Optimizing anti-TNF therapy in inflammatory bowel disease
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This thesis illustrates how the use of inflammatory markers and drug concentrations may help to optimize anti-TNF therapy in patients with IBD. Of all currently available biomarkers, serum CRP and fecal calprotectin have been the most informative and frequently used in CD and UC.

Chapter 2 demonstrates how fecal calprotectin levels depend on the disease location in patients with active CD. Likewise, fecal calprotectin levels correlate better with the inflammatory burden, as detected by leukocyte SPECT-CT, in patients with more distal left sided UC compared to patients with pancolitis (Chapter 3).

These results might be explained by the degradation of calprotectin throughout the colon by bacterial proteases, like matrix metalloproteinases, trypsine, neutrophil elastase and cathepsins, that are abundantly present in stool of patients with IBD.

Moreover, increased concentrations of particularly matrixproteinase-1 and -3 have been correlated with disease activity. Proteolysis could thereby play a significant role in the breakdown of biomarkers for mucosal disease activity and therapeutic proteins. This confounding factor along with the location of the inflammation should hence be taken into consideration when interpreting the results of fecal calprotectin measurements.

Most current markers require time consuming laboratory procedures, so it takes relatively long before results become available. Hence, even less invasive, but rapidly available, that correlate with mucosal disease activity would be clinically useful. To this extend, numerous innovative tools have been studied lately, such as the detection of volatile organic compounds in exhaled breath or fecal gas analysis by 'electric nose', which we correlated with disease activity.

Currently, an ongoing study in the AMC investigates heart rate variability as an alternative measure for inflammation, with a device that has been used in trials for vagal nerve stimulation in patients with rheumatoid arthritis and IBD.

The detection of Technetium stained leukocytes in the colon (Chapter 3) represents an interesting diagnostic modality to quantify the anti-inflammatory load and the effect of drugs in clinical trials. The imaging by leukocyte SPECT-CT might be of particular interest to estimate the effect of leukocyte trafficking inhibitors, such as vedolizumab.

Technetium labelling with nuclear scintigraphy was also used to display anti-IFX complexes in patients with rheumatoid arthritis and the accumulation of immunoglobulins in the colon of patients with UC.

Patients with IBD exhibit increased clearance of proteins and immunoglobulins, which enhances during active and severe disease. This phenomenon of fecal immunoglobulin loss was also observed with IFX in patients with severe UC (Chapter 4). Recently, our findings of therapeutic antibody loss in stool have been replicated with the detection of (anti-)adalimumab in stool of IBD patients with elevated fecal calprotectin levels.

Future initiatives include full validation of the fecal IFX assay and collaborative study with the Cincinnati IBD Center and Cincinnati Children’s Hospital and other hospitals affiliated with the pediatric IBD network PRO-KIDS (funded by Broad Medical).
This thesis illustrates how the use of inflammatory markers and drug concentrations may help to optimize anti-TNF therapy in patients with IBD. Of all currently available biomarkers, serum CRP and fecal calprotectin have been the most informative and frequently used in CD and UC. Chapter 2 demonstrates how fecal calprotectin levels depend on the disease location in patients with active CD. Likewise, fecal calprotectin levels correlated better with the inflammatory burden, as detected by leukocyte SPECT-CT, in patients with more distal left sided UC compared to patients with pancolitis (Chapter 3). These results might be explained by the degradation of calprotectin throughout the colon by bacterial proteases, like matrix metalloproteinases, trypsin, neutrophil elastase and cathepsins, that are abundantly present in stool of patients with IBD. Moreover, increased concentrations of particularly metalloproteinase-1 and -3 have been correlated with disease activity. Proteolysis could thereby play a significant role in the breakdown of biomarkers for mucosal disease activity and therapeutic proteins. This confounding factor along with the location of the inflammation should hence be taken into consideration when interpreting the results of fecal calprotectin measurements.

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Research Program of the Crohn’s & Colitis Foundation North American). This ARCH trial (Anti-TNF Therapy for Refractory Colitis in Hospitalized Children) aims to study pharmacokinetic determinants of infliximab non-response in children hospitalized with severe steroid-refractory colitis. Frozen stool samples obtained during the first 24 hours after IFX administration will be checked for fecal IFX levels, in 36 pediatric patients with severe acute steroid-refractory UC.

Proteolytic degradation of therapeutic antibody in the mucosa may contribute to lack of response to IFX. Matrix metalloproteinases 3 and 12 were shown to degrade IFX to F(\(ab\'))2 fragments in patients with IBD.\(^9\) Mucosal proteins disturb the structure and effect of IFX, which can be prevented by the addition of matrix metalloproteinase inhibitors. To further investigate the possible role of proteolysis of IFX, a validation study for fecal IFX is currently ongoing in the AMC. In this study, IFX is added to diarrheic stool samples of hospitalized UC patients that do not use IFX. The main objective is to compare the concentration of the supplemented IFX with the predefined concentrations (‘spiking’) at different temperatures (allowing different protease activity) and with or without protease inhibitors.

Besides fecal concentrations of anti-TNF, there is a rising interest in therapeutic biomarkers at the level of the actual target tissue. Previously, mucosal TNF at baseline, quantified by PCR in biopsies, was found to be predictive of long-term outcome of IFX in patients with steroid refractory CD.\(^9\) This finding was reproduced in a technical highly sophisticated study by the Neurath group. Baseline mucosal membrane bound TNF, detected with confocal laser endomicroscopy, predicted therapeutic response in CD.\(^9\) Furthermore, the recent ATLAS study looked at the balance of mucosal TNF to anti-TNF concentrations and associated endoscopic disease activity. Severely inflamed mucosa was characterized by a relatively high TNF to anti-TNF rate.\(^9\) Extensive local inflammation with accompanying high concentrations of TNF may thereby serve as a ‘sink’ for the local and systemically available anti-TNF. An ongoing study at the AMC integrates (anti-)TNF serum, tissue and feces levels in order to establish a comprehensive and complete pharmacokinetic model of the 3 separate compartments in patients with moderate to severe UC in correlation with response.

Anti-drug antibodies, detected by a drug tolerant assay, already appear during induction treatment and increase clearance of IFX in patients with moderate to severe UC.\(^9\) (Chapter 5) Most previous studies used assays that are unable to detect anti-IFX antibodies (ATI) in the presence of high circulating drug concentrations and its immunogenicity might thereby have been underestimated. A wide variety in assays with variable affinity, sensitivity and drug tolerance makes it impossible to compare results between different studies.\(^9\) Sanquin laboratories have compared 3 different in-house developed drug tolerant ATI assays. The acid dissociation radioimmunoassay (ARIA) was more sensitive than the pH-
shift-anti-idiotype antigen binding test (PIA) or 37°C radioimmunoassay in the presence of high drug concentrations. In an effort to harmonize anti-infliximab antibody assays, the Leuven group has developed a calibration assay, possibly allowing for future comparisons of ATI results from different clinical trials.

Another limitation of most commonly used drug intolerant assays is that current therapeutic drug monitoring is mainly based on trough measurements. The trough level is measured in a serum sample collected just before the next infusion, the moment that drug concentrations are lowest, potentially allowing for the detection of anti-drug antibodies. Since trough level results can technically only be obtained days to weeks after sample collection, drug dosing cannot be adjusted until the next infusion, 4-8 weeks later. The reliability to predict trough levels based on non-trough, intermediate measurements, taken weeks before infusion was investigated in the PREDIX trial. In this prospective, observational study, sequential serum IFX measurements were performed at week 4, 6 and 8 in patients with CD in clinical remission on 8-weekly maintenance therapy. An excellent correlation between IFX concentration at 4 and 6 weeks from the last infusion and the trough level 8 weeks after the latest infusion was observed. Determination of non-trough concentrations can thereby facilitate earlier concentration-guided dose adjustments of IFX in Crohn's disease. Even more ideal would be the availability of an easy sampling method via capillary puncture and concentration analysis with dry blood spot. Such a point-of-care test would allow for 'on the spot' measurement and immediate dose adjustment and is currently being validated.

Subcutaneous anti-TNF agents show a different pharmacokinetic profile, not only by different dosing regimen, but also by a different pattern of distribution, namely lymphatic absorption. Furthermore, the humanisation of subcutaneous antibodies is thought to result in lower immunogenicity. Although pharmacokinetics of golimumab was already studied in the GEMINI registration studies, real life clinical data, from patients that were previously anti-TNF exposed, are still lacking. Therefore, we are currently studying the pharmacokinetics of golimumab induction and maintenance therapy in patients with moderate to severe ulcerative colitis, analogous to the KINETIC study, as described in Chapter 5. (GO-KINETIC, ClinicalTrials.gov Identifier: NCT02277470). Additionally, we are performing a population pharmacokinetic study of adalimumab, similar to the study described in Chapter 6, to determine which patient, disease and treatment factors influence serum concentrations and clearance of adalimumab in patients with IBD.

In contrast to patients with high inflammatory burden, possibly receiving too little IFX when standard doses are administered (Chapter 5 and 8), some patients that achieve clinical and biochemical remission might receive too much drug. In these patients supratherapeutic IFX levels might lead to impaired quality of life, whilst their bowel disease is under control, as demonstrated in Chapter 7. Decreasing doses could be considered in
Chapter 10

those patients. Guided by trough levels, dose de-escalation can be successful IBD patients, whilst retaining disease control.\textsuperscript{34} Similarly, doses could be tapered in patients suffering from spondyloarthritis with low disease activity, without increased rates of relapse.\textsuperscript{35} In patients with low rheumatoid arthritis activity dose reduction of anti-TNF guided by disease activity was non-inferior to usual care.\textsuperscript{36} Doses could be successfully reduced in the majority of these patients and was even stopped in one-fifth of all patients.

Besides the evolving knowledge about optimizing anti-TNF therapy, by dose-intensification or de-intensification, it is still unclear in which IBD patients anti-TNF can be (definitely) stopped. High rates of relapse are reported in trials evaluating CD patients that stop anti-TNF and continue thiopurines monotherapy.\textsuperscript{37} In the STORI trial, about half of the CD patients relapsed on immunomodulator within one year after infliximab therapy was discontinued. Elevated inflammatory parameters (leukocyte counts, C-reactive protein and fecal calprotectin) at time of discontinuation were the most important factors predicting relapse in these patients.\textsuperscript{38} In contrast, if IFX is discontinued in patients with quiescent disease, a considerable amount of patients remain in clinical remission after a decade on immunomodulator monotherapy.\textsuperscript{39}

After a so called ‘drug holiday’ patients are often restarted on a different drug. Finally, Chapter 9 advocates that retreatment with IFX should be considered in patients with refractory CD.\textsuperscript{40} In a historical cohort, therapeutic drug monitoring was not yet routinely done during the first IFX treatment episode and IFX was primarily discontinued for a variety of reasons. Response could then be regained at the second treatment episode, possibly by the more institutionalised use of therapeutic drug monitoring by that time. Indeed higher IFX trough levels and the absence of ATI at re-initiation of IFX correlated with response and safety of retreatment in another retrospective cohort of IBD patients.\textsuperscript{41} Concomitant use of immunomodulators seems advisable at restart of IFX.\textsuperscript{42}

In 2013, the expiration of the patent on IFX gave rise to the introduction of two generic IFXs: Inflectra and Remsima.\textsuperscript{43} These biosimilars were tested for equivalence in an efficacy and safety trial in rheumatologic conditions.\textsuperscript{44, 45} Without any further trials, these results were immediately extrapolated to all registered indications for IFX, including CD and UC, by approval of the European Medicines Agency (EMA).\textsuperscript{46} Cost savings are the major advantage of those drugs and limited reports indicate similar efficacy in IBD, however differences or similarities in pharmacokinetics and immunogenicity are to be investigated.\textsuperscript{47, 48} Indeed, antibodies to the originator IFX equally recognise and cross-react with innovator IFX.\textsuperscript{49}

Numerous drugs with different therapeutic targets are in the future pipe-line for CD and UC.\textsuperscript{50, 51} Lessons can be learned from the recent discoveries in immunology or in the field of other auto-immune mediated diseases, such as rheumatoid arthritis and psoriasis.
Tofacitinib, for example, a JAK-kinase inhibitor, which also has demonstrated efficacy in rheumatoid arthritis, will probably receive registration for ulcerative colitis in the next coming years.\textsuperscript{52} Ustekinumab, a monoclonal antibody targeting the common p40 subunit of IL12 and IL23, already registered for the treatment of psoriasis, is particularly effective in anti-TNF refractory CD patients and might thereby offer a future alternative in these patients.\textsuperscript{53}

We performed a phase I and II study of the efficacy and safety of low level light therapy (LLLT) on healthy subjects and patients with ulcerative proctitis. (Photopill Ltd) LLLT has been used for tissue healing in many mucosal diseases, similar to IBD, that involve wounds, ulcers and inflammation, oral chemotherapy-induced mucositis in particular. Firstly, photo biostimulation with LLLT was found to exert a positive effect on disease progression in mice with Dextran Sodium Sulphate (DSS) colitis.\textsuperscript{54} Consecutively, Photopill (low level light with wavelength of 850nm) treatment was tested in 4 healthy volunteers and was well tolerated and safe (no adverse effects or mucosal damage). (data on file) Finally an open-label, interventional, clinical trial (Phase 2) was designed for assessment of the safety and feasibility of the Photopill capsule treatment in patients with mild to moderate Ulcerative Proctitis. (ClinicalTrials.gov Identifier: NCT01837615). Although treatment with LLLT by Photopill appears to be save (again no adverse events), clinical and biochemical parameters and mucosal appearance did not improve significantly during this study. (data on file)

Similar to Chapter 3 in human IBD, we have performed studies in transfer colitis mice (CD45RB\textsuperscript{hi} subpopulation of CD4+ T cells transferred to severe combined immunodeficiency mice) comparing in vivo mice endoscopy and histology.\textsuperscript{55-57} We aimed to validate histology and endoscopy in mice, by blinded central reading, as was previously done in humans.\textsuperscript{58-61} A good inter and intra observer variability was found for both histologic and endoscopic evaluation in mice with different severity of colitis. (data on file) Furthermore, we did a dose-titration pilot experiment to assess effects of different dosages of anti-TNF in a murine transfer colitis model, which was evaluated by mice endoscopy, to support our findings of Chapter 5. Indeed, a dose-dependent effect of anti-mouse TNF was observed in transfer colitis, with suboptimal dosing resulting in suboptimal mucosal healing. (data on file) Finally, these results will be used to find new drugs that might help to further optimize treatment with anti-TNF in mouse models. Preliminary data from a large in vitro drug screen identified several candidate drugs to potentiate IFX in the formation of wound healing macrophages, thereby potentially increasing its therapeutic effect.\textsuperscript{52}

Findings from this thesis support the use of inflammatory markers, serum concentrations and detection of anti-drug antibodies for optimizing anti-TNF therapy in patients with IBD. Exposure to insufficient anti-TNF leads to lower drug levels in patients with a high baseline inflammatory load and may thereby increase the risk of early formation of anti-drug antibodies. Similarly, low serum IFX trough levels preceded the formation of anti-
drug antibodies and subsequent treatment failure in patients with rheumatoid arthritis. Increased doses of IFX resulted in better response in rheumatoid arthritis patients with high inflammatory load compared to patients with a lower inflammatory burden. Selected patients should possibly receive higher doses initially, through which immunogenicity may be prevented. High dose tolerance and inverse dose-response with respect to immunogenicity has been observed with anti-TNF agents. The mechanism how high inflammatory burden (or TNF/anti-TNF ratio) could cause early antibody formation is still unclear. Speculatively, a more active immune system by suboptimal dosing might render patients prone to develop an immunogenic reaction. Alternatively, the size of immune complexes may also alter their immune activating capacity. Whether rapid clearance and low drug concentrations are the cause or consequence of early anti-drug antibody formation and loss of response however remains to be determined. Changes in serum drug concentrations precede regain of response and correlate with consecutive response, suggesting that optimization of drug concentrations really improve outcomes.

An accelerated infliximab induction strategy has already retrospectively found to decrease early colectomy rates in patients with severe ulcerative colitis. Nevertheless, this strategy warrants prospective evaluation, guided by inflammatory parameters and drug concentrations. Several trials are presently performed that might provide valuable insights in this approach. Higher versus standard adalimumab dosing regimens are being investigated in an ongoing double-blind, randomized, multicenter study comparing induction and maintenance therapy in patients with moderately to severely active UC (SERENE UC, AbbVie Humira, Protocol M14-033) Furthermore, the results of the EaSiFx study by Mahadevan et al., will be awaited with great interest (ClinicalTrials.gov Identifier: NCT01971814). In this study, hospitalized patients with severe ulcerative colitis received infliximab 10mg/kg IV and early IFX levels were measured in the first 2 weeks (including day 1,2 and 3) of therapy. Many answers to the remaining questions in this thesis are to be expected from the recently completed TAILORIX trial, a randomized controlled trial investigating tailored treatment with IFX for active CD. (ClinicalTrials.gov Identifier: NCT01442025) This protocol aims to investigate whether sustained trough levels of IFX can be obtained by algorithm guided dose adjustment based on IFX trough measurements compared to 'standard conventional' IFX treatment (symptom based dosing) and to compare the effects on clinical and endoscopic outcomes. Finally, we are currently performing randomized controlled multicenter study comparing precision dosing of IFX maintenance therapy, based on a Bayesian PK model with targeted trough serum IFX of 3g/ml (taken in account: body weight, individual clearance and inflammatory activity) to conventional dosing in IBD patients that are in clinical remission. (PRECISION study, ClinicalTrials.gov Identifier: NCT02453776) Endpoints of this study include sustained clinical remission and biochemical
Discussion and future perspectives

remission (defined by serum CRP and fecal calprotectin) at week 52 and costs of IFX treatment.

Ultimately, informative baseline patient, disease and treatment factors should be incorporated into clinical algorithms for individualising dosing of anti-TNF biologics in order to achieve early optimal drug exposure. Predictive model guided dosing by using biomarkers and drug concentrations will conceivably improve symptoms, intestinal mucosa and quality of life in patients with IBD.
REFERENCES


