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

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

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

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

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

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

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

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

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

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

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

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

The relationship between variations in dosing, risk of an immunogenic response and immune tolerance is an interesting topic. Patients with a transient immunogenic response were reported in chapter 2, in a study of RA patients treated with adalimumab and is supported by data of natalizumab and infliximab. The mechanism behind the development of immune tolerance is currently unknown, but it seems to be a state of immune unresponsiveness specific to a particular antigen induced by previous exposure to that antigen. For therapeutic antibodies it has been shown that ADA originated from different naive B-cells and underwent extensive hypermutation; resulting in ADA (IgG) with high avidity for the idotype of the biological therapeutic. For this maturation process, activation of T- and B-cells is required.

In contrast, if higher dose is associated with reduced risk of immunogenicity, a dose reduction and/or interval prolongation might be associated with a higher risk of evoking an immunogenic response. This is an important topic, because dose reduction of a biological is currently broadly studied and this topic will be further discussed during the summary of chapter 6.

In Chapter 5 the relationship between serum tocilizumab trough level and clinical outcome was studied in patients with RA. We expected to identify immunogenicity
as main pharmacokinetic factor of clinical relevance, like was found for TNF-inhibitors.1,2,4,8,10,12 Interestingly, undetectable serum tocilizumab trough levels could not be explained by immunogenicity. This suggest that another pharmacokinetic factor plays a more important role, which is probably target-binding and this is discussed below.

A serum tocilizumab trough level above 1 mg/L is sufficient for systemic blockade of the IL-6 pathway. Firstly, because it is sufficient to normalize C-reactive protein (CRP), which is produced by the liver and can be considered as a surrogate marker for membrane bound IL-6 receptor saturation.66,67 Secondly, this drug level is sufficient to bind more than 95% of systemically present soluble IL-6 receptor.69-72 Nonetheless, it might be that bioavailability of tocilizumab is slightly different in target tissue as compared to the systemic compartment but, to our knowledge, data regarding this issue is lacking. In IBD, one preliminary study has investigated the correlation between serum and tissue TNF-inhibitor drug level and endoscopic remission to identify the ‘therapeutic tissue drug level’. Overall correlation between serum drug level and endoscopic remission was good, but some patients with active IBD had a higher ‘serum to tissue anti-TNF mismatch’ suggesting serum drug levels are not always a good predictor for local bioavailability. This could be explained by increased local target load, or possibly, delay in saturation of target cells as was seen in our tocilizumab study. Nevertheless, these data cannot be extrapolated to chronic inflammatory rheumatic diseases, because in active IBD there will be an increased protein clearance, including losses in the stool.36,71

Our study shows that some patients have undetectable serum tocilizumab trough levels despite receiving standard dose, which suggests that they have more target load (i.e. IL-6 receptors) than others, and therefore, might benefit from a higher tocilizumab dose at start of treatment to saturate all IL-6 receptors. In addition, most of these patients obtain sufficient levels over time, suggesting that some process of receptor down-regulation occurs. Nevertheless, little is known about variations in target between patients (e.g. polymorphism of the IL-6 receptor)74-76 or up and down regulation of IL-6 receptors during disease course and treatment.

Due to the direct relationship between the IL-6 pathway and CRP, tocilizumab is ideally suited to investigate the therapeutic window for effective target pathway blockade. Currently, optimal ranges are known for adalimumab (RA, PsA and psoriasis) and infliximab (IBD), but these are often based on conventional measurements of disease activity. Only for IBD preliminary results show that higher serum anti-TNF levels are associated with endoscopic remissions, but the therapeutic window for endoscopic remission has not been studied.77-79

Conventional measures to assess disease activity in chronic inflammatory rheumatic diseases might not always be a good representation of amount of target blockade.31,39,80-82 These measurements (e.g. disease activity score (DAS) or Bath AS Disease Activity Index (BASDAI)) represent several domains, like active inflammation, clinical symptoms and patient perception on well-being and quality of life. These
composite measurements are suitable to assess the overall treatment goal, but not to discriminate between the role of the target molecule versus other factors, like the influence of other inflammatory mediators, the contribution of non-inflammatory domains or potential misdiagnosis. To optimize biological treatment in a rational, personalized and cost-effective manner it will be important to approach this kind of treatment as molecular targeted therapy, thus, dosing guided by amount of target inhibition. If, target is not measurable in a valid manner, a surrogate marker could be used, like CRP as surrogate marker for systemic IL-6 pathway inhibition or CD86 for monitoring belatacept treatment in adult kidney transplant recipients. In the absence of suitable surrogate markers, TDM can add value to clinical measurements alone to optimize treatment, because detectability of functional drug levels provide additional information with regard to inhibition of the target pathway; not only in case of under treatment but also with regard to over treatment.

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

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

Our study shows promising results, with a good correlation between adalimumab and ADA levels measured in serum obtained by venipuncture compared with the measurements obtained by finger prick. Precision and accuracy are within acceptable limits as described by Food and Drug Administration (FDA) and European Medicines Agency (EMEA) guidelines. For further development it will be necessarily to investigate, if material collected by the patient at home will be of sufficient quality to
Concluding remarks and future research

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

Although, the clinical impact of immunogenicity is mostly determined by the remaining functional drug level, it remains important to characterize the immunogenic response. Several initiatives are currently being conducted, like the Anti-Biopharmaceutical Immunization: prediction and analysis of clinical relevance to minimize the RISK (ABIRISK)\textsuperscript{90} and the Impact of immunogenicity on anti-TNF response after switch (INTENT) study (EudraCT Number: 2015-002284-42).\textsuperscript{91}

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

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

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

The above mentioned RCT is based on the therapeutic window of adalimumab as assessed in the concentration effect curve.\textsuperscript{31,80,81} However, the efficacious dose of a biological therapeutic is probably highly individual; for example, serum adalimumab range of 5-8 mg/L applies to RA patients with active disease in general. For patients with sustained low or minimal disease activity or remission the overall range is probably lower. However, in individual cases the optimal range may be higher or lower depending on the amount of TNF and TNF-receptors present in the system. Therefore, tapering possibilities do not depend on drug level itself, but on excess of anti-TNF compared to target load and its pathway. If, both could be measured reliably, this might be an opportunity to predict the chance of successful dose tapering in patients treated with biological therapy. To date, such a predictor has not been identified.

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

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

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