*Parts of this introduction have been published:
Biological medications include a wide range of medicinal products created by biological instead of chemical processes. Biologicals may consist of proteins, nucleic acids or complex combinations of substances, or may be living entities such as cells and tissues. They are isolated from natural sources or are produced by biotechnology methods. Over the past decades, biologics with variable targets have been developed for treatment of inflammatory rheumatic diseases. These biologics appeared similar effective in patients with rheumatic diseases in whom conventional treatment was not sufficient. One of the targets is tumour necrosis factor (TNF), a pro-inflammatory cytokine and one of the key factors in inflammation and matrix destruction in inflammatory diseases.

This thesis will focus on TNF-inhibitors in rheumatoid arthritis (RA). RA is an auto-immune disease characterised by inflammation of the joints, if not treated adequately, resulting in cartilage and bone destruction and related functional disability, decreased quality of life and shortened life expectancy. The TNF-inhibitors available for the treatment of RA are infliximab, a chimeric monoclonal antibody of the IgG1 subclass, adalimumab and golimumab, fully human monoclonal antibodies also of the IgG1 subclass, etanercept, a recombinant fusion protein receptor and certolizumab, a pegylated Fab’ fragment of an immunoglobulin.

**Immunogenicity of biologics**

Biological therapeutics can be recognised by the human immune system as ‘non self’ and induce an immune response, also known as immunogenicity. Therapeutic endogenous proteins, such as erythropoietin and growth factors, have an amino acid sequence identical to the human equivalent but may be immunogenic due to glycosylation or conformational changes, whereby new epitopes emerge. Therapeutic antibodies carry unique complementarity determining regions (CDR), containing stretches of sequences that frequently will be recognized as foreign and induce formation of antidrug antibodies (ADA).

**Assay techniques**

Various classes of immunoglobulins, with variable affinity, are produced. In addition, the formation of ADA fluctuates over time and immunological tolerance might develop at a certain time point during treatment. On the other hand, with longer follow-up duration a higher percentage of patients might test positive for ADA. Also, timing of blood sampling during treatment can affect the detection of ADA: the measurement of ADA in trough
samples reduces drug interference in ADA assays. These issues will be discussed in this thesis.

Several assay formats are available for the detection of ADA. None of the available assays is able to detect all of the subclasses or idiotypes of ADA. IgM antibodies have low avidity, and are therefore difficult to detect. Affinity matured IgG1 and IgG4 antibodies are easier to detect. IgE has a high avidity, but excess of IgG makes detection of the relatively low amounts of IgE challenging.

Commonly used assays include direct enzyme linked immunosorbent assays (ELISAs), which are straightforward but suffer from nonspecific binding. Two-site or bridging assays are sensitive and specific, but do not detect IgG4 antibodies and are susceptible to drug interference, since both binding sites of the ADA need to be available for detection. Antigen binding tests (ABT) have been shown to be specific and are less susceptible to drug interference. However, for these radioimmunoassays there is a need for laboratory facilities for radioactivity. A fourth type is the cell-based assays which are not frequently used for immunogenicity testing, because they are time consuming, complex and variable.

Confounding factors: false positive

Numerous factors could lead to false-positive results when assessing the immunogenicity of therapeutic antibodies. Binding to the plate may induce conformational changes of the therapeutic protein that may result in nonspecific signals. In particular, Fc interactions of IgG4 to coated IgG may give rise to background problems. In patients with RA, low avidity IgM (or IgG) rheumatoid factor can also lead to false positive results, because rheumatoid factors bind to the Fc part of antibodies. Therefore, the use of F(ab')2 fragments of therapeutic antibodies in assays can eliminate this background, but it introduces another source of false-positive results due to anti-hinge antibodies (found in sera from some healthy individuals as well as some patients with RA). However, this background can be blocked by addition of polyclonal F(ab')2 fragments prepared from intravenous immunoglobulin (IVIG).

Confounding factors: false negative

Although the two-site assay is the most sensitive for the detection of ADA, a smaller number of patients test positive in the two-site assay compared with the ABT. This discrepancy is caused by the susceptibility for drug interference (Figure 1). The two-site assay typically measures ADA in the absence of detectable drug levels, which occurs when the amount of
ADA exceeds the concentration of the drug present in the serum. In the presence of functional drug levels, drug-antidrug antibody complexes are formed and as a result, the assay cannot detect the ADA. The ABT is able to measure a small part of the ADA in complex with the drug. A pH-shift-anti-idiotype ABT enabled the measurement of ADA in the presence of drug by dissociation of the complexes. This will be further discussed in this thesis. Confounding factors of ADA assays and different test results for different assay types could lead to confusion or misinterpretation when the user is unaware of these aspects.

In this thesis, for the studies describing the clinical relevance of immunogenicity, ADA were measured using an ABT. ADA detected with this assay are clinically relevant and the assay is less sensible for drug interference compared with an ELISA. To study the immune response against TNF-inhibitors into more detail a Ph-shift-anti-idiotype ABT is used.

**Figure 1.** The detection of antidrug antibodies (ADA) is dependent on the assay used for detection and its susceptibility for drug interference. This graph shows the drug level, detected with an ELISA, and the amount of ADA detected with a Ph-shift-anti-idiotype antigen binding test (PIA), an antigen binding test (ABT), which is a radioimmunoassay, and an ELISA, respectively. Most clinically relevant situation is depicted on the right side of this figure: high ADA levels and no functional drug.
Drug level measurement
The measurement of functional drug levels is, in comparison to the measurement of ADA, more straightforward and costs are low. In general, a drug specific ELISA is used for the measurement of these levels. For TNF-inhibitors, the drug is captured with this assay via its ability to bind TNF. The measurement of trough levels is important in the standardisation process and interpretation of the results. Trough levels are measured directly before the next injection or infusion with a biologic. Drug levels reflect pharmacokinetics of a drug and can be influenced by the amount of ADA formed via the formation of drug-antidrug complexes. (Figure 1)

Clinical implications: efficacy
Therapeutic antibodies are removed from the circulation at similar or slightly faster rate compared with endogenous IgG. The production of ADA can cause faster clearance due to the formation of immune complexes. In addition, drug in complex with ADA will not be biologically active if the ADA are directed to the antigen-binding site, which is usually the only foreign part of humanised or fully human therapeutic antibodies. As a consequence, TNF can be active again (Figure 2). These two mechanisms lead to decreased functional drug levels and consequently to impaired treatment responses.

ADA detection in clinical practice is registered in several long-term observational cohort studies. For example, in 2006 we already demonstrated that in a cohort of 51 infliximab-treated RA patients, nearly half of the patients developed ADA within one year of treatment and this interfered with clinical response. In addition, in 105 infliximab-treated RA patients, after 14 weeks of treatment, non-responders had lower infliximab trough levels than responders. In 121 adalimumab-treated RA patients, 17% had detectable ADA after 6 months of therapy. The presence of ADA was associated with low serum adalimumab levels and non-response to treatment.
Antidrug antibodies (ADA) interfere with tumour necrosis factor (TNF) binding. On the left panel two TNF molecules bind to a therapeutic monoclonal antibody (Drug). On the right panel, ADA interfere with the binding of a TNF molecule to the therapeutic antibody (Drug), whereby TNF can be active again.

Clinical implications: adverse events

The presence of ADA might be associated with adverse events, of which infusion reactions are most common.

A well known example of an adverse reaction that occurred after the administration of an erythropoietin product was a pure red cell aplasia, in which ADA against the erythropoietin product depleted not only the drug itself but also the endogenous erythropoietin. This very rare phenomenon, caused by conformational changes of the ADA, is typical for endogenous therapeutic proteins with a biological function, potentially with severe consequences for patients.

Infusion reactions are common adverse events in patients treated with biological medications, with limited consequences in some of the cases and major ones in others. In infliximab treated Crohn’s disease patients, the presence of higher concentrations of ADA predicted a higher risk of infusion reactions (relative risk 2.40; 95%CI 1.65-3.66). Adverse events associated with the formation of drug-antidrug complexes, are probably rare. In some adalimumab-treated patients who previously developed ADA, serious thromboembolic events occurred. No firm conclusions can be drawn because this study was underpowered and other risk factors could have contributed to the occurrence of events.
Factors influencing immunogenicity

Strategies to reduce the immunogenicity of biologic medications have mainly involved alterations to the molecule. For instance, the humanization of biologic agents of murine origin has greatly reduced the immunogenicity of these drugs. However, even fully human biologic agents may evoke an immunogenic reaction. Another potential strategy for reducing immunogenicity is the modification of immunogenic peptide fragments (T-cell epitopes) on the therapeutic antibodies. An important consideration with this strategy is that the protein has to remain stable and active, despite the modifications that have been undertaken to reduce the immunogenicity.\(^\text{23}\)

In RA patients treated with biologics, efficacy is in general enhanced when given in combination with methotrexate.\(^\text{24-26}\) There appears to be a favourable effect of co-treatment with methotrexate on the immunogenicity of biologicals, as will be further discussed in this thesis. Suppression of early T-cell and B-cell expansion by methotrexate might be responsible for the modulation of the immune response, whereby the formation of ADA is reduced in quantity and subsequently functional drug levels are increased.\(^\text{27 28}\) Other investigators demonstrated that trough drug levels are higher in patients concomitantly treated with methotrexate because methotrexate reduces inflammation and consequently the TNF-load might be lower, resulting in higher functional drug levels.\(^\text{29}\) This might indicate that the state of the immune system, an active inflammatory state, influences the immune response against ‘non self’ therapeutic antibodies.

Objectives of this thesis

Data discussed in this thesis is derived from two well-defined, simultaneously-running observational cohort studies of RA patients, treated with either adalimumab or etanercept, during long-term follow-up.

Although the impact of immunogenicity on clinical response to TNF-inhibitors, mainly infliximab and adalimumab, has emerged from a growing number of observational studies over the past decade, those studies generally comprise a short-term follow-up of 6 to 12 months. Our first aim was to investigate the long-term effect of immunogenicity of adalimumab on important clinical outcome parameters. In addition, the relationship between etanercept serum levels and clinical response was studied during 6 months of follow-up to obtain more insight in the pharmacokinetics of etanercept. Furthermore, long-term response rates in both cohorts were compared, since at that time head-to-head trials were lacking. In
this study, the presence or absence of ADA was emphasised to identify the best responding patients.

To optimise treatment of RA with TNF-inhibitors, the effect of methotrexate on the immunogenicity of adalimumab and infliximab was reviewed, since observational studies showed a favourable effect of immunosuppressive co-treatment on the development of ADA. In addition, a more practical question was what methotrexate dose to use to achieve a beneficial effect on immunogenicity.

To explore other ways to optimise treatment with biologicals, a concentration-effect curve of adalimumab was constructed to study the relationship between drug level and clinical response and whether a therapeutic window could be identified. Knowledge gained from observational studies could be used to adapt treatment in a controlled setting, not only based on clinical response, but also on drug level testing, and we hypothesised that this might be a cost-effective treatment strategy.

At last, the Ph-shift-anti-idiotype ABT, might help in analysing the magnitude of an immune response, since with this assay is was possible to measure ADA in complex with drug. We wanted to investigate the clinical relevance of ADA in complex with drug and the benefit of measuring ADA with this assay compared to the regular ABT.

Outline of this thesis

Chapter 2 describes the long-term (3 year) clinical outcome of 272 adalimumab treated patients with RA and the negative effect of the immunogenicity of adalimumab on a range of clinical outcome parameters.

In chapter 3 ADA detected with a pH-shift-anti-idiotype antigen binding test give more insight in the immune response to adalimumab. The clinical relevance of the results of this assay is discussed.

In chapter 4 the concentration-effect curve of adalimumab in patients with RA is shown. This curve might provide a basis for therapeutic drug monitoring.

Chapter 5 is a cost-effectiveness analysis of personalised treatment with adalimumab. An algorithm in which treatment decisions are based on adalimumab serum levels and clinical response is defined and compared to usual care in which treatment adaptation decisions are solely based on clinical response.
In **chapter 6** etanercept serum levels are measured in a cohort of 292 patients with RA followed for 6 months. It is investigated whether serum levels of etanercept correlate with clinical response to treatment.

**Chapter 7** compares the clinical outcome of anti-TNF-naive patients with RA treated with either etanercept or adalimumab, with regard to the presence of ADA detected with a radioimmunoassay.

In **chapter 8a** evidence of the beneficial effect of methotrexate and other immunosuppressive drugs on the immunogenicity of various monoclonal therapeutic antibodies is reviewed. This effect is shown for several inflammatory diseases. **Chapter 8b** investigates the methotrexate dose needed to reduce the immunogenicity of adalimumab in patients with RA.

In **chapter 9** the research agenda is discussed. Attention is paid to the importance of therapeutic drug levels and if, how and when therapeutic drug monitoring can be implemented in daily clinical practice. The concept of dose-to-target will be discussed.
Reference list


