Optimizing anti-TNF therapy in inflammatory bowel disease
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CHAPTER 1

General introduction and outline of this thesis
Inflammatory bowel disease; Crohn's disease and ulcerative colitis

The term inflammatory bowel disease (IBD) is used for chronic inflammatory disease of the colon and small intestine. The most important IBDs are Crohn's disease (CD) and ulcerative colitis (UC).

The prevalence of IBD has increased in the past 50 years, up to 5/1000 persons for UC and 3/1000 persons for CD in developed countries.

In Europe an estimated 3 million people, mostly young adults (15-40 years), are affected by IBD, with a direct healthcare cost of €4.6-5.6 billion per year.

The two different IBDs can be distinguished by genetic predisposition, risk factors, and clinical, endoscopic, and histological features. The exact cause of IBD is unknown; however, IBD is generally thought to be caused by a continuing inappropriate intestinal mucosal immune response, possibly related to (commensal) gut microbiota, in genetically susceptible hosts, resulting in intestinal inflammation.

Crohn's disease is characterized by chronic mucosal inflammation, which can occur in any part of the gastrointestinal tract (from mouth to anus), with predominance for the ileocecal region (terminal ileum). (Figure 1) Patients with CD typically present with symptoms of (cramping) abdominal pain, persisting diarrhea and weight loss. Symptoms depend on the location and severity of disease.

Most cases of Crohn's disease manifest with intestinal inflammation, but the disease tends to evolve over time from luminal disease into clinical patterns that are primarily stricturing (obstructive narrowing of the lumen) or penetrating (fistulizing). 7-9

Figure 1: the anatomic locations of CD and UC

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![Figure 1: The anatomic locations of CD and UC](https://www.gi.jhsps.org)
Chapter 1

In ulcerative colitis, in contrast, inflammation is restricted to the colon, with the rectum almost always affected, spreading proximally in a continuous and diffuse manner through part of or the entire colon. Besides abdominal pain and weight loss, UC often manifest with bloody diarrhea, urge and sometimes fecal incontinence. Dependent on the anatomic extent of involvement, patients can be classified as having proctitis (with inflammation limited to rectum), left-sided colitis (up to the splenic flexure), or pancolitis (beyond the splenic flexure). (Figure 1) A typical periappendicular red patch is observed in some patients with proctitis or left-sided colitis.\textsuperscript{10, 11}

**Measures for disease activity: clinical disease activity, endoscopy and histology**

The diagnosis of IBD is made on the basis of medical history and physical examination, supplemented with objective findings from endoscopic, radiological, laboratory, and histological studies.\textsuperscript{12} The course of disease is unpredictable with alternating episodes of relapse and remission.\textsuperscript{13} Several scores, which consist of symptoms like abdominal pain, bowel frequency, rectal bleeding and (subjective) physician’s assessment, have been developed to measure clinical disease activity, such as the Harvey Bradshaw Index (HBI) and the (more complex) Crohn’s Disease Activity Index (CDAI) for CD and the Mayo score or Simple Clinical Colitis Activity Index (SCCAI) for UC.\textsuperscript{14-17}

Nonetheless, clinical disease activity (symptoms of disease) has shown to poorly correlate with mucosal disease activity, particularly in CD.\textsuperscript{14, 16} Therefore, endoscopy is considered as the gold standard for assessing disease activity.\textsuperscript{16, 20} The Crohn’s Disease Endoscopic Index of Severity (CDEIS) is often used to score endoscopic activity in clinical trials for CD.\textsuperscript{21} For UC, the more simple endoscopic Mayo score (0-3: inactive, mild, moderate, severe ulcerative colitis) scores mucosal impressions, such as: loss of vascular pattern, erythema, friability, erosion, ulcerations and spontaneous bleeding.\textsuperscript{16} Recently, a new and more detailed endoscopic score was developed for UC: the Ulcerative Colitis Endoscopic Index of Severity (UCEIS).\textsuperscript{22}

**Figure 2:** Colonic mucosa of patient with severe ulcerative colitis
Figure 2 depicts colonic mucosa of a patient with severe ulcerative colitis. Severity is marked by complete loss of vascular pattern, erythema and continuous purulent and deep ulceration. The need for objective endpoints and centralized reading of endoscopy was emphasized in a comparative therapeutic trial of mesalamine versus placebo in ulcerative colitis. In this trial ‘objective’ centralized reading of endoscopies by observers, who were blinded to treatment and patient, yielded a bigger and significant effect of mesalamine (in the treatment arm vs. the placebo arm) compared to unblinded endoscopic scoring performed by the treating physicians.

Finally, histology of mucosal biopsies remains important in the diagnosis of IBD. For UC, Geboes et al. proposed a histopathological score considering microscopic features like crypt destruction, chronic cellular infiltrate, neutrophils and architectural changes (such as erosion or ulceration).

Inflammatory markers

Both mucosal bowel imaging and biopsy collection require an endoscopic procedure, which (including bowel preparation) is often experienced as invasive to patients, thereby not allowing frequently repeated examinations. Consequently, less invasive surrogate markers are needed for identification of and screening for IBD, to monitor (mucosal) disease activity, to predict response or relapse and to guide therapeutic decisions in patients with IBD.

Several biomarkers have been proposed and thus far the general inflammatory marker serum C-reactive protein (CRP) is most commonly used in clinical practice. However, this marker is not specific for bowel inflammation. Moreover, CRP elevation is present in more than half of the patients with active CD, but only one third of active UC patients show elevated CRP levels. Another general inflammatory marker, the blood leukocyte count, is influenced by immunosuppressive therapies, such as corticosteroids and thiopurines. Serum albumin is a prognostic marker in patients with UC, but albumin levels are influenced by the nutritional state of the patient. Inconsistent findings have been reported on circulating serum or tissue TNFα levels correlated with inflammation or response in IBD.

Last decade an increasing amount of ‘gut specific’ fecal biomarkers have been suggested for the diagnosis and management of IBD. The stool marker that is commonly used in clinical practice, fecal calprotectin, makes up 60% of the total cytosolic protein concentration in neutrophil granulocytes and macrophages. Calprotectin is thought to be resistant to enzymatic degradation and therefore stable in stools at room temperature for several days. A small sample can be assessed by a simple ELISA. The accuracy of fecal calprotectin is generally better in UC than in CD. Cut-offs have been proposed of >50 µg/g for differentiating IBD from irritable bowel syndrome and >250 µg/g for the presence of ulcers. Still, there are some pitfalls causing false positive (NSAID use, the presence of
malignancy or gastrointestinal infection) and negative results (diluted sample) and interpretation of fecal calprotectin levels are hampered by considerable inter- and intra-assay variability.\textsuperscript{38,40}

Thus, although several markers are available and commonly used to measure disease activity, it remains unclear which of these measures best reflects the ‘inflammatory load’ and could therefore be best used in a model for therapeutic decision making.

**Therapies**

Treatment objectives in IBD have evolved from the treatment of symptoms and induction of clinical remission to more stringent outcomes, including sustained steroid-free remission, restoration of nutritional state, ‘mucosal healing’, prevention of hospital admission and bowel resection and improving quality of life. A ‘step-up’ approach is used for the treatment of IBD with the prescription of the least toxic, but also the least potent, drugs for mild disease and stronger immunosuppressant therapy for refractory severely active and relapsing IBD.

The first-line therapy for patients with mild to moderate ulcerative colitis is 5-aminosalicylic acid (5-ASA, mesalazine), a derivative of salicylic acid, which includes oral and rectal mesalazine formulations. Mesalazine is the preferred initial treatment for induction and maintenance in patients with mild to moderate colitis\textsuperscript{41,42} For distal UC, such as proctitis, mesalazine suppositories or enemas can be an effective ‘add-on’ to oral therapy with mesalazine.\textsuperscript{43,44} Proctitis and left-sided ulcerative colitis might respond better to rectal mesalazine or topical steroids rather than oral 5-aminosalicylic acid compounds or systemic corticosteroids. Systemic corticosteroids are appropriate if symptoms of active colitis do not respond to mesalazine.\textsuperscript{45}

The optimum first-line therapy for mild Crohn’s disease is dependent on the disease location. Patients with ileal or ileocecal Crohn’s disease are usually treated with budesonide at 9 mg daily to induce remission.\textsuperscript{46,47}

IBD patients who do not respond to first-line therapy generally receive induction therapy with oral or intravenous prednisolone (up to 40–60 mg daily). A first episode of disease or exacerbation is, depending on the severity, typically treated by a short course of these steroids. The efficacy of steroids was first reported by Truelove in 1954. This study was already placebo controlled and even used sigmoidoscopy as an endpoint.\textsuperscript{48} Although most patients with CD or UC initially respond to corticosteroids, many patients become steroid dependent, which comes with substantial short and long term side-effects, such as diabetes mellitus and osteoporosis.\textsuperscript{49} The response to an intravenous course of steroids is best evaluated around the third day of administration.\textsuperscript{50} Treatment options including colectomy should be discussed with patients with severely active UC or Crohn’s Colitis not
responding to intravenous steroids. In case of persisting symptoms on this regime or recurrence of complaints with tapering treatment, alternative therapeutics should be considered.

Azathioprine (AZA) or 6-mercaptopurine, both nucleoside analogues and immunomodulators, were traditionally used to treat leukemia (albeit in higher doses). Currently, these thiopurines are recommended for maintaining clinical and endoscopic remission and avoiding steroid requirement in the treatment of steroid dependent CD or UC. For some patients, who have infrequently relapsing moderately active IBD failing on first-line treatment, restarting steroids with an immunomodulator may be appropriate. However, treatment with thiopurines is limited by a delayed onset of therapeutic action and frequent adverse events.

In the past decades, several biological drugs that target the inflammatory cytokine tumor necrosis factor-alpha (TNF-α) have improved the management and outcome of patients with CD and UC. Infliximab (IFX), a chimeric (mouse/human) monoclonal antibody against TNFα, is administered intravenously at 5mg/kg with an induction phase in week 0-2-6 and maintenance therapy every 8 weeks thereafter. Infliximab binds with high affinity to soluble and membrane-bound TNF to form a stable complex that blocks the association of this cytokine to its receptor. Binding of anti-TNF antibodies to membrane-bound TNF has been associated with induction of T cell apoptosis and formation of wound healing macrophages. This mechanism of action of anti-TNF is subject to variation in the Fc tail of the antibody and the presence of costimulatory molecules.

Van Deventer et al. first reported, in a letter to Lancet, about a 12-year old girl with Crohn’s disease, who was unresponsive to medical therapy, in whom clinical remission was achieved by treatment with anti-TNF monoclonal antibodies (chimeric monoclonal cA2, later known as infliximab). Almost a decade later, The ACCENT-1 trial provided the rationale for the final registration of IFX as induction and maintenance therapy for patients with CD. Three years later, IFX got approved for UC by the publication of the ACT study by Rutgeerts et al. Notably, in this study 10mg/kg IFX dosing was almost equally effective as 5mg/kg (clinical response at week 8: 5mg: 64%, 10 mg: 69%, placebo: 29%) and hospitalized or steroid refractory patients were excluded.

Adalimumab, a humanized anti-TNF antibody, which is administered subcutaneously, represents the second anti-TNF antibody that induces and maintains clinical remission and mucosal healing in patients with CD or UC. Thirdly, Golimumab, also a subcutaneous humanized anti-TNF antibody, has recently been approved for the treatment of moderate to severely active Ulcerative Colitis. Anti-TNF therapy, with or without immunomodulator, should be considered as an alternative for patients with objective evidence of active disease who have previously been steroid-refractory, steroid-dependent, or steroid-intolerant.
luminal CD, IFX and adalimumab appear to have generally similar efficacy and adverse-event profiles, so the choice depends on availability, route of delivery, patient preference, cost and national guidelines.47

The combination therapy of IFX with azathioprine results in higher rates of mucosal healing than the treatment with either one of those agents in Crohn’s disease. Also in UC, the combination of IFX and AZA was found to be superior to monotherapy.64 Arguably this combination therapy of IFX with an immunomodulators can still be considered the most powerful drugs that are available for UC.

Intravenous cyclosporine, a calcineurin inhibitor originally developed to prevent organ rejection in renal transplant patients, was shown to be effective particularly in steroid refractory patients with severe UC.65, 66 The CYSIF trial reported equal effectiveness of IFX compared to cyclosporine for the treatment of severe colitis. However, cyclosporine dosing was based on serum concentrations, whereas for IFX therapeutic ranges for the induction phase were still unknown.67 Moreover, cyclosporine cannot be used as maintenance therapy, due to toxicity concerns, whereby patients should be continued on thiopurine monotherapy.

In view of similar effectiveness, IFX might be preferred over cyclosporine, because it can be continued as maintenance treatment in responding patients, particularly in those for whom thiopurines as maintenance therapy has been ineffective.

Finally, just recently, Vedolizumab, an α4β7 integrin antagonist, which limits the migration of leukocytes specifically to the gut, was added to the medical armamentarium, with its approval for active CD and UC.68, 69

Despite all available therapies, many patients are still refractory to medical treatment and will eventually need surgery. Loss of response or intolerance to medical therapy should lead to re-evaluation of disease activity, exclusion of complications and discussion of surgical options with the patient. The decision to intensify or switch therapy and consider surgery in patients with severely active IBD should preferably be discussed in a multidisciplinary meeting involving both the gastroenterologist’s and the surgeon’s perspective. Up to half of all patients with CD will eventually need some kind of bowel surgery, whereas an estimated one third of UC patients will need colonic resection at some point.4 For ileal Crohn’s disease (short segment) small bowel resections or (laparoscopic) ileocecal resections are often required to reduce the inflammatory burden, thereby increasing the efficacy of subsequent anti-inflammatory treatment. Patients with steroid and/or anti-TNF refractory ulcerative colitis typically undergo laparoscopic (sub)total colectomy with temporarily ileostomy and consecutive ileal-pouch anal anastomosis procedure. Complicated IBD surgery should only be performed in medical and surgical expertise centers.47
Optimizing anti-TNF therapy: immunogenicity, pharmacokinetics and therapeutic drug monitoring

Clearly, there is a need to optimize medical therapies for patients with IBD. Although being amongst the most efficacious drugs for active IBD, anti-TNF therapies have considerable limitations. Substantial healthcare society costs are involved with the prescription of these biological agents. Regular dosing of anti-TNF costs about €20,000 per patient per year. Anti-TNF is estimated to account for 2/3 of all costs in CD and 1/3 of all costs in UC patients.\textsuperscript{70} Adalimumab (Humira, AbbVie) and infliximab (Remicade, MSD) were both ranked in US top 10 drugs sales of 2013, with $5.4 billion and $3.9 billion sales, respectively. Moreover, about one third of IBD patients do not respond to these antibodies in the first 3 months, during induction phase, a phenomenon called primary non-response.\textsuperscript{58, 59} In addition, another 20-40 % of initial responders lose response over time: so called secondary non-response.\textsuperscript{71} (Figure 3)

![Graph showing primary and secondary non-response to anti-TNF](image)

**Figure 3:** Estimated primary and secondary non-response to anti-TNF in IBD

The mechanism of primary non-response to anti-TNF remains poorly understood.\textsuperscript{72} Secondary non-response has been associated with several factors: Particularly in CD, non-inflammatory complications like the formation of fibrostenotic strictures can cause loss of response.\textsuperscript{71} But loss of response has also been attributed to the formation of anti-drug antibodies, which have been shown to neutralize the drug and can cause acute infusion reactions.\textsuperscript{73} Furthermore, a low serum trough level of the drug, the serum concentration just before the next infusion or injection (also without the presence of anti-drug antibodies), has been correlated with secondary non-response.\textsuperscript{74, 75}

The immunogenicity of anti-TNF antagonists is influenced by structural properties of the drug, such as: the extent of humanisation (chimeric or humanized construction), aggregates, glycosylation or the affinity to the human neonatal Fc Receptor.\textsuperscript{76-78} The
The formation of anti-drug antibodies represents the major form of immune mediated clearance. Neutralisation by these antibodies causes rapid clearance and thereby loss of function of the drug. Infrequent or episodic exposure to anti-TNF agents stimulates the appearance of those anti-drug antibodies. Patients that restart treatment after a drug holiday are at risk for developing anti-drug antibodies. Therefore, treatment should not (‘electively’) be interrupted or discontinued in patients with active disease. Thus, anti-TNF should be administered at scheduled occasions with induction followed by maintenance treatment. The formation of antibodies to IFX (ATI) can be prevented by the concomitant treatment with immunomodulators. For adalimumab, it remains uncertain whether concomitant immunomodulators are needed to prevent anti-drug antibody formation, although a retrospective study did observe significant less flares in the first semester in patients that used co-immunomodulators. Likewise, pretreatment hydrocortisone or concomitant administration of corticosteroids appears to prevent the development of ATI.

The appearance of anti-drug antibodies seems to be a dynamic phenomenon: anti-drug antibodies gradually disappear after definite discontinuation of anti-TNF. Two types of anti-drug antibodies have been identified: persistent and transient anti-drug antibodies. Transient anti-drug antibodies, which seem of little clinical significance, can appear randomly at any time during treatment. Nevertheless, the temporal evolution of ATI has also been correlated with clinical loss of response. Most patients develop antibodies to IFX within the first 12 months of therapy. Concomitant use of an immunomodulator prolongs ATI-free
survival, even in scheduled-treated patients.\textsuperscript{96} In patients with rheumatoid arthritis, suppression of ATIs was observed after IFX dose intensification.\textsuperscript{97} Finally, a small case-series from Israel demonstrated a gradual decline of anti-drug antibodies after addition of immunomodulators.\textsuperscript{98} Unfortunately, various anti-drug antibody detection assays are used in different studies, making it impossible to compare results. Traditionally, drug intolerant assays were used that cannot measure anti-drug antibodies in the presence of high circulating drug concentrations.\textit{(Figure 4)} Recently, a more sensitive assay has been developed to detect anti-drug antibodies in the presence of high circulating drug concentrations.\textsuperscript{99}

\textbf{In presence of IFX: anti-IFX antibody not able to bind to ELISA}

![Drug intolerant assay](image)

\textbf{Figure 4:} Drug intolerant assay\textsuperscript{110}

Low serum trough concentrations, also without the presence of these neutralizing anti-drug antibodies, have been correlated with therapeutic response in both CD and UC.\textsuperscript{74, 75} This has provided a rationale for optimization of anti-TNF therapy by therapeutic drug monitoring in patients with IBD. Several studies have indicated that the therapeutic range for IFX trough concentrations during maintenance therapy should range between 3-7 µg/ml for therapeutic response.\textsuperscript{100-103} For adalimumab, therapeutic concentrations above 5 µg/ml have been correlated with improved clinical outcomes and mucosal healing.\textsuperscript{104, 105}

![Concept of therapeutic window](image)

\textbf{Figure 5:} Concept of therapeutic window (www.thebody.com/content/art13513.html)

Definition of these therapeutic ranges has already led to the first dose optimisation study. In the so called TAXIT trial, all patients with IBD were dose optimized within serum IFX trough concentrations of 3-7 µg/ml in the first phase of the study. Departing from there,
patients were then randomized to conventional dosing based on clinical symptoms or dosing based on the targeted trough range 3-7 µg/ml during maintenance therapy. Level based dosing resulted in longer ‘relapse free survival’, but not in significant higher rates of clinical remission. Nevertheless, successful dose de-escalation was established in patients with supratherapeutic levels, whilst retaining disease control.\textsuperscript{103} A different randomized, controlled trial demonstrated that individualized algorithm based therapy is more cost-effective than ‘blind’ dose intensification in patients with Crohn’s disease with loss of response to IFX.\textsuperscript{106} Dose intensification is able to regain response in 45-75% of patients with CD.\textsuperscript{107, 108} Whether dose increase or interval decrease is best to regain response remains unsure. Halving intervals was not found to be superior to dose-doubling in a multicenter retrospective cohort.\textsuperscript{109} Dose increase does not require an additional infusion visit, with associated costs and patient inconvenience, whereas decreasing the dosing interval allows for more precise titration based on the timing of the recurrence of symptoms. Currently, therapeutic drug monitoring has been established and incorporated in algorithms for patients with symptoms suggestive for loss of response on anti-TNF maintenance therapy.\textsuperscript{71, 110-112}

Several factors have been identified to affect serum concentrations of anti-TNF antibodies, such as body weight, neutralizing anti-drug antibodies and concomitant use of immunomodulators.\textsuperscript{73, 88, 113}

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<tr>
<td>High Body weight</td>
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<td>Neutralizing anti-drug antibodies</td>
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<td>High serum CRP?</td>
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<td>Low serum Albumin?</td>
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Figure 6: Factors that have been identified or suggested to affect anti-TNF drug levels.

Furthermore, high CRP and low serum albumin, both reflecting a high inflammatory load, have also been suggested to have an impact on anti-TNF drug levels, but clinical data, especially regarding IFX induction in ulcerative colitis, are scarce.\textsuperscript{74, 79, 114} Most pharmacokinetic data available are derived from IFX maintenance therapy studies, pharmacokinetic population computer modelling or from studies involving patients with rheumatoid arthritis.\textsuperscript{102, 113-116} Specifically in patients with severe UC, the situation seems to be considerably different with massive presence of (mucosal) inflammation, severe ulceration and potential fecal loss of proteins.
Outline of this thesis: therapeutic drug monitoring based on inflammatory markers

Clinical observations suggest that patients with severe UC may benefit from higher than standard doses of anti-TNF.\textsuperscript{117, 118} Those patients likely have a higher serum and mucosal TNF burden that acts as a ‘sponge’ or antigen sink to quickly absorb and bind anti-TNF monoclonal antibodies and may lead to insufficient therapeutic exposure.

We hypothesize that patients with severe IBD exert rapid clearance and might not receive sufficient anti-TNF to suppress the amounts of TNF, resulting in primary non-response.\textsuperscript{119, 120} Furthermore, we postulate that in patients with severe ulcerative colitis, therapeutic antibodies may be lost in feces, through ulcerated and denuded mucosa. This might increase clearance and affect pharmacokinetics of anti-TNF agents. Understanding of the inflammatory burden and drug levels in these patients is needed to individualize drug dosing and optimize anti-TNF therapy in patients with IBD.

This thesis will therefore focus on the research question: \emph{Can assessment of the inflammatory load and drug levels help to optimize the outcomes of anti-TNF therapy in patients with IBD?}

\textbf{Chapter 2} discusses several markers to measure inflammatory load in patients with UC, compared to leukocyte scintigraphy. \textbf{Chapter 3} demonstrates that disease localization influences fecal calprotectin levels in patients with ileal or (ileo)colonic Crohn’s disease. \textbf{Chapter 4} introduces a new concept of fecal loss of IFX which may contribute to primary non-response in severe colitis. \textbf{Chapter 5} reports on early formation of anti-drug antibodies and how inflammatory load affects the pharmacokinetics of IFX induction therapy in UC. \textbf{Chapter 6} presents the analysis of several factors that influence the PK in a large cohort of IBD patients. \textbf{Chapter 7} shows that supratherapeutic anti-TNF levels are correlated with impaired quality of life in patients with IBD in clinical and biochemical remission on anti-TNF maintenance therapy. \textbf{Chapter 8} establishes the correlation between IFX serum levels and disease activity in children with IBD. \textbf{Chapter 9} investigates the effect of IFX retreatment after consecutive discontinuation of IFX and adalimumab in refractory CD. \textbf{Chapter 10} discusses the contribution of this thesis to the optimization of anti-TNF therapy based on markers for response and how inflammatory load affects the pharmacokinetics of infliximab and provides future perspectives.
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