalimumab (n=21)</th>
<th>Placebo (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>55 ± 12</td>
<td>57 ± 10</td>
</tr>
<tr>
<td>Sex (%female)</td>
<td>62</td>
<td>75</td>
</tr>
<tr>
<td>Rheumatoid factor (%&gt;10 IE)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Disease duration (yrs)</td>
<td>13 ± 8</td>
<td>9 ± 6</td>
</tr>
<tr>
<td>Number of previous DMARDs</td>
<td>6 (4-8)</td>
<td>5 (3-6)</td>
</tr>
<tr>
<td>NSAID use (%)</td>
<td>95</td>
<td>100</td>
</tr>
<tr>
<td>Prednisone use (% of patients)</td>
<td>71</td>
<td>75</td>
</tr>
<tr>
<td>Disease Activity Score</td>
<td>5.4 ± 0.8</td>
<td>5.3 ± 0.9</td>
</tr>
<tr>
<td>Swollen joints 44/66 *</td>
<td>18.8/21.6</td>
<td>25.5/20.3</td>
</tr>
<tr>
<td>RAI/Tender joints 68 *</td>
<td>25.3/37</td>
<td>25.5/29.3</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>53 ± 42</td>
<td>71 ± 32</td>
</tr>
<tr>
<td>ESR (mm/hr)</td>
<td>33 ± 25</td>
<td>32 ± 20</td>
</tr>
<tr>
<td>EULAR response after two weeks</td>
<td>58%</td>
<td>0%</td>
</tr>
</tbody>
</table>

* in study 1 Ritchie Articular Index and swollen 44 were measured, in study 2 swollen 66 and tender joints 68 were used.
No statistically significant differences were present at baseline.

In-vivo neutrophil function

Scintigraphic evaluation with radiolabeled leukocytes was performed in 4 patients which had been randomised to anti-TNFα (n = 2) and placebo (n = 2). At baseline, a polyarticular pattern of uptake mirroring the clinically inflamed joints was observed in all patients (Figure 1). Scintigraphic joint scores obtained at baseline (absolute values ranging from 6 to 18) correlated with swollen joint counts (within-patient values ranging from $R = 0.3$ to 0.64; $P = 0.008$ to <0.0001).

Scintigraphic images obtained two weeks after the first dose of anti-TNFα showed a markedly decreased uptake into inflamed joints (Figure 1). In the treated patients 30% of the joints showing clearly increased leukocyte uptake (score 2) at baseline had became normal at week two (score 0) and 64% decreased to score 1. In contrast, no change from baseline values could be demonstrated in patients receiving placebo. Scintigraphic scores normalised for the baseline values were 53% and 33% in treated patients and 100 and 106% in the placebo group respectively.

The effect of anti-TNFα therapy on the physiological skin inflammatory reaction to UVB light was assessed in 12 patients all of whom had been randomised to anti-TNFα. Irradiation of the skin with UVB resulted in local erythema and, histologically, in a significant influx of neutrophils, lymphocytes and monocytes into the dermis ($P = 0.05$; table 3). The minimal dose of UVB required to reach skin erythema (MED) was 0.2 (0.1-0.2) Joules/cm² at baseline and did not change during the study. Immunohistological examination of the UVB irradiated skin before and 2 weeks after the first showed that the influx of neutrophils into the dermis did not decrease after administration of anti-TNFα (Figure 2). The same was true for the lymphocyte and monocyte infiltration (table 3).
Table 2. Neutrophil chemotaxis and ROS production, patients vs controls (n=25), treated (n=18) vs placebo (n=7).

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After</th>
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<tbody>
<tr>
<td></td>
<td>Patients</td>
<td>Controls</td>
</tr>
<tr>
<td></td>
<td>All patients</td>
<td>Anti-TNFα</td>
</tr>
<tr>
<td>Superoxide production</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.6 ± 2.3</td>
<td>8.5 ± 2.5</td>
</tr>
<tr>
<td>Chemiluminescence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spont. AUC</td>
<td>5.7 (3.7-11.5)</td>
<td>6.4 (3.1-11.6)</td>
</tr>
<tr>
<td>Spont. Peak</td>
<td>2.0 (1.0-2.7)</td>
<td>2.1 (0.9-3.5)</td>
</tr>
<tr>
<td>STZ AUC</td>
<td>25.8 ± 10.1</td>
<td>23.5 ± 8.6</td>
</tr>
<tr>
<td>STZ Peak</td>
<td>12.5 ± 3.9</td>
<td>12.2 ± 3.5</td>
</tr>
<tr>
<td>PMA AUC</td>
<td>11.6 ± 3.7</td>
<td>11.2 ± 3.5</td>
</tr>
<tr>
<td>PMA Peak</td>
<td>8.6 ± 3.2</td>
<td>8.5 ± 3.4</td>
</tr>
<tr>
<td>Chemotaxis</td>
<td>53.6 ± 18.5</td>
<td>49.1 ± 18.8</td>
</tr>
</tbody>
</table>

Superoxide production is expressed as nmol/minute/10^6 neutrophils, chemiluminescence is expressed as RLU/sec for peak value and AUC of the RLU/sec for AUC, chemotaxis is expressed as percentage of cells that passed the membrane. STZ serum treated zymosan

† P < 0.05, patients compared to controls at baseline

‡ P < 0.05, comparison patient/control ratio after treatment vs before treatment and treated patients vs placebo group
Table 3. UVB induced inflammation in the dermis before and after treatment with adalimumab (n=12)

<table>
<thead>
<tr>
<th></th>
<th>Control biopsy</th>
<th>Baseline</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td>0.0 (0.0-0.3)</td>
<td>1.0 (1.0-1.8)†</td>
<td>1.0 (1.0-1.0)</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>1.0 (1.0-2.0)</td>
<td>3.0 (2.0-3.0)†</td>
<td>2.3 (2.0-3.0)</td>
</tr>
<tr>
<td>Monocytes</td>
<td>2.0 (2.0-2.0)</td>
<td>3.0 (2.3-3.0)†</td>
<td>2.5 (2.0-2.9)</td>
</tr>
<tr>
<td>Langerhans cells</td>
<td>2.0 (1.0-2.3)</td>
<td>2.0 (1.3-2.0)</td>
<td>2.0 (1.0-2.0)</td>
</tr>
</tbody>
</table>

Cell counts are expressed on a semi quantitative scale from 0-4
† p<0.05, compared to control biopsy
No significant differences were found between baseline and day 14

Figure 1.
Scintigraphic image of a hand taken 4 hours after administration of Tc-99m labeled autologous leukocytes in a patient with RA. Before treatment (A) uptake is seen in the wrists (closed arrows) the second metacarpophalangeal joint of the left hand and several proximal interphalangeal joints (open arrows). Two weeks after the first injection of anti-TNFα (B), the activity in the wrists almost normalized and the increased leukocyte uptake in the finger joints disappeared.
Figure 2.
Skin biopsies after UVB irradiation in a patient with RA before (A) and after (B) the first administration of anti-TNFα. Staining for elastase (neutrophils) is performed. Irradiation with UVB leads to increased presence of neutrophils after 24 hours. This effect is maintained after treatment with anti-TNFα.
Discussion

Our results show that treatment with adalimumab drastically reduces leukocyte migration into synovial joints shortly after therapy initiation without inducing an increase in neutrophils in peripheral blood. The decreased homing of neutrophils to the target organ is not the result of a downmodulation in leukocyte function. Adalimumab does neither impair the production of ROS nor the chemotactic capacity of neutrophils ex-vivo or in-vivo. The physiological migration of neutrophils and mononuclear cells to an acute inflammatory focus in a non-target organ was not hampered by anti-TNFα therapy.

In our study, the reduction of neutrophil homing to the joints, was observed using scintigraphy with radiolabeled WBC. This is a sensitive method for assessing joint inflammation and correlates with clinical signs (42,43), in our case especially with joint swelling scores. Although only a limited number of patients were studied, the scintigraphic results were consistent (observed only in treated patients and not in the placebo group) and concomitant with the clinical response and the decrease in acute phase reaction (data not shown). Moreover, similar findings have been reported with another anti-TNFα MoAb (25). The decreased joint influx of neutrophils after anti-TNFα may be mediated through deactivation of the synovial endothelium, as suggested by the decrease of local and systemic levels of adhesion molecules (26,27) or by a decreased local production of chemoattractants such as IL-8 (44,45).

In view of the results in our study, a direct effect on the intrinsic chemotactic capacity of neutrophils seems very unlikely.

In contrast to the scintigraphic results, the acute inflammatory reaction in a non-target organ was not impaired by therapy. The acute skin model that was chosen for our study shares several features with a chronic inflammatory reaction such as that found in RA. An enhanced production of TNFα (46), upregulation of chemokines (e.g. IL-8) and adhesion molecules play a pivotal role in both processes. In spite of these similarities, TNF neutralisation does not hamper this acute reaction. Potential explanations for this paradox could be either that the production of TNFα exceeds the blocking capacity of the locally present anti-TNFα or that TNF-independent pathways play a major role in acute inflammation (47). Nonetheless, our results put forward the fact that in vivo TNFα blockade in therapeutic dosages does not interfere with the physiological inflammatory response.

The findings described here have several potential consequences. Firstly, it does not seem probable that suppression of neutrophil function or motility is implicated either in the mechanisms of action or in some of the potential adverse events of anti-TNFα therapy. This is especially true for the susceptibility to bacterial infections since the killing of these microorganisms by neutrophils and other phagocytes is largely dependent on the production of reactive oxygen metabolites.

Secondly, the knowledge that TNF blockade does not downregulate neutrophil functions can be used to design combination therapies with synergistic effects. In this context, several studies do suggest that some anti-rheumatic drugs such as steroids,
leflunomide and methotrexate and certain NSAIDs can modulate neutrophil ROS production and migration (20-23).

Some other observations in our study deserve further mention. Comparison of the neutrophil ROS production and migration between RA patients and healthy controls did not show important differences. Only the peak production of ROS after stimulation by receptor-independent pathways (PMA) was slightly higher in RA than in controls. This difference was not apparent two weeks after the first administration of anti-TNFα and was also not detected using time-integrated measures (AUC) or by the cytochrome-c reduction assay. Our findings suggest the neutrophil function in RA patients is not impaired and corroborates findings in several previous studies (48). Potential bias due to concomitant therapies cannot be definitively ruled out but these remained unchanged during the whole study period.

One potential limitation of the present study might be the short follow-up period. Therefore, these observations cannot be directly extrapolated to the situation during long-term TNF neutralisation. Nevertheless, the effect of this anti-TNFα MoAb and other TNF blocking agents is extremely rapid and the drug half-life of adalimumab is approximately 12 days (49). Moreover the life span of neutrophils is only a few hours for non-activated neutrophils and somewhat longer under inflammatory conditions (50). This motivates the time-schedule used in our studies and the hypothesis that short- and long-term anti-TNFα treatment will not differ much concerning their effects on neutrophils.

In conclusion, we found that treatment with the human anti-TNFα MoAb adalimumab effectively suppresses neutrophil migration into inflamed joints in RA. This therapy does neither interfere with the physiologic response to acute inflammatory foci nor with other neutrophil functions such as oxidative burst and chemotaxis.

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Chapter IX

A nephrotic syndrome as complication of anti-TNFα in a patient with rheumatoid arthritis.

Alfons A den Broeder, Karel JM Assmann, Piet LCM van Riel, Jack FM Wetzels
Abstract
Monoclonal antibodies directed against Tumour Necrosis Factor α (TNFα) have been developed in recent years for the treatment of patients with rheumatoid arthritis. We describe a patient with RA who developed a nephrotic syndrome during treatment with a recombinant human monoclonal antibody against TNFα. The proteinuria disappeared spontaneously after cessation of anti-TNF treatment. Upon rechallenge, the nephrotic syndrome reappeared, pointing to anti-TNFα as the culprit. Renal biopsy disclosed a membranous glomerulopathy. Clinically, the nephrotic syndrome was characterised by a highly selective proteinuria and immediate responsiveness to steroid treatment, more compatible with a diagnosis minimal lesions glomerulopathy than with membranous glomerulopathy. This is the first report of a glomerulopathy as adverse effect of anti-TNFα treatment. The potential mechanisms of this side effect of anti-TNFα are discussed.

Introduction
TNFα is a proinflammatory cytokine that plays a pivotal role in the inflammation in rheumatoid arthritis (RA). Increased levels of TNFα were found in the synovial fluid of patients with RA and in several animal models, the abrogation of TNFα activity was associated with a decrease of joint inflammation. These and other findings have stimulated the development of anti-TNFα strategies. Blocking the action of TNFα either with monoclonal antibodies directed against TNFα or TNF receptor constructs has been shown to yield fast and impressive responses in patients with RA. (1-3). Adverse effects were usually mild and no specific side effects have been reported except for reactions during infusion and at the local injection site. Two anti-TNFα agents have now reached clinical practice. We describe a patient who developed a nephrotic syndrome as adverse effect of treatment with anti-TNFα.

Case report
Our patient was diagnosed with a rheumatoid factor positive, ANA negative erosive rheumatoid arthritis (RA) in 1982 at the age of 48 yrs. His medical history was unremarkable, except for the presence of Chronic Obstructive Pulmonary Disease (COPD). In the following years he was treated with D-penicillamin followed by parenteral aurothioglucose. This was stopped in 1984 because of proteinuria up to 1.5 g/l. After cessation of intramuscular gold administration, the proteinuria completely disappeared. DMARD therapy was continued with methotrexate, followed by aurothioglucose that was stopped in 1990 because of exacerbation of the RA. Proteinuria did not appear during this second episode of gold therapy nor did it later. Sulfasalazine treatment was initiated, later combined with methotrexate and prednisone. The latter combination therapy was stopped due to lack of efficacy. In light of the persistent disease activity the patient was recruited in May 1997 for a placebo-controlled phase I trial with a fully human monoclonal antibody against TNFα.
Treatment with anti-TNFα was started in a dose of 1.0 mg/kg body weight every two weeks i.v. and later increased to 3.0 mg/kg according to the protocol. Concomitant medication consisted of prednisone 1 dd 10mg, diclofenac 2 dd 75mg, alternating calcium and etidronic acid, omeprazol 2 dd 20mg, paracetamol as needed 500 mg, ipratropiumbromide 4 dd 40 µg, salmeterol 2 dd 100 µg and doxycycline 1 dd 100mg. The latter drugs were prescribed for treatment of his COPD. There was an excellent clinical response to anti-TNFα treatment: the Disease Activity Score (DAS, a combined disease activity measure for RA) dropped from 5.89 to 2.41, swollen joint counts and tender joint counts (Ritchie Articular Index) decreased from 20 and 33 to 6 and 12 respectively, and C-reactive protein levels went from 142.8 to 24.2 mg/l. No serious adverse events were noted and treatment was continued uneventfully. Prednisone was gradually reduced to 2.5 mg/day.

In April 1998, the patient developed rapidly progressive pitting oedema of both lower extremities. He also noticed facial oedema and a weight gain from 73 to 84 kg in 2 weeks. Diuretic treatment was initiated but when his complaints worsened, he was admitted. On admission, blood pressure was 150/80 mmHg. Physical examination revealed bilateral leg oedema and evidence of pleural and peritoneal fluid. Laboratory investigations: serum creatinine 82 µmol/l, serum albumin 15 g/l, cholesterol 7.0 mmol/l, haemoglobin 5.5 mmol/l. ANA negative, normal complement C3 an C4. Proteinuria averaged 16.7 g/24 hrs, selectivity index was 0.07 (highly selective).

A renal biopsy was performed. By light microscopy of silver stained sections all glomeruli demonstrated segmental irregularities of the glomerular basement membrane (GBM) suggestive for a membranous glomerulopathy, such as vacuolisation - especially on tangential sections -, spike formation or some ring-like structures. Podocytes, endothelial cells and mesangial areas were normal (figure 1, A and B). Congo red staining did not reveal amyloid. By immunofluorescence fine granular deposits of particularly IgG and C3 were present along the capillary wall in a characteristic membranous pattern. The granules were unevenly distributed in some capillary segments (figure 2). No AA amyloid deposits were found using a monoclonal antibody. By electron microscopy podocytes were hypertrophic and showed retraction of their foot processes (figure 3). In some segments of the GBM irregularly spaced subepithelial electron dense deposits accompanied by spike formation were observed. Other segments displayed a broad and abnormal GBM, incorporating many complexes that were less electron dense and resolving (figure 3). Several segments of the GBM, however, did not show any alterations or electron dense aggregates.
Figure 1. Light microscopy of a part of a glomerulus. Some segments of the GBM show extensive vacuolisation (A; arrows), minor irregularities and spike-like conformations (B; arrows) on the epithelial side or bead like structures (B; arrowheads). A,B *900. Silver methenamine staining.

Figure 2. Direct immunofluorescence using FITC-labelled antihuman IgG antibody showing fine granular deposits of IgG along the capillary wall in a characteristic membranous pattern. In some capillary loops only a few granules are present (*400).
The patient was treated with diuretics and an Angiotensine Converting Enzyme (ACE) inhibitor, and anti-TNFα treatment and diclofenac were withdrawn. The subsequent course is depicted in figure 4. The proteinuria remained difficult to control up to three month after cessation of diclofenac and anti-TNFα treatment but eventually disappeared. Interestingly, symptoms of RA, which had been mild before and during the phase that the nephrotic syndrome was active, relapsed in the same week the nephrotic syndrome disappeared. We then tried to find out if drug treatment was the cause of the nephrotic syndrome. Diclofenac was started, however, proteinuria did not reappear until after restart of anti-TNFα treatment. The patient was treated with prednisone 60mg daily and cyclophosphamide 100mg daily. Within one week, proteinuria had completely disappeared. Cyclophosphamide was stopped and prednisone quickly tapered to 10mg daily. At this timepoint, anti-TNFα was reintroduced. The nephrotic syndrome did not reappear until prednisone was reduced to a dose of 5 mg daily. Again, the patient responded to a higher dose of prednisone. Since December 1998, the patient is being treated with anti-TNFα in combination with 10mg prednisone daily. He has retained an excellent clinical response and proteinuria has remained absent.

Discussion
In our patient a nephrotic syndrome developed during treatment with anti-TNFα. Renal biopsy disclosed a membranous glomerulopathy. The clinical course strongly incriminated anti-TNFα as the culprit, since proteinuria disappeared after withdrawal of anti-TNFα treatment and reappeared upon rechallenge. Furthermore, we could exclude that the other likely candidate, the NSAID diclofenac, was the cause of the nephrotic syndrome. To our knowledge, this is the first report of a nephrotic syndrome as adverse effect of anti-TNFα treatment.

Membranous nephropathy has been frequently reported in patients with RA. In a series of 110 biopsies in patients with RA, membranous nephropathy was observed in 19 (17%) (4). In most patients with RA and membranous nephropathy, treatment with gold salts or D-penicillamin has been incriminated as the cause. However, recent reports have suggested that patients with RA may be prone to the development of membranous nephropathy, even in the absence of gold or D-penicillamin treatment (5). It is however unclear if in these latter patients the use of NSAIDs as causative agents was excluded, as also an association between NSAIDs and membranous nephropathy was recently found (6).
Figure 3. Electron microscopy reveals subepithelial electron dense deposits in some segments of the GBM (A; arrows) In other segments of the capillary wall the GBM is thickened and extensively altered with incorporation of many resolving deposits showing variable lesser electron density (B,C; arrows). Extensive retraction of the foot processes of the podocytes (P) is present. A,B,C; *17000.
Figure 4. Clinical course of the patient. Proteinuria is depicted over time. Injection of anti-TNFα is indicated by the arrows. There is a clear relationship between the level of proteinuria, the use of anti-TNFα and lowering of prednisone.
Our patient may represent a case of membranous nephropathy caused by anti-TNFα treatment. One might speculate on the mechanism of action of this side effect. TNFα has been incriminated in glomerular diseases. In vitro studies have shown that TNFα can increase glomerular permeability, possibly by inducing oxygen radical production (7). Glomerular visceral epithelial cells can produce TNFα (8). Of particular interest for our case are the observations of Neale et al (9). These investigators have demonstrated the unique presence of TNFα in the visceral epithelial cells and in the subepithelial deposits in patients with membranous nephropathy. TNFα was not found in other forms of glomerular disease. Since membranous nephropathy is the result of an interaction between antibodies and antigens present on the surface of the glomerular epithelial cells (10), one can speculate that in our patient the membranous nephropathy was caused by interaction of the anti-TNFα antibodies with TNFα present on visceral epithelial cells. However, to the best of our knowledge, TNFα is only present in the cytoplasm of glomerular epithelial cells and an increased production is only found in patients with established membranous glomerulopathy. Furthermore, we found no evidence for TNFα in the glomerular basement membrane in our patient using biotin labelled IgG1 anti-TNFα MoAb or a polyclonal anti human TNFα by indirect immunofluorescence.

We feel that the clinical data points to an alternative explanation for the cause of the nephrotic syndrome. The clinical picture in our patient mimicked the nephrosis that is associated with minimal change nephropathy (11). Proteinuria was highly selective and there was an immediate and brisk response to treatment with steroids. In patients with established membranous nephropathy proteinuria disappears quite slowly if at all, even during treatment with immunosuppressive agents (12). In 34 patients with membranous nephropathy who were treated with high dose prednisone, we never observed a complete remission of proteinuria within three months after start of treatment (13). Likewise, in patients with RA with membranous nephropathy caused by either gold or D-penicillamin proteinuria disappears gradually, in most patients the maximal proteinuria is even reached 1-2 month after stopping the offending drug (14). In such patients, treatment with prednisone apparently does not influence the duration of proteinuria (15).

The finding of subepithelial deposits certainly does not prove that the proteinuria is caused by the membranous nephropathy. In the rat model of membranous nephropathy, injection of monoclonal antibodies against the podocyte antigen megalin results in the formation of small subepithelial deposits that are not paralleled by the development of proteinuria (12). Likewise, a membranous nephropathy with subepithelial deposits in the absence of proteinuria has been observed in hypertensive patients treated with captopril (16). Notably, in the study mentioned above in patients with RA, three patients with membranous nephropathy were described with no proteinuria (4). Furthermore, it is well established that in patients with membranous nephropathy the deposits can remain visible after remission has occurred, even for many years (17). In our patient, many deposits seem to be old, incorporated in the GBM and dissolving, suggesting that they may have been caused
by the previous gold therapy. Alternatively, the deposits may have been induced by the diclofenac treatment.

As noted, the nephrotic syndrome in our patient was highly steroid sensitive but also steroid dependent. A dose of 10 mg prednisone was able to prevent the reoccurrence of proteinuria. This latter aspect deserves attention. Since a considerable number of patients who receive anti-TNFα are concurrently being treated with prednisone, the potential of anti-TNFα to induce proteinuria may be masked. It remains to be established if this side effect becomes more frequent if anti-TNFα treatment is instituted in patients who do not receive steroids.

In conclusion, we have presented a patient with a nephrotic syndrome during treatment with anti-TNFα monoclonal antibodies. Clinically, the course of the disease closely resembled a steroid-dependent minimal change nephropathy.

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Chapter X

Dose titration using the Disease Activity Score (DAS28) in rheumatoid arthritis patients treated with anti-TNFα.

Alfons A den Broeder, Marjonne CW Creemers, Anke M van Gestel, Piet LCM van Riel
Abstract

Background Anti-TNFα therapy yields high response rates shortly after institution of therapy, and on theoretical grounds large differences in the effective dose between patients can be expected. Together with the high costs, these differences warrant new approaches to the way patients are dosed.

Patients and methods We used the Disease Activity Score (DAS28), a composite disease activity index, to titrate the dose of anti-TNFα (adalimumab, D2E7, Knoll) in 21 patients with low disease activity in an open extension study during 40 weeks. The dose of anti-TNFα was reduced stepwise and dosing intervals were kept stable. Disease activity and flares were assessed using the DAS28. Patients who flared received the previous effective dose.

Results Dose reduction was accomplished in 15 patients. The total amount of anti-TNFα given to the patients was reduced by 67 %. At study end the mean DAS28 had not changed and no patients dropped out due to persistent worsening of the RA.

Conclusion Dose titration of anti-TNFα treatment using the DAS28 is feasible and leads to overall dose reduction while maintaining clinical efficacy. This approach will save costs and possibly prevent long-term side effects.

Introduction

Treatment of rheumatoid arthritis (RA) has traditionally been centred round the use of Disease Modifying Anti Rheumatic Drugs (DMARDs) as being the main agents to reduce disease activity and influence the course of the disease. Although effective, no single agent from this group is able to fully control the disease in the majority of patients. Furthermore, the use of DMARDs is often limited by toxicity, leading to dose adjustments or cessation of therapy (1). In recent years, the increased knowledge of the mechanism underlying the inflammation as seen in RA has led to the development of new agents. In contrast to conventional DMARDs, which have mostly been discovered empirically, these so-called biologicals have specifically been designed to block either the action of immune cells or the cytokines produced by these cells (2). A number of these agents have been developed and clinically tested, the most promising being the agents targeting TNFα. The latter are expected to dramatically change current therapeutic strategies to control RA due to their impressive efficacy and low toxicity profile, although its’ exact place in the treatment of RA has yet to be established (3).

TNFα blocking agents have unique properties, distinguishing them from conventional DMARDs. Firstly, clinical effects occur within days to weeks compared to DMARDs, in which responses are seen no earlier than after four weeks to three months. Secondly, due to dose dependant toxicity it is often not possible for DMARDs to reach the dose that is necessary to obtain optimal clinical efficacy. For anti-TNFα agents in general no dose dependant toxicity has been found, although a study with infliximab suggested more upper airway infections in the higher dose groups (4-7). A third important difference is the expected large variation between patients regarding
the optimal dose of anti-TNFα. For conventional DMARDs the differences between the lowest and highest effective dose does not often exceed one order of magnitude; for example doses of methotrexate (MTX) range from 5 mg/week to 30 mg/week representing a 6-fold increase. In vivo levels of cytokines often differ with one or several order of magnitudes between RA patients (8). TNFα blocking agents bind TNFα in a fixed ratio (9), therefore, it is conceivable that also large differences can be found in the required doses of these agents. For obvious reasons in randomised controlled trials a restricted number of fixed doses are being studied. The use of standard dosing schedules in daily clinical practice will however induce gross under- and/or overtreatment. Prevention of the latter might reduce long-term side effects, since the first cases of diseases associated with impaired immune response have already been reported (10).

Tailoring treatment to the individual needs of the patient will also reduce the high costs, which are another hallmark of TNFα blocking agents compared to traditional DMARDs. The first TNFα blocking agent that has been approved for the treatment of RA (etanercept) is over 200 times more expensive than for example MTX (etanercept 25 mg twice a week: 14,000 Euro a year; MTX: 15 mg/week, 60 Euro a year).

For titration of the dose of anti-TNFα based on disease activity, it is necessary to monitor disease activity continuously. The Disease Activity Score (DAS28) is a continuous composite index for measuring disease activity in RA, which has been well documented and validated (11,12).

In order to test the hypotheses that there is a large difference between patients in the required dose of anti-TNFα and that titration of anti-TNFα treatment is feasible, a dose titration regimen using the DAS28 in patients with RA, treated with anti-TNFα, was studied.

**Patients and methods**

Patients with RA, treated in a clinical trial with anti-TNFα in our centre, were studied for 40 weeks. Patients had originally been enrolled in a 6 week phase I study and had been treated subsequently in an extension study for two years with intravenously administered anti-TNFα (adalimumab, D2E7, Knoll). During the second year of this extension study, the patients had been treated with a fixed dose of 3.0 mg/kg. Dosing intervals were either every two or four weeks depending on the clinical effect in the first year of anti-TNFα treatment, but were fixed for each patient and remained unchanged in the second year and during the present study. All patients were responding to treatment and showed a stable level of relatively low disease activity in the second year preceding this study. Treatment with other DMARDs was not allowed. Steroids equivalent to up to 10 mg/day prednisone were accepted and kept stable during the study.

Patients were seen every eight weeks to evaluate disease activity and toxicity. Disease activity was measured using the DAS28 (11). This disease activity index ranges from 0 to 10 and includes the 28 tender and swollen joint counts, the ESR
(Westergren, mm/hr) and the patient's general health (or patients' disease activity) measured using a Visual Analogue Scale (100 mm). A DAS28 > 5.1 means the patient has a high disease activity and a DAS28 < 3.2 means that the disease activity is low. A change in DAS28 > 0.6 constitutes a change greater than the measurement error of the DAS28. A change in DAS28 of > 1.2 (two times the measurement error) is a clinical significant change in the DAS28. Criteria for a disease flare were defined as an increase of the DAS28 of > 1.2 or an increase of the DAS28 of 0.6-1.2 if this resulted in a DAS28 > 5.1. The DAS28 is calculated as follows: DAS28 = 0.56*Vtender28 + 0.28*Vswwolen28 + 0.70*LnESR + 0.014*General Health.

Doses of anti-TNFα were decreased stepwise at every 8-weekly visit from 3.0 to 1.0, 0.5 and eventually to 0.25 mg/kg. In case a flare of the disease occurred, the dose of anti-TNFα was increased one step to the previous dose of anti-TNFα. In between the visits, the patients visited the outpatient clinic every two or four weeks for intravenous administration of anti-TNFα. A DAS28 was assessed during these drug administration visits only in case a patient reported a disease flare. This way, a prolonged flare of the RA was prevented.

The minimal individual required dose to sustain response was assessed using this protocol. The total weekly dose for each patient was calculated taking into account the different dosing intervals. A paired t-test was used to test DAS28 values at baseline and at the end of the study.

Results
Twenty-one patients were included in this study. Patient characteristics are shown in table 1. At start of the study the mean DAS28 was 3.5 (range 1.4-4.7). None of the patients had a high level of disease activity at the start of the study (DAS28 > 5.1).

Table 1. Patient baseline characteristics

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<tr>
<th>N=21</th>
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<tr>
<td>Age (yrs)</td>
<td>54 ± 14</td>
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<tr>
<td>Sex (f/m)</td>
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<tr>
<td>Disease duration (yrs)</td>
<td>14 ± 10</td>
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<tr>
<td>Number of previous DMARDs</td>
<td>4.7 ± 1.9</td>
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<tr>
<td>Prednisone use (mg daily dose)</td>
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<td>Rheumatoid Factor&gt;10IE/ml</td>
<td>96%</td>
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<tr>
<td>DAS28 at baseline</td>
<td>3.5 ± 0.8</td>
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In figure 1 the use of the DAS28 for titration of therapy is shown in one patient. At week 0 the dose of anti-TNFα was 3.0 mg/kg. This was lowered to 1.0 mg/kg at week 8. At week 16 the dose was further lowered to 0.5 mg/kg. This dose reduction resulted after two weeks in a flare with an increase of the DAS28 >1.2. The dose of
anti-TNFα was increased again to 1.0 mg/kg and remained unchanged for the rest of the study. The DAS28 quickly returned to baseline level.

Figure 1. DAS28 course and anti-TNFα dose titration in one patient. After a decrease in dose of anti-TNFα from 3.0 to 1.0 and subsequently to 0.5 mg/kg a flare of the disease occurs, reflected in an increase of the DAS28. After increasing the dose of anti-TNFα to 1.0 mg/kg the DAS28 returns to the previous low level.

The results of the dose titration are shown in figure 2. Six out of 21 patients were placed back on the original dose of 3.0 mg/kg after flaring on 1.0 mg/kg whereas 9, 3 and 3 patients respectively reached a dose of 1.0, 0.5 and 0.25 mg/kg. Required weekly doses of anti-TNFα ranged from 4.1 mg to 130 mg. The differences between the weekly required absolute doses of anti-TNFα were caused by differences in dose (3.0-0.25 mg/kg, factor 12), differences in dosing interval (every 2 or 4 weeks, factor 2) and differences in bodyweight (53-90, factor 1.7). As a result of this dose reduction, the median of the calculated weekly dose of anti-TNFα administered to these patients was reduced with 67% from 97.5 mg/week (range 46.3-135) to 32.5 mg/week (range 4.1-130). One protocol violation was noted: in one patient the dose was escalated while the increase of the disease activity did not meet the criteria for a disease flare with an increase of the DAS28 of 0.83 and a resulting DAS28 of 4.84.

Eighteen patients flared during the study, with a mean increase of the DAS28 of 1.3. Eight of these patients reported a flare in between the regular 8-weekly evaluations, the other ten patients were dose adjusted at the regular evaluation. Three patients
did not experience a flare even on the lowest dose of 0.25 mg/kg anti-TNFα. At study end, one patient had a DAS28 that was > 1.2 higher than the DAS28 at baseline. No patients had dropped out during the study due to persistent increase of the disease activity. The mean DAS28 at study end was 3.8 (range 2.0-5.1), which was somewhat higher but not significantly different compared with the DAS28 of 3.5 (range 1.4-4.7) at study start.

**Figure 2.** Results of the anti-TNFα dose titration. The total weekly doses of the individual patients (n=21) before and after the dose titration phase are shown in mg/week. Medians are depicted with a horizontal bar. The differences before the dose titration study between individual patients are caused by differences in dosing interval (every 2 or 4 weeks) and differences in body weight.

**Discussion**
To our knowledge this is the first report of the use of a dose titration regimen to tailor anti-TNFα treatment in the individual patient. Although our study included only a limited number of patients, it demonstrates the Proof of Principle of dose titration and the advantages of this approach compared to the common ‘one size fits all’ standard dosing schemes.
The importance of monitoring disease activity in daily clinical practice has recently been recognised and the first monitoring protocols are currently under investigation (13,14). To implement a dose titration regimen, measurement of the actual disease activity is necessary, preferably on a continuous scale. Improvement criteria like ACR and Paulus criteria are not suitable for this purpose as these criteria define response by a relative improvement, disregarding the actual disease activity (15,16). This hampers the use of these criteria in daily clinical practice, although these criteria have shown their value in randomised clinical trials.

A disease flare was defined using a reversed version of the EULAR criteria for response (12). The measurement error of the DAS28 is 0.6, therefore a change greater than two times the measurement error (1.2) can be considered as a clinical relevant change. A DAS28 of > 5.1 indicates high disease activity according to clinical judgement of rheumatologists. Combination of these values leads to a definition of a disease flare as an increase in DAS28 with 1.2 in case of a low disease activity or an increase in DAS28 with 0.6 to 1.2 and a resulting DAS28 > 5.1 (high disease activity).

The use of the DAS28 is feasible and time-effective using a pre-programmed calculator, spreadsheet or web-based calculator. One should take notice, however, that the cut-off levels for high and low disease activity mentioned in this report are calculated with the DAS28. The original DAS that is based on the Ritchie Articular index for tender joints and the 44 swollen joint count uses 3.7 as cut-off level for high disease activity and 2.4 for low disease activity instead. The measurement error is the same for both.

In spite of the relatively small number of patients, a large variation in required dose of anti-TNFα was found. Required doses ranged from 4.1 mg/week to 130 mg/week. Using the above described titration regimen, the median weekly amount of anti-TNFα given to these patients could be lowered with 67% from 97.5 mg/week to 32.5 mg/week. Since no lower dose steps than 0.25 mg/kg were included, one could speculate that even further reduction is possible for individual patients. This is supported by the remarkable long duration of response seen in some patients after only one administration of anti-TNFα, documented for both adalimumab (up to 14 weeks EULAR response) and infliximab (up to approximately 18 weeks Paulus 20 response) (17,18). Because the patients in this study already had a stable and low disease activity on 3.0 mg/kg for one year, it was not necessary to include a higher dose of anti-TNFα in this titration regimen.

The other possible approach to dose titration is time-interval titration. In our centre a group of 12 patients with exceptionally refractory RA and high baseline disease activity (median number of used DMARDs of 6, mean DAS28 at baseline 6.2) have been treated with infliximab infusions using a time-titration regimen based on the same flare criteria used by the present study. Infusions were delayed until a disease flare occurred. The new time-interval was based on the time to flare derived from the last infusion, with a minimum time interval of 4 weeks and a maximum of 16 weeks. Even in this selected group of patients with severe RA substantial differences in
required dosing intervals could be demonstrated (median time interval 5.1 weeks, range 4-7 weeks). The calculated weekly required doses were between 24 mg/week and 76 mg/week (factor 3.2). It is possible that some patients might have benefited from an even shorter dosing interval, but this was not allowed by the study protocol. A drawback of a step down dose titration is the inevitable disease flare in the titration phase. Eighteen out of 21 patients experienced a flare of the disease, but the mean DAS28 after the dose titration phase was not significantly higher than the DAS28 at start of the study. Moreover, no patients dropped out of the study due to a persistent flare. However, it should be noted that in case of an increase of the disease activity the dose of anti-TNFα could be adjusted at a drug administration visit (every 2 or 4 weeks). This way, prolonged deterioration of the RA was prevented.

In conclusion, dose titration of anti-TNFα treatment using the DAS28 is feasible and leads to a substantial median dose reduction while maintaining clinical efficacy. A large variation in the individual dose of anti-TNFα needed to maintain clinical efficacy is found. This approach will save costs and possibly prevent long-term side effects.

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Chapter XI

Summary
Summary
Rheumatoid Arthritis (RA) is a common disease, characterised by a symmetric chronic polyarthritis. Treatment traditionally consists of second line antirheumatic agents (or Disease Modifying Anti Rheumatic Drugs, DMARDs) in combination with NSAIDs and steroids if needed. The second line antirheumatic drugs are a heterogeneous group of chemical drugs. These agents have limited efficacy in some patients and frequently side effects are seen.
In the last decade, the knowledge about the pathogenesis of RA has increased. This is especially true concerning the role of proinflammatory cytokines like Interleukin β (IL-1β) and tumour necrosis factor α (TNFα) and other soluble mediators of inflammation. Based on these insights, new agents have been designed to specifically block the action of TNFα or IL-1β. TNFα blockade can be accomplished using monoclonal antibodies or with TNF receptor fusion proteins. These agents bind with TNFα, prevent its binding to membrane receptors and neutralise therefore the biological activity of TNFα. Both approaches have been shown to be effective in the treatment of RA and have found their way to the clinics in recent years.
The first monoclonal anti-TNFα antibodies that were clinically tested contained both human and murine protein sequences. The use of such, so-called, chimeric or humanised antibodies is potentially limited by the formation of human anti-chimeric antibodies (HACA) which may lead to a shortened half-life and therefore shortened effect or to adverse reactions. To circumvent these drawbacks, a fully human anti-TNFα antibody (Adalimumab (D2E7, Knoll) has been developed which is now being studied in phase III rheumatoid arthritis clinical trials.
The subject of this thesis is the evaluation of several aspects of the short and long-term treatment of patients with RA with adalimumab, a human anti-TNFα MoAb adalimumab and lenercept, a p55 receptor fusion protein [Ro 45-2081, lenercept, Roche]. These aspects include among others the efficacy, mode of action, safety and the use in daily clinical practice of this biological agent.

Chapter II
In this chapter the current state-of-the-art in disease assessment in rheumatoid arthritis is reviewed. To study the effects of any therapeutic intervention in patients with RA, the disease activity needs to be measured. There is however no "golden standard" for disease assessment in RA. Therefore, a wide variety of variables have been used in rheumatoid arthritis clinical trials, which of course hampers the comparison of results obtained in different studies. In the last decade, much effort has been directed towards improving and standardising disease assessment. Consensus has been reached on a minimum - so-called core set - of variables that should be measured in clinical trials. This consensus is based on the validity and sensitivity to change of these variables. The methods that should be used to measure these variables (for instance which type of joint counts should be used or which questionnaire is more suitable to assess a certain aspect of the disease) have however not yet been standardised.
Using this core set of variables, improvement criteria have been developed. These are the American College of Rheumatology improvement criteria (ACR criteria) and the European League Against Rheumatism (EULAR). ACR criteria are dichotomous improvement criteria based on all coreset variables. The EULAR response criteria are based on the Disease Activity Score (DAS). The DAS is a combined disease activity index that is calculated using the number of swollen and tender joints, the Erythrocyte Sedimentation Rate (ESR) and the patient’s judgement of global health. This results in a numerical value that may range from 0-10. Using the EULAR response criteria, patients can be classified as good, moderate or no responder. Both the ACR and EULAR criteria have proven to be powerful response criteria that can be used to assess the response to a given treatment.

The use of the aforementioned coreset and response criteria was studied in recently published clinical studies. To this aim, clinical studies comparing two or more interventions that have been published in 1999 in leading journals were reviewed. To our surprise, it was found that the majority of the studies used incorrect or incomplete disease activity variables or response criteria. Possible causes for these inconsistencies and suggestions for future improvement are discussed.

Chapter III

In chapter three the short-term efficacy and toxicity of intravenous treatment with increasing doses of a fully human anti-TNFα antibody were studied in a randomised, placebo-controlled study in 120 patients. These patients suffered from a longstanding disease and had been previously treated with a large number of DMARDs. They were randomly assigned to one of five dose groups or placebo. The ACR and the EULAR response criteria were used to judge individual response percentages at the timepoints day 1 and week 1 to 4. Our results show that single doses of adalimumab up to 10 mg/kg were well tolerated. The pharmacokinetics properties of adalimumab could best be fitted to a non-compartment model. The mean half-life was approximately 12 days. The efficacy ranged from 70 to 100% response according to both ACR and EULAR response measures for doses from 1.0 mg/kg or higher (in the group receiving placebo the response was of 27%). The dose group of 0.5 mg/kg showed less efficacy. These data demonstrate the short-term safety and efficacy of adalimumab.

Chapter IV

Radiologic progression is an important outcome variable in RA. The first reports on the effect of TNFα blocking strategies on this variable show that radiologic progression is retarded with these therapies. Radiologic damage can however only be judged after a prolonged period of time and this induces an undesired delay in therapeutic decisions. Therefore, there is a need for markers that can predict radiologic outcome at an early timepoint.

We studied the effect of long-term treatment with anti-TNFα on radiological outcome in 47 patients. Patients that had been treated with anti-TNFα for two years were
compared with patients that dropped out earlier because of toxicity or lack of efficacy and were treated with other DMARDs. Furthermore, a number of biological markers were measured at baseline to assess their predictive value for radiologic progression. These markers were chosen either for their relation with bone and cartilage turnover (HC gp-39, COMP and the metalloproteinases 1 and 3) or based on their involvement in endothelial activation (sE-selectin and sICAM-1), which plays an important role in the inflammation that characterises RA.

Our results show that the proportion of patients who showed radiologic progression was smaller in those that were treated with anti-TNFα during the whole study period compared to the patient group that dropped out during the study. In addition, we found that the time-integrated measurements of disease activity during the study were correlated to the radiological damage. Less disease activity resulted therefore in less radiological damage. The radiological outcome can to a certain extend be predicted using baseline sICAM-1 and COMP levels.

Chapter V
The exact mode of action of TNFα blockade in RA is not yet fully understood. Studies with another IgG1 anti-TNFα monoclonal antibody (infliximab) showed that blockade of TNFα reduces the acute phase reaction and decreases the local and systemic levels of adhesion molecules in RA patients. Whether these therapies also downregulate the synovial expression of the key proinflammatory cytokines IL-1 and TNFα in RA has not been fully elucidated yet, although some data supports this theory.

In this chapter, the effects of the first dose of adalimumab or placebo on the homeostasis of the two most important pro-inflammatory cytokines IL-1β and TNFα were studied. This was assessed both systemically (n=50) and at the level of the inflamed joint (n=25) using percutaneously obtained knee biopsies.

Two weeks after the first dose of anti-TNFα a clear decrease in acute phase related factors was seen (CRP, IL-6, IL-1ra, circulating TNF receptors). The level of IL-1 in the circulation was low and did not change in this period. The systemic level of TNFα was low before treatment but increased after administration of anti-TNFα. This is probably due to the formation of TNFα:anti-TNFα complexes. Despite a clear reduction in joint swelling and pain already 2 weeks after therapy initiation, no clear changes with respect to the expression of proinflammatory cytokines and inflammatory cells were seen in synovial biopsies. The expression of TNFα and IL-1 and the number of inflammatory cells were correlated within individual biopsies but demonstrated large inter-individual variation.

The short-term clinical effects of anti-TNFα can therefore not be explained by changes at the synovial level but seem to be caused by systemic effects of anti-TNFα.
Chapter VI
The Hypothalamic-Pituitary-Adrenal axis (HPA-axis) could play a role in the mechanism of action of anti-TNFα. Although acute TNFα or IL-1 administration is known to stimulate the HPA-axis, the HPA axis does not seem to be activated in patients with RA. We hypothesised that TNFα could have a suppressive effect on the HPA axis in a chronic inflammatory state. In this scenario, blockade of TNFα could lead to an increased activity of the HPA axis. The latter would be concordant with the rapid clinical and laboratory effects of anti-TNFα treatment that mimic treatment with corticosteroids.

The effect of treatment with anti-TNFα on the HPA axis was assessed in eighteen patients receiving adalimumab (n=8), lenercept (n=5) or placebo (n=5). Several variables that reflect the activity of the HPA axis were measured, including ACTH levels and levels of cortisol in serum and saliva. In order to measure the effect of anti-TNFα on the HPA axis and not the effects of prolonged disease ameliorating during therapy, all assessment were performed at baseline and one day after the first administration of anti-TNFα.

Results show that the disease activity, measured using the DAS, had significantly decreased already one day after the first does of anti-TNFα. However, all HPA axis related variables remained unchanged in this period and no correlation was found between these variables, acute phase reaction, and clinical parameters. Therefore, it can be concluded that HPA axis modification is not part of the short-term mechanism of action of anti-TNFα treatment.

Chapter VII
Except for involvement of synovial joints, extra-articular pathology can also accompany RA. Vasculitis is one of the more frequent occurring extra-articular manifestations of RA. It usually affects small vessels, ranging from nail-fold infarcts to more severe digital gangrene and leg ulcers. Vasculitis can also affect larger vessels leading to organ damage. The effect of TNFα-blockade on RA related vasculitis is unknown.

Chapter 7 describes the positive effect of treatment with lenercept (a p55:IgG1 fusion protein) on skin vasculitis in one patient with RA. This patient had persistent nailfold lesions that disappeared within days after administration of anti-TNFα. This phenomenon was repeatedly observed during several cycles of anti-TNFα administrations. This is the first report on the effect of TNFα blocking agents on extra-articular manifestations of RA.

Chapter VIII
Neutrophilic granulocytes (neutrophils) play an important role in the inflammation that characterises RA. These cells are abundantly present in RA synovial fluid and increased production of reactive oxygen species (ROS) by these cells can induce cartilage damage. Modulation of neutrophil functions could therefore be beneficial in RA. TNFα is involved in the in-vivo priming of neutrophils and in the induction of ROS
production and chemotaxis. It has been suggested that reduction of neutrophil priming and ROS production may be implicated in the mechanism of action of several antirheumatic drugs, including methotrexate and leflunomide. Whether this also holds true for TNFα blocking agents is still unknown.

In contrast, modification of neutrophil activity by TNFα blocking agents could yield adverse effects. Data derived from experimental animal models suggest increased susceptibility for infections and impaired clearance of (pre) malignant cells after TNFα neutralisation. These adverse effects are partially mediated through an impaired function of phagocytic cells, including neutrophils.

Neutrophil ROS production and chemotactic capacity were measured ex-vivo in RA patients and healthy controls (n=29). Moreover, two in vivo models were used to study neutrophil migration in RA patients. Migration into the synovial joints was measured using scintigraphy with radiolabelled autologous leukocytes (n=4). Neutrophil migration to a non-target organ (the skin) was assessed using an ultraviolet type B skin inflammation model (n=12).

Our results show that ROS production in RA patients was only slightly increased compared to healthy controls and this difference disappeared two weeks after the first injection of anti-TNFα. Ex-vivo measured chemotaxis did not change after treatment. The scintigraphic studies revealed a reduction in neutrophil migration into inflamed joints of approximately 50% compared to baseline in patients treated with anti-TNFα, whereas placebo treated patients remained unchanged. The normal migration of neutrophils and other inflammatory cells to the skin after UVB radiation induced acute inflammation was not significantly influenced by anti-TNFα treatment.

In conclusion, anti-TNFα therapy rapidly decreases the influx of neutrophils into RA joints. However, the physiological migration of neutrophils to a non-target organ, such as the skin, and their chemotaxis and ROS production are not significantly impaired after treatment.

Chapter IX

No clearly related specific adverse effects of anti-TNFα treatment have been reported yet. In this chapter a patient is described that develops a nephrotic syndrome during treatment with anti-TNFα. The proteinuria disappeared spontaneously after cessation of anti-TNF treatment. After rechallenge, the nephrotic syndrome reappeared, establishing a causal relationship with anti-TNFα treatment. Renal biopsy disclosed a membranous glomerulopathy but clinically, the nephrotic syndrome was characterised by a highly selective proteinuria and immediate responsiveness to steroid treatment. This clinical picture is more compatible with a minimal lesion glomerulopathy. The potential pathophysiology of this side effect of anti-TNFα is discussed.
Chapter X

Although two anti-TNFα agents have recently reached clinical practice, their exact place in the treatment of RA has yet to be established. Anti-TNFα strategies possess some distinct characteristics compared to traditional antirheumatic drugs. The onset of action is very rapid, especially after intravenous administration. In addition, it is conceivable that the inter-individual variation in effective dose of anti-TNFα is large. This conception is based on the blocking characteristics of these agents – blocking TNFα with a fixed ratio - and the large inter-individual variation in cytokine levels in patients with RA. This large inter-patient variation could theoretically result in gross over- or undertreatment when standard dosing schedules are applied. Moreover, in view of the high costs, individual dosing is warranted.

We developed a treatment protocol to find the minimal effective dose of anti-TNFα for each individual patient that can be used in daily clinical practice. This protocol was tested for 40 weeks in 21 patients that had been treated with intravenously anti-TNFα every 2 or 4 weeks for two years and had stable disease activity. The disease activity was monitored using the Disease Activity Score (DAS28), a combined disease activity index, ranging from 0-10. A disease flare was defined using a reversed version of the EULAR response criteria. An increase in DAS28 greater than 1.2 or an increase between 0.6 and 1.2 but a resulting DAS28 higher than 5.1 were considered a disease flare. The dose of anti-TNFα was lowered every eight weeks in steps from 3.0 through 1.0, 0.5 to 0.25 mg/kg. When a patient had a flare of the disease that fulfilled the flare criteria, the dose was increased to the previously effective dose. The dosing intervals were kept unchanged.

At study end, three patients remained on the highest initial dose whereas nine, three and three patients could be downtitrated to 1.0, 0.5 and 0.25 mg/kg respectively. The highest individual total weekly dose of anti-TNFα was 130 mg, the lowest 4 mg, more than 40 times lower. No patients dropped out from the study due to a persistent flare of the disease activity.

It can be concluded that inter-individual variation in effective anti-TNFα dosages is high. Dose titration of anti-TNFα treatment using the DAS28 is feasible in daily clinical practice and leads to overall dose reduction while maintaining clinical efficacy, thus saving costs.
Chapter XII

Nederlandse samenvatting
Reumatoïde artritis ("chronisch reuma" in de volksmond, RA), is een ziekte die gekenmerkt wordt door een chronische ontsteking van meerdere gewrichten. Naast de stijfheid en pijn die dit veroorzaakt, kan de ziekte op den duur ook leiden tot beschadiging en functieverlies van gewrichten. Bij de medicamenteuze behandeling van RA spelen naast ontstekingsremmende pijnstillers (NSAIDs) en bijnierschorshormonen (b.v. prednison) de langzaam werkende antireumatische middelen (DMARDs) de belangrijkste rol. Van deze medicijnen is het gunstig effect op de RA meestal bij toeval ontdekt, zij werden hiervoor niet specifiek ontwikkeld. Hoewel behandeling met DMARDs de RA vaak positief beïnvloedt is dit niet bij alle patiënten het geval. Ook wordt behandeling met DMARDs vaak bemoeilijkt door het ontstaan van bijwerkingen.

In de afgelopen jaren is er meer bekend geworden over de mechanismen die ten grondslag liggen aan de ontsteking die bij RA plaatsvindt. Bepaalde cytokinen, eiwitten die geproduceerd worden door cellen en die andere cellen aanzetten tot het vormen van een ontsteking, lijken hierbij een belangrijke rol te spelen. De belangrijkste hiervan bij RA zijn interleukine-1 en tumor necrose factor α (TNFα). Gebaseerd op deze kennis zijn medicijnen ontwikkeld die zeer specifiek deze stof neutraliseren.

In het menselijk lichaam kunnen de effecten van TNFα op verschillende manieren worden geblokkeerd. Dit kan worden gedaan met antistoffen die specifiek binden aan TNFα. Ook kunnen voor dit doel TNF receptoren worden gebruikt. De eerste gepubliceerde resultaten van beide benaderingen laten een goed effect zien op RA. De antistof tegen TNFα die in deze onderzoeken werd bestudeerd, is een eiwit dat een combinatie is van mensen en muizeneiwit. Dit is op theoretische gronden minder aantrekkelijk dan een geheel menselijk eiwit. Niet-menselijke eiwitten zouden namelijk na toediening aan de mens allergische reacties zouden opwekken.

Adalimumab is een antistof gericht tegen TNFα dat geheel uit menselijke eiwitten bestaat. Het is ontwikkeld door Knoll BASF. Vanaf 1997 wordt dit middel bij patiënten met RA getest. In dit proefschrift worden diverse aspecten van de behandeling van patiënten met RA met adalimumab belicht. De werkzaamheid, veiligheid en onderliggende werkingsmechanismen worden bekeken.

Om de werkzaamheid van een behandeling bij patiënten met RA te kunnen meten, moet de activiteit van de ziekte gemeten worden. Omdat er niet één gouden standaard is waarmee de ziekteactiviteit in RA gemeten kan worden, werd in het verleden een veelheid aan variabelen gemeten die met de ziekteactiviteit samenhangen. Hieronder vallen bijvoorbeeld het aantal pijnlijke en gezwollen gewrichten, de duur van de ochtendstijfheid, de grijpkracht van de handen et cetera. In de laatste jaren is onder meer door onze groep veel werk verricht om de meting van de ziekteactiviteit betrouwbaarder en beter vergelijkbaar te maken. In hoofdstuk 2 wordt de huidige stand van zaken op dit gebied geïnventariseerd. Ook wordt bestudeerd of de bestaande richtlijnen in wetenschappelijke publicaties worden gevolgd.
Er blijkt overeenstemming te bestaan over welke variabelen minimaal gemeten zouden moeten worden in patiënt gebonden onderzoeken om de ziekteactiviteit bij RA vast te stellen. Twee gecombineerde respons criteria (criteria, die een aantal van voornoemde variabelen combineren in één maat) zijn ontwikkeld in de afgelopen jaren. Dit zijn de ACR criteria en de EULAR criteria. De laatstgenoemde zijn gebaseerd op de DAS. Dit is een gecombineerde ziektemaat voor RA, die berekend wordt uit het aantal gezwollen en pijnlijke gewrichten, de bezinking en de ziekteactiviteit volgens de patiënt zelf. De schaal loopt van 0 tot 10. Zowel de EULAR als de ACR criteria zijn goed bruikbaar voor het vastleggen van welke individuele patiënten met RA goed reageren op een behandeling. Over een aantal zaken is echter nog geen duidelijkheid, bijvoorbeeld met welke gewrichtscore het aantal aangedane gewrichten geteld zou moeten worden.

Bij revisie van de medische literatuur blijken de bovengenoemde richtlijnen bij opzet en verslaglegging van onderzoek nog onvoldoende of incorrect gebruikt te worden. Van 12 klinische studies die in 1999 werden gepubliceerd gebruikt het grootste deel onjuiste of onvolledige ziektematen. Deze ontwikkeling zal in de toekomst nauwlettend gevolgd dienen te worden.

De effectiviteit en veiligheid van een éénmalige toediening van verschillende doseringen adalimumab in 120 patiënten wordt in hoofdstuk 3 beschreven. Deze patiënten waren eerder zonder succes behandeld met andere middelen tegen RA, maar hadden nog niet adalimumab gekregen. Verschillende doseringsgroepen, waaronder ook een placebogroep (een niet werkzaam medicijn), worden met elkaar vergeleken.

Doseringen van 1 tot 10 mg per kilogram lichaamsgewicht blijken goed verdragen te worden door alle patiënten en effectief te zijn bij ongeveer 70% tot 100% van de behandelde patiënten. De dosering van ½ mg per kilogram heeft echter een lagere effectiviteit na éénmalige toediening. Na toediening van placebo laat slechts 27% van de patiënten een verbetering zien. De halfwaardetijd bedraagt ongeveer 12 dagen. Op de korte termijn is behandeling met adalimumab veilig en effectief.

Bij patiënten met RA is, naast het behandelen van de huidige klachten, het voorkomen van kraakbeen- en botschade op de langere termijn en daarmee gepaard gaande verlies aan functionaliteit een belangrijk doel van de behandeling. De gouden standaard voor het vervolgen van deze schade zijn de röntgenfoto’s van handen en voeten. Het aantal beschadigingen op deze foto’s kan worden geteld en zo kan de röntgenschade in getal uitgedrukt worden. Deze informatie is echter altijd pas achteraf beschikbaar, en het zou aantrekkelijker zijn uit het oogpunt van behandeling om hierover eerder geinformeerd te kunnen worden. Hoofdstuk 4 laat de effecten van twee jaar behandeling met anti-TNFα op de röntgenschade in 47 patiënten met RA zien. Ook wordt de voorspellende waarde van zes verschillende bloedbepalingen (metalloproteinases, adhesiemoleculen, COMP en HC gp-39) op het ontstaan van
röntgenschade na twee jaar onderzocht. Deze variabelen zijn uitgekozen op basis van hun (mogelijke) relatie met kraakbeenschade.

De patiënten die de gehele periode zijn behandeld met anti-TNFα ontwikkelen minder röntgenschade na twee jaar dan patiënten die gedurende de studie stopten en met andere middelen behandeld werden. Ook is de gemiddelde ziekteactiviteit gedurende deze 2 jaar gerelateerd aan de uiteindelijke röntgenschade, dat wil zeggen: minder ziekteactiviteit, minder schade. Aangezien behandeling met anti-TNFα de gemiddelde ziekteactiviteit duidelijk verminderd, pleit deze bevinding voor een positief effect van behandeling met anti-TNFα op het ontstaan van röntgenschade. Het beloop van de röntgenschade in patiënten groepen kan tot op zekere hoogte worden voorspeld middels de COMP en de sICAM-1 spiegels aan het begin van de studie.

Het werkingsmechanisme van TNFα blokkade in patiënten met RA is nog niet geheel opgehelderd. Er is nog zeer weinig bekend over het effect van deze behandeling op de hoeveelheden van de belangrijkste ontsteking cytokines (TNFα en IL-1β) in het bloed en in de gewrichten. Hoofdstuk 5 beschrijft de effecten van een éénmalige toediening van anti-TNFα op de hoeveelheid en productiesnelheid van TNFα en IL-1β en hun natuurlijke tegenhangers (IL-1ra en TNF receptoren) in het bloed van 50 patiënten met RA. Ook wordt in 25 patiënten met behulp van kniebiopten het effect van deze behandeling op het aantal ontstekingscellen en de aanwezigheid van TNFα en IL-1β in het kniegewricht bestudeerd.

De behandeling met anti-TNFα leidt tot een duidelijke daling in het bloed van factoren die gerelateerd zijn aan ontsteking (CRP, IL-6, IL-1ra, TNF receptoren). De hoeveelheid IL-1β in het bloed blijft laag en onveranderd; de aanmaak van IL-1β neemt echter wel af. De concentratie TNFα is voor behandeling laag en neemt toe na behandeling. Dit is het gevolg van het binden van TNFα aan de antistof in het bloed. Ondanks duidelijke afname van gewrichtspijn en zwelling en de algemene ziekteactiviteit waren in het weefsel van de knie bovengenoemde effecten niet aantoonbaar. De hoeveelheden TNFα, IL-1β en ontstekingscellen in één biopt blijken wel duidelijk aan elkaar gerelateerd. Allen worden echter niet beïnvloed door twee weken behandeling met anti-TNFα. De klinische effecten van een éénmalige toediening van anti-TNFα lijken derhalve niet verklaard kunnen worden door beïnvloeding van IL-1β en TNFα lokaal aanwezigheid in het gewricht maar eerder door systemische activiteit van anti-TNFα.

Hormonale regelsystemen kunnen ook een rol spelen bij het effect van anti-TNFα. Ofschoon een acute toediening van TNFα de natuurlijke productie van bijnierschorshormoon productie activeert, wordt in patiënten met RA geen verhoogde of juist een verlaagde productie gezien. Van bijnierschorshormoon is bekend dat het een sterk onderdrukkend effect heeft op de TNFα productie. Het zou zo kunnen zijn dat patiënten met RA een relatief verlaagde bijnierschorshormoon productie hebben. Omgekeerd is het mogelijk dat TNFα de bijnierschorshormoon productie verlaagd bij
de chronische ziekte RA. Blokkade van TNFα zou dan leiden tot toename van de bijnierschorshormoon productie. Dit zou goed kunnen passen bij één van de belangrijkste kenmerken van anti-TNFα behandeling, het snelle optreden van het klinisch effect. Dit wordt namelijk eveneens gezien na behandeling met bijnierschorshormonen (prednison).

In hoofdstuk 6 wordt bestudeerd of toediening van anti-TNFα effect heeft op de regulatie van de productie van bijnierschorshormoon (de zogenaamde hypofysesyijnier as). Bij 18 patiënten (13 anti-TNFα en 5 placebo) worden een aantal bepalingen verricht die een maat zijn voor de activiteit van de eigen bijnierschorshormoon productie, zoals ACTH en cortisol in het bloed, en cortisol in het speeksel en in de urine. Deze bepalingen worden herhaald op de eerste dag na toediening van de eerste dosering anti-TNFα.

Er wordt geen verschil gezien tussen de resultaten voor en na behandeling en tussen patiënten die placebo of anti-TNFα krijgen. Ook het klinisch effect van anti-TNFα is niet gerelateerd aan de hoogte van bijnierschorshormoon productie of uitscheiding. Geconcludeerd kan worden dat de het snelle klinische effect van anti-TNFα niet te maken heeft met beïnvloeding van de bijnierschorshormoon productie.

Naast de gewrichtsontsteking die wordt gezien bij patiënten met RA, kunnen ook ziekteverschijnselen buiten de gewrichten optreden, zogenaamde extra-articulaire symptomen. De bekendste hiervan zijn de onderhuidse reumanoduli ("reumaknobbels"). Ook kunnen ontstekingen van de kleine bloedvaatjes optreden (vasculitis). De gevolgen van het laatste kunnen variëren van kleine wondjes aan de huid tot grote beschadigingen van inwendige organen zoals longen en nieren. Er is weinig bekend over de effecten van behandeling met anti-TNFα op extra articulaire manifestaties van RA.

In Hoofdstuk 7 wordt één patiënt beschreven die een typische ontsteking aan de kleine bloedvaatjes van de vingertoppen heeft. Deze patiënt laat herhaaldelijk een goede reactie zien op toediening van anti-TNFα, waarbij de verschijnselen van de vasculitis eveneens verdwijnen. Deze waarneming suggereert dat behandeling met anti-TNFα extra-articulaire verschijnselen zoals vasculitis van de huid of andere organen gunstig kan beïnvloeden.

Naast de werkzaamheid van een nieuw medicijn speelt de veiligheid ervan ook een belangrijke rol. Van blokkade van TNFα in proefdieren is bekend dat er een verhoogde gevoeligheid voor bepaalde infecties kan ontstaan. Dit wordt mede veroorzaakt door afgenomen activatie van neutrofiele segmentkernigen, een soort witte bloedcellen. Deze cellen hebben belangrijke functies bij de afweer tegen micro-organismen. Zij kunnen zich naar de ontstekingshaard verplaatsen (chemotaxie) en micro-organismen onschadelijk maken middels de aanmaak van bepaalde stoffen (zuurstofradicaal productie). Of deze functie beïnvloedt worden door behandeling met anti-TNFα is nog niet geheel duidelijk. Dit mogelijke effect van de behandeling
zou een deel kunnen zijn van het werkingsmechanisme maar ook de gevoeligheid voor infecties kunnen doen toenemen.

In hoofdstuk 8 worden de effecten van een éénmalige dosering anti-TNFα op de aanmaak van zuurstof radicalen en chemotaxie door neutrofielen beschreven in 29 patiënten met RA. Aansluitend hierop wordt in enkele patiënten door middel van beeldvorming bekeken, of de instroom van deze witte bloedcellen in ontstoken gewrichten afneemt na behandeling. Tenslotte wordt middels een huidonderzoek na UVB belichting in 12 patiënten onderzocht of ook buiten de gewrichten een verandering van instroom van neutrofielen wordt gezien.

De resultaten laten zien dat de zuurstofradicaal productie door neutrofielen bij patiënten met RA slechts iets hoger is dan bij gezonde vrijwilligers. Dit verschil verdwijnt echter na toediening van anti-TNFα. De instroom van witte bloedcellen in de gewrichten neemt wel duidelijk af na behandeling. Het vermogen tot chemotaxie van de cellen zelf blijft echter onveranderd na behandeling. In het huid wordt geen effect gevonden. Dit leidt tot de conclusie dat er geen grote beïnvloeding van neutrofiel functie door behandeling met anti-TNFα bestaat. De afname van de instroom van cellen in de gewrichten wordt waarschijnlijk veroorzaakt door afname van ontstekingsfactoren in het gewricht, niet door functieverlies van de bloedcellen zelf.

In hoofdstuk 9 wordt een patiënt beschreven met een nog niet eerder beschreven bijwerking van behandeling met anti-TNFα. Enkele maanden na de start van behandeling met anti-TNFα ontwikkelde deze patiënt een nieraandoening, die gekenmerkt wordt door verlies van eiwit via de urine. Na staken van de behandeling met anti-TNFα verdween dit zogenaamde nefrotisch syndroom binnen een aantal weken. Herstart van de toediening van anti-TNFα resulteerde in een hernieuwde opvlamming van het nefrotisch syndroom, zodat de oorzakelijke relatie met de anti-TNFα behandeling duidelijk werd. De anti-TNFα behandeling werd voortgezet, omdat het nefrotisch syndroom goed reageerde op een lage dosering prednison. De mogelijke onderliggende mechanismen van dit nefrotisch syndroom veroorzaakt door anti-TNFα worden belicht.

De behandeling van RA met anti-TNFα verschaft in belangrijke mate van de behandeling met conventionele DMARDs. Het effect van anti-TNFα treedt snel op en is sterk. Daarnaast valt op basis van de grote verschillen in TNFα spiegels en productie tussen patiënten een groot verschil in werkzame dosering te verwachten. Ook gezien de zeer hoge kosten die verbonden zijn aan behandeling met anti-TNFα is het van belang om per individu de juiste dosering te kunnen vaststellen. In hoofdstuk 10 worden 21 patiënten bestudeerd waarbij met behulp van de DAS score de individueel benodigde dosis anti-TNFα wordt bepaald. Terwijl elke acht weken een DAS score wordt bepaald (en eventueel vaker wanneer de ziekteactiviteit toeneemt), wordt de dosering anti-TNFα verlaagd van 3.0 naar 1.0, 0.5 en tenslotte 0.25 milligram per kilogram lichamsgewicht.
De resultaten laten zien dat het mogelijk is middels de DAS score de behandeling per patiënt te optimaliseren. De gemiddelde wekelijkse dosering van anti-TNFα kon worden gehalveerd zonder duidelijke toename van de ziekteactiviteit. De doseringen die nog effectief waren varieerden tussen patiënten met een factor 40. Hieruit blijkt dat er grote verschillen tussen patiënten bestaan in behoefte aan anti-TNFα.
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