Optimizing biological treatment in rheumatoid arthritis with the aid of therapeutic drug monitoring

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LIST OF PAPERS

1. Eng G, Stoltenberg MB, Szkudlarek M, Bouchelouche PN, Christensen R, Bliddal H, Bartels EM. Efficacy of treatment intensification with adalimumab, etanercept and infliximab in rheumatoid arthritis: a systematic review of cohort studies with focus on dose. Semin Arthritis Rheum 2013;43(2):144-51.

2. Eng GP, Bendtzen K, Bliddal H, Stoltenberg M, Szkudlarek M, Fana V, Lindegaard HM, Omerovic E, Højgaard P, Jensen EK, Bouchelouche PN. Antibodies to infliximab and adalimumab in patients with rheumatoid arthritis in clinical remission: a cross-sectional study. Arthritis 2015;2015:784825.

3. Eng GP, Bouchelouche P, Bartels EM, Bliddal H, Bendtzen K, Stoltenberg M. Anti-Drug Antibodies, Drug Levels, Interleukin-6 and Soluble TNF Receptors in Rheumatoid Arthritis Patients during the First 6 Months of Treatment with Adalimumab or Infliximab: A Descriptive Cohort Study. PLoS One 2016;11(9):e0162316.

ABBREVIATIONS

ACR	American College of Rheumatology
Anti-TNFi Abs	Anti-TNF inhibitor antibodies
CI	Confidence interval
CRP	C-reactive protein
DAS28	Disease activity score in 28 joints
DMARD	Disease-modifying anti-rheumatic drug
ELISA	Enzyme linked immuno sorbent assay
ESR	Erythrocyte sedimentation rate
EULAR	European League Against Rheumatism
HAQ	Health assessment questionnaire
hs-CRP	High sensitivity C-reactive protein
IgM-RF	Immunoglobulin-M rheumatoid factor
IL-6	Interleukin-6
IQR	Interquartile range
LDA	Low disease activity
MTX	Methotrexate
NSAID	Non-steroid anti-inflammatory drug
RA	Rheumatoid arthritis
RCT	Randomized controlled trial
s.c.	Subcutaneous
SJC	Swollen joint count
sTNF-R	Soluble TNF receptor
TJC	Tender joint count
TNF	Tumor necrosis factor-α
TNFi	TNF inhibitor
VAS	Visual analogue scale

BACKGROUND

Pathogenesis and inflammatory mediators in rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic disease targeting 0.5-1% of the adult population in western countries[1-3]. The autoimmune inflammation in RA affects primarily joints and adjacent soft tissue. The joints are damaged through bone resorbing osteoclasts and cartilage-damaging fibroblast-like synoviocytes[4]. During rheumatoid inflammation, mononuclear immune cells are recruited to the synovial membrane, where they produce cytokines and chemokines; some of these cells may differentiate into osteoclasts and play a role as co-activators of T-lymphocytes[4]. Although abundant in arthritic joints, the precise role of T-cells in RA is not yet fully understood. However, their importance is supported by the strong genetic association between RA and the HLA class II phenotype of HLA-DRB1*04, as the main function of HLA class II molecules is to present antigenic peptides to T-cells, hereby activating them. Furthermore, therapeutic targeting of T-cells has been somewhat effective in the treatment of RA[5]. The contribution of B-cells to the pathogenesis of RA is supported by the presence of autoantibodies in seropositive RA, and the associated effective treatment with the B-cell depleting drug rituximab[6]. Osteoclasts and fibroblast-like synoviocytes are stimulated by inflammatory stimuli, among these TNF produced primarily by activated macrophages, but also by B- and T-cells[7]. TNF exerts its effects through

two trans-membrane receptors, TNF-R1 (p55) and TNF-R2 (p75), expressed on a variety of different cells[8]. Enzymatic cleavage of these receptors results in shedding of the TNF receptors from the cell surface, creating soluble forms of the receptors, sTNF-R1 and sTNF-R2. The soluble receptors are still capable of binding TNF, and the soluble TNF receptors therefore function as natural TNF inhibitors. Additionally, the shedding decreases the amount of membrane-bound receptors, making the cells less responsive to stimuli with TNF[8]. sTNF-R1 and sTNF-R2 are increased in patients with RA when compared to healthy individuals[9-12], and sTNF-R2 in serum and synovial fluid correlates with RA disease activity[9,12].
The pivotal role of TNF in the pathogenesis of RA has been partly derived from observations following the introduction of pharmaceutical inhibitors of TNF (TNFi)[13]. These studies have shown that TNF among its numerous pro-inflammatory effects[14] increases interleukin (IL)-6 production[15]. IL-6 is crucial in activating B-cells, hereby stimulating immunoglobulin production. IL-6 also stimulates the hepatic acute-phase response, including the production of C-reactive protein (CRP), which among other effects, activates complements C3 and C4[16] and increase the production of pro-inflammatory cytokines[17]. Higher serum levels of IL-6 have been correlated with joint destruction and increased disease activity in RA[18], and inhibition of the IL-6 receptor is effective in the treatment of RA[19].

Treatment of rheumatoid arthritis with TNFi
Since the etiology of RA is unknown, and because the pathogenic processes involved in the autoimmune inflammation in RA are not fully known, treatment is not yet capable of curing this disease, but rather aims at controlling it through suppression of inflammation. For this, treatment with non-steroid anti-inflammatory drugs (NSAID) or glucocorticoids are often first choice. For long-term treatments, disease-modifying anti-rheumatic drugs (DMARDS) are frequently used[20]. This category includes cytostatic drugs such as methotrexate (MTX). Since the turn of the millennium, a new treatment option has become available, and these biological DMARDS include inhibitors of TNF (TNFi)[21-25]. Other biological DMARDS are inhibitors of IL-6[19], B-cells[6], IL-1[26], and CD80/86 of T-cells[5], and the novel janus kinase inhibitor (JAK)[27] which has not yet been approved for treatment in Denmark (Figure 1).
Biological DMARDs are protein constructs, as opposed to chemically derived pharmaceuticals. In rheumatology, biological DMARDs include therapeutic monoclonal antibodies and receptor constructs. Three TNFi are the focus of this thesis, two monoclonal antibodies (adalimumab (Humira®) and infliximab (Remicade®)), and one TNF receptor fusion protein (etanercept (Enbrel®)) (Figure 2).
The binding in vivo of all three drugs to TNF results in the formation of immune complexes which are then cleared, preventing TNF from binding to its receptors and thus inhibiting intracellular signalling[30]. *In vitro* studies have shown that adalimumab, etanercept and infliximab may act cytotoxic, most likely through the process of antibody-dependent cell cytotoxicity[31,32].

Clinical use of TNFi
As previously mentioned, inhibiting TNF has proven effective in preventing structural damage and lowering disease activity in RA[21,22,24,25,33,33]. Following the recommended treatment regimens, adalimumab is administered subcutaneously (s.c.) with 40 mg taken every 2 weeks[34], etanercept is administered s.c. with 50 mg once weekly (alternatively 25 mg twice weekly)[35] and infliximab is given as intravenous infusions with 3 mg/kg every 8 weeks, following an intensified induction regimen[36]. Intensification of drug regimen can be effectuated either by changing the dose of TNFi or by administering the TNFi with a frequency different from the recommended one. Intensification of treatment regimens is recommended for adalimumab (40 mg s.c. once weekly) and infliximab (up to 10 mg/kg up to every 4 weeks), but not for etanercept. Current national and international guidelines do not advise on the intensification of treatment[20,37], but clinicians do attempt to improve reduced clinical responses by intensifying drug regimens[38-41].

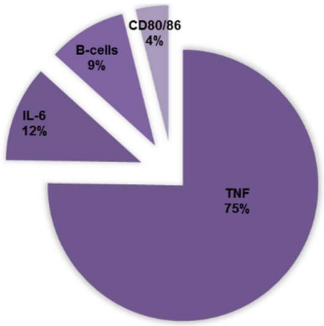

Figure 1. Overview of target molecule in biological treatment of rheumatoid arthritis by share of RA patients receiving treatment in Denmark[28].

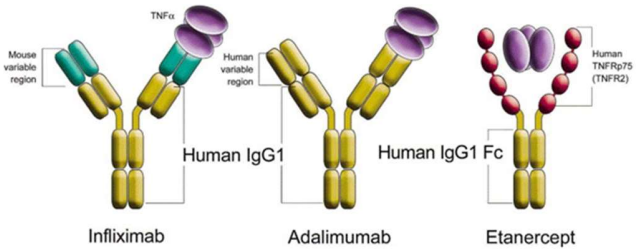

Figure 2. Schematic structure of the TNF inhibitors adalimumab, etanercept and infliximab[29] (with permission). Infliximab is comprised of a human IgG-κ chain and a murine variable Fab fragment holding the TNF binding region. The molecule is expressed in a line of murine cells. Adalimumab is an anti-TNF IgG1 monoclonal antibody developed by phage display resulting in a gene construct of human TNF binding Fab and human IgG1 Fc backbone. The gene construct is expressed in a line of Chinese hamster cells. Etanercept is a recombinant fusion protein comprised of the extracellular part of the human TNF-R2 coupled to a human IgG1 Fc.

Clinical response to TNFi
Efficacy of TNFi treatment is for example evaluated by composite measures of disease activity such as the Disease Activity Score in 28 joints (DAS28), the European League Against Rheumatism (EULAR) response criteria and the American College of Rheumatology (ACR) response rates (for further definitions, please consult the Methods section). Frequency of achievement of EULAR good response for patients on standard treatment regimens ranges from 40 to 60% with little difference between adalimumab, etanercept and infliximab[39]. This leaves approximately half the patients with a non-response or incomplete response to treatment[42]. Based on

the EULAR response criteria, two types of non-response can be described.

Primary non-response can be defined as the failure of the treatment to reduce clinical disease parameters and attain moderate or good EULAR response within the first three months of TNFi treatment. In Denmark, approximately one third of RA patients on TNFi may be classified as primary non-responders[39]. Primary non-response may reflect that the inflammation in these patients does not depend exclusively on TNF[43], or that the dose of TNFi is insufficient to suppress the inflammation[44], or that no inflammation is present.

Secondary non-response describes the situation where an initial decrease in clinical disease activity is lost. Secondary non-response can occur at any time in treatment and the increase in inflammatory activity may be due to a change in composition of inflammatory mediators or changes in pharmacokinetics and/or pharmacodynamics of the TNFi[45].

Concentration of TNFi

Regarding both adalimumab, etanercept and infliximab, observations have been made on an association between increased serum concentration and greater clinical efficacy[46-48]. It seems that increased serum concentrations, at least partly, depend on increased dose or frequency of TNFi administration[48-50] and in turn, that higher dose or more frequent administration of TNFi correlate to higher response rates[23,25,51]. Likewise, undetectable serum concentrations associate with diminished treatment efficacy or treatment failure[47,52,53]. These findings are the rationale for intensifying TNFi treatment in patients with a poor treatment response, and this is the subject of Paper I of this thesis.

Immunogenicity of TNFi

TNF inhibitors contain protein sequences foreign to our immune system, and may therefore be immunogenic, i.e. they are capable of eliciting an immune response directed towards any non-self sequences or residues on the drugs[54]. This may lead to production of anti-TNF inhibitor antibodies (anti-TNFi Abs). The antibodies directed towards adalimumab target human amino-acid residues, primarily the idiotypes responsible for the TNF binding to the Fab part of the drug molecule, whereas the antibodies towards the chimeric infliximab may target the murine components, such as the murine derived epitopes on the Fab fragment[55]. For both adalimumab and infliximab, antibodies may further be directed towards the foreign glycosylation on the drugs, resulting from the expression in a non-human cell line, or they may be directed towards neo-epitopes formed by drug aggregation[45]. For both adalimumab and infliximab, more than 90% of the anti-TNFi Abs target the TNF binding region[56]. Depending on binding kinetics, these anti-TNFi Abs may neutralize the TNF-inhibitory activity of the drugs[55]. Furthermore, circulating immune complexes may be formed, leading to an increased drug clearance, again depending on binding affinity and association/dissociation kinetics[57,58]. This increased clearance lowers the concentration of active drug, hereby impairing the efficacy of treatment[53,59-62].

Methotrexate (MTX) acts in synergy with several TNFi, among these adalimumab[63] and infliximab[33]. Observational studies report the use of MTX to be associated with decreased levels of anti-TNFi Ab and increased levels of TNFi in the circulation[33,59,64]. This effect may be exerted through an inhibitory effect of MTX on drug clearance[57,65], by inhibition of anti-TNFi Ab production by B-cells[66] or simply by the anti-inflammatory effect of MTX. Down-regulation of Fcγ-receptors on monocytes may contribute to this synergy[67]. Finally, the known synergistic apoptotic effect of MTX on lymphocytes may play a role as well[68,69].

Despite recent efforts, neutralizing anti-etanercept Abs have not yet been positively identified, which is why they will not be investigated further in this thesis[47,70-72].

Clinical observations on anti-TNFi Ab formation

In RA patients, reports on the incidence of anti-adalimumab Abs range from 26 to 31%[53,59,73], while anti-infliximab Abs occur more frequently, with reports ranging from 33 to 54%[62,74-76]. RA patients with longstanding and more severe disease, e.g. longer disease duration, erosive disease, higher ESR, higher CRP and higher DAS28, seem more prone to development of anti-TNFi Abs[59] and this may be explained by low drug levels associated with the kinetics of anti-infliximab Ab formation[60,77]. Between one half and two thirds of the patients who develop antibodies over a period of several years do so within the first 6 months[59,60]. Furthermore, RA patients with anti-TNFi Abs are more at risk of developing infusion-related reactions[58,74,77] and possibly other anti-drug Ab related adverse events[78], and as a result, withdraw form therapy[75]. At present, it is not possible to identify patients at risk of developing anti-TNFi Ab. Study III of this thesis was conducted in an attempt to investigate biochemical markers predicting anti-TNFi Ab development.

Current clinical strategies

Reaching low disease activity or remission is the aim of the current treatment principles[20], the treat-to-target strategy. Reaching this target may require frequent monitoring and treatment adjustments. Current national and international recommendations do not advise on the use of therapeutic monitoring of serum concentration of TNFi or immunogenicity in treatment with TNFi[20,37]. Algorithms using therapeutic drug monitoring have been developed in an attempt to optimize response rates[54] and improve cost-effectiveness[79], but their use in a clinical setting has yet to be established in RA patients; the cost-effectiveness of this approach has been demonstrated in infliximab-treated patients with Crohn's disease[80]. Regarding discontinuing or tapering of TNFi, the recommendations state that this may be attempted in case of persistent glucocorticoid-free remission[20], although most trials on the subject regard patients achieving low disease activity (LDA). In these trials, persistent TNFi-free LDA or remission is only sustained in less than half the patients discontinuing TNFi[81-84]. Predictors of TNFi-free remission differ throughout the studies investigating this area and include low baseline disease activity, early treatment with TNFi, shorter disease duration and rapid treatment response[81-85]. Reinstitution of therapy in case of flare, restore LDA or remission in most patients[81,84] and is largely without complications, although 10% of patients have to discontinue infliximab treatment because of infusion-related reactions[86]. Discontinuing treatment solely on the basis of low drug levels as a predictive marker has not been successful[87]. These results suggest that the reason for the low drug level has to be taken into account in order to distinguish sub-populations in whom the cause of low drug level could be a high inflammatory load, pharmacodynamics issues, compliance problems or accelerated clearance due to anti-TNFi Ab.

From the clinical observations regarding presence of anti-TNFi Abs, low drug levels and impaired clinical efficacy, algorithms propose discontinuation of further TNFi treatment in patients with a good clinical response/low disease activity and presence of anti-

TNFi Abs and low drug levels[79,88]. In Paper II of this thesis, this group of patients is studied.

OBJECTIVES

The objective of this thesis is to explore if biomarkers can be used to improve treatment of RA patients with biological TNF inhibitors. Improvement refers not only to ways in which the clinical outcome of treatment or the safety of treatment may be increased. Improvement also refers to ways to avoid redundant treatment or over treatment, which potentially can reduce costs and increase safety. This is investigated in three independent studies with the following objectives:

- To review and summarize the present scientific evidence for the effect of treatment intensification on clinical outcomes in patients with RA treated with adalimumab, etanercept or infliximab.
- To investigate the potential of anti-TNFi Abs as a biomarker of TNFi-free remission in RA patients treated with TNFi and in clinical remission.
- To identify biomarkers predictive of anti-TNFi Ab development in RA patients, by investigating early development of anti-TNFi Abs in relation to levels of bioactive TNFi, sTNF-R1, sTNF-R2 and IL-6.

METHODS

To reach the objectives, the following standards and techniques were applied in Papers I-III. Details are shown in the Methods sections of the respective papers.

GRADE

The GRADE system is developed as a tool for grading quality of evidence and strength of recommendations[89]. Through an evaluation of the overall quality of the evidence presented in the studies, the system indicates to which extent one can be confident that an estimate of effect is correct[90]. The levels of quality are designated high, moderate, low or very low. To determine the quality of a given study included in our review, we assessed factors that may increase or decrease the quality level, starting at a level according to the present study design

Identification of patients through DANBIO

Patients for the study presented in Paper II were selected using the national Danish database, DANBIO. In DANBIO, 89% of the Danish RA patients treated with biological DMARDs are registered and monitored[28]. DANBIO compiles demographic data, as well as data regarding medication and disease activity. For the study presented in Paper II, a search was conducted including diagnosis, medication, geographical location and disease activity, in order to identify possible participants.

Measures of clinical disease activity and treatment response

To measure the disease activity of patients with RA, the DAS28(CRP)[92] was used in both of the clinical studies. DAS28 is a combined index and is calculated using the number of tender joints (TJC28) and swollen joints (SJC28) in the patient, the level of CRP, and the patients' global health assessment (GH) on a visual analogue scale (VAS) of 100 mm.

At the point of inclusion into the study for Paper II, a doctor assessed all patients and obtained TJC and SJC and the patient filled in the Health Assessment Questionnaire (HAQ) (see Appendix IV) including GH. The cut-point of DAS28(CRP) < 2.6 corresponds with an increased likelihood of being in remission[93], and therefore this cut-point was chosen as an inclusion criteria for the study.
Clinical response to treatment can be evaluated based on the DAS28(CRP) and treatment response can be defined as good, moderate or non(-existing) by a method developed by the European League Against Rheumatism (EULAR)[94].
In Paper I, other means of evaluating disease activity are reported, among these the American College of Rheumatology (ACR) response criteria (ACR20, ACR50, ACR70)[95].

Para-clinical measures

CRP is a component of our innate immune response, and is synthesized in the liver[96]. It is routinely used as a biochemical marker of inflammatory activity and is a part of the composite score of DAS. A highly sensitive CRP assay (hs-CRP) has become available, enabling the measurement of low values of CRP, improving assessment of inflammatory activity in patients with low levels of CRP[97].
For Paper II, quantification of hs-CRP was performed at The Department of Clinical Biochemistry at Copenhagen University Hospital at Køge, Denmark, using their standard hs-CRP kit (Abbott Laboratories, Copenhagen, Denmark).
For Paper III, quantification of CRP was performed at The Department of Clinical Biochemistry at Copenhagen University Hospital at Frederiksberg, Denmark (Roche/Hitachi cobas-Csystems, Roche Diagnostics GmbH, Mannheim, Germany).
Immunoglobulin M rheumatoid factor (IgM-RF) is an auto-antibody directed towards the Fc part of immunoglobulins[96]. Many patients with RA have detectable IgM-RF and its presence is incorporated in the ACR classification criteria for RA[98]. Presence of IgM-RF has been correlated to a more severe disease course and radiographic progression in RA[99,100]. Quantification of IgM-RF for Paper II was performed at The Department of Clinical Biochemistry at Slagelse Hospital, Denmark, using their standard analysis. Patients were considered IgM-RF positive at values above 14 international units/ml.
None of the experimental analysis were performed in duplicates, which were in accordance with the practice of the involved laboratories. It is certain, that the results and following conclusions would stand more firm if all analyses had been performed in duplicates, and in future studies, measures to ensure this will be taken.

Concentration of TNFi

For both studies reported in Paper II and Paper III, the levels of bioactive TNFi were measured by reporter gene assays (RGAs) (iLite™ Infliximab Bioassay and iLite™ Adalimumab Bioassay, respectively, Biomonitor, Copenhagen, Denmark). These assays measure drug-induced TNF neutralizing capacity in the blood[101]. Upon activation by TNF, a luciferase reporter gene construct is activated within TNF sensitive cells and the enzyme activity is then determined by luminescence assessment. Patients were classified as having detectable or undetectable levels of TNFi according to the detection limit of both assays of 0.7 µg/ml.
For the study reported in Paper II, the initial 20 patients included had their drug levels assessed through a radio immuno-assay (RIA) (Biomonitor), but as the functional assays became available, RGAs were preferred. As expected, the results obtained from the

two different analyses, RIA and RGA, were not directly comparable, and the results obtained with RIA were therefore omitted from the concentration analyses in Paper II.

Anti-TNFi Abs

Anti-infliximab and anti-adalimumab Abs were measured by fluid-phase radioimmunoassay (RIA) (Biomonitor)[60]. This method exploits the fact that adalimumab and infliximab are composed of κ light chains. Anti-human λ light chain Abs may therefore be used to distinguish between free TNFi and TNFi in complex with human anti-TNFi Abs. Patients were classified as anti-TNFi Ab-positive or -negative according to whether anti-TNFi Abs were detectable or not. The limit of quantification was 10 arbitrary units/ml for both anti-adalimumab and anti-infliximab Abs. For the used assay, inter- and intraassay variations were <20% and <10% respectively, according to previous studies describing the assay[60].

Concentration of sTNF-R1, sTNF-R2 and IL-6

sTNF-R1, sTNF-R2 and IL-6 were quantified using commercially available assays and according to the manufacturers' instructions, at The Institute for Inflammation Research, Rigshospitalet University Hospital, Copenhagen, Denmark.

sTNF-R1 and sTNF-R2 were measured using a solid phase Enzyme Linked Immuno Sorbent Assay (ELISA). In this assay, monoclonal Abs against recombinant sTNF-R1 and sTNF-R2, respectively, were used to capture sTNF-R1 or -2 in the serum samples. Unbound material was washed away, and a second enzyme-labelled monoclonal Ab specific for the sTNF-R1 or -2, respectively, and linked to an enzyme was added. Following a second washing cycle, the substrate for the enzyme was added and the colour developed in proportion to the quantity of sTNF-R1 or -2 and was measured in an ELISA-reader.

IL-6 was measured using the principle of Bio-Plex, a sandwich immunoassay built on coloured microspheres. Briefly, in this assay antibodies against the investigated biomarker (capture antibodies) were coupled to fluorescently dyed microspheres. When the antibody and biomarker was coupled, a detection antibody was added and the complex was coupled to a fluorescent reporter. The colour of the microspheres attached to the fluorescent complex were then read using two lasers in a flowcytometer (Luminex) distinguishing microsphere colour and fluorescence intensity. This allowed information regarding biomarker and concentration to be extracted.

Statistics and data presentation

Paper I

Evidence synthesis was mainly descriptive with reported numbers and frequencies and short descriptions. Due to the extent of heterogeneity in both study designs and reported outcomes, the planned meta-analysis could not be performed.

Paper II and Paper III

Continuous variables were reported as means and interquartile ranges (IQR), and comparisons between groups were carried out using the Mann-Whitney test for unpaired data, and the Wilcoxon matched pair test for paired data. Categorical data were reported as numbers and percentages and 95% confidence interval (CI) and analysed by Fisher's exact test. Two-sided *p*-values less than 0.05 were considered significant.

For the analysis of functional TNFi levels, values below detection limit were truncated at detection limit. For analysis of level of sTNF-R2, values above detection limit were truncated at the upper detection limit. For analysis of level of IL-6, values below detection limit were truncated at half of the detection limit.

For both studies, a priori power calculations were considered, but not incorporated in the study design. The study reported in Paper II was an explorative cross-sectional study including all available samples in a field with very little research, which is why we could not apply any estimate of power. For the study reported in Paper III we tried to do power calculations, but it was in our view not possible to estimate a reliable effect size and standard deviation of the change in the outcome(s). We decided to use the available fixed sample size from a previously defined cohort in spite of the lack of estimate of power.

Ethics

The study reported in Paper II was approved by the local ethics committee of Region Zealand (SJ-196), and the study for Paper III was approved by the local ethics committee of the Capital Region of Denmark (KF-01-045/03). In accordance with the Helsinki Declaration (http://www.wma.net/en/30publications/10policies/b3/), all patients gave written informed consent prior to inclusion, and both studies were approved by the Danish Data Protection Agency.

PAPER PRESENTATION

Paper I

Efficacy of treatment intensification with adalimumab, etanercept and infliximab in rheumatoid arthritis: A systematic review of cohort studies with focus on dose

Aim

In this systematic literature review we wanted to evaluate the effect of treatment intensification with a TNFi on outcome measures related to clinical disease activity in patients with RA.

Methods

The review was performed according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) guidelines[102] and the risk of bias was assessed using the GRADE approach[89,90]. Prior to the literature search, the protocol was registered in the PROSPERO database of protocols for systematic reviews (http://www.crd.york.ac.uk/prospero/ registration number 42011001850). The structured search was conducted on January 16th 2012 in the bibliographic databases Medline (via PubMed from 1966), EMBASE (via OVID from 1980), Web of Science (from 1990) and Cochrane Central Register of Controlled Trials using the following search strategy:

Adalimumab OR Humira OR etanercept OR Enbrel OR infliximab OR Remicade
AND rheumatoid
AND dose OR treatment interval OR treatment intensification

We included clinical trials and observational studies in which a minimum of 12 adult patients with RA were subject to treatment intensification for at least 12 weeks. The intensification could be either dose increase or reduction of time interval between medication or both, and disease activity had to be assessed prior to and following the intervention. Our main outcome was changes in disease activity following treatment intensification.

Results
Of 1135 retrieved records, we included 11 studies with 627 patients comprised of 8 clinical trials and 3 observational studies. Adalimumab and etanercept were used for treatment in two studies each, and none of these studies found a significant decrease in clinical disease activity following treatment intensification. Infliximab was the choice of drug in nine studies, out of which five clinical trials and one observational study reported a decrease in clinical disease activity following intensification, and data from two studies favoured frequency increase as opposed to dose increase. The risk of bias in the majority of studies was high, and the results somewhat conflicting. There was no evidence of an increased safety risk.

Strengths and limitations
A systematic review is considered the strongest form of medical evidence[103], based on the systematic approach summarizing and appraising the current literature on a specific subject. Our systematic review was strengthened by a structured search in all the appropriate bibliographic databases in the field, and the inclusion of both clinical trials and cohort studies in an effort to include as many research data as was available on the subject. An additional strength of this review is the adherence to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)-guidelines. These includes the registration of a protocol, which should diminish or prevent publication bias and duplicate works and promote transparency[104].

A review is none the less no better than the included studies, and therefore the quality of these and the risk of bias was assessed. We used the GRADE approach as recommended by the Cochrane Collaboration[105], which considers within-study risk of bias, directness of evidence, heterogeneity of results, precision of effect estimates, and risk of publication bias. When using the principles of GRADE, we are aware that the system only to a certain extent can objectify the quality of the included studies, and that the factors influencing the quality level are still considered in a somewhat subjective manner. As an example, the study be Breedveld et al. may be mentioned. For meeting the main objective of this RCT comparing adalimumab treatment with or without combination therapy with MTX, the study was well designed and the reporting of outcomes very adequate. In the study, a dose increase was available for ACR20 non-responders. In the context of evaluating effect of dose increase, the reporting of results was not as thorough as for the main objective. This resulted in a downgrading of the quality level of the study to moderate, when it was considered in the context of effect of dose increase.
The overall risk of bias was high, mainly due to selection and performance bias in the allocation and blinding of participants, but examples of detection bias were found, and reporting bias was also suspected. These observations resulted in an overall low quality of evidence. Furthermore, the studies were quite heterogeneous with regard to patient group, allocation to intervention group, use of control group, duration of intervention and reported outcome measures, hindering a meta-analysis of the obtained results. Furthermore, the observational design of several studies raised the question, that without a proper control group an observed effect might be a result of regression towards the mean[106]. These considerations were especially relevant regarding several studies on infliximab. In these studies, several forms of bias and the conflicting results weaken our conclusions, and we conclude that further studies would strengthen our findings.

In the search process, we tried to foresee publication bias by contacting authors of papers whom we suspected could have information regarding our intervention and outcome. This did not yield further data. Language bias was not considered an issue, as only one article was not in English but in German, which was mastered by several of the authors.
One could suspect selection bias as only one author assessed the full-text articles for eligibility. Any doubts regarding eligibility were discussed with the last author, and consensus was reached easily, leading us to believe that chances of selection bias were minimal.

Conclusion
The evidence regarding treatment intensification of adalimumab and etanercept is scarce and shows no clinical benefit. Regarding infliximab, the included studies were heterogeneous, and although the majority found a beneficial clinical effect of treatment intensification with infliximab, this review highlights the need for further studies.

Paper II
Antibodies to infliximab and adalimumab in patients with rheumatoid arthritis in clinical remission; a cross-sectional study

Aim
The aim of this cross-sectional study was to assess frequency of anti-TNFi Ab formation in a cohort of patients with RA treated with infliximab or adalimumab and in clinical remission.

Methods
This observational study recruited patients with RA from 6 different out-patient clinics in Denmark, selected through the DANBIO database. Patients had been treated with infliximab or adalimumab for a minimum of 12 months and had a DAS28(CRP) < 2.6 at inclusion. Blood sampling was arranged prior to next scheduled administration of TNFi, and samples were analysed for presence of anti-TNFi Abs, and for level of TNFi and hs-CRP. Presence of anti-TNFi Abs was the main outcome, level of TNFi was the secondary outcome.

Results
Ninety-three patients were included, 44 treated with infliximab and 49 treated with adalimumab. Patients had been in remission for a mean of 2 years and 88% were co-medicated with MTX. We found anti-TNFi Abs in a total of 10% of the patients; 18% (8/44) of patients treated with infliximab and 2% (1/49) of patients treated with adalimumab. The presence of anti-infliximab Abs correlated with impaired levels of infliximab (Figure 4). Shorter disease duration at initiation of TNFi treatment predicted anti-TNFi Ab development. Due to the use of dissimilar laboratory tests, results from 20 patients were omitted from the final analysis regarding drug concentration.

Strengths and limitations
Observational studies are in general less expensive and time consuming, but are more vulnerable to methodological issues when compared to clinical trials[107].
We assessed remission through a DAS28 cut-off- point of 2.6, which may not guarantee that the patients were without inflammatory activity, but never the less was the cut-off point for remission at the time the study was initiated. At present, the definition

of true remission is debated, and being able to establish that a patient is in true remission may require the use of imaging[108,109].

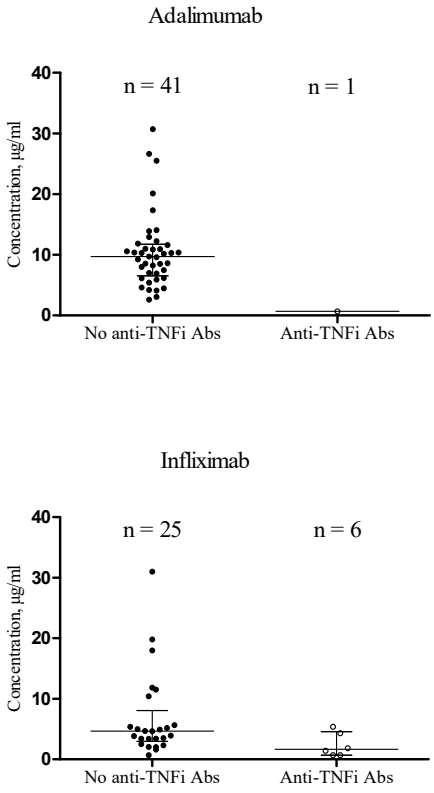

Figure 4. Level of adalimumab and infliximab in relation to presence of anti-TNFi antibodies in 73 patients with rheumatoid arthritis. For patients treated with infliximab, presence of anti-infliximab Abs correlated with low infliximab levels (p = 0.048).

Since the protocol for the study reported in Paper II was written and the study was conducted, the DAS28(CRP) cut-off point for remission at < 2.6 has been abandoned, and new analyses suggest that DAS28(CRP) < 2.6 rather represents what may be termed "minimal disease activity"[108]. If remission, rather than minimal disease activity, is maintained as the starting point when assessing possible discontinuation of TNFi treatment, our study may overestimate the size of this target population. One of the strengths of our study was that the DAS28 was confirmed at inclusion. A limitation was that in a minority of patients, the observer was not the principal investigator, but a single investigator affiliated with the local out-patient clinic. An inter-observer variation may be present, but we have attempted to minimize this source of bias by having a limited number of observers.
In the present cross-sectional study, results may have been biased by confounding by indication[110]. Regarding the difference in immunogenicity between the two TNFi, the characteristics that have prompted the treating physician to prescribe either of the drugs may not have been recorded in the data for the study. Even though none of the recorded variables differed between the patients treated with either drug, there may have been an unidentified determinant. We have, however, tried to foresee this by including as many variables as seemed appropriate in the analysis. Likewise there is a risk that we have overlooked determinants of anti-TNFi Ab development, although we have included numerous variables in the comparison between anti-TNFi Ab-positive and negative patients. Bias may also have been present in the event of effect modification for example by MTX. In our analysis, we have looked at the use of MTX at the time of initiation of treatment and at the time of inclusion in the study, and have not found MTX to impact on the development of anti-TNFi Ab. We might have differentiated patients differently, possibly with different outcome.
Our study has a somewhat limited population, and only a total of 9 patients had developed anti-TNFi Abs. This increases the risk of a type II error in some of the analyses and we could wish for a similar study to be conducted in a larger patient group.
Several methods for the detection of anti-TNFi Abs have been developed using different principles[101,111,112]. A concern regarding detection of anti-TNFi Abs has been the risk of false-negative samples owing to the presence of residual TNFi in the sample[113]. We have attempted to account for this by sampling blood at trough level of TNFi, that is, immediately prior to the next administration of medication. Furthermore, we chose a commercially available fluid-phase radioimmunoassay (RIA), which has been shown to be robust[111] and comparable to other available assays[114]. In spite of our efforts, at least one sample has been drawn form a patient only 9 days following infliximab infusion, and no anti-TNFi Ab were detected in the sample, and the level of infliximab was extremely high.
Likewise, the timing of blood sampling of patients on self-administered s.c. adalimumab gave rise to some concern, as it was not possible to control that the sampling indeed took place just prior to drug administration. Therefore, there may be doubts if all samples for evaluation of TNFi were indeed samples taken at the lowest concentration of TNFi (trough level). If samples were not trough samples, the trough levels of TNFi might have been lower than the ones reported, and an effect of anti-TNFi Abs on drug levels may have been underestimated.
A further limitation of this study was that concentration of TNFi was measured at one time-point only, which is when concentrations were at their lowest. Hereby we have no way of knowing if drug levels were adequate through most of the interval between drug administrations and merely were low just before next dose of TNFi, hereby providing the patient with adequate amounts of TNFi throughout most of the treatment intervals.
In the study, we have not recorded the weight of the patients, and therefore we have not been able to characterize the patients according to weight-adjusted dose. From information regarding dosing intervals, we may report that infliximab was administered at a standard interval of 8 weeks in 48% of patients, while it was administered less frequently in 23% and more frequently in 29% of patients. None of the patients with a reduced infusion frequency had developed anti-TNFi Ab . For adalimumab, 74% of patients received a standard treatment of 40 mg biweekly, 21% had less frequent administration and 5% had more frequent administration.

Conclusion

In our study, anti-TNFi Abs were present in a total of 10% of RA patients treated with infliximab or adalimumab and in clinical remission. Anti-TNFi Abs occurred more frequently in patients treated with infliximab. In patients with shorter disease duration, anti-TNFi Ab development was more frequent in our cohort, and anti-infliximab Abs correlated with low levels of infliximab. Prospective studies with larger patient populations are needed to

show if low drug levels and/or the presence of anti-TNFi Abs in RA patients in clinical remission are predictors of the possibility of continued drug free remission.

Paper III

Anti-drug antibodies, drug levels, interleukin-6 and soluble TNF receptors in rheumatoid arthrits patients during treatment with TNF-α inhibitors: A 6 months cohort study of treatment with adalimumab or infliximab.

Aim

The aim of this study was to assess the development of anti-TNFi Abs during the first 6 months of treatment with adalimumab or infliximab in RA patients, and to relate this to levels of the drugs, changes in biomarkers and to possible predictive parameters.

Methods

Blood samples were included from an original cohort of 114 patients[115], wherefrom a subgroup of 26 patients with available baseline and 6 months follow-up blood samples were included. The blood samples were analysed for level of TNFi by RGA, for the presence of anti-TNFi Abs by RIA, for levels of sTNF-R1 and sTNF-R2 by ELISA, and for levels of IL-6 by a Bio-Plex sandwich immunoassay.

Results

The included 26 patients consisted of 15 patients who had been treated with infliximab, and 11 patients who had been treated with adalimumab. During the 6 months treatment period, 23%, 6/26, (95% CI 11-42%) of the patients developed anti-TNFi Abs, and they were evenly distributed between the two treatment groups. Equal to this, 23% had undetectable levels of drug at follow-up, although the patients with anti-TNFi Abs did not all have undetectable drug levels, and vice versa. DAS28(CRP) and levels of sTNF-R2 and IL-6 decreased significantly in patients without anti-TNFi Abs (Figure 5), and in patients retaining detectable drug levels. Higher baseline levels of IL-6 were associated with undetectable levels of TNFi at follow-up (p = 0.031). Anti-TNFi Abs were associated with decreased levels of adalimumab (p = 0.043) or infliximab (p = 0.037), but no predictors for anti-TNFi Ab development could be found.

Strengths and limitations

Selection bias must be considered in this study, as inclusion in the original study happened at a time when biological therapy was not as readily available as it is today. This may result in the included patients representing a different group from the patients entering into biological therapy today, but it does not hinder a comparison between patients who did or did not develop anti-TNFi Abs in the present study.

Another source of selection bias is the follow-up period of 6 months. Patients not adhering to therapy for 6 months, and therefore not contributing with a 6-months blood sample, were not included in the present study. Patients with anti-TNFi Ab development have a higher risk of treatment failure and of withdrawal due to adverse events[75], and hence patients withdrawing early from the original study may be at a higher risk and represent a different phenotype than the remaining participants. This may lead to an underestimation of the risk of anti-TNFi Ab development and of the impact it has on levels of various biomarkers, as it may be the least affected patients who have remained in the study.

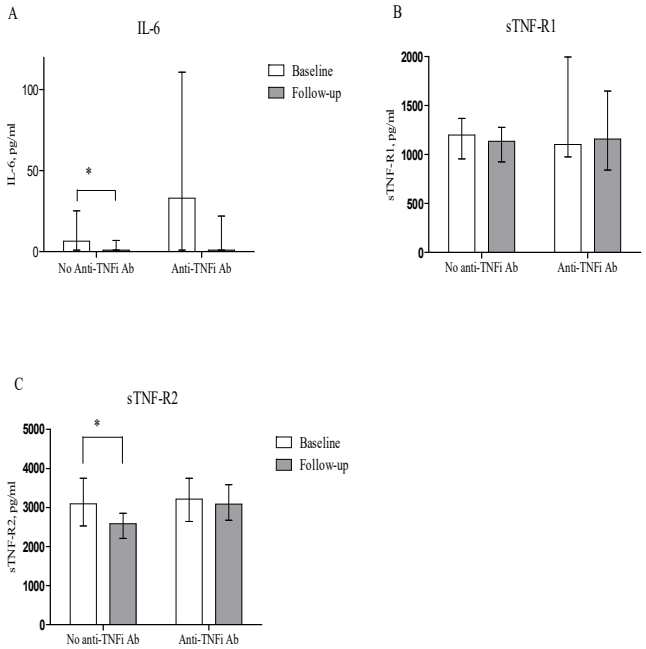

Figure 5. Changes in levels of IL-6, sTNF-R1 and sTNF-R2 in patients with and without anti-TNFi Ab development during the first 6 months of treatment with adalimumab or infliximab. Patients without anti-TNFi Abs experienced a decline in levels of IL-6 (A) and sTNF-R2 (C), while levels of sTNF-R1 remained unchanged in both groups (B).* $p < 0.05$. Median and IQR depicted.

These speculations underline some of the pitfalls in using historic material for contemporary analyses. In this case, the reasons for the missing samples in the available biobank, could not be evaluated. From the initial 75 patients treated with infliximab or adalimumab, only 15/18 (infliximab) + 27/45 (adalimumab) seemed to have at some point delivered a 6-months sample. If the missing samples reflect discontinuation of treatment, the drop-out rate is 44 %, which must be considered extremely high. Following, it may be suspected that only the patients with a good treatment response would be left to be included in our study.

Contributing to a possible underestimation of the frequency of anti-TNFi Ab formation is the short treatment period of 6 months. Several studies have shown that although anti-TNFi Ab formation is most frequent during the initial 6 months of treatment, the risk is still imminent in the subsequent months and years[59,75].

We are aware of the concerns regarding the influence of IgM-RF on detection of soluble biomarkers[116]. Blood samples from the original cohort have previously been analysed for levels of certain cytokines, including IL-6 by ELISA (Bartels et al. unpublished data). We have compared our results with the ones previously obtained, and although there were minor differences in the individual measurements, there were no systematic differences. This reassures us that the standardized method for blocking interference from IgM-RF in our commercial assay is comparable with other techniques[117].

Unfortunately, the analyses of sTNF-R1, sTNF-R2 and IL-6, were conducted without the use of controls, which is why recovery cannot be reported. For the detection of sTNF-R2, 29 of 52 measured samples were above the upper limit of the standard curve of

the applied assay. Unfortunately, further analysis on diluted material was not performed, which would have produced more accurate quantitative data. The results would have been further strengthened, had they been performed in duplicates.
To be able to include censored data in a continuous analysis, we choose to truncate measurements. This may have introduced bias, the magnitude of which cannot be estimated, as we do not know the true values of the measurements. To limit bias, measurements of TNFi and IL-6 below detection limit and measurements of sTNF-R2 above detection limit were substituted with fictive values. For measurements of TNFi below detection limit, 6/26 (23%) we substituted with the value of the detection limit. Based on previous studies, we expected that anti-TNFi Abs would be associated with lower levels of TNFi. To minimize the risk of bias by the truncation, and in an attempt to avoid overestimating an impact, we chose to truncate the levels at a value as high as possible. This may have overestimated the median levels of TNFi, and thereby underestimated an impact of anti-TNFi Abs on the levels of TNFi. For non-detectable results of IL-6, 26/52 (50%), the results were replaced by half the detection limit. Values beyond the calibration interval of sTNF-R2, 12/52 (23%) were substituted for the value of the upper detection limit.

Conclusion
In this study treatment with TNFi lowered DAS28(CRP) and circulating levels of sTNF-R2 and IL-6 in patients who retained measurable levels of TNFi, and in patients who did not develop anti-TNFi Abs. These findings support the previously observed negative impact of anti-TNFi Abs and reduced levels of TNFi on clinical effect. Baseline inflammatory activity assessed by level of IL-6 was associated with reduced levels of TNFi. Assessing baseline inflammatory activity may predict depletion of TNFi during treatment, and may identify patients at risk of later impaired drug levels and possible therapeutic failure.

DISCUSSION

Principal findings

The systematic review (Paper I) highlights the need for further studies on the clinical effect of treatment intensification with adalimumab, etanercept and infliximab. From the existing research, it seem that clinical efficacy increase when treatment with infliximab is intensified, but results are conflicting. There are no increased clinical efficacy when treatment with adalimumab or etanercept is intensified.
The cross-sectional second study (Paper II) finds that anti-TNFi Abs develop in 10% of seemingly well-treated patients, and in this population, infliximab is more immunogenic than adalimumab. Results from the third and final study (Paper III) indicate that treatment with adalimumab or infliximab decrease levels of IL-6 and sTNF-R2 depending on anti-TNFi Ab status and drug level. This study also reveal that baseline levels of IL-6 may predict depletion of TNFi early in the treatment course.

Comparison with previous findings

Efficacy of treatment intensification

Several early studies find that higher serum concentrations of TNFi correlate to increased clinical efficacy[23,48,49], and further, that increased serum concentration of TNFi depend on dose and frequency of medication[48-50,118]. Due to the link between concentration of TNFi and clinical efficacy, several studies have proposed possible target concentrations for treatment[118,119]. The notion of a target concentration may have formed the basis for the widespread use of treatment intensification for patients with an inadequate response to standard therapy[38,41,120-123]. Reflecting clinical practice[38,106,124], the majority of studies addressing the impact of treatment intensification on clinical disease activity have not measured concentration of TNFi prior to treatment adjustment[63,71,118,125-129]. In clinical studies, this approach may have led to patients with an intermediate or high concentration of TNFi, having their already therapeutic levels of TNFi increased, and therefore not adding any further clinical effect. The seeming lack of additional clinical effect following treatment intensification may be the result of the disregard of a subgroup of patients with sub-therapeutic concentrations of TNFi. Only one clinical trial has measured the concentration of TNFi prior to treatment adjustment[130]. In this small-scale, open-label study, personalized treatment with infliximab was accomplished by increasing the dose in patients with low concentration of infliximab and impaired treatment response. The result was an increase in concentration of infliximab and an improved control of disease activity[130]. In order to attain higher concentrations of infliximab, pharmacokinetic modelling supports that increasing frequency is superior to increasing dose[48]. This observation has not been thoroughly confirmed in the clinic, as results are conflicting[126,127].
The reason for the conflicting results may be, that thus far the terminology and understanding regarding patients with an incomplete response to treatment may have been somewhat simplified. Some studies fail to differentiate between what may be two completely different groups of patients. Patients who have a consistently incomplete response to TNFi treatment and patients, who have an initial good response, but loose it before the next scheduled infusion. Both are termed partial responders and are in many studies treated alike, although the mechanism for the treatment failure is most likely very different. The former group, whose inflammation is at best only partially controlled by TNFi therapy, may reflect a disease mechanism where inflammation is driven only partially by TNF. The latter group including patients with an appropriate response to treatment for the initial weeks of the treatment interval, may reflect a group who, due to individual differences in drug clearance and maybe due to differences in inflammatory load, need more frequent drug administration to attain a sustained clinical response. When including these two groups in studies of dose escalation of infliximab without trying to determine the underlying mechanism for failure, e.g. by measuring the infliximab concentration prior to dose escalation, the poor results from the former group may mask any positive effect observed by the latter group.
The conflicting results from the trials published by Pavelka et al. and Takeuchi et al. may reflect this. Although both trials are well performed and very interesting, they deal with two different populations. Looking at demographics, it is striking that the better clinical results in the study by Takeuchi et al., are obtained in a population with a longer disease duration, a higher baseline DAS28(CRP), higher tender and swollen joint count at baseline. As mentioned in the Background section of this thesis, longer disease duration and higher DAS28 have been associated with the formation of anti-TNF Abs. One could speculate that the patients in the study by Takeuchi et al. may have been more prone to anti-TNF Ab-development than the patients in the study by Pavelka et al., and that the following reduced concentration of infliximab then would be overcome by the dose-increase.

Frequency of anti-TNFi Ab development

In Papers II and III, the difference observed in immunogenicity between adalimumab and infliximab, is in accordance with studies on prospective cohorts of patients with mixed treatment responses. In these, infliximab is more immunogenic than adalimumab. The studies report the frequency of anti-TNFi Ab formation to range from 33-54% for infliximab[53,60,62,74-76,119] and to be approximately 30% for adalimumab[53,59,73]. When considering the construction of infliximab, it does consist of non-human material, in contrast to adalimumab, which could explain the increased immunogenicity[29].

Patients in whom anti-TNFi Abs have been found, have less effect from their TNFi treatment, as anti-TNFi Abs correlates with impaired treatment efficacy[53,59-62]. In the remission cohort of Paper II, we expected to find the overall frequency of anti-TNFi Ab development to be lower than in mixed treatment response cohorts, as patients in remission supposedly have exhibited a good treatment response and have low disease activity. Our detection of anti-infliximab Abs in 18% of the infliximab-treated patients concurs with a study reporting anti-infliximab Abs in 13% of patients with DAS ≤ 2.6 in a larger cohort[76].

The anti-TNFi Ab-positive patients may be in remission independent of the current TNFi treatment. This would explain how they have managed to reach and sustain remission despite the presence of anti-TNFi Abs. We do not know whether the remission has occurred spontaneously or if it has been induced by the TNFi treatment , but we do know that the autoimmune disease sometimes subside for a shorter or longer period of time[131]. If we continue to medicate these patients as if they are in a stage of active disease, the medication may be superfluous.

In the patients initiating treatment in the cohort in Paper III, the frequency of anti-TNFi Ab formation was comparable to the frequency in other mixed treatment response cohorts[59,60,62].

Anti-TNFi Abs and drug levels

The correlation between anti-TNFi Abs and clinical efficacy is likely mediated by the impact anti-TNFi Abs has on circulating TNFi levels. Apart from binding to the TNF binding site on the drug and hereby directly blocking the TNF binding effect of the drug[56,132], presence of anti-TNFi Abs increases clearance of TNFi[57]. The correlation between anti-TNFi Abs and impaired drug levels has been demonstrated in several studies concerning infliximab[53,60,61], and adalimumab[53,59,61].

Anti-TNFi Abs and TNFi efficacy

Presence of anti-TNFi Abs have been associated with treatment failure in RA patients[53,59-62].

In Paper II, EULAR response and initial decline in DAS28(CRP) did not reflect whether the patients had developed detectable anti-TNFi Abs at the time of the study. This indicates that initial treatment efficacy does not guarantee that anti-TNFi Abs have not been or will not be formed. In the remission cohort presented in Paper II we do not know when anti-TNFi Ab formation occurred. One additional study has, in spite of a good EULAR response, found presence of anti-infliximab Abs following six months of treatment[62]. Studies with information regarding timing of anti-TNFi Ab formation reveal that most patients who do form anti-TNFi Abs, do so within the first six months of treatment[59]. As our cohort was in remission, they may have exhibited a different pattern of development of anti-TNFi Abs. In our cohort, anti-TNFi Ab might have developed at a later stage. This could be at a time when variations in endogenic or exogenic factors have influenced the immune system of the patients in a way, which has prompted them to develop anti-TNFi Abs. Individual differences in immune composition and pharmacokinetics may determine development of anti-TNFi Abs, and may also hold the explanation for the differences in the timing of anti-TNFi Ab occurrence.

In the third study (Paper III), the presence of anti-TNFi Abs was associated with treatment failure, as would be expected from the literature.

Treatment impact on level of soluble biomarkers

Mutations in the gene encoding TNF-R2 are associated with an impaired clinical response to TNFi treatment[133], implicating that the effects of TNFi mediated by TNF-R2, possibly both in its transmembrane and soluble form, are important. Our finding that treatment with TNFi reduces levels of sTNF-R2 *in vivo* is novel compared with previous findings[134]. *In vitro,* infliximab reduces the expression of TNF-R2 on monocytes and increases the extracellular release of the receptor, hereby lowering the cellular response to TNF and increasing TNF neutralizing activity around the cells[135]. This may lead to an increase in sTNF-R2 at treatment initiation, as the receptors are shed, and then to a later decrease as expression is reduced. This initial rise and then decline may explain the conflicting results regarding impact of TNFi treatment on sTNF-R2 levels[134,136].

Reflecting on the impairment of efficacy by anti-TNFi Abs, we found that patients with anti-TNFi Abs failed to decrease in their levels of inflammatory biomarkers during treatment. This is in contrast to the patients who did not develop anti-TNFi Abs. In these, DAS28(CRP) decreased during treatment along with decreased levels of sTNF-R2 and IL-6. Previously levels of IL-6 have been found to decrease in response to infliximab treatment in a dose dependent manner[134]. This corresponds with our observation that a decrease in IL-6 level was greater in patients retaining detectable levels of TNFi.

We would have expected CRP to decline during treatment[23], and we did see a decline, it was just not large enough to establish statistical significance. This may be explained in part by the relatively low mean baseline level of CRP, and by the limited size of the cohort.

Predictors of immunogenicity

Previously, higher DAS28, higher CRP, low drug levels and longer disease duration have been associated with an increased formation of anti-TNFi Abs[59,60]. MTX has also been proposed to decrease or postpone anti-TNFi Ab formation[59,64]. In our remission cohort (Paper II), we did not find any association with baseline disease activity and concomitant use of MTX therapy. We found that patients with anti-TNFi Abs had shorter disease duration at initiation of TNFi treatment, which is in contrast to the findings by other groups[59,137]. This discrepancy may be explained by the difference in cohort composition, as our cohort consisted of patients in long-time remission. Further, the populations differed in median disease duration, as our cohort had a considerably higher median disease duration (14 years) than the ones we compare with (8 years)[59]. Patients with shorter disease duration at initiation of TNFi therapy may be more inflammatory active, reflecting that more ill patients are treated more aggressively, e.g. with TNFi, and as previously mentioned, higher inflammatory activity is associated with development of anti-TNFi Ab[59]. The population of RA patients who are able to retain remission on TNFi treatment may for example comprise a subgroup with certain characteristics in inflammatory drive. This or these characteristics may influence

development of anti-TNFi Abs, and results derived from a remission cohort may therefor differ from a cohort of mixed treatment response types and the underlying mixed RA phenotypes. Regarding MTX, the inhibitory effect on anti-TNFi Ab formation in other cohorts, may be exerted by the anti-inflammatory properties of this compound[66]. At the time of inclusion, inflammation in our cohort was already very low, if present at all, as the patients were in remission. Thus, inflammation may have been abated at the time of anti-TNFi Ab formation, which is why this was not influenced by MTX therapy.
We did not find any predictors of immunogenicity in the cohort study of patients initiating TNFi therapy (Paper III), but rather found predictive factors associated with low drug levels. This may partly be explained by the fact that our cohort was limited in numbers.

Predictors of impaired drug levels
Baseline CRP and baseline DAS28 have been identified as predictors of impaired drug levels and impaired treatment efficacy[60,65,138].
In Paper III, looking towards baseline predictors of impaired drug levels following six months treatment, we found that impaired drug levels were associated with higher baseline levels of IL-6. It has previously been found that higher baseline inflammatory activity leads to an increased clearance of TNFi, using CRP as an indicator of inflammatory activity[65]. Our findings regarding CRP show a tendency towards this, with a *p*-value of 0.089.

CONCLUSIONS
From Paper I we may conclude that treatment intensification with the TNFi, adalimumab, etanercept and infliximab is not very well researched, the available studies are far from ideal, and so treatment intensification practice is generally not evidence-based. Few studies have looked into intensification with adalimumab or etanercept, and none have found a beneficial effect of intensification. Regarding intensification with infliximab, results are conflicting but with an overweight of studies reporting improved clinical outcome following intensification. The sparse evidence regarding intensification of infliximab treatment regimens points towards frequency increase being more efficient than dose increase.

In Paper II we find that 10% of RA patients in remission have developed anti-TNFi Abs, and that infliximab is more immunogenic than adalimumab in this population. As seen in mixed response cohorts, presence of anti-TNFi Abs are associated with decreased levels of active TNFi.

In Paper III we find that treatment with adalimumab or infliximab in RA patients result in 23% of patients developing anti-TNFi Abs during the first 6 months of treatment, and that drug levels and the presence of anti-TNFi Abs have an impact on disease activity and inflammatory biomarkers. In addition, we find that baseline inflammatory activity predict impairment of drug levels.

FUTURE PERSPECTIVES
The results of this thesis prompt for further research. The following studies could be valuable to gain insight into measures that might improve, and possibly personalize, treatment with TNFi.
The results from the systematic review highlight the lack of a prospectively designed clinical trial investigating intensified treatment to sub-groups of patients with different levels of TNFi, and with and without presence of anti-TNFi Abs. A concentration-steered strategy would test the hypothesis, that efficacy of treatment depends on concentration of TNFi, and that anti-TNFi Abs impair efficacy through an impact on concentration. Impact of intensification on the level of TNFi and on clinical, paraclinical and radiographic disease activity should be investigated. The trial should include patients with both high and low drug levels, and within these two groups should be both patients with and without anti-TNFi Abs. In addition, differences in the type of previous response to TNFi treatment, e.g. primary- or secondary non-response, should be differentiated between. In patients with Crohn's disease, this approach has proven to be cost effective without compromising clinical outcome[80].
The ambition that our treatment practices should be evidence-based is clearly not fulfilled according to the results of the review. In spite of this, clinical practice is rarely questioned, as clinicians presumably experience some patients to decrease in clinical disease activity following intensified treatment. To ascertain that this effect is not just a result of regression towards the mean[106], clinical trials, including comparable control groups, are warranted.
Furthermore, initiatives to try to determine the minimum effective concentration of the different TNFi's may be useful. If such concentrations can be established, they may provide clinicians with a mark they can aim for when adjusting dose or frequency. This has already been attempted in a post hoc analysis[52,119], but the results need to be confirmed in prospectively designed clinical trials, and the cut-off concentrations need to be established in the different available assays.
The finding in Paper II, of a large proportion of patients in remission having low drug levels and circulating anti-TNFi Abs, invites to testing the hypothesis that patients who are in remission despite impaired drug levels are redundantly treated. These patients may be included in a randomized clinical trial, in which TNFi is tapered or discontinued. In such a trial, patients should be stratified according to reason for the low drug levels, e.g. presence of detectable anti-TNFi Abs.
Several cohort studies regarding tapering or discontinuation of TNFi are presently including participants (EudraCT no. 2007-006657-63, 2012-004631-22 and 2012-004482-40), and hopefully these will contribute to knowledge regarding how to manage TNFi treatment in a long-term perspective.
Finally, the results from Paper III call for confirmation in larger cohorts. Future studies should address the ability of baseline levels of IL-6, and possibly CRP, to predict later impairment of drug levels. Knowledge regarding the patients at risk of treatment failure may alert physicians and prevent longer periods of active disease in patients losing response. Baseline parameters may also aid in deciding which of the ever-expanding array of bio-DMARDs is suitable for a particular patient. If further observational studies confirm that baseline inflammatory activity determine drug levels, prospectively designed clinical trials could help evaluate different treatment strategies, incorporating baseline inflammatory activity in treatment algorithms, in an attempt to test an individualized treatment approach.

SUMMARY
The treatment of rheumatoid arthritis has greatly improved with the use of biological TNF inhibitors (TNFi). These biopharmaceuticals target the inflammatory cytokine TNF, and hereby decrease the autoimmune inflammation, which may otherwise lead to permanent joint damage in the afflicted patients. Although TNFi de-

crease clinical disease activity in the majority of the treated patients, they are not always effective. Some patients have a partial response, some lose their initial response to treatment, and others never experience effect at all. The concentration of TNFi in the patients' bloodstreams, or the generation of antibodies directed towards the TNF inhibitor (anti-TNFi Abs), are known to have an impact on treatment efficacy. Furthermore, in patients with a good treatment response, strategies for how to tamper or discontinue treatment are lacking.

In this PhD thesis, ways to improve treatment with TNFi are explored in three studies.

The first study describe current knowledge on the effect of intensifying treatment with TNFi as a way to increase treatment efficacy. The results from this literature review do not convincingly support that intensified treatment increase efficacy in patients with RA in general, although an effect may be seen in patients treated with infliximab. The diverging results on the efficacy of infliximab intensification may be explained by effects on subgroups of patients being masked in mixed cohorts. We suspect that if patients are sub-grouped according to factors such as blood concentration of TNFi or presence of anti-TNFi Abs, an effect of treatment intensification on clinical outcome may bee more convincing.

The second study assesses the frequency of anti-TNFi Ab formation in patients with RA in remission in an effort to identify patients for whom continued treatment is superfluous. If anti-TNFi Ab and low drug concentrations in patients in remission are predictors of TNFi-free remission, the impact on treatment and economic costs may be considerable. The finding that 10% of the patients in remission have developed anti-TNFi Abs shows that the potential is substantial.

The third study investigates if baseline values of various biomarkers and other variables can predict development of anti-TNFi Abs or the emergence of sub-therapeutic drug levels. From the results, it seem that baseline inflammatory activity, judged from the level of interleukin-6 and possibly C-reactive protein, predicts low drug levels after six months of treatment. This may lead to early identification of patients at risk of treatment failure owing to inadequate drug levels, with the opportunity to take measures to prevent this.

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