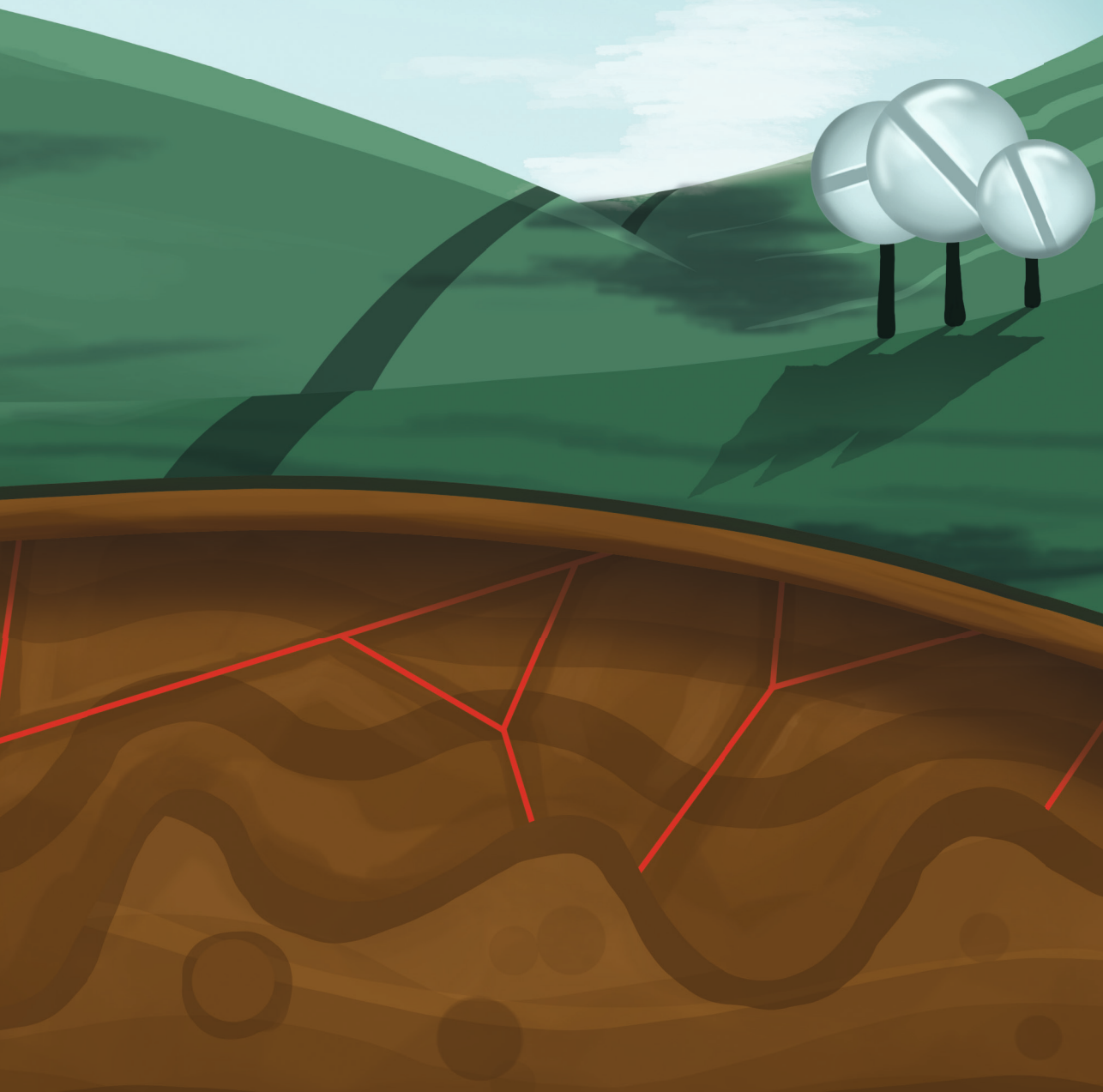
Journey to improve pediatric Crohn's disease treatment

Martinus Arend (Maarten) Cozijnsen



Journey to improve pediatric Crohn's disease treatment

Martinus Arend (Maarten) Cozijnsen

Journey to improve pediatric Crohn's disease treatment

ISBN: 978-94-6375-947-2

The research described in this thesis was conducted at the department of Pediatrics, division Gastroenterology and Nutrition, Erasmus Medical Center, Rotterdam, the Netherlands.

Financial research support: ITSKids and TISKids were supported by the Netherlands Organisation for Health Research and Development (ZonMw) project number 113202001. Additionally, TISKids was supported by Crocokids (Dutch fund-raising organization to support research on IBD in children) and Hospira, now Pfizer. Infliximab (Inflectra®) vials were provided by Pfizer for the first year after randomization.

Printing of this thesis was financially supported by:

Cover design and layout: © evelienjagtman.com
Printing: Ridderprint

Journey to improve pediatric Crohn's disease treatment

Zoektocht naar verbetering behandeling ziekte van Crohn bij kinderen

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof.dr. R.C.M.E. Engels

en volgens besluit van het College voor Promoties.

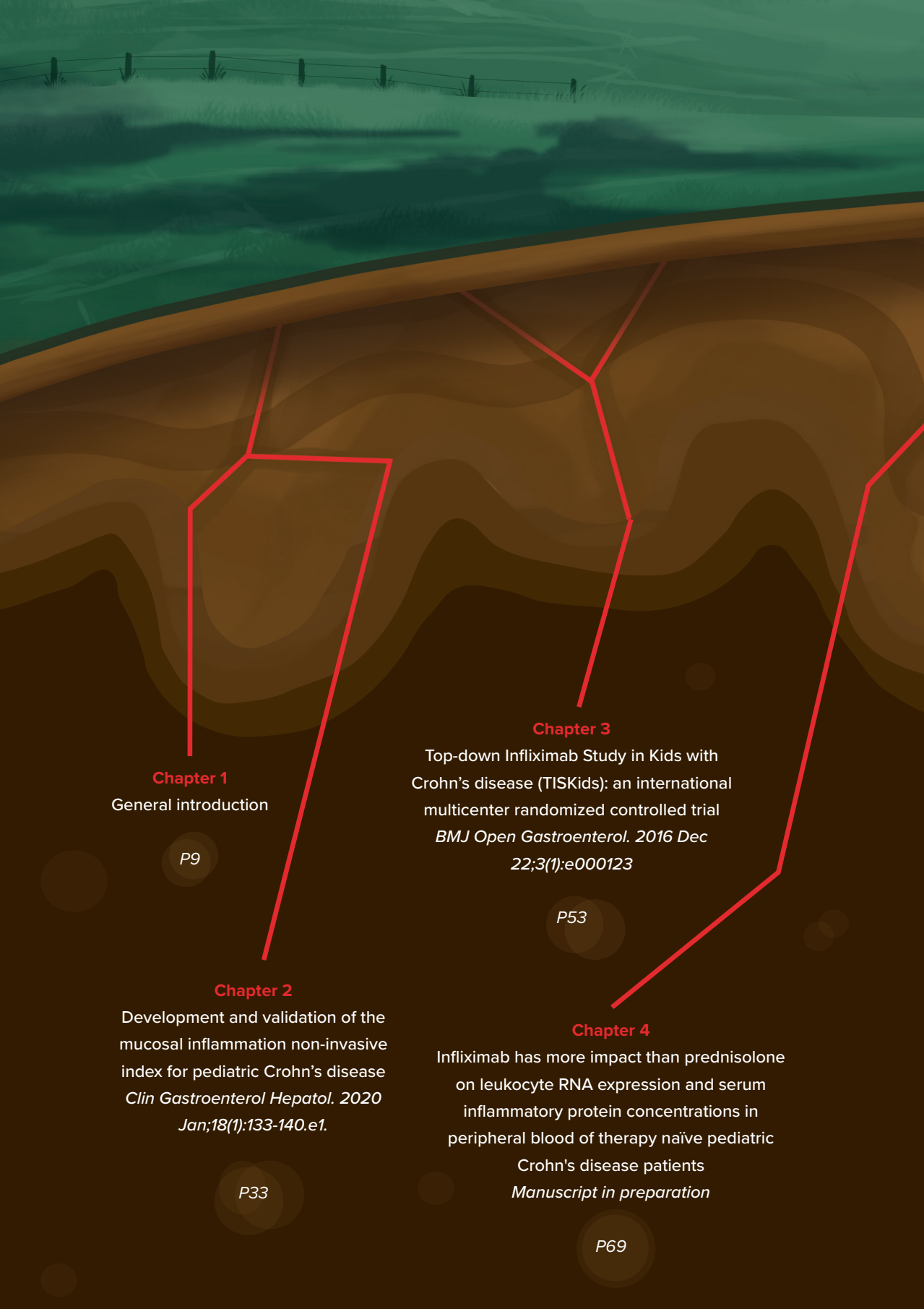
De openbare verdediging zal plaatsvinden op
Woensdag 2 september 2020

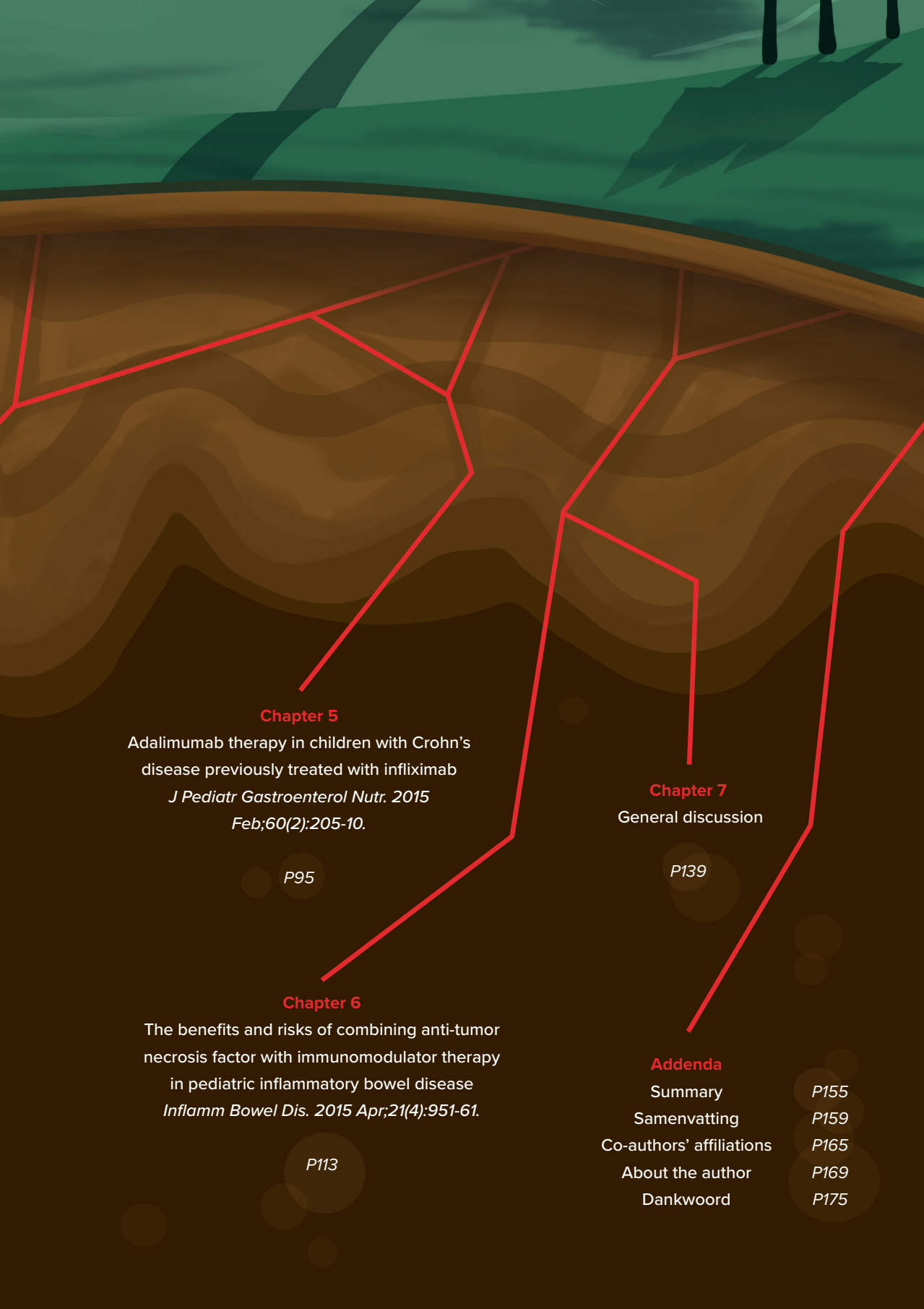
door
Martinus Arend Cozijnsen
geboren te Apeldoorn

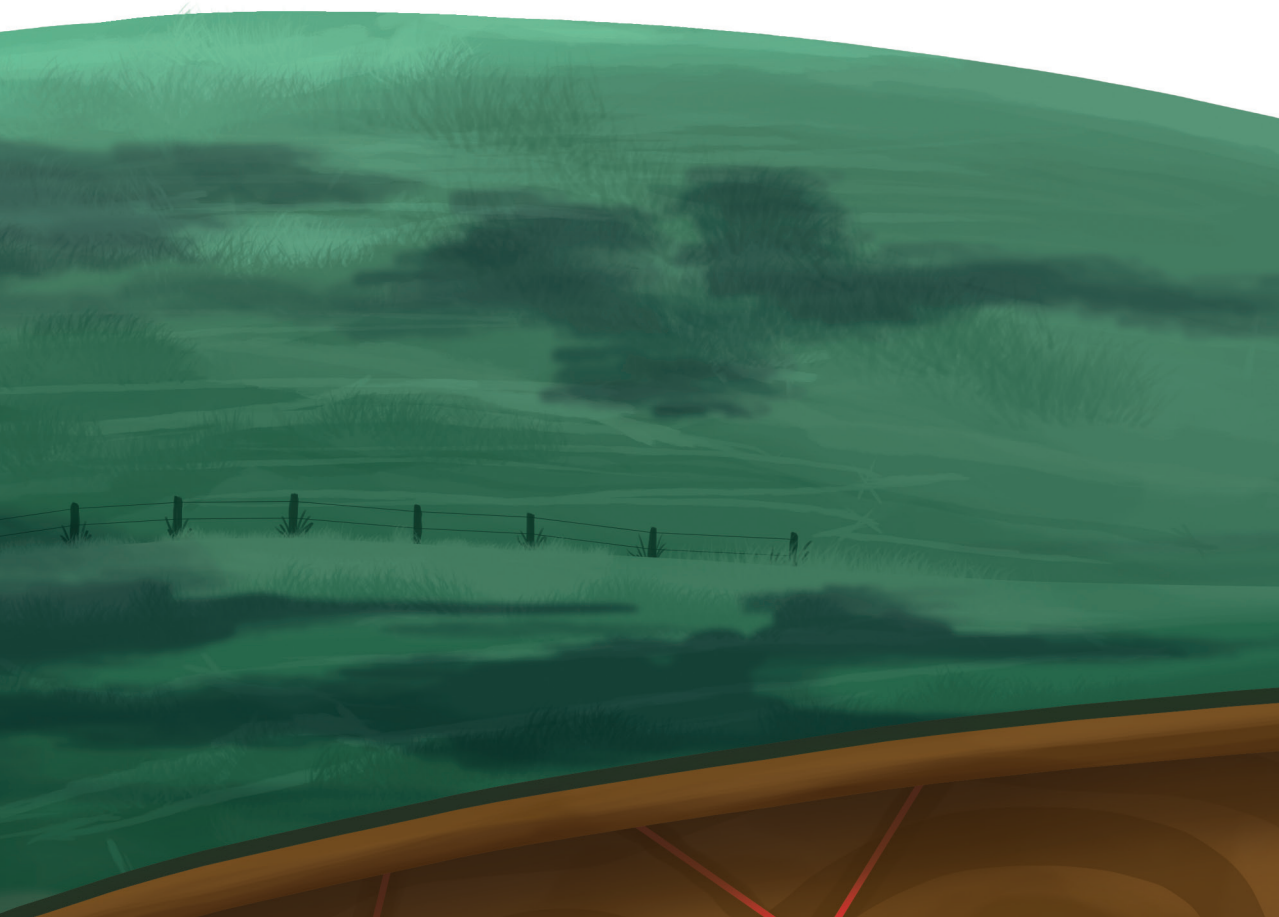
Erasmus University Rotterdam

Promotiecommissie:

Promotor:	Prof. dr. J.C. Escher
Overige leden:	Prof. dr. C.J. van der Woude
	Prof. dr. E.E.S. Nieuwenhuis
	Dr. S.S.M. Kamphuis
	Prof. dr. E.H.H.M. Rings
	Prof. dr. J.E. Van Limbergen
Copromotor(en):	Dr. L. de Ridder
	Dr. J.N. Samsom







Chapter 1

General introduction

Based on the article *Anti-TNF therapy for pediatric CD: improved benefits through treatment optimisation, deeper understanding of its risks, and reduced costs due to biosimilar availability.*

Cozijnsen MA, Samsom JN, de Ridder L.

Pediatr Drugs. 2018 Feb;20(1):19-28.

Crohn's disease (CD) characteristics and pediatric CD

Crohn's disease (CD) is an inflammatory bowel disease (IBD) of unknown origin. It is a chronic, relapsing-remitting disease characterized by gastrointestinal symptoms (e.g. abdominal pain, watery and/or bloody stools), fatigue, weight loss and impaired longitudinal growth. The inflammation can result in increased concentrations of C-reactive protein (CRP) and an increased erythrocyte sedimentation rate (ESR) in patients' blood and increased calprotectin concentrations in patients' stool. The inflammation is mostly located in the terminal ileum, colon or both, but can be located throughout the gastrointestinal tract (*Figure 1*). Although less common, the inflammation can even present outside the gastrointestinal tract, commonly referred to as extraintestinal manifestations. Additionally, CD inflammation can give rise to the formation of penetrating fistulas or intestinal strictures. Ulcerative colitis (UC) – the other mayor IBD subtype – in comparison is limited to the colon and does not lead to fistulas or strictures.

The prevalence of IBD is around 0.3% in western countries, of which approximately 40% is CD.[1] Where the prevalence of CD in adults seems to be stable in Western countries, the incidence of CD in children and adolescents seems to be rising.[1,2] The incidence of childhood-onset CD is approximately 4 per 100,000 patient years.[3] When CD manifests during childhood or in adolescents, its course usually is more extensive and progressive than adult-onset CD. As a result more intensive treatment is required.[4,5] In the Netherlands, according to publicly available data at DIS opendata, pediatricians treat approximately 3,000 IBD patients annually, and physicians that treat adult IBD patients – mostly gastroenterologists and some internists – treat approximately 92,000 IBD patients, of which half are diagnosed with CD.

Patients suspected of IBD undergo ileocolonoscopy, which allows for visual inspection of the gut mucosa and taking of biopsies. Besides disease location, the presence of aphthous ulcers, cobblestoning, and so called "skip" lesions helps distinguish CD from UC. Histologic signs of CD include epithelial damage, architectural changes, infiltration of mononuclear and polymorphonuclear cells in the lamina propria and / or epithelium, the presence of erosions, ulcers or granulomas.[6]

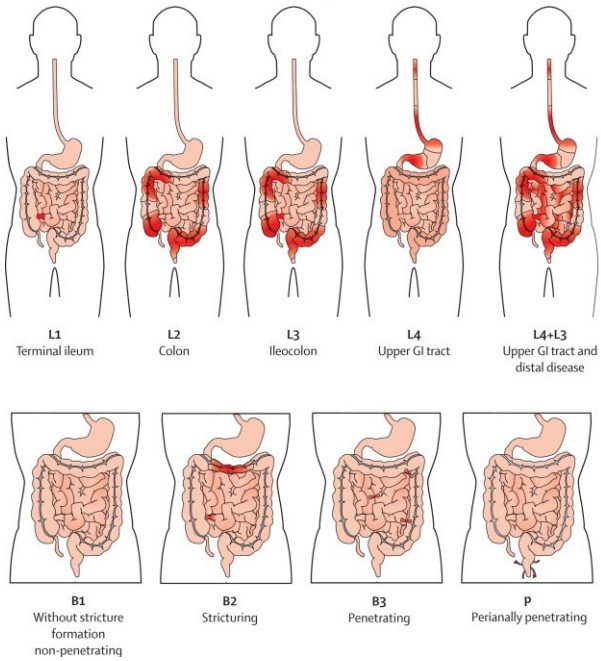


Figure 1. Classification of Crohn's disease location and behavior

Crohn's disease can manifest in different location throughout the gastrointestinal tract. In some patients, CD can cause penetrating or structuring disease and / or present with perianal fistulas. Modified version of an original figure published in The Lancet 2012;380(9853):1590-1605.

Pathogenesis of CD and the role of TNF

The pathogenesis of CD is highly complex and still not fully understood. CD is a multifactorial disease in which genetic predisposition, microbial and dietary environmental pressure and susceptibilities of the immune system lead to aberrant inflammatory responses to luminal microbiota and concomitant autoimmune responses. Although a cure has not yet been found, manipulating one of these factors does alleviates disease. For example, diversion of luminal content with ileostomy, which drastically alters environmental pressure, reduces mucosal inflammation in the bowel distal to the stoma. In addition, dietary intervention with exclusive enteral nutrition (EEN), which affects luminal microbial composition, also inhibits inflammation and can restore the integrity of the mucosal layer. Lastly, inhibiting the immune response also has strong beneficial effects, as most clearly evidenced by the effect of immune suppressive and immune modulating interventions on CD. A key problem in the chronicity of CD is the development of immune memory driven by T-lymphocytes (T-cells) that reside in the intestinal lamina propria, secrete interferon-gamma and cause reactivation of the disease upon recognition of their environmental activating trigger.[7] Effective elimination or inhibition of this cell population may reduce the chance of disease re-activation and explains why T cells are an important target in CD treatment strategies.

Tumor necrosis factor alfa (TNF-alfa) is an inflammatory cytokine mainly produced by macrophages although it can also be produced by many other leukocytes amongst which T cells. It is produced as a transmembrane protein (tmTNF) and a soluble form (sTNF). TNF-alfa is an important factor for orchestrating cellular immune responses and plays a crucial role in host-defense to pathogens and killing of malignant cells. TNF signals via two receptors: TNF receptor type 1 (TNFR1), expressed in almost all cell types, and TNFR2, expressed on leukocytes only. Ligation of the receptor results in a complex signaling cascade leading to the production of wide variety of proteins involved in cell survival, proliferation, differentiation, migration, and apoptosis. When TNF concentrations in blood become very high, an acute phase reaction in the liver ensues causing fever and cachexia.

CD treatment and endoscopic remission

Immunosuppressive treatment is required for inducing and maintaining disease remission and preventing development of disease complications. It focuses on relieving symptoms, restoring longitudinal growth and pubertal development. Furthermore, it focuses on suppressing the inflammatory immune response leading to macroscopically detectable repair of the mucosal surface, also known as endoscopic remission.[8] Acquiring endoscopic remission is important since it predicts a favorable disease outcome, and reduces the need for steroids, the risk of complications, of hospitalization and the need for surgery.[9] Endoscopic remission can be assessed with ileocolonoscopy. However, frequent assessment of endoscopic remission with endoscopy has several limitations given its invasiveness, cost and potential risks, including the requirement of anaesthesia.[10] Therefore, non-invasive measures of endoscopic remission are desirable for tight monitoring of CD patients. In **Chapter 2** we describe the mucosal-inflammation non-invasive (MINI) index we developed and validated. This non-invasive index identifies children with endoscopic remission with high sensitivity and specificity.

Pediatric CD guidelines instruct physicians, in most cases, to start treatment with EEN or prednisolone to induce disease remission, and at the same time start with a thiopurine, such as azathioprine (AZA), or methotrexate (MTX) to maintain remission.[8] Patients refractory to these treatments can step up to anti-tumor necrosis factor alfa (anti-TNF) antibody treatment. Additionally the guideline suggests starting with anti-TNF treatment as initial treatment in patients with high risk for poor outcome and in patients with active perianal fistulizing disease.[8]

Anti-TNF treatment has shown to be very effective in inducing and maintaining remission in therapy refractory pediatric CD patients.[11,12] It not only induces remission of clinical symptoms, but also heals the mucosa, restores mucosal tissue integrity, denoted as endoscopic remission.[13] Since the market approval of the first anti-TNF[14] treatment – infliximab (IFX) – researchers have searched for ways to optimize anti-TNF antibody usage, to increase response rates and to prolong the duration of disease remission. Based on research findings, the use of anti-TNF treatment in managing pediatric CD has significantly evolved over time.

Step-up versus top-down treatment strategy

Both IFX and adalimumab (ADA) are approved for a restricted population of pediatric CD patients, namely the therapy refractory patients with moderately-to-severely active disease. Yet, their benefit seems higher when given earlier in the course of disease.[15] If more effective treatment is given early, it may prevent disease complication. It may therefore be more beneficial to start anti-TNF antibodies right after diagnosis rather than delay the initiation.

This is especially true for patients that are not effectively treated with—i.e. do not respond to or quickly relapse under—the conventional non-biologic treatment options (prednisolone or EEN, combined with AZA or MTX). However, it remains difficult to predict responsiveness to these therapeutic options, so further research is needed to assess the benefits (and risks) of starting anti-TNF antibodies as first-line treatment option.

Patients at greater risk of disease complication, such as strictures and fistulas, would benefit most from an early initiation with anti-TNF antibodies. For this purpose, the current guidelines lists the following seven factors as potentially predictive of poor outcome – mostly based on clinical experience:[8]

- deep colonic ulcerations on endoscopy
- persistent severe disease despite adequate induction therapy
- extensive (pan-enteric) disease
- marked growth retardation N−2.5 (minus 2.5) height Z scores),
- severe osteoporosis
- stricturing and penetrating disease (B2 and/or B3 disease behavior at onset
- severe perianal disease

Recently, new results of the RISK study were published (*Risk Stratification and Identification of Immunogenetic and Microbial Markers of Rapid Disease Progression in Children with Crohn's Disease*).[16] This prospective inception cohort followed 913 pediatric CD patients from disease onset up to 3 years after. Baseline predictive factors for stricturing or penetrating disease at 3 years, were older age, African-American race, isolated ileal disease, and ASCA and CBir1 serum-positivity. However, their combined sensitivity and specificity were low (66% [95% CI 51%–82%] and 63% [55%–71%]). The authors state that the accuracy was low because of the low prevalence of complications within those 3 years in their cohort. Due to the low accuracy, the significance of these predictive factors in clinical decision making is limited. Thus, it remains difficult to accurately determine patients at high risk of complications.

Starting with anti-TNF treatment after patients lose response to other treatment options - the so-called step-up treatment strategy – has several disadvantages. Although prednisolone and EEN both induce clinical remission effectively (in ~80% of patients), prednisolone has considerable side effects, and EEN necessitates a complete refrain from normal food for a long period of time which is unpleasant and hard to comply with. Furthermore, prednisolone rarely induces endoscopic remission.[8,17,18] Once in clinical remission, approximately 60% of patients maintain remission during the first year of AZA treatment. [19–22] One registry showed that 54% (55/102) of pediatric patients with CD had received either an additional corticosteroid course or had started infliximab (IFX) within the first year after diagnosis.[23]

Thus, a large proportion of pediatric patients require more intensive treatment in the first year after diagnosis. For these patients, the step-up strategy delays the initiation of effective treatment and increases the risk of CD progression and complications.

In **Chapter 3** of this thesis we describe the international multicenter randomized controlled trial (RCT) we set up to compare the efficacy and safety of top-down treatment (starting with IFX from diagnosis) with the conventional step-up treatment strategy in newly diagnosed pediatric CD patients.

Mechanism of action of anti-TNF treatment in CD

Multiple mechanisms of action may contribute to the beneficial effect of anti-TNF antibody therapy in CD (Figure 2). Both the antibody's binding fragment (FAB) region and the fragment crystallizable (FC) region exert immunomodulatory properties. The FAB regions of IFX and adalimumab (ADA) specifically bind to TNF-alpha molecules. Upon binding with its FAB region, anti-TNF antibodies block and neutralize the signaling potential of TNF. Additionally, anti-TNF antibodies bound to a tmTNF-expressing target cell suppress pro-inflammatory cytokine production or induce apoptosis in the target cell, a process denoted as reverse signalling.[24–26]

Although it was anticipated that anti-TNF antibodies would primarily exert their beneficial function in CD by neutralizing TNF function through its FAB regions, it is now recognized that the FC tail of the antibody is important for effectiveness. Etanercept—a TNF receptor/immunoglobulin G fusion protein, capable of neutralizing sTNF—has been shown to be ineffective in CD.[27] Secondly, certolizumab pegol—a PEGylated FAB fragment of an anti-TNF antibody that lacks an FC region—had only low efficacy in CD.[28] The poor efficacy of these biologicals that are effective for the treatment of other chronic inflammatory diseases—rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis among others—may suggest that the FC region has a crucial role in inducing immunomodulation in CD. The FC region enables bound antibodies to elicit complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC).[29] Secondly, it enables an antibody-antigen complex to bind with cells presenting an FC receptor, such as macrophages. Based on in vitro experiments it is suggested that TNF-anti-TNF immune complexes may lead to the induction of immunosuppressive macrophages, able to produce anti-inflammatory proteins, inhibit T-cell proliferation and promote wound healing.[30,31] The induction of these immunosuppressive macrophages may partly explain the higher effectiveness of anti-TNF antibodies that possess an FC region, but this hypothesis still needs to be proven. In a pilot analysis of the Infliximab Top-down Study in Kids with Crohn's disease (ITSKids) multicenter randomized trial in **Chapter 4,** we demonstrate that IFX treatment has a strong effect on blood leukocyte mRNA expression and protein concentrations by reducing Th1 and neutrophil signatures, and tissue remodeling proteins.

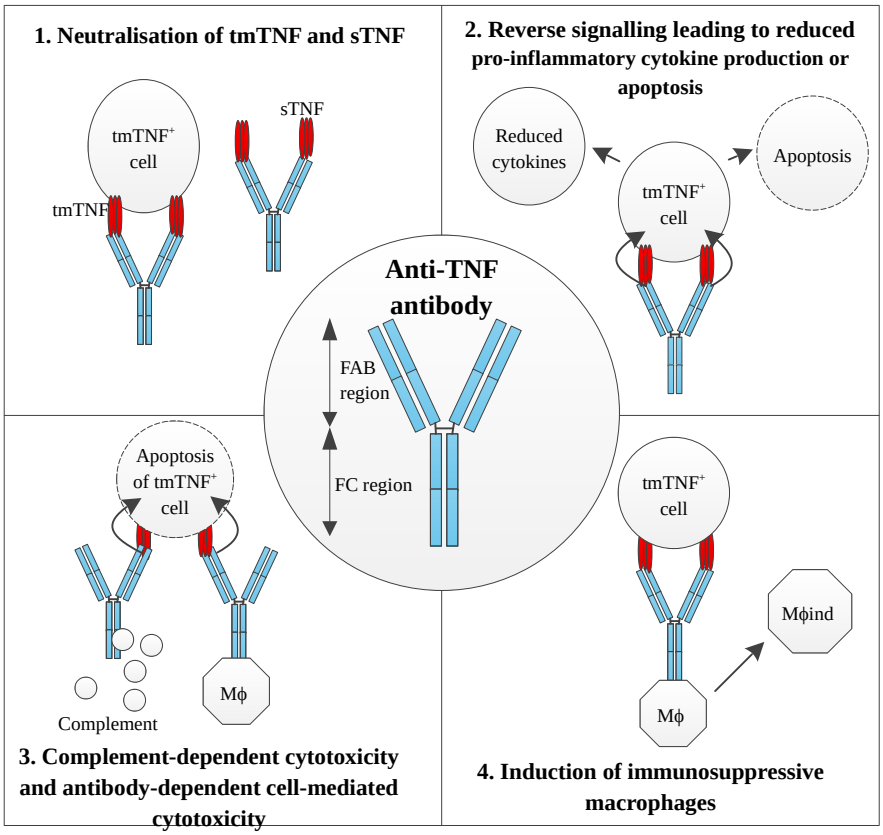


Figure 2. Overview mechanisms of action anti-TNF antibodies

Displaying four mechanisms of action of anti-TNF antibodies in treating CD. Via its binding fragment (FAB) region, anti-TNF antibodies can (1) neutralize both soluble (s)TNF and transmembrane (tm) TNF, and (2) elicit reverse signaling that can reduce pro-inflammatory cytokine production of the tmTNF^{+} cell or induce apoptosis. Through its fragment crystallizable (FC) region, (3) complement and natural killer (NK) cells—among others—can bind to the antibodies and can elicit apoptosis through complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC). Moreover, (4) macrophages (Mφ) can bind to the antibody-antigen complex which leads to the induction of immunosuppressive macrophages (Mφind), able to produce anti-inflammatory proteins, inhibit T-cell proliferation and promote wound healing.

Relative effectiveness of anti-TNF treatment

Both IFX and ADA are efficacious in treatment of pediatric CD and are considered equally effective – although a head-to-head comparison is lacking.[8] They can both be used to induce and maintain remission in pediatric CD patients. The available prospective trials demonstrate that more than 80% of therapy refractory patients respond to induction

treatment with anti-TNF antibodies.[11,12,32] These trials further demonstrate that anti-TNF antibodies are able to maintain remission up to 1 year in approximately 45% to 83% of patients. The variation in these remission rates largely depends on patient or treatment factors, as will be discussed further below. Anti-TNF antibodies are also effective in closing perianal fistulas in children with CD: After 2 to 4 months of treatment, approximately 64% show complete fistula closure (range 54% to 100% [33–35]) and after 1 year of treatment 40% to 68% show complete closure.[12,35] In **Chapter 5** we describe a nationwide, observational cohort study into the real-world effectiveness of ADA treatment for children and adolescents with CD who had previously failed IFX treatment.

However, a head-to-head comparison of the efficacy of anti-TNF therapy to that of the alternative therapies in use—exclusive enteral nutrition (EEN) or corticosteroids for remission induction and thiopurines or MTX for remission maintenance – is still lacking.[8] The pivotal trials of both IFX and ADA in pediatric CD did not have a control group, and since their approval no prospective trial has been published that compares the effectiveness of anti-TNF treatment with alternative treatments. Thus, there is currently no reliable way to compare their effectiveness. In the international multicenter RCT we set up, described in **Chapter 3** of this thesis, we aim to compare the efficacy and safety of remission induction with IFX, prednisolone or EEN in newly diagnosed pediatric CD patients.

Optimizing treatment effectiveness

Patient characteristics impacting effectiveness

Patient characteristics can have a high impact on drug effectiveness. In the phase 3 ADA trial, IFX experienced patients were only half as likely to achieve disease remission during follow-up than IFX naïve patients.[12] Secondly, the authors reported that younger age and shorter disease duration were associated with higher remission rates, a finding confirmed by several observational trials.[33,36–38] The third factor influencing remission rates in this trial was baseline C-reactive protein (CRP).[12] Patients with a lower CRP were more likely to achieve remission during follow-up. However, this finding conflicts with literature in adult CD patients, where several trials found high baseline CRP to be associated with higher remission rates.[39–41]

Combination therapy and therapeutic drug monitoring

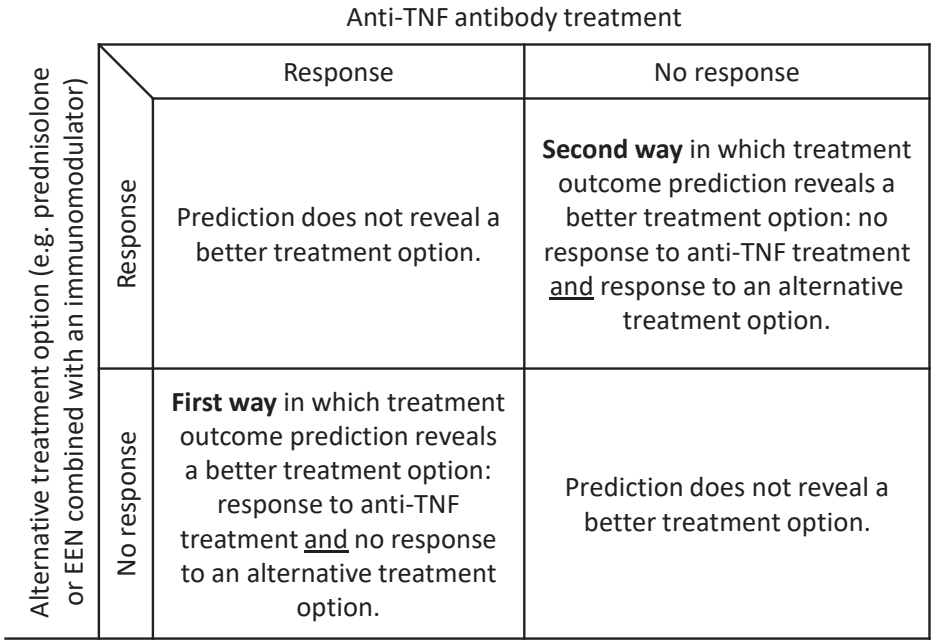
Besides patient characteristics, some treatment options are known to impact treatment effectiveness and allow further treatment optimization. Currently there are two methods being used to improve the effectiveness of anti-TNF antibodies: combination therapy with an immunomodulator and monitoring of therapeutic drug levels. **Chapter 6** is a review in which we compare the benefits and risks of combining anti-TNF treatment with immunomodulator therapy based on published evidence. In short, although evidence of

increased effectiveness is lacking in pediatric CD, based on adult CD literature it is likely that combination therapy is more effective, at the cost of increased risk of adverse effects. The current CD treatment guideline thus suggests to "allow concomitant AZA treatment in the first 6 months of IFX therapy and then consider stopping AZA, but individualization of the strategy is required based on prediction variables".[8]

Another method used to increase effectiveness is monitoring of therapeutic drug levels (TDM). Drug level measurements are typically timed preceding an infusion, resulting in trough levels. IFX trough levels are considered therapeutic when roughly between 3 and 7 µg/ml based on adult CD literature.[42–44] Whether TDM increases the effectiveness of IFX has not been tested in pediatric CD. A prospective RCT in adult IBD patients with stable response to maintenance IFX, demonstrated increased remission rates in patients with sub-therapeutic levels when doses were routinely screened and optimized. [45] It did however not increase 1 year remission rates—the primary efficacy endpoint. Thus, in adult CD patients, TDM hasn't proven to overall increase the effectiveness of IFX. However, TDM may be more beneficial for children than for adults, as the risk of sub-therapeutic IFX levels is higher in pediatrics.[46]

Predicting treatment response

Predicting patients' chances to respond to available treatment options can improve overall treatment success by enabling physicians to directly choose the treatment option that offers the highest chance for response—also known as precision treatment. There are three different ways in which treatment outcome prediction can improve overall treatment success (Figure 3). Firstly, by predicting—before treatment initiation—which patients respond to anti-TNF treatment and do not respond to alternative treatment options. Since 80-90% of pediatric CD patients respond to anti-TNF antibodies, research should focus on predicting who does not respond to alternative treatment options, e.g. as steroids, EEN and immunomodulators, to limit the delay of effective treatment initiation. Unfortunately, there is only very limited data published on this matter. Two trials assessed predictive markers for steroid responsiveness in adult IBD patients.[47,48,49] Thiopurines effectiveness can be predicted to some extent by measuring thiopurine S-methyltransferase (TPMT) enzyme activity and not commencing thiopurines in patients with extremely-low TPMT activity.[50] Some data on predicting MTX responsiveness is available in the field of adult rheumatology[51], but not for IBD patients and neither is data available for predicting response to EEN. Thus, only very limited data is available on predicting responsiveness to the alternative non-biologic treatment options.



Third way: Once anti-TNF treatment is initiated, patients may benefit from a timely recognition of a disease relapse. Prediction which patients have high risk of losing response helps to timely recognize and act on a relapse.

Figure 3. Ways in which patients may benefit from treatment outcome prediction

Displaying the three ways in which CD patients may benefit from treatment outcome prediction related to anti-TNF treatment. The goal or object of the first two ways of treatment prediction are the same—to prevent treatment non-response. This can only be achieved by an accurate prediction of the chance to respond to anti-TNF treatment and a prediction of the chance to respond to an alternative treatment option. The third way in which treatment outcome prediction can be beneficial is by predicting which patients are at high risk to lose response.

The second way is predicting who does not respond to anti-TNF antibodies and may better receive an alternative treatment option. Research on this topic is complicated by the low chance of primary non-response in pediatric CD, and demands a relatively large patient sample in order to be studied. As we discussed previously, some patient characteristics are known to impact anti-TNF treatment success, i.e. no previous anti-TNF exposure, younger age and shorter disease duration are associated with higher anti-TNF response rates. However, these features are currently not used to determine who should or should not receive anti-TNF treatment, since they cannot accurately predict anti-TNF primary non-response—one exception being IFX non responders, who are not switched to ADA but to a drug that does not target TNF). In adult CD, not in pediatric CD, several trials have sought for baseline biomarkers that can predict anti-TNF response. Response to anti-TNF antibodies has been associated with baseline RNA expression of several genes in mucosal biopsies[52] and peripheral blood[53], and with the patients' genetic make-up.[54–59] Arijs et al demonstrated that RNA expression profiles of mucosal biopsies from adult colonic CD patients were able to accurately distinguish all IFX responders from IFX non-responders—response was determined based on change in endoscopic disease severity at week 4-6.(42) The authors reported that the top 5 differentially expressed genes alone reached perfect accuracy, i.e. 100% (top 5 genes: TNF-a-induced protein 6 [TNFAIP6], S100 calcium-binding protein A8 [S100A8], interleukin-11, G0/G1switch 2 [G0S2], and S100 calcium-binding protein A9 [S100A9])—no such predictive gene set was identified in ileal CD patients. More recently, West et al reported high Oncostatin M expression in mucosal tissue to be associated with anti-TNF response, which may be a promising marker in the future.[60] These findings now require replication in a separate cohort of pediatric CD patients before they can be used in clinical practice to guide treatment choices.

Thirdly, it would be beneficial to predict patients at risk of losing response to anti-TNF antibodies during treatment, since these patients may need intensified treatment and more frequent follow-up. There are multiple trials that addressed this topic. Typically, they have a follow-up period of 1 year and measure a certain marker after the induction period (roughly at 2-4 months from anti-TNF antibody initiation) and relate these results to their 1 year effectiveness outcomes. When measured after the induction period, lower clinical disease activity[12], lower endoscopic disease activity[62], lower calprotectin concentrations[63], lower disease activity measured by sonography[64] or by magnetic resonance enterography[65,66], and higher IFX trough levels[44,67] are associated with longer disease remission. In short, all available evidence indicates that more effective induction treatment results in more durable disease remission. Assessment of most of these factors are already part of routine clinical assessment and assist in timely discovery of treatment inefficacy.

Treatment side effects and risk of malignancy

Anti-TNF antibodies are in use for about two decades and most adverse effects are well established. Serious side-effects include acute and delayed infusion reactions, serious infections and opportunistic infections.[8,11,12,68,69] More uncertainty remains for rare but serious adverse events. These include rare cases of malignancies and mortality. Mortality in IBD patients is primarily linked to serious infections, followed by malignancy or uncontrolled disease.[70] The risk of malignancies was thought to be increased by anti-TNF treatment, as cases of lymphoma and hepato-splenic T-cell lymphomas (HSTCLs) were being reported in CD patients treated with both anti-TNF antibodies and immunomodulators.[71,72] This was one of the reasons why, next to the increased serious infection risk, anti-TNF antibodies were only approved for therapy refractory CD patients, because of a presumed lower benefit-risk ratio in this population.[73]

Recently, new evidence suggests that the risk of lymphoma seems more linked to thiopurine use (+/- in combination with anti-TNF) than anti-TNF treatment in itself. A large industry-sponsored long-term observational registry of pediatric patients with IBD (DEVELOP; NCT00606346) was initiated in 2007 to evaluate the long-term safety profile of IFX and other therapies prescribed to pediatric IBD patients. In their first publication, using data from 5766 patients with a median follow-up of 4.7 years and a total of 18 malignancy events, the authors report that they did not find an increased risk of malignancy and hemophagocytic lympho-histiocytosis (HLH) in IFX treated patients compared to a non-CD control population. Instead these risks were increased in thiopurine treated patients—with or without biologic exposure.[74] Notably, all (5) HLH cases were patients exposed to thiopurine and either a primary Epstein Barr virus infection (4/5) or a cytomegalovirus infection (1/5)–none had been exposed to anti-TNF antibodies. For malignancies, 4 out of 15 cases were thiopurine related, and without anti-TNF antibodies exposure, in the remaining 11 malignancy cases patients were exposed to both thiopurines as anti-TNF antibodies. Note that these conclusions were based on exposure defined as ‘ever exposed’, and in their discussion the authors acknowledged that, based on their data, cessation of thiopurine treatment for more than 1 year reduced the malignancy risk approaching the baseline risk. Nevertheless, infliximab alone did not significantly increase the malignancy risk, this was only the case when patients were also—previously or currently—exposed to thiopurine. This was also the conclusion of a case-control study on the risk of lymphomas, which reported an increased risk of T-cell lymphoma for combination therapy (anti-TNF treatment plus thiopurines), but not for anti-TNF treatment alone.[75] These findings imply a somewhat more favorable benefit-risk ratio of anti-TNF treatment than previously assumed, especially when given as monotherapy—without thiopurines.

Biosimilars

Biosimilars of IFX have become available on the European market since the expiration of the patent of the IFX originator. The similarity of IFX biosimilar CT-P13 with IFX originator was extensively tested. First in pre-clinical tests that compared their physicochemical characteristics, and by comparing their biological activities in several models related to their mechanisms of action. Afterwards, their similarity was confirmed clinically in two of the indications of IFX: ankylosing spondylitis and rheumatoid arthritis.[76,77] Based on these results, CT-P13 received market approval for all IFX's indications, including pediatric CD.

Only recently, the results of a randomized, double-blind, non-inferiority trial were published comparing the efficacy and safety of continuing on IFX originator with switching to CT-P13 in patients with various diseases including CD on stable treatment with IFX originator.[78] A total of 482 patients (155 CD patients [32%]) with stable conditions under IFX treatment, were randomized to continue on IFX originator or switch to CT-P13. After 1 year follow-up, they reported similar rates of disease worsening (IFX originator vs CT-P13: 26% vs 30%) and similar rates of adverse events (AE: 70% vs 68%, SAE: 10% vs 9%). Notably, the study was not powered to show non-inferiority in CD specifically, but in the overall population. Additionally, multiple observational trials assessed the effects of switching from IFX originator to CT-P13, and these were recently combined in a systematic review.[79] The authors combined the data from 11 observational trials and 1007 IBD patients, and compared these results—i.e. efficacy, safety and immunogenicity rates of CT-P13—with the results of IFX originator as reported in preciously published trials. Again, they reported no significant differences. Currently, only one observational trial assessed the effect of switching to CT-P13 in pediatric CD.[80] A total of 32 pediatric CD patients—and 7 UC—were switched from IFX originator to CT-P13. The authors report that switching seemed to be safe and did not impact efficacy. Thus, the early results confirm the expected similarity of IFX originator and CT-P13 in CD. Yet studies on both long-term outcome and switching from the originator to the biosimilar in pediatric CD are still required.

Aim of this thesis

The primary aim of this thesis is to compare the efficacy and safety of the top-down and step-up treatment strategies.

Additional aims of this thesis are:

- to develop a novel, Mucosal Inflammation Non-invasive (MINI) index that correlates with mucosal inflammation, and accurately discriminates endoscopic remission from active inflammation in children with CD.
- to study differences in the immune responses of newly diagnosed pediatric CD patients to infliximab or prednisolone treatment.
- to evaluate the real-world efficacy of ADA in pediatric CD patients and compare its efficacy in patients that were prior IFX non-responders or had lost response to IFX.
- to review the scientific international literature to determine the benefits and risks of combining anti-TNF with immunomodulator therapy in pediatric IBD.

Outline of this thesis

In **Chapter 2** we describe the MINI index we developed and validated. This non-invasive index identifies children with endoscopic remission with high sensitivity and specificity.

In **Chapter 3** we describe the international multicenter randomized controlled trial (RCT) we set up to compare the efficacy and safety of top-down treatment (starting with IFX from diagnosis) with the conventional step-up treatment strategy in newly diagnosed pediatric CD patients.

In a pilot analysis of the Infliximab Top-down Study in Kids with Crohn's disease (ITSKids) multicenter randomized trial in **Chapter 4,** we demonstrate that IFX treatment has a strong effect on mRNA expression and protein concentrations by reducing Th1 and neutrophil signatures, and tissue remodeling proteins.

In **Chapter 5** we describe the real-world effectiveness of ADA treatment for children and adolescents with CD who had previously failed IFX treatment in a nationwide, observational cohort study.

In **Chapter 6** we review the benefits and risks of combining anti-TNF treatment with immunomodulator therapy based on published evidence.

References

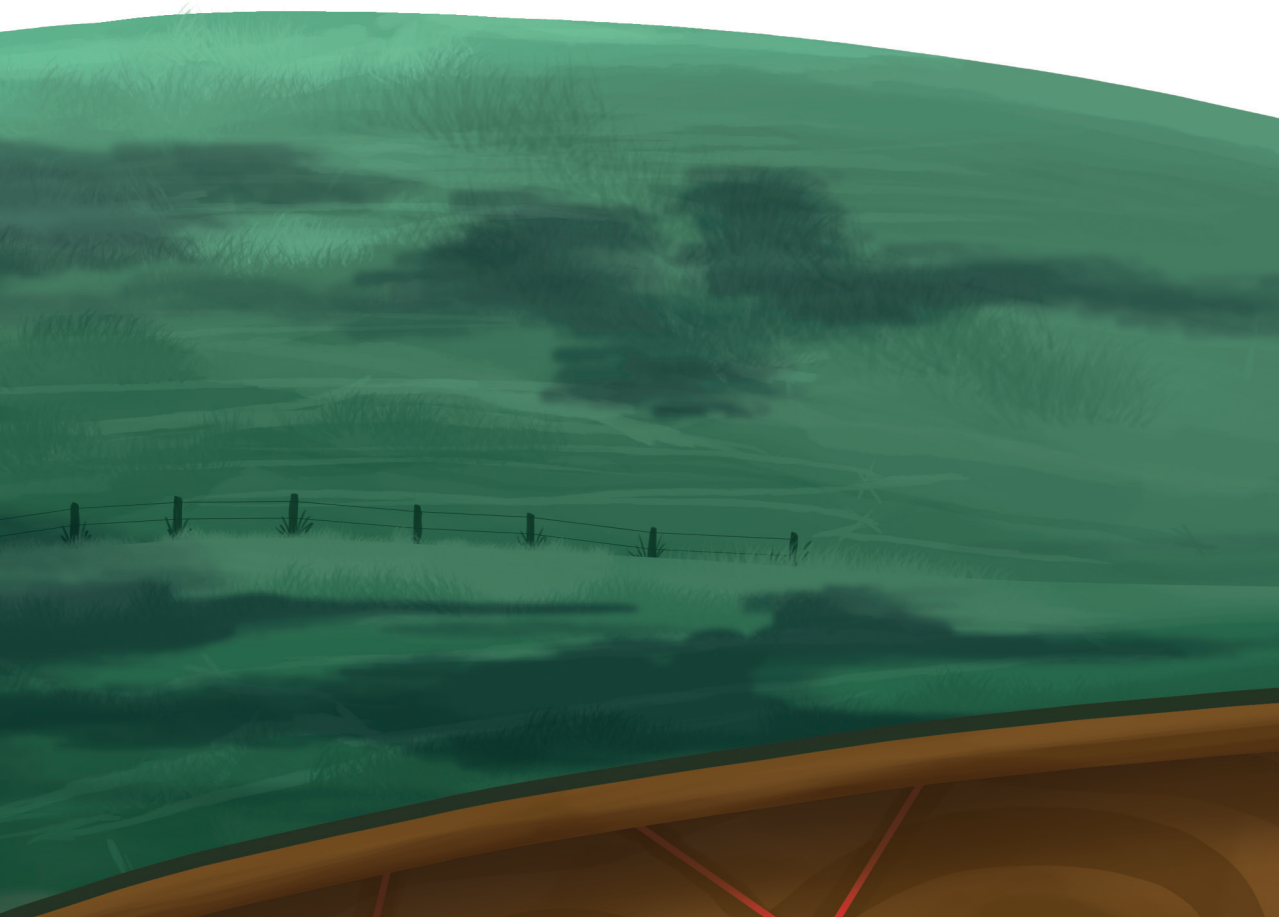
1. Ng SC, Shi HY, Hamidi N, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. Lancet 2018;390:2769-2778.
2. Ghione S, Sarter H, Fumery M, et al. Dramatic Increase in Incidence of Ulcerative Colitis and Crohn's Disease (1988-2011): A Population-Based Study of French Adolescents. Am J Gastroenterol 2018;113:265-272.
3. Benchimol EI, Fortinsky KJ, Gozdyra P, et al. Epidemiology of pediatric inflammatory bowel disease: a systematic review of international trends. Inflamm Bowel Dis 2011;17:423-439.
4. Van Limbergen J, Russell RK, Drummond HE, et al. Definition of phenotypic characteristics of childhood-onset inflammatory bowel disease. Gastroenterology 2008;135:1114-1122.
5. Pigneur B, Seksik P, Viola S, et al. Natural history of Crohn's disease: comparison between childhood- and adult-onset disease. Inflamm Bowel Dis 2010;16:953-961.
6. Geboes K, Rutgeerts P, Opdenakker G, et al. Endoscopic and histologic evidence of persistent mucosal healing and correlation with clinical improvement following sustained infliximab treatment for Crohn's disease. Curr Med Res Opin 2005;21:1741-1754.
7. Mannon PJ, Fuss IJ, Mayer L, et al. Anti-interleukin-12 antibody for active Crohn's disease. N Engl J Med 2004;351:2069-2079.
8. Ruemmele FM, Veres G, Kolho KL, et al. Consensus guidelines of ECCO/ESPGHAN on the medical management of pediatric Crohn's disease. J Crohns Colitis 2014;8:1179-1207.
9. Vatn MH. Mucosal healing: impact on the natural course or therapeutic strategies. Dig Dis Basel Switz 2009;27:470-475.
10. Schoepfer AM, Beglinger C, Straumann A, et al. Fecal calprotectin correlates more closely with the Simple Endoscopic Score for Crohn's disease (SES-CD) than CRP, blood leukocytes, and the CDAI. Am J Gastroenterol 2010;105:162-169.
11. Hyams J, Crandall W, Kugathasan S, et al. Induction and maintenance infliximab therapy for the treatment of moderate-to-severe Crohn's disease in children. Gastroenterology 2007;132:863-873.
12. Hyams JS, Griffiths A, Markowitz J, et al. Safety and efficacy of adalimumab for moderate to severe Crohn's disease in children. Gastroenterology 2012;143:365-74.e2.
13. Fan R, Zhong J, Wang ZT, et al. Evaluation of "top-down" treatment of early Crohn's disease by double balloon enteroscopy. World J Gastroenterol 2014;20:14479-14487.
14. Derkx B, Taminiau J, Radema S, et al. Tumour-necrosis-factor antibody treatment in Crohn's disease. Lancet 1993;342:173-174.
15. Cozijnsen MA, de Ridder L. Infliximab More Effective in Therapy-Naive Than in Therapy-Refractory Patients. J Pediatr Gastroenterol Nutr 2015;61:e15.
16. Kugathasan S, Denson LA, Walters TD, et al. Prediction of complicated disease course for children newly diagnosed with Crohn's disease: a multicentre inception cohort study. Lancet 2017;389:1710-1718.
17. Lichtenstein GR, Feagan BG, Cohen RD, et al. Serious infections and mortality in association with therapies for Crohn's disease: TREAT registry. Clin Gastroenterol Hepatol 2006;4:621-630.
18. Lichtenstein GR. Approach to Steroid-Dependent and Steroid-Refractory Crohn's Disease. J Pediatr Gastroenterol Nutr 2001;33:S27-S35.
19. Barabino A, Torrente F, Ventura A, et al. Azathioprine in paediatric inflammatory bowel disease: an Italian multicentre survey. Aliment Pharmacol Ther 2002;16:1125-1130.

20. Jaspers GJ, Verkade HJ, Escher JC, et al. Azathioprine maintains first remission in newly diagnosed pediatric Crohn's disease. Inflamm Bowel Dis 2006;12:831-836.
21. Riello L, Talbotec C, Garnier-Lengliné H, et al. Tolerance and efficacy of azathioprine in pediatric Crohn's disease. Inflamm Bowel Dis 2011;17:2138-2143.
22. Prefontaine E, Sutherland L, Macdonald J, et al. Azathioprine or 6-mercaptopurine for maintenance of remission in Crohn's disease. Cochrane Database Syst Rev 2009.
23. Markowitz J, Hyams J, Mack D, et al. Corticosteroid therapy in the age of infliximab: acute and 1-year outcomes in newly diagnosed children with Crohn's disease. Clin Gastroenterol Hepatol 2006;4:1124-1129.
24. Tracey D, Klareskog L, Sasso EH, et al. Tumor necrosis factor antagonist mechanisms of action: a comprehensive review. Pharmacol Ther 2008;117:244-279.
25. ten Hove T, van Montfrans C, Peppelenbosch MP, et al. Infliximab treatment induces apoptosis of lamina propria T lymphocytes in Crohn's disease. Gut 2002;50:206-211.
26. Mitoma H, Horiuchi T, Hatta N, et al. Infliximab induces potent anti-inflammatory responses by outside-to-inside signals through transmembrane TNF-alpha. Gastroenterology 2005;128:376-392.
27. Sandborn WJ, Hanauer SB, Katz S, et al. Etanercept for active Crohn's disease: a randomized, double-blind, placebo-controlled trial. Gastroenterology 2001;121:1088-1094.
28. Schreiber S, Rutgeerts P, Fedorak RN, et al. A randomized, placebo-controlled trial of certolizumab pegol (CDP870) for treatment of Crohn's disease. Gastroenterology 2005;129:807-818.
29. Nesbitt A, Fossati G, Bergin M, et al. Mechanism of action of certolizumab pegol (CDP870): in vitro comparison with other anti-tumor necrosis factor alpha agents. Inflamm Bowel Dis 2007;13:1323-1332.
30. Vos AC, Wildenberg ME, Duijvestein M, et al. Anti-tumor necrosis factor-alpha antibodies induce regulatory macrophages in an Fc region-dependent manner. Gastroenterology 2011;140:221-230.
31. Vos AC, Wildenberg ME, Arijs I, et al. Regulatory macrophages induced by infliximab are involved in healing in vivo and in vitro. Inflamm Bowel Dis 2012;18:401-408.
32. Ruemmele FM, Lachaux A, Cézard J-P, et al. Efficacy of infliximab in pediatric Crohn's disease: a randomized multicenter open-label trial comparing scheduled to on demand maintenance therapy. Inflamm Bowel Dis 2009;15:388-394.
33. Lionetti P, Bronzini F, Salvestrini C, et al. Response to infliximab is related to disease duration in paediatric Crohn's disease. Aliment Pharmacol Ther 2003;18:425-431.
34. Cezard JP, Nouaili N, Talbotec C, et al. A prospective study of the efficacy and tolerance of a chimeric antibody to tumor necrosis factors (remicade) in severe pediatric crohn disease. J Pediatr Gastroenterol Nutr 2003;36:632-636.
35. Crandall W, Hyams J, Kugathasan S, et al. Infliximab therapy in children with concurrent perianal Crohn disease: observations from REACH. J Pediatr Gastroenterol Nutr 2009;49:183-190.
36. Cozijnsen MA, de Ridder L. Infliximab More Effective in Therapy-Naive Than in Therapy-Refractory Patients. J Pediatr Gastroenterol Nutr 2015;61:e15.
37. Kugathasan S, Werlin SL, Martinez A, et al. Prolonged duration of response to infliximab in early but not late pediatric Crohn's disease. Am J Gastroenterol 2000;95:3189-3194.
38. Cameron FL, Altowati MA, Rogers P, et al. Disease Status and Pubertal Stage Predict Improved Growth in Antitumor Necrosis Factor Therapy for Pediatric Inflammatory Bowel Disease. J Pediatr Gastroenterol Nutr 2017;64:47-55.

39. Sandborn WJ, Colombel JF, D'Haens G, et al. Association of baseline C-reactive protein and prior anti-tumor necrosis factor therapy with need for weekly dosing during maintenance therapy with adalimumab in patients with moderate to severe Crohn's disease. Curr Med Res Opin 2013;29:483-493.
40. Reinisch W, Wang Y, Oddens BJ, et al. C-reactive protein, an indicator for maintained response or remission to infliximab in patients with Crohn's disease: a post-hoc analysis from ACCENT I. Aliment Pharmacol Ther 2012;35:568-576.
41. Vermeire S, Van Assche G, Rutgeerts P. C-reactive protein as a marker for inflammatory bowel disease. Inflamm Bowel Dis 2004;10:661-665.
42. Levesque BG, Greenberg GR, Zou G, et al. A prospective cohort study to determine the relationship between serum infliximab concentration and efficacy in patients with luminal Crohn's disease. Aliment Pharmacol Ther 2014;39:1126-1135.
43. Cornillie F, Hanauer SB, Diamond RH, et al. Postinduction serum infliximab trough level and decrease of C-reactive protein level are associated with durable sustained response to infliximab: a retrospective analysis of the ACCENT I trial. Gut 2014;63:1721-1727.
44. Bortlik M, Duricova D, Malickova K, et al. Infliximab trough levels may predict sustained response to infliximab in patients with Crohn's disease. J Crohns Colitis 2013;7:736-743.
45. Vande Casteele N, Ferrante M, Van Assche G, et al. Trough concentrations of infliximab guide dosing for patients with inflammatory bowel disease. Gastroenterology 2015;148:1320-9 e3.
46. Hofmekler T, Bertha M, McCracken C, et al. Infliximab Optimization Based on Therapeutic Drug Monitoring in Pediatric Inflammatory Bowel Disease. J Pediatr Gastroenterol Nutr 2017;64:580-585.
47. Montero-Meléndez T, Llor X, García-Planella E, et al. Identification of Novel Predictor Classifiers for Inflammatory Bowel Disease by Gene Expression Profiling. PLoS One 2013;8.
48. Honda M, Orii F, Ayabe T, et al. Expression of glucocorticoid receptor beta in lymphocytes of patients with glucocorticoid-resistant ulcerative colitis. Gastroenterology 2000;118:859-866.
49. Fujishima S, Takeda H, Kawata S, et al. The relationship between the expression of the glucocorticoid receptor in biopsied colonic mucosa and the glucocorticoid responsiveness of ulcerative colitis patients. Clin Immunol 2009;133:208-217.
50. Gisbert JP, Nino P, Rodrigo L, et al. Thiopurine methyltransferase (TPMT) activity and adverse effects of azathioprine in inflammatory bowel disease: long-term follow-up study of 394 patients. Am J Gastroenterol 2006;101:2769-2776.
51. Urano W, Taniguchi A, Yamanaka H, et al. Polymorphisms in the methylenetetrahydrofolate reductase gene were associated with both the efficacy and the toxicity of methotrexate used for the treatment of rheumatoid arthritis, as evidenced by single locus and haplotype analyses. Pharmacogenetics 2002;12:183-190.
52. Arijs I, Quintens R, Van Lommel L, et al. Predictive value of epithelial gene expression profiles for response to infliximab in Crohn's disease. Inflamm Bowel Dis 2010;16:2090-2098.
53. Mesko B, Poliska S, Vancsa A, et al. Peripheral blood derived gene panels predict response to infliximab in rheumatoid arthritis and Crohn's disease. Genome Med 2013;5:59.
54. Pierik M, Vermeire S, Steen KV, et al. Tumour necrosis factor-alpha receptor 1 and 2 polymorphisms in inflammatory bowel disease and their association with response to infliximab. Aliment Pharmacol Ther 2004;20:303-310.
55. Matsukura H, Ikeda S, Yoshimura N, et al. Genetic polymorphisms of tumour necrosis factor receptor superfamily 1A and 1B affect responses to infliximab in Japanese patients with Crohn's disease. Aliment Pharmacol Ther 2008;27:765-770.

56. Billiet T, Papamichael K, de Bruyn M, et al. A Matrix-based Model Predicts Primary Response to Infliximab in Crohn's Disease. J Crohns Colitis 2015;9:1120-1126.
57. Vermeire S, Van Assche G, Rutgeerts P. Role of genetics in prediction of disease course and response to therapy. World J Gastroenterol 2010;16:2609-2615.
58. Repnik K, Koder S, Skok P, et al. Transferrin Level Before Treatment and Genetic Polymorphism in HFE Gene as Predictive Markers for Response to Adalimumab in Crohn's Disease Patients. Biochem Genet 2016;54:476-486.
59. Barber GE, Yajnik V, Khalili H, et al. Genetic Markers Predict Primary Non-Response and Durable Response To Anti-TNF Biologic Therapies in Crohn's Disease. Am J Gastroenterol 2016;111:1816-1822.
60. West NR, Hegazy AN, Owens BMJ, et al. Oncostatin M drives intestinal inflammation and predicts response to tumor necrosis factor-neutralizing therapy in patients with inflammatory bowel disease. Nat Med 2017;23:579-589.
61. Atreya R, Neumann H, Neufert C, et al. In vivo imaging using fluorescent antibodies to tumor necrosis factor predicts therapeutic response in Crohn's disease. Nat Med February 2014.
62. af Bjorkesten CG, Nieminen U, Sipponen T, et al. Mucosal healing at 3 months predicts long-term endoscopic remission in anti-TNF-treated luminal Crohn's disease. Scand J Gastroenterol 2013;48:543-551.
63. Boschetti G, Garnero P, Moussata D, et al. Accuracies of serum and fecal S100 proteins (calprotectin and calgranulin C) to predict the response to TNF antagonists in patients with Crohn's disease. Inflamm Bowel Dis 2015;21:331-336.
64. Ripolles T, Paredes JM, Martinez-Perez MJ, et al. Ultrasonographic Changes at 12 Weeks of Anti-TNF Drugs Predict 1-year Sonographic Response and Clinical Outcome in Crohn's Disease: A Multicenter Study. Inflamm Bowel Dis 2016;22:2465-2473.
65. Buisson A, Hordonneau C, Goutte M, et al. Diffusion-weighted magnetic resonance enterocolonography in predicting remission after anti-TNF induction therapy in Crohn's disease. Dig Liver Dis 2016;48:260-266.
66. Naganuma M, Okuda S, Hisamatsu T, et al. Findings of ulceration and severe stricture on MRE can predict prognosis of Crohn's disease in patients treated with anti-TNF treatment. Abdom Radiol NY 2017;42:141-151.
67. Stein R, Lee D, Leonard MB, et al. Serum Infliximab, Antidrug Antibodies, and Tumor Necrosis Factor Predict Sustained Response in Pediatric Crohn's Disease. Inflamm Bowel Dis 2016;22:1370-1377.
68. Rahier JF, Ben-Horin S, Chowers Y, et al. European evidence-based Consensus on the prevention, diagnosis and management of opportunistic infections in inflammatory bowel disease. J Crohns Colitis 2009;3:47-91.
69. Veereman-Wauters G, de Ridder L, Veres G, et al. Risk of infection and prevention in pediatric patients with IBD: ESPGHAN IBD Porto Group commentary. J Pediatr Gastroenterol Nutr 2012;54:830-837.
70. de Ridder L, Turner D, Wilson DC, et al. Malignancy and mortality in pediatric patients with inflammatory bowel disease: a multinational study from the porto pediatric IBD group. Inflamm Bowel Dis 2014;20:291-300.
71. Siegel CA, Marden SM, Persing SM, et al. Risk of lymphoma associated with combination anti-tumor necrosis factor and immunomodulator therapy for the treatment of Crohn's disease: a meta-analysis. Clin Gastroenterol Hepatol 2009;7:874-881.

72. Kotlyar DS, Osterman MT, Diamond RH, et al. A systematic review of factors that contribute to hepatosplenic T-cell lymphoma in patients with inflammatory bowel disease. Clin Gastroenterol Hepatol 2011;9:36-41 e1.
73. CHMP. Remicade-H-C-240-II-0075 : EPAR - Scientific Discussion - Variation., 2007.
74. Hyams JS, Dubinsky MC, Baldassano RN, et al. Infliximab Is Not Associated With Increased Risk of Malignancy or Hemophagocytic Lymphohistiocytosis in Pediatric Patients With Inflammatory Bowel Disease. Gastroenterology February 2017.
75. Deepak P, Sifuentes H, Sherid M, et al. T-cell non-Hodgkin's lymphomas reported to the FDA AERS with tumor necrosis factor-alpha (TNF-alpha) inhibitors: results of the REFURBISH study. Am J Gastroenterol 2013;108:99-105.
76. Yoo DH, Hrycaj P, Miranda P, et al. A randomised, double-blind, parallel-group study to demonstrate equivalence in efficacy and safety of CT-P13 compared with innovator infliximab when coadministered with methotrexate in patients with active rheumatoid arthritis: the PLANETRA study. Ann Rheum Dis 2013;72:1613-1620.
77. Park W, Hrycaj P, Jeka S, et al. A randomised, double-blind, multicentre, parallel-group, prospective study comparing the pharmacokinetics, safety, and efficacy of CT-P13 and innovator infliximab in patients with ankylosing spondylitis: the PLANETAS study. Ann Rheum Dis 2013;72:1605-1612.
78. Jorgensen KK, Olsen IC, Goll GL, et al. Switching from originator infliximab to biosimilar CT-P13 compared with maintained treatment with originator infliximab (NOR-SWITCH): a 52-week, randomised, double-blind, non-inferiority trial. Lancet May 2017.
79. Radin M, Sciascia S, Roccatello D, et al. Infliximab Biosimilars in the Treatment of Inflammatory Bowel Diseases: A Systematic Review. BioDrugs 2017;31:37-49.
80. Sieczkowska J, Jarzebicka D, Banaszkiewicz A, et al. Switching Between Infliximab Originator and Biosimilar in Paediatric Patients with Inflammatory Bowel Disease. Preliminary Observations. J Crohns Colitis 2016;10:127-132.

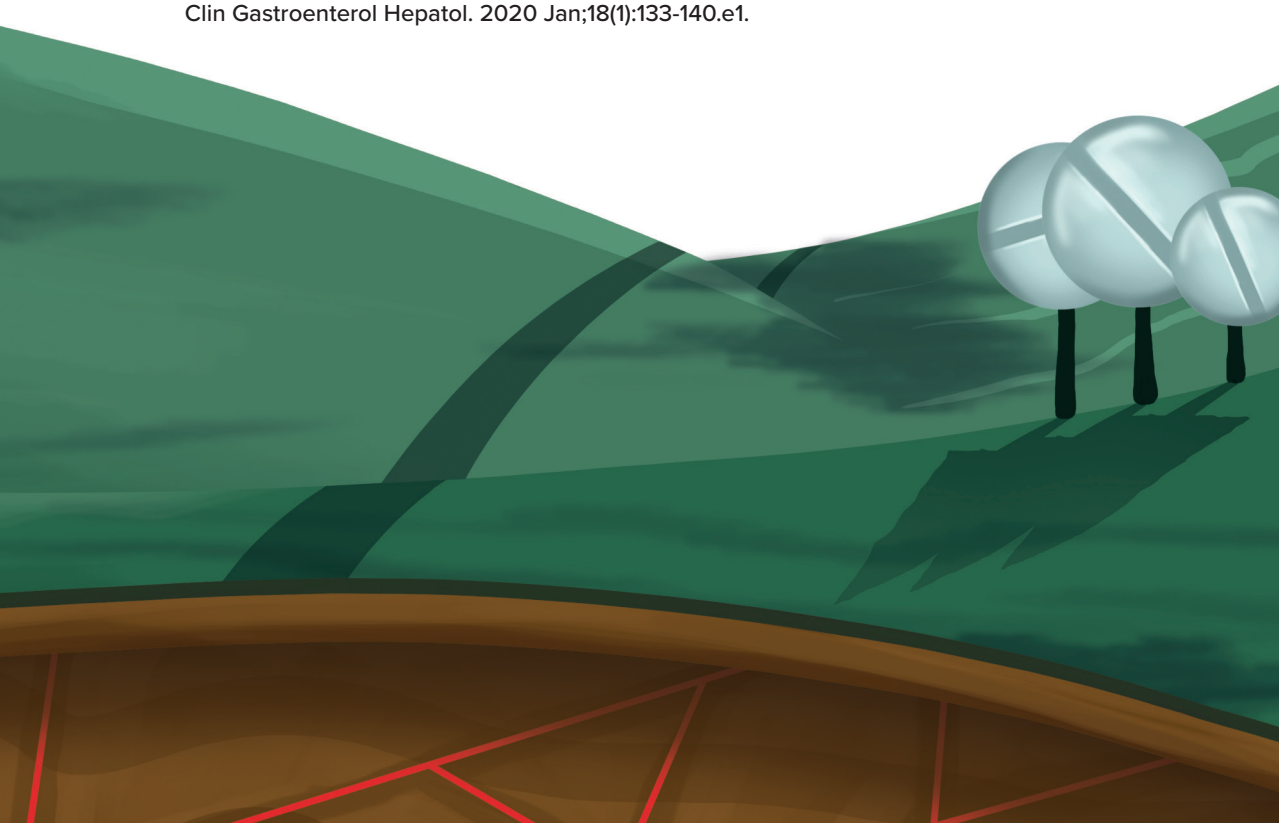


Chapter 2

Development and validation of the mucosal inflammation non-invasive index for pediatric Crohn's disease

Cozijnsen MA, Ben Shoham A, Kang B, Choe BH, Choe YH, Jongsma MME, Russell RK, Ruemmele FM, Escher JC, de Ridder L, Koletzko S, Martín-de-Carpi J, Hyams J, Walters T, Griffiths A, Turner D.

Clin Gastroenterol Hepatol. 2020 Jan;18(1):133-140.e1.



Abstract

Background & Aims: Mucosal healing (MH) has become a goal of therapy for Crohn's disease (CD), but frequent endoscopies are not feasible. We aimed to develop and validate a non-invasive index to assess mucosal inflammation in children with CD.

Methods: We collected data from the multi-center prospective ImageKids study, in which children with CD underwent ileocolonoscopy with magnetic resonance enterography. We investigated the association of pediatric CD activity index (PCDAI) items and laboratory test results with the simple endoscopic score for CD (SESCD). We used these data in a blended mathematical judgmental clinimetric approach to develop a weighted categorized index to identify children with CD who have MH, which we called the MINI index. We validated the index using data from 3 independent patient cohorts. The derivation and validation cohorts included 154 and 168 children, respectively (age 14.1±2.5 years and 14.2±3.9 years), of whom 16% and 36% had MH (defined as SESCD<3).

Results: In multivariable models, the stooling item of the PCDAI, erythrocyte sedimentation rate, and level of fecal calprotectin were associated with SESCD (all $P<.05$). We added data on level of C-reactive protein to develop the MINI index. MINI scores below 8 identified children with MH with 88% sensitivity and 85% specificity in the derivation cohort and with 84% sensitivity and 87% specificity in the validation cohorts. Ninety percent of the patients in the validation cohort with scores of 8 or more had active mucosal inflammation, yet 78% of patients with scores below 8 had MH. Scores below 6 increase the positive predictive value to 86%.

Conclusions: We developed an index to non-invasively assess mucosal inflammation in children with CD. This index, called the MINI index, identifies children with MH with high sensitivity and specificity. The added benefit of MINI over measurement of fecal calprotectin was small but significant, especially for patients with concentrations of fecal calprotectin from 100 to 599 μg/g.

ClinicalTrials.gov no: NCT01881490.

Introduction

It is widely accepted that treating Crohn's disease (CD) to the target of mucosal healing (MH), may be associated with improved long-term outcomes and may reduce the risk of bowel damage[1–3]. The visualized degree of mucosal inflammation is quantified by endoscopic scores, such as the simple endoscopic score for Crohn's disease (SESCD)[4,5]; it scores each bowel segment for ulcerations, affected surface area and luminal narrowing. However, the use of endoscopic evaluation as a target to treatment has several limitations given its invasiveness, cost and potential risks, including the requirement of anesthesia[6]. Therefore, non-invasive measures of MH are desirable for tight monitoring of CD patients.

Clinical disease activity indices correlate poorly with endoscopic disease activity in CD[4,7–10]. In children, the reported correlation of the pediatric Crohn's disease activity index (PCDAI) and the weighted PCDAI (wPCDAI) with the SESCD, whilst higher than reported for the adult clinical disease activity index (CDAI), still does not surpass 0.3–0.45[9,11]. In every day practice, physicians regularly use serum markers, including erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), to monitor disease activity given their correlation with mucosal disease activity (SESCD or CDEIS) that ranged between 0.12 and 0.54 in different studies[4,6,9,12–14]. While the specificity of both markers is high they lack sensitivity and approximately half of patients with normal serum markers may still have significant mucosal inflammation[15]. Fecal calprotectin (FC) is increasingly used as a superior measure of mucosal inflammation with correlation coefficients ranging from 0.45 to 0.76[6,16–18]. However, its large inter-patient variability prevents determining a clear cutoff value to reflect MH[1,15]. In addition, FC proportionally reflects histological rather than macroscopic inflammation and thus intermediate values (e.g. 100-300) do not necessarily reflect macroscopic mucosal inflammation[19].

We hypothesized that a combination of clinical symptoms with serum and fecal inflammatory markers can reflect mucosal inflammation if weighted mathematically on a large cohort of patients[9]. We thus aimed to develop a novel, Mucosal-Inflammation Non-Invasive (MINI) index that correlates with SESCD, and accurately discriminates MH from mucosal inflammation in children with CD.

Methods

The MINI index was derived and then validated on data from four independent prospective cohorts of children with CD, utilizing a blended mathematical–judgmental clinimetric approach.

Derivation cohort

The derivation of the MINI index utilized data from the ImageKids study: a multicenter, prospective cohort (22 medical centers in 9 countries) aimed to develop and validate magnetic resonance enterography (MRE)-based indices of inflammation and intestinal damage (ClinicalTrials.gov: NCT01881490). A total of 240 children with an established diagnosis of CD were enrolled at the time of performing ileocolonoscopy and MRE at disease onset or thereafter. Explicit demographic and clinical data were recorded, including PCDAI, serum biochemical tests, and stool for FC. Endoscopic disease activity was captured using the SESCD[4], and mucosal healing was defined as a SESCD<3. MRE assessment of disease severity, performed within 2 weeks of the endoscopy, was captured using overall radiologist assessment (RGA) of bowel inflammation by two independent radiologists, with RGA score<20 mm considered radiographic remission.[20] Deep healing was defined as a combination of RGA<20 mm and SESCD<3. We excluded patients in whom the terminal ileum was not reached during endoscopy, who lacked FC measurement, and patients with isolated L4a or L4b disease as per the Paris classification[21]. A total of 154 children from the ImageKids cohort fulfilled the eligibility criteria and were included in the derivation cohort (Table 1).

Validation cohorts

The validation of the MINI index utilized three cohorts of children with CD. We applied the same eligibility criteria in the validation cohorts as in the derivation cohort. The first was a prospective cohort assembled at two medical centers in South Korea. Children in clinical remission 1-2 years after diagnosis underwent scheduled ileocolonoscopy or sooner in case of a relapse. The second validation cohort was from a bio-bank registry at Shaare Zedek Medical Center, Jerusalem. All children with CD undergoing ileocolonoscopy at disease onset or thereafter were included, when endoscopic, laboratory and clinical data were prospectively recorded, and stool collected for FC. The third validation cohort was from a clinical trial that randomized 100 children, aged 3-17 years, with new-onset moderate-to-severe CD disease into top-down and step-up treatment groups (TISKids, ClinicalTrials.gov: NCT02517684).[22] We used the ileocolonoscopic, clinical and laboratory data from baseline prior to randomization.

In all three cohorts endoscopic activity was captured using the SESCD and explicit demographic and laboratory data, including FC, were collected at the time of ileocolonoscopy (but not during the bowel preparation), as well as PCDAI. A total of 168 children were included in the validation cohorts (Table 1).

Statistical analyses

The derivation of the MINI index was based on the individual PCDAI items and the following laboratory items: hematocrit, albumin, ESR, CRP, platelets, white blood cell count, and FC. We explored various models to associate with the SESCD including when laboratory tests were entered to the models as continuous variables or grouped into categories. We used a blended mathematical–judgmental approach to determine the items; those with a p-value>0.1 in the multivariate analyses were considered for exclusion by an advisory board of 10 international experts in pediatric CD (see authors of this manuscript), thus ensuring content and face validity. Discriminative validity was assessed by the area under the receiver operating characteristic (ROC) curve (AUROC) which was also used for exploring the best cutoff to identify MH (SESCD<3), and a second cutoff to discriminate mild (SESCD 3-9) from moderate-to-severe (SESCD > 9) mucosal inflammation.

Data are reported as mean ± standard deviation or median (inter quartile range [IQR]) as appropriate. Continuous data were compared using Student's t test, or the Wilcoxon rank sum test as per the distribution normality. Spearman or Pearson correlations were used as appropriate. Categorical variables were compared using χ^2 or Fisher's exact tests, as appropriate. McNemar's test was used to compare the accuracy of MINI<8 with FC<300 µg/g to detect MH. Of the entire derivation dataset, there were 26 missing values of any individual blood test (13 CRP, 8 ESR, 4 albumin, 1 platelets) which were imputed by a regression analysis using the other blood tests corrected for age, gender and FC value. For the validation dataset, 19 missing values were imputed (2 CRP, 11 ESR, 6 albumin). The ethics committees of all centers approved the Imagekids study and the validation cohorts. Consent, and when appropriate also assent, were obtained in all cases. All authors had access to the study data and approved the final manuscript.

Table 1. Basic characteristics of the derivation and validation cohorts (mean±SD, median (IQR) and percentage displayed as appropriate)

	Derivation cohort (n=154)	Validation cohort 1 (n=86)
Females (%)	42%	44%
Age (years)	14.1±2.5	15.4±2.5
Disease-duration (years)	2.2 (0.3-4.3)	1.5 (1.1-3.4)
Albumin (g/dL)	4.0±0.6	4.5±0.3
ESR (mm/hr)	18 (10-35)	10 (4-21)
CRP (mg/L)	6.2 (1.8-20.5)	0.4 (0.3-0.9)
FC (µg/g)	632 (163-1287)	107 (34-711)[1]
FC<300µg/g	31%	62%
wPCDAI		
Remission (PCDAI<10 / wPCDAI<12.5)	33%	88%
Mild (PCDAI 10-27.5 / wPCDAI 12.5-40)	47%	7%
Moderate to severe (PCDAI≥30 / wPCDAI>40)	20%	5%
SESCD score	9 (4-15)	1 (0-3)
Remission (<3)	16%	65%
Mild (3-9)	36%	23%
Moderate-to-severe (>9)	49%	11%

[1]In the validation cohort, FC results are capped at 2000µg/g.
Validation cohort 1=prospective cohort South Korea; Validation cohort 2= Bio-bank registry Shaare Zedek Medical Center; Validation cohort 3= multicenter RCT TISKids
ESR=erythrocytes sedimentation rate; CRP=C-reactive protein; FC=fecal calprotectin; PCDAI=pediatric Crohn's disease activity index; SESCD=simple endoscopic score for Crohn's disease; MRE=magnetic resonance enterography; NA=not available.

	Validation cohort 2 (n=44)	Validation cohort 3 (n=38)	All validation cohorts (n=168)	p-value (derivation vs all validation)
	41%	58%	46%	0.411
	12.7±5.5	14.6±3.4	14.2±3.9	0.777
	0.1 (0-2.8)	0 (0-0)	1.1 (0-2.4)	<0.001
	3.9±0.5	3.6±0.6	4.1±0.6	0.005
	27 (15-44)	35 (26-54)	22 (9-38)	0.952
	16.6 (6.3-44)	27.0 (15.5-54.8)	2.5 (0.3-26)	0.021
	2100 (555-2100)	835 (622-1130)	620 (68-1067)	0.388
	18%	5%	38%	0.187
	32%	0%	54%	<0.001
	30%	3%	12%	<0.001
	39%	97%	35%	0.002
	11 (6-17)	15 (9-21)	6 (0-15)	0.001
	9%	3%	36%	<0.001
	34%	29%	27%	0.107
	57%	68%	36%	0.025

Results

Derivation of the MINI index

We first constructed a regression model where the SESCD served as the dependent variable and the explanatory variables included the total PCDAI score, CRP and FC. The PCDAI and FC were associated with SESCD (both $p<0.001$), while CRP was not ($p=0.32$) ($R^2=0.45$).

Next, we aimed to identify key items of the PCDAI reflecting mucosal inflammation in a model with and without CRP and FC (Supplementary table 1). We judgmentally excluded the items 'well-being' which is poorly defined and lacks reliability, 'abdominal examination' which lacks reliability and 'height velocity' which is an important determinant of mucosal inflammation but has poor responsiveness over time and not relevant to adolescents who completed their growth period. These three items were previously proven to be redundant in a multivariable regression analysis of the PCDAI[23]. The stooling item of PCDAI and FC were strongly associated with SESCD in all models (Supplementary table 1). Hypoalbuminemia (<3 g/dL) was associated with low SESCD in the multivariate analyses, counterintuitive to the expected direction and thus was excluded – in univariate analyses the association was opposite, as expected. We constructed further models where we substituted the laboratory PCDAI items (i.e. hematocrit, albumin and ESR) with their absolute values (rather than the categorized values), but this did not improve the model fit ($R^2=0.476$). We also analyzed models with additional laboratory measures associated with inflammation (i.e. platelets, white blood cells) but again without an added value ($R^2=0.469$).

ESR and the weight items of the PCDAI were significant in some of the models, especially without FC or CRP (Supplementary table 1). Indeed, FC, CRP and ESR, were in collinearity with each other and FC crowded out the association of ESR and CRP. Nonetheless, the advisory board reached consensus to retain CRP in order to improve face and content validity, considering the extensive literature and vast clinical experience demonstrating the importance of CRP in reflecting mucosal inflammation in CD, and since at times only CRP and not ESR is available or vice versa.

To construct an intuitive tool, we grouped continuous laboratory measures (FC, CRP and ESR) into categories by plotting the variable against categories of endoscopic disease severity (Figure 1). The advisory board opted to exclude the weight item since it is a longitudinal measure, has limited responsiveness to change, it can be artificially affected by other factors like steroids and its contribution to the overall model fit was negligible (Supplementary table 2).

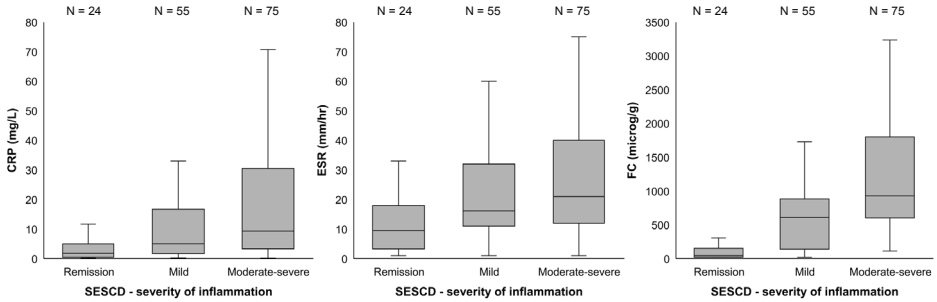


Figure 1. CRP (1a), ESR (1b) and fecal calprotectin (1c) across the different severity categories of endoscopic inflammation, as measured by the SESCD in the derivation cohort

The final MINI index was constructed based on the final model which eventually achieved the best performance (R^2=0.78) (Table 2 and Supplementary table 3). The advisory board used the beta scores of the variables as a general guide to assign weights to the items of the MINI index.

We then set the threshold of the MINI that corresponds to MH by exploring the best cutoff values on a ROC curve (AUROC to predict MH was excellent 0.92 [95%CI 0.86 – 0.97], p<0.001; rho=0.70). A cutoff <8 best balanced sensitivity (88%) and specificity (85%) for MH; 41 out of 154 patients (27%) had a MINI index score <8.

Validation of the MINI index

The median MINI index score of the 168 patients from the validation cohorts, was 11.5 (IQR 1 to 17, range -3 to 25), with 103 (61%) having a score <8 points. MH was detected using MINI<8 as a cutoff with 84% sensitivity and 87% specificity, PPV 78% and NPV 90% (AUROC 0.93 [95%CI 0.89 - 0.97], p<0.001; rho=0.82) (Table 3).

The implication of PPV 78%, is that 22% of the 65 patients with MINI<8 did not have MH (n=14). However, of those, 12 (86%) had merely mild inflammation (SESCD 3-9) and only 2 (1.2% of the entire cohort) had moderate-to-severe inflammations. A lower cutoff of <6 was more reliable to diagnose MH; 86% of children with that cutoff had a SESCD<3 (48/56). Of the remaining 14% (n=8), 88% (n=7) had merely mild inflammation (SESCD 3-9) and only one child had moderate-to-severe inflammation (SESCD>9). Approaching it from the other side, a MINI≥8 score was highly accurate to diagnose inflammation: 90% of MINI≥8 (93/103) had mucosal inflammation, of whom 59 (63%) with moderate-severe inflammation.

The MINI index not only detected MH but also managed to categorize degree of mucosal inflammation. A score of 8-11 reflected mild inflammation and >11 points moderate-to-severe inflammation (Table 3, Figure 2, Figure 3).

Table 2. The Mucosal-Inflammation Non-Invasively (MINI) index

Item	Points
1. Stool	
0-1 Normal or liquid stools, no blood	0
≤ 2 Semi-formed with small blood, or 2-5 liquid	4
Gross bleeding, or ≥ 6 liquid, or nocturnal diarrhea	8
2. Fecal calprotectin (µg/g)	
<50	-3
50-99.9	0
100-299.9	5
300-599.9	7
600-899.9	9
≥900	12
3. ESR (mm/hr) and CRP (mg/L)	
ESR<10 and CRP<5	0
30>ESR≥10 or 10>CRP≥5	1
50>ESR≥30 or 30>CRP≥10	2
ESR≥50 or CRP≥30	5
Sum of MINI	-3 to 25

User guide: While it is possible to score the MINI index with either CRP or ESR, both are preferred. Score the highest of CRP or ESR. The stool item: The intent is to score the stool pattern during the preceding week. First categorize the subject as having blood in the stool or not. If there is no blood in the stool, score as follows: Formed stools or up to 1 loose stool daily = 0; 2-5 liquid or very loose stools on 1 or more days = 4; 6 or more liquid or very loose stools on 1 or more days or any nocturnal diarrhea = 8. If blood is present in the stool, score as follows: Small amounts of blood (on toilet paper or small spots in stool) = 4; Any gross bleeding (large amounts on stool or colors the water in the toilet) = 8.

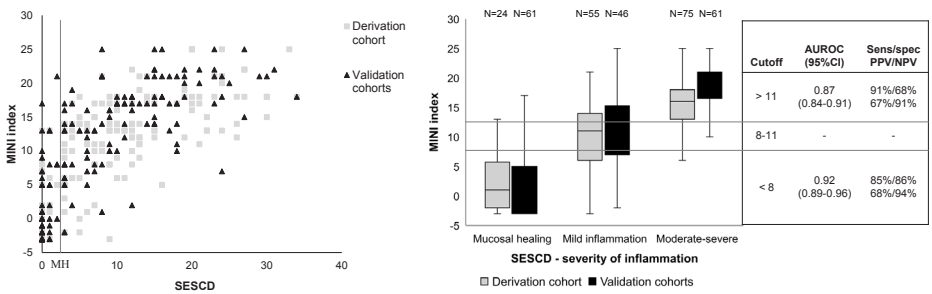


Figure 2. The MINI index stratified by severity of endoscopic inflammation, as measured by the SESCD in the derivation and validation cohorts

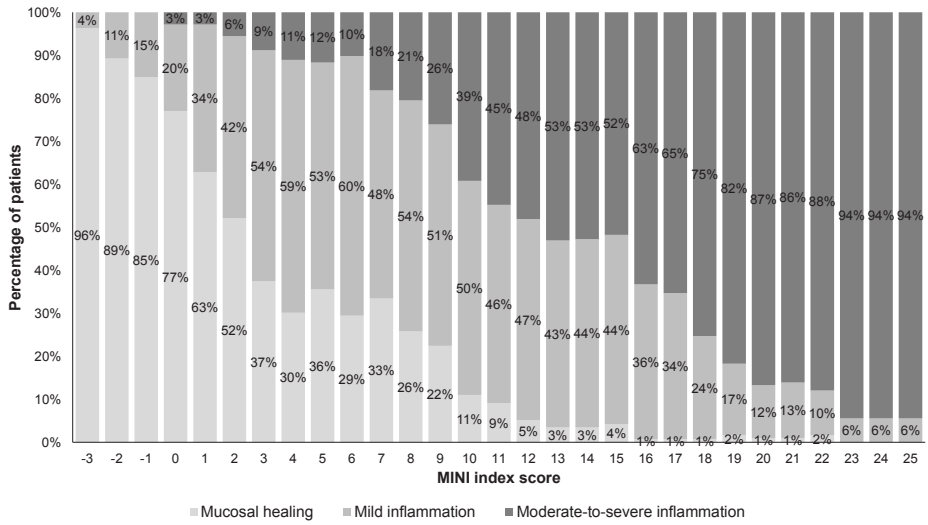


Figure 3. Rates of MH (SESCD<3), mild inflammation (SESCD 3-9) and moderate-to-severe inflammation (SESCD>9) for each MINI index score in all cohorts combined (n=322).

Secondary analyses

FC alone was slightly less accurate in detecting MH than the MINI index (derivation: AUROC 0.92, rho=0.63. validation: AUROC 0.92, rho=0.73, at an optimal cutoff of <300 µg/g,) (Table 3). Of the 322 children in the entire cohort, 275 (85%) were diagnosed correctly (i.e. either a true positive or true negative test result) by MINI<8 vs. 265 (82%) by FC<300 µg/g (p=0.013). The difference in accuracy between the MINI index and FC was highest among the 76 patients with FC concentrations between 100 and 599 µg/g – a gray range of FC with low discriminatory accuracy. Of these 76 patients, 50 (66%) were diagnosed correctly by MINI<8 and 41 patients (54%) were diagnosed correctly by FC<300 µg/g (p=0.022, number needed to screen 9).

PCDAI alone (score <10) had a lower performance (combined data: AUROC 0.81, rho=0.65), as CRP (combined data: AUROC 0.85, rho=0.59) and ESR (combined data: AUROC 0.75, rho=0.46).

The accuracy of MINI<8 to detect MH varied slightly between disease location categories of the Paris classification. Sensitivity and specificity were slightly lower in ileal CD (L1), than in colonic and ileocolonic CD (L2/L3) on the combined data (L1: n=77, sensitivity 76%, specificity 77%, AUROC 0.77; L2/L3: n=231, sensitivity 86%, specificity 89%, AUROC 0.87).

Table 3. Accuracy of cutoffs for the MINI index, FC, PCDAI, CRP and ESR

Cohort	Measure	MINI index				FC (µg/g)	PCDAI/ wPCDAI	CRP (mg/L)	ESR (mm/ hr)
	Cutoff	<6		≥8	>11	<300	<10 / <12,5	<5	<20
	Detect	**MH**	**DH**	**MI***	**MSI**	**MH**	**MH**	**MH**	**MH**
Derivation (n=154)	Sensitivity	75%	82%	85%	89%	83%	67%	75%	79%
	Specificity	90%	88%	88%	61%	79%	73%	64%	47%
	PPV	58%	45%	97%	68%	43%	31%	28%	22%
	NPV	95%	98%	51%	86%	96%	92%	93%	92%
Validation (n=168)	Sensitivity	79%	NA	87%	93%	80%	93%	93%	79%
	Specificity	93%	NA	84%	73%	87%	69%	69%	64%
	PPV	86%	NA	90%	66%	78%	63%	63%	56%
	NPV	88%	NA	78%	95%	89%	95%	95%	84%

**For MINI≥8, we set SESCD≥3 as positive result (thus NPV and PPV are switched). CRP=C-reactive protein, ESR=erythrocyte sedimentation rate, FC=fecal calprotectin; MH=mucosal healing (SESCD<3), DH=deep healing (i.e. SESCD<3 and remission by MRE as defined by radiologic global assessment<20 mm), MI=mucosal inflammation (SESCD≥3), MSI=moderate-to-severe inflammation (SESCD>9), PCDAI=pediatric Crohn's disease activity index; wPCDAI=mathematically weighted PCDAI; PPV=positive predictive value, NPV=negative predictive value, NA=not applicable.*

In the derivation cohort, the MINI index had good accuracy to detect the 17 children with deep healing (Table 3); a cut-off value of < 6 points detected deep healing with 82% sensitivity, 88% specificity, 45% PPV and 98% NPV (AUROC 0.82). The low PPV is a result of the low deep healing rate in the derivation cohort (17/154 [11%]). Fourteen out of 17 children with deep healing (82%) had a MINI < 6, versus 17 out of 137 (12%) without deep healing ($p < 0.001$).

Discussion

We developed the MINI index, a Mucosal Inflammation- Non-Invasive index that strongly correlates with SESCD and that can accurately assess mucosal inflammation. The MINI index was generated on a large prospective cohort of pediatric CD, and validated on three independent prospective cohorts. A cutoff of <8 best balanced sensitivity and specificity in reflecting. A cutoff of <6 had a higher PPV to reflect MH (86%) and ≥8 was most accurate to diagnose mucosal inflammation (PPV 90%).

Although the index was significantly more accurate than FC (p=0.013), its clinical benefit over FC was modest for the entire dataset. However, one of the largest weaknesses of FC is the low discriminatory accuracy in the gray range of 100-599 µg/g, which may reflect severe inflammation in some patients or near mucosal healing in others. The MINI index significantly improved the utility of FC in this gray zone, adding to the correct classification of at least half of these patients with a number needed to screen of 9. Furthermore, the items added to FC are collected in routine clinical practice, and thus the use of the index should be relatively easy and intuitive.

In our study, the PCDAI correlated moderately with SESCD (rho=0.59) and higher than previously reported in children (0.3–0.45)[9,11]. Of the individual items, the stooling and weight items were most significant, and abdominal pain and fatigue were not.

The correlation between CRP and SESCD was 0.59, slightly higher than the range of previous reports (i.e. rho~0.12-0.54)[4,6,9,12–14,18]. In the multi-variable models, CRP had little contribution to the overall fit, due to strong collinearity with FC and ESR. The latter was similarly crowded out in the presence of the other biomarkers. Guided by strong judgmental input from our international advisory board, we incorporated both CRP and ESR with low weights. Indeed, a similar study to ours among adult CD patients[24] and a recent post-hoc analysis of the CALM randomized controlled trial of 244 adults with CD[25] both affirmed that adding CRP to FC increases the accuracy of detecting MH. Another recent retrospective study reported improved accuracy in diagnosing CD among 128 children with elevated FC levels by considering ESR, CRP and albumin as additional markers to FC.[26]

We used a blended mathematical–judgmental clinimetric approach. An analytic account of the clinical phenomena that is observed, judged and decided by clinicians and patients themselves, is often missing from psychometric outcome measures[27]. Mathematical modeling alone is not a sine-qua-non for accuracy, since the psychometric approach has been criticized for lack of face validity and sensibility in developing scales[28]. To optimize the utility of scores in day-to-day clinical practice, a thoughtful combination of both mathematical

methods with strong clinical input should be the basis of developing outcome measures, as done here[29–32]. All judgmental decisions have been, however, justified by our results and prior literature while providing strong clinical rationale.

This is by far the largest dataset of pediatric CD yet to study the association between non-invasive activity measures and mucosal inflammation. Data were gathered in multiple countries, giving rise to inter-observer and cross-cultural diversity, which is strongly encouraged when developing outcome measures. Furthermore, there was room for site-dependent and observer-dependent variations in our results: CRP and ESR were performed locally, endoscopies were not centrally read and both endoscopy and lab work-up was not always conducted on the same day, but up to 2 weeks intervals were allowed as long as treatment was unchanged. These variations, however, reflect clinical practice, and the fact that the MINI index performed well even outside the “sterile” condition of clinical trials lends further support for its real world clinical utility.

The MINI index was validated on three independent prospective cohorts and, reassuringly, its accuracy to reflect mucosal inflammation remained good. Due to its non-invasive nature, the MINI index allows tight monitoring of mucosal inflammation and facilitating appropriate selection of children for colonoscopic assessment. It may also serve as an outcome measure in clinical trials instead or in addition to ileocolonoscopy to increase feasibility and enrollment rates. It may now also be tested in adults as the included items are not specific to children.

References

1. Ruemmele FM, Veres G, Kolho KL, et al. Consensus guidelines of ECCO/ESPGHAN on the medical management of pediatric Crohn's disease. J Crohns Colitis 2014;8:1179-1207.
2. Kierkus J, Dadalski M, Szymanska E, et al. The impact of infliximab induction therapy on mucosal healing and clinical remission in Polish pediatric patients with moderate-to-severe Crohn's disease. Eur J Gastroenterol Hepatol 2012;24:495-500.
3. Vatn MH. Mucosal healing: impact on the natural course or therapeutic strategies. Dig Dis Basel Switz 2009;27:470-475.
4. Daperno M, D'Haens G, Van Assche G, et al. Development and validation of a new, simplified endoscopic activity score for Crohn's disease: the SES-CD. Gastrointest Endosc 2004;60:505-512.
5. Annese V, Daperno M, Rutter MD, et al. European evidence based consensus for endoscopy in inflammatory bowel disease. J Crohns Colitis 2013;7:982-1018.
6. Schoepfer AM, Beglinger C, Straumann A, et al. Fecal calprotectin correlates more closely with the Simple Endoscopic Score for Crohn's disease (SES-CD) than CRP, blood leukocytes, and the CDAI. Am J Gastroenterol 2010;105:162-169.
7. Modigliani R, Mary JY, Simon JF, et al. Clinical, biological, and endoscopic picture of attacks of Crohn's disease. Evolution on prednisolone. Groupe d'Etude Therapeutique des Affections Inflammatoires Digestives. Gastroenterology 1990;98:811-818.
8. Jones J, Loftus EV, Panaccione R, et al. Relationships between disease activity and serum and fecal biomarkers in patients with Crohn's disease. Clin Gastroenterol Hepatol 2008;6:1218-1224.
9. Zubin G, Peter L. Predicting Endoscopic Crohn's Disease Activity Before and After Induction Therapy in Children: A Comprehensive Assessment of PCDAI, CRP, and Fecal Calprotectin. Inflamm Bowel Dis 2015;21:1386-1391.
10. Cellier C, Sahmoud T, Froguel E, et al. Correlations between clinical activity, endoscopic severity, and biological parameters in colonic or ileocolonic Crohn ' s disease. A prospective multicentre study of 121 cases. Gut 1994;35:231-235.
11. Turner D, Levine A, Walters TD, et al. Which PCDAI Version Best Reflects Intestinal Inflammation in Pediatric Crohn Disease? J Pediatr Gastroenterol Nutr 2017;64:254-260.
12. Thompson D, Milford-Ward A, Whicher JT. The value of acute phase protein measurements in clinical practice. Ann Clin Biochem 1992;29 (Pt 2):123-131.
13. D'Haens G, Ferrante M, Vermeire S, et al. Fecal calprotectin is a surrogate marker for endoscopic lesions in inflammatory bowel disease. Inflamm Bowel Dis 2012;18:2218-2224.
14. Meuwis MA, Vernier-Massouille G, Grimaud JC, et al. Serum calprotectin as a biomarker for Crohn's disease. J Crohns Colitis 2013;7:e678-83.
15. Kopylov U, Yablecovitch D, Lahat A, et al. Detection of Small Bowel Mucosal Healing and Deep Remission in Patients With Known Small Bowel Crohn's Disease Using Biomarkers, Capsule Endoscopy, and Imaging. Am J Gastroenterol 2015;110:1316-1323.
16. Foell D, Wittkowski H, Roth J. Monitoring disease activity by stool analyses: from occult blood to molecular markers of intestinal inflammation and damage. Gut 2009;58:859-868.
17. Aomatsu T, Yoden A, Matsumoto K, et al. Fecal calprotectin is a useful marker for disease activity in pediatric patients with inflammatory bowel disease. Dig Dis Sci 2011;56:2372-2377.
18. Sipponen T, Karkkainen P, Savilahti E, et al. Correlation of faecal calprotectin and lactoferrin with an endoscopic score for Crohn's disease and histological findings. Aliment Pharmacol Ther 2008;28:1221-1229.

19. Rheenen PF. Role of fecal calprotectin testing to predict relapse in teenagers with inflammatory bowel disease who report full disease control. Inflamm Bowel Dis 2012;18:2018-2025.
20. Nakarl I, Focht G, Church P, et al. Associations among mucosal and transmural healing and fecal level of calprotectin in children with Crohn's disease. Clin Gastroenterol Hepatol 2018;16:1089-1097.
21. Levine A, Griffiths A, Markowitz J, et al. Pediatric modification of the Montreal classification for inflammatory bowel disease: the Paris classification. Inflamm Bowel Dis 2011;17:1314-1321.
22. Cozijnsen MA, van Pieterson M, Samsom JN, et al. Top-down Infliximab Study in Kids with Crohn's disease (TISKids): an international multicentre randomised controlled trial. BMJ Open Gastroenterol 2016;3:e000123.
23. Turner D, Griffiths AM, Walters TD, et al. Mathematical weighting of the pediatric Crohn's disease activity index (PCDAI) and comparison with its other short versions. Inflamm Bowel Dis 2012;18:55-62.
24. Minderhoud I, Steyerberg E, van Bodegraven A, et al. Predicting Endoscopic Disease Activity in Crohn's Disease: A New and Validated Noninvasive Disease Activity Index (The Utrecht Activity Index). Inflamm Bowel Dis 2015;21:2453-2459.
25. Reinisch W, Panaccione R, Bossuyt P, et al. OP015 Biomarker correlation with endoscopic outcomes in patients with Crohn's disease: data from CALM. Abstr 13th Congr ECCO February 2018.
26. Daniluk U, Daniluk J, Krasnodebska M, et al. The combination of fecal calprotectin with ESR, CRP and albumin discriminates more accurately children with Crohn's disease. Adv Med Sci 2018;64:9-14.
27. Feinstein A. An additional basic science for clinical medicine: IV. The development of clinimetrics. Ann Intern Med 1983;99:843-848.
28. Feinstein A. "Clinical Judgment" revisited: the distraction of quantitative models. Ann Intern Med 1994;120:799-805.
29. Streiner D. Clinimetrics vs. psychometrics: an unnecessary distinction. J Clin Epidemiol 2003 Dec;56(12):1142-5; discussion 1146-9.
30. Marx RG, Bombardier C, Hogg-Johnson S, et al. Clinimetric and psychometric strategies for development of a health measurement scale. J Clin Epidemiol 1999;52:105-111.
31. Ribera A, Permanyer-Miralda G, Alonso J, et al. Is psychometric scoring of the McNew Quality of Life after Myocardial Infarction questionnaire superior to the clinimetric scoring? A comparison of the two approaches. Qual Life Res 2006;15:357-365.
32. Beaton D, Wright JG, Katz J, et al. Development of the QuickDASH: comparison of three item-reduction approaches. J Bone Joint Surg Am 2005;87:1038-1046.

Supplementary material

Supplementary table 1. Linear regression model for deriving the MINI-index

	B	P-value	B	P-value	B	P-value	B	P-value
Constant	4.2	0.00	4.0	0.00	-8.8	0.00	-9.5	0.00
Abdominal pain 5	2.0	0.14	1.9	0.19	1.5	0.23	1.7	0.17
Abdominal pain 10	0.8	0.69	0.7	0.70	1.0	0.57	1.0	0.54
Stool 5	4.2	0.00	4.3	0.00	3.2	0.01	3.0	0.02
Stool 10	9.9	0.00	9.9	0.00	7.7	0.00	7.6	0.00
Weight 5	3.0	0.03	2.6	0.07	2.4	0.05	2.9	0.03
Weight 10	6.4	0.04	6.2	0.04	5.3	0.05	5.5	0.04
Perianal disease 5	3.4	0.15	3.5	0.14	3.3	0.11	3.2	0.12
Perianal disease 10	2.7	0.42	3.0	0.37	3.0	0.31	2.6	0.37
EIM 2.5	-1.3	0.60	-1.3	0.60	-1.1	0.61	-1.1	0.62
HCT 2.5	2.0	0.17	1.9	0.19	0.8	0.53	0.8	0.53
HCT 5	5.3	0.18	5.0	0.21	3.4	0.33	3.7	0.29
Albumin 5	1.9	0.29	1.7	0.35	0.9	0.58	1.1	0.51
Albumin 10	-6.2	0.03	-6.1	0.03	-6.3	0.01	-6.3	0.01
ESR 2.5	1.8	0.16	1.2	0.39	0.2	0.88	0.8	0.51
ESR 5	5.2	0.02	4.1	0.10	3.0	0.13	4.2	0.05
Log CRP			0.4	0.36			-0.6	0.18
Log FC					2.5	0.00	2.7	0.00
Adjusted R^2	0.36		0.36		0.51		0.51	

SESCD score served as the dependent variable in all models. All items – except Log CRP and Log FC – are from the PCDAI and scored accordingly. B = unstandardized coefficient; EIM = extraintestinal manifestations; HCT = hematocrit; ESR = erythrocytes sedimentation rate; CRP = C-reactive protein; FC = fecal calprotectin.

Supplementary table 2. Derivation of the MINI-index in the second regression stage

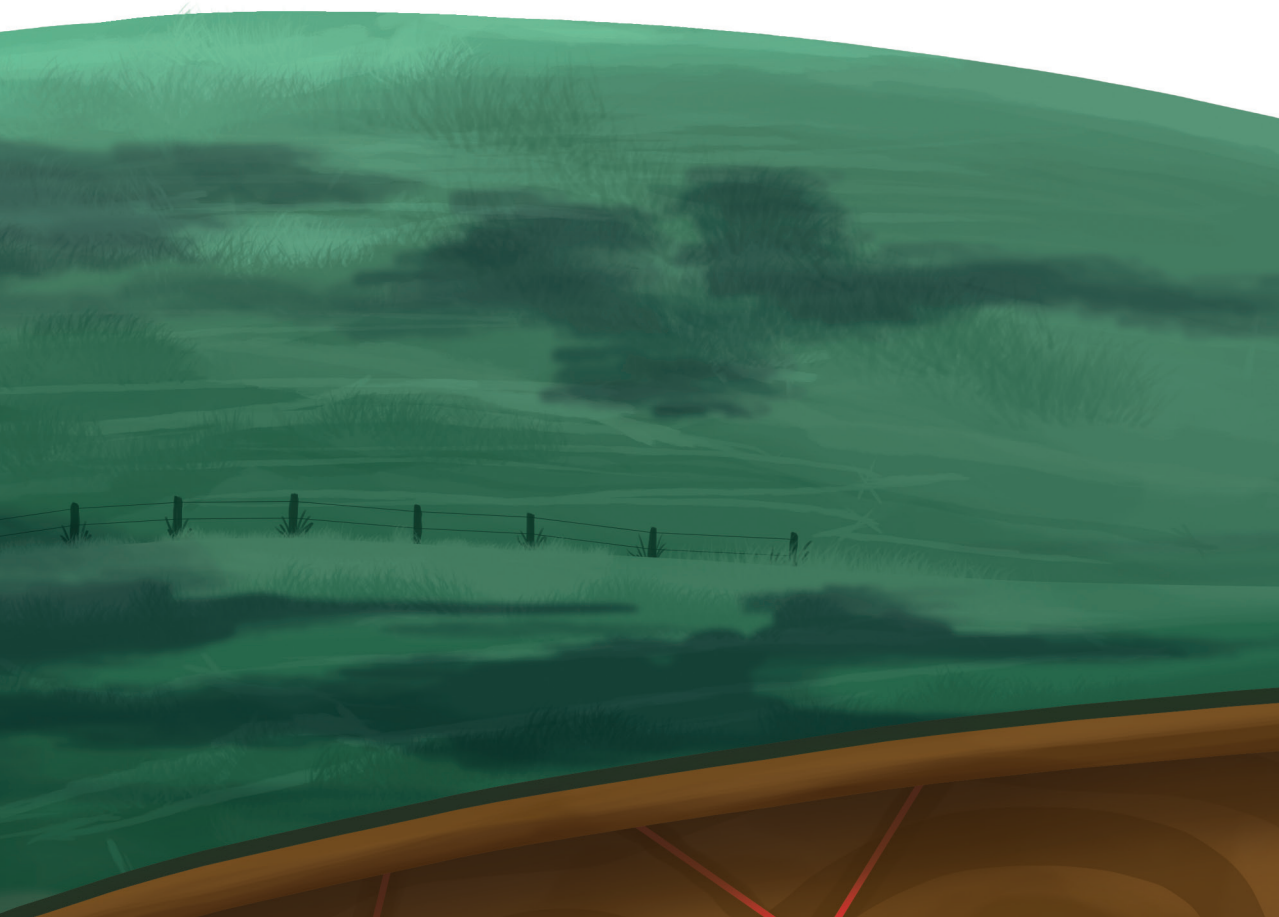
	B	P-value	B	P-value	B	P-value
Stool 5	3.9	0.00	4.7	0.00	4.7	0.00
Stool 10	7.3	0.00	9.3	0.00	8.9	0.00
Weight 5	2.4	0.05				
Weight 10	6.4	0.01				
Albumin < 3 g/dL			-2.2	0.38		
FC < 50	-0.3	0.90	-0.4	0.84	-0.3	0.86
FC 50-99.9	1.8	0.45	1.4	0.57	1.5	0.55
FC 100-299.9	4.5	0.01	4.4	0.09	4.5	0.01
FC 300-599.9	5.7	0.01	5.9	0.06	5.8	0.01
FC 600-899.9	8.6	0.00	8.7	0.00	8.6	0.00
FC ≥ 900	11.3	0.00	11.1	0.00	11.2	0.00
30 > ESR ≥ 10 Or 10 > CRP ≥ 5	0.2	0.92	0.7	0.69	0.6	0.70
50 > ESR ≥ 30 or 30 > CRP ≥ 10	0.1	0.95	0.9	0.65	0.8	0.66
ESR ≥ 50 or CRP ≥ 30	0.7	0.71	2.4	0.23	2.1	0.28
Adjusted R^2	0.79		0.78		0.78	

SESCD score served as the dependent variable in all models. The Stool and Weight items are based on the PCDAI and scored accordingly. B = unstandardized coefficient; FC = fecal calprotectin; ESR = erythrocytes sedimentation rate; CRP = C-reactive protein.

Supplementary table 3. Final weighting of the MINI-index compared with the raw regression coefficients (based on the derivation dataset, n = 154)

	B	P-value	MINI
Stool 5	4.7	0.00	4
Stool 10	8.9	0.00	8
FC < 50	-0.3	0.86	-3
50 ≤ FC < 100	1.5	0.55	0
100 ≤ FC < 300	4.5	0.01	5
300 ≤ FC < 600	5.8	0.01	7
600 ≤ FC < 900	8.6	0.00	9
FC ≤ 900	11.2	0.00	12
30 > ESR ≥ 10 Or 10 > CRP ≥ 5	0.6	0.70	1
50 > ESR ≥ 30 Or 30 > CRP ≥ 10	0.8	0.66	2
ESR ≥ 50 Or CRP ≥ 30	2.1	0.28	5
Adjusted R^2	0.78		

SESCD score served as the dependent variable in the model. The Stool items are based on the PCDAI and scored accordingly. B = unstandardized coefficient; FC = fecal calprotectin; ESR = erythrocytes sedimentation rate; CRP = C-reactive protein.



Chapter 3

Top-down Infliximab Study in Kids with Crohn's disease (TISKids): an international multicenter randomized controlled trial

Cozijnsen MA, van Pieterson M, Samsom JN, Escher JC, de Ridder L.

BMJ Open Gastroenterol. 2016 Dec 22;3(1):e000123

Abstract

Rationale: Crohn's disease (CD) is a chronic inflammatory disease predominantly affecting the gastrointestinal tract. CD usually requires lifelong medication and is accompanied by severe complications, such as fistulae and strictures resulting in surgery. Infliximab (IFX) is very effective for treating pediatric CD patients, but is currently only registered for therapy refractory patients – the so-called step-up strategy. We hypothesize that using IFX first-line, i.e. top-down, will give more mucosal healing, fewer relapses, less complications, need for surgery and hospitalization.

Objectives: Compare efficacy and safety of top-down IFX versus conventional step-up treatment in pediatric CD patients.

Methods and analysis: This international multicenter open-label randomized controlled trial includes children, aged 3-17 years, with new-onset, untreated, CD with moderate-to-severe disease activity (weighted Pediatric Crohn's Disease Activity Index (wPCDAI)>40). Eligible patients will be randomized to top-down or step-up treatment. Top-down will consist of 5 IFX infusions combined with azathioprine (AZA). After these 5 infusions, patients will continue AZA. Patients randomized to step-up will receive standard induction treatment, either oral prednisolone or exclusive enteral nutrition, combined with AZA as maintenance treatment. Primary outcome is clinical remission (wPCDAI<12.5) at 52 weeks without need for additional CD related therapy or surgery. Total follow-up is 5 years. Secondary outcomes include clinical disease activity, mucosal healing by endoscopy (at week 10 and optionally week 52), faecal calprotectin, growth, quality of life, medication use, and adverse events.

Ethics: Conducted according to the Declaration of Helsinki and Good Clinical Practice. Medical-ethical approval will be obtained for each site.

Trial registration number: NCT02517684

Background

Crohn's disease (CD) is a chronic inflammatory disease predominantly affecting the gastrointestinal tract. The disease pathogenesis is not fully known, but involves an aberrant immune response to the patients' intestinal microbiota. Because of the inflammation, patients may present with symptoms such as abdominal pain, diarrhea, fatigue and weight loss, and further investigation may reveal increased inflammatory products in the patients' blood and feces. The diagnosis is based on the patients' history, physical examination, endoscopic and radiologic imaging of the bowel as well as microscopic evaluation of mucosal biopsies.(1)

Approximately 4 per 100.000 children develop CD during childhood or adolescence.(2) Compared with adult onset CD, patients with childhood onset may present with more extensive and progressive disease, and generally require more intensive treatment.(3, 4) Pediatric CD treatment focusses on relieving symptoms, restoring longitudinal growth and pubertal development, and on suppressing the inflammatory immune response leading to macroscopically detectable repair of the mucosal surface, also known as mucosal healing.(5) Acquiring mucosal healing is important since it predicts a favorable disease outcome, and reduces the need for steroids, the risk of complications, of hospitalization and need for surgery.(6) Current pediatric CD guidelines instruct physicians to start treatment with exclusive enteral nutrition (EEN) or prednisolone to induce disease remission, and at the same time start with a thiopurine, such as azathioprine (AZA), or methotrexate (MTX) to maintain remission.(5) Only patients refractory to these treatments can step-up to anti-tumor necrosis factor (TNF) antibody therapy. However, this so-called step-up treatment strategy has disadvantages. Although prednisolone and EEN both induce clinical remission effectively (in approximately 80% of patients), prednisolone has considerable side-effects, and EEN necessitates a complete refrain from normal food for a long period of time which is unpleasant and hard to comply to.(5) Furthermore, prednisolone only rarely induces mucosal healing.(5, 7, 8) Once in clinical remission, 60-70% of patients maintain remission during the first year of AZA treatment.(5) One registry showed that 54% (55/102) of pediatric CD patients had received either an additional corticosteroid course or had started IFX within the first year after diagnosis.(9) Thus a large proportion of pediatric patients requires more intensive treatment in the first year after diagnosis. For these patients, the step-up strategy delays the initiation of effective treatment and increases the risk of CD progression and complications.

Since its introduction, infliximab (IFX) – the first anti-TNF antibody registered for CD – has shown to be very effective for treating refractory pediatric CD patients.(10) In the REACH trial – the pivotal IFX trial in pediatric CD patients refractory to azathioprine treatment – 88%

of patients responded to infliximab after 10 weeks of therapy, of whom the majority achieved and maintained remission on IFX throughout week 54. Subsequent research showed that IFX efficacy can be improved through individualized dose optimization to ensure therapeutic levels and by combination therapy with AZA or MTX to avoid immunogenicity.(11-14) Notably, IFX was also demonstrated to be more effective the sooner it is initiated after diagnosis. Three retrospective trials, assessing the efficacy of IFX, demonstrated that patients receiving IFX '*early*' after diagnose (either directly after diagnosis or less than 1 or 2 years afterwards) had longer remission duration and increased fistula closing rates than those receiving IFX '*late*'.(15-17) Postponing IFX could thus reduce its efficacy. IFX has also shown to induce mucosal healing in a large proportion of patients: In the ACCENT 1 trial in adult CD patients, 31% (10/32) of the patients receiving IFX maintenance treatment had mucosal healing (absence of ulcers) at week 10 and 50% (13/26) had mucosal healing at week 54 – a post-hoc analysis of week 2 IFX responders who had mucosal ulcerations at baseline.(18) Giving IFX early as part of the top-down strategy may thus optimize IFX efficacy and may offer a good chance for restoration of the gut's mucosa, which in turn can reduce risks of disease relapse, hospitalization and need for surgery.

Evidence on the efficacy of top-down treatment as compared to step-up treatment is however limited. Currently, two prospective trials compared both strategies in adult CD patients. In the first trial(19), 133 adult CD patients were randomized to start with either step-up treatment (steroids only) or top-down therapy (three IFX infusions and AZA maintenance therapy). Top-down therapy resulted in higher remission rates (week 26: 39/65 [60%] vs 23/64 [36%]; week 52: 40/65 [62%] vs 27/64 [42%]), and led more often to mucosal healing (absence of ulcers at week 104: 19/26 [73%] vs 7/23 [30%]). In the second trial(20), 77 patients were randomized to receive either 6 IFX infusions and AZA or prednisone and AZA. At week 30, top-down treatment resulted in higher remission rates (26/38 [68%] vs 17/39 [44%]) and mucosal healing rates (17/38 [45%] vs 7/39 [18%]).

There are no prospective randomized controlled trials in pediatric CD patients, only several retrospective, observational studies. The first retrospective study found that patients who – by either patient's or physician's choice – had started top-down treatment had lower relapse rates at 1 year than those who had started with step-up treatment (3/13 [23%] vs 8/13 [62%]).(21) A second cohort demonstrated that IFX is more effective in therapy naïve than refractory patients (relapse-free rates at 3 years: 36% vs 15% [survival curve, no absolute numbers]).(15, 22, 23) Results from a third retrospective cohort, using propensity scores analysis to correct for baseline differences, showed that early IFX monotherapy resulted in higher remission rates at 1 year than early immunomodulator monotherapy (thiopurine or methotrexate) (58/68 [85%] vs 152/248 [55%]).(24) The available literature thus suggests that starting IFX therapy early is more effective in pediatric CD patients, but this needs to

be confirmed in a prospective randomized trial. Also, the top-down strategy by definition aims at stopping IFX therapy and stepping down to immunomodulator monotherapy. This is to reduce risks associated with combination therapy(13), limit healthcare expenses while – hopefully – not compromising in efficacy. Whether this approach truly offers the best risk/cost/benefit balance still needs to be tested. This study therefore aims to compare the efficacy and safety of the top-down IFX treatment with conventional step-up treatment in pediatric CD patients with moderate-to-severe disease.

Methods

Trial design

We designed an international multicenter open-label randomized controlled trial (RCT) with two parallel treatment arms.(Figure 1) In addition, one of these arms (step-up) contains two initial treatment options to choose from (prednisolone and EEN). This decision is made by the treating physician together with the patient and/or parents. This allocation based on choice was chosen over randomized allocation, because of two reasons. Firstly, this choice mimics the current clinical practice of the step-up strategy and is therefore a better comparator. Secondly, a strong aversion to one of the step-up treatment may prevent patients from participating in this trial.

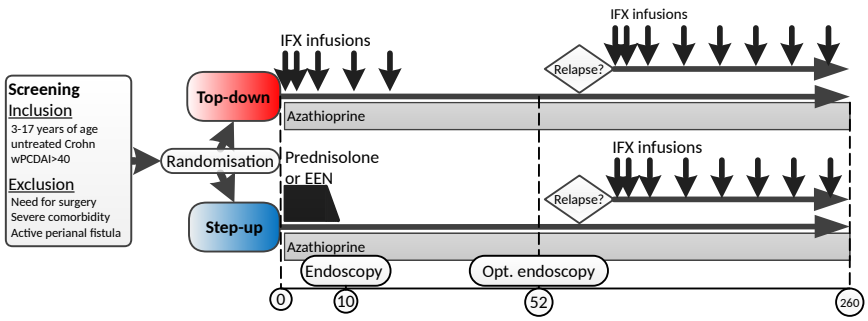


Figure 1. TISKids study design

IFX = infliximab; EEN = Exclusive Enteral Nutrition; wPCDAI = weighted Pediatric Crohn's Disease Activity Index (25)

Eligible patients willing to participate in this trial will be randomized with concealed group allocation, resulting in two comparable groups. Although a double-blind design is considered ideal for treatment comparison, an open-label design was chosen instead, because the former was not feasible due to the use of three treatments with different routes of administration – IFX is given intravenously, prednisolone are tablets and EEN is a liquid formula either ingested by mouth or by nasogastric tube – which makes using placebos very complex and costly. As a consequence of the open-label design, our results could potentially be influenced by performance bias and detection bias. However, since a double-blind design is not feasible, our open-label RCT is the optimal design for this research question.

Eligibility criteria and recruitment

Newly diagnosed CD patients, according to revised Porto criteria (1), are eligible if untreated, aged 3 up to and including 17 years, with bodyweight above 10 kg, presenting with moderate-to-severe disease activity (weighted Pediatric Crohn's Disease activity Index [wPCDAI] above 40).(25) Patients are excluded in case of a need for primary surgery, such as symptomatic bowel stenosis or stricture, active perianal fistulas, or if they have serious co-morbidity, such as a severe infection, sepsis, opportunistic infection, positive stool culture (*Salmonella enterica/Shigella* species*/Yersinia enterocolitica/Campylobacter* species), positive *Clostridium difficile* toxin assay, positive tuberculosis screening, or if they present with a suspected or definite pregnancy.

Patients suspected of CD and undergoing routine diagnostic work-up are potential candidates and screened for this trial when presenting to one of the participating sites. After a CD diagnosis is established and eligibility criteria are met, patients and/or parents/guardians are informed about the trial and asked to consider participation. After a waiting period of a minimum of 2 days, written consent is asked by the treating physician or researcher. Note that before the initial, diagnostic endoscopy and study consent, preliminary consent is sought for the collection of additional biopsies, which will be used for search for biomarkers predictive for treatment response – one of the additional study objectives.

Randomization, blinding and treatment allocation

Eligible patients are equally (1:1 ratio) randomized by a computer-generated list into two treatment groups, stratified by center. Randomization is incorporated in the web-based Case Record Form database used for this trial (Castor EDC).(26) Collaborators at each site have access to this database and can register and randomize their patients.

Treatment groups

Participants are randomized into two groups, either the experimental '*top-down*' group or the control group named '*step-up*', which is the current standard treatment strategy (5). The top-down group will receive five IFX infusions (Inflectra®, IFX induction at week 0, 2 and 6, followed by 2 maintenance infusions every 8 weeks, dosed at 5 mg/kg) combined with oral AZA as maintenance treatment (once daily, dosed 2-3 mg/kg). Step-up treatment consists of standard induction treatment with either oral prednisolone (1 mg/kg daily with a maximum of 40 mg for 4 weeks, followed by tapering down 5 mg per week until stop) or EEN (polymeric feeding for 6-8 weeks after which normal diet is gradually reintroduced within 2 to 3 weeks). (2) Similar to the top-down group, both prednisolone and EEN will be combined with oral AZA as maintenance treatment (2-3 mg/kg, once daily). AZA dosing may be altered based on TPMT genotype, but TPMT testing is not obligatory. Following its initiation, routine complete blood count are performed as part of routine clinical care (weekly in 1st month, monthly in 2nd and 3rd month, and thereafter once every three months) and AZA metabolites are measured about the

time of induction treatment cessation. In both groups, methotrexate may be given instead of AZA for instance in patients with low or absent TPMT activity. Screening for serum positivity to varicella zoster virus – as well as Epstein Barr virus and hepatitis B – is part of routine clinical care, and if vaccination is required and if time allows, treatment initiation will be postponed.

The top-down and step-up groups differ in the type of induction treatment that is started after diagnosis, but may switch to similar treatments during follow-up. Treating physicians are allowed to change treatment or increase dosing during follow-up when clinically indicated, for instance in case of drug inefficacy (non-response or loss of response) or intolerance. IFX may thus also be given to a step-up patient, but only as second-line treatment. Note that the step-up group may thus include patients stepping-up to IFX early after diagnosis, which, as explained in the introduction, was associated with better efficacy in retrospective trials than starting IFX late. Overall, this study thus compares two treatment strategies and not two different drugs, like in regular drug-trials.

Study endpoints

Comparative efficacy and safety

In total, patients will be followed for 5 years from randomization (Figure 1). The primary endpoint is clinical remission at week 52 (defined as a wPCDAI score<12.5) without need for additional CD-related therapy or surgery, i.e. additional to the treatment scheme described in the previous section.(Table 1)

Secondary endpoints include assessment off endoscopic disease activity, growth, quality of life and medication use. Endoscopic disease activity is an important outcome in this trial due to the expected difference between the two treatment strategies. To assess endoscopic disease activity, an endoscopic examination is scheduled at week 10, and another offered at week 52. The week 52 endoscopy is performed on a voluntary basis, as most patients may not benefit from this assessment while it does pose risk and discomfort. Endoscopic disease activity is also indirectly assessed via measuring the fecal marker calprotectin. To address longitudinal growth during follow-up, height and BMI Z-scores will be calculated at baseline and during follow-up for all patients with use of age and gender specific anthropometric reference values (preferably country specific, otherwise global reference values). Additionally, bone age will be measured with hand X-ray, and pubertal development will be assessed. Safety endpoints include the rate of adverse events and complications during follow-up.

Besides comparing top-down with step-up, we planned two sub-analyses. Firstly, we aim to compare both the efficacy and safety of the two step-up treatment options, and secondly, to assess the correlation between clinical and endoscopic disease activity measures.

Table 1. Study endpoints

	Time (weeks)
Primary endpoint	
Remission[1] without need for additional CD-related therapy or surgery	52
Secondary efficacy endpoints	
Remission[1] and response[2]	10, 52
Endoscopic disease activity (presence of ulcers, SES-CD)	10, optionally 52
Fecal calprotectin	10, 52
Height and BMI Z-scores, bone age and pubertal development	52
Quality of life (IMPACT-III)	14, 52
Cumulative therapy use and therapy failure	52
Secondary safety endpoints	
Adverse events and complications	52
Long-term endpoints	
Remission[1] without need for additional CD-related therapy or surgery	104, 156, 208, 260
Remission[1] and response[2]	104, 156, 208, 260
Fecal calprotectin	104, 156, 208, 260
Number of flares	104, 156, 208, 260
Quality of life (IMPACT-III)	260
Cumulative therapy use and therapy failure	260
Adverse events and complications	260
Sub analyses	
Comparing efficacy and safety of prednisolone plus AZA with EEN plus AZA	
Correlations between wPCDAI, fecal calprotectin and endoscopic disease severity (SES-CD)	
Additional objectives	
Comparing cost-effectiveness of top-down with step-up	
Identifying predictive biomarkers for treatment response	
Assessing the pharmacokinetic and pharmacodynamic properties of IFX in children	

[1]Remission = wPCDAI<12.5; [2]Response = wPCDAI decrease>17.5; wPCDAI = weighted Pediatric Crohn's Disease Activity Index (25); SES-CD = Simplified Endoscopic activity Score for Crohn's Disease (27); IMPACT-III (28, 29); IFX = infliximab; EEN = Exclusive Enteral Nutrition; AZA = azathioprine

Health-care costs, response prediction, and evaluation of the kinetic and dynamic properties of IFX

Three additional objectives are set. First additional objective is to compare the health-care related cost of both treatment strategies. This is an important outcome, because of the large difference in costs between biologic and non-biologic drugs. The recent introduction of an IFX biosimilar to the market has strongly reduced the costs of IFX therapy, while the costs of

top-down therapy may be further reduced compared to step-up by its hypothesized higher efficacy, which may reduce medication use, hospitalization and surgery.(6) We therefore hypothesize that after 5 years of follow-up healthcare related costs of top-down therapy will be comparable to those of step-up therapy.

We will also look for biologic markers that may predict treatment response. Additional biopsies and blood samples are collected from patients to measure RNA and protein expression, both before the start of treatment and during follow-up (additional biopsies are taken in pairs from affected and unaffected mucosal tissue in the ileum and colon with a maximum of 8 biopsies). This may help unravel the underlying mechanisms of treatment response of both strategies and preferentially lead to markers predictive of treatment response. The ability to predict treatment response prior to its initiation would allow for tailored treatment, aimed at maximal effect and safety hereby decreasing health-care cost.

Lastly, the pharmacokinetic and pharmacodynamic properties of IFX in children will be assessed during follow-up. Currently, only few controlled trials assessed these properties of IFX in children and by gathering more, high-quality data, we expect to further optimize IFX dosing in children. Based on clinical experience, we hypothesize that younger patients will obtain lower trough levels and lower drug efficacy with fixed IFX dosing of 5 mg/kg.

Sample size calculation

Our sample size calculation was based on week 52 remission ratios in three studies; two retrospective trials in pediatric CD patients and one prospective RCT among adult CD patients. The first retrospective trial compared top-down infliximab use with conventional step-up in pediatric CD and found a remission difference at week 52 of 38% (15/18 [83%] vs 5/11 [45%]).(23) The second trial compared early IFX use versus early immunomodulator use and found a remission difference of 24% (58/68 [85%] vs 152/248 [61%]).(24) The only prospective RCT, comparing top-down versus step-up in adult CD patients, reported a remission difference of 19% at week 52 (40/65 [61.5%] vs 27/64 [42.2%]).(19) Based on this data, we calculated to need 100 inclusions (50 patients in each arm, considering a drop-out rate of 2%) to find a 25% difference in clinical remission at week 52 with a power of 80% (2-sided α 0.05). A low drop-out rate was considered appropriate, because there are only few reasons for drop-out: only if the patient wishes to, or if after randomization the assigned treatment is not started.

Data collection and monitoring

Data are collected in Castor EDC (26), a web-based CRF database enabling the central study coordinators to follow and check the CRF input of each of the collaborating centers online. Additionally, a certified monitor will visit each site every year. A Data Safety Monitoring Board was not appointed, as the risks of adverse events associated with this study are considered low, because only approved therapies are used and treatment is not blinded.

Statistic methods

Subject baseline and demographic data as well as baseline disease characteristics data will be summarized by treatment group. Parametric variables will be described by their mean and standard deviation, and compared with use of the T-test, and non-parametric variables will be described by their median and interquartile range and compared using the Mann-Whitney U test. Categorical variables will be summarized using counts and percentages, and compared using the Chi-squared test, or the Cox proportional hazard test in case of time-dependent categorical variables. Correlations will be assessed using either the Pearson correlation coefficient (parametric) or the Spearman's rank correlation coefficient (non-parametric). Analyses will be performed on an intention-to-treat basis. All statistical testing will be 2-sided and significant at the 0.05 level. Missing data will be reported and left out of the analyses.

Discussion

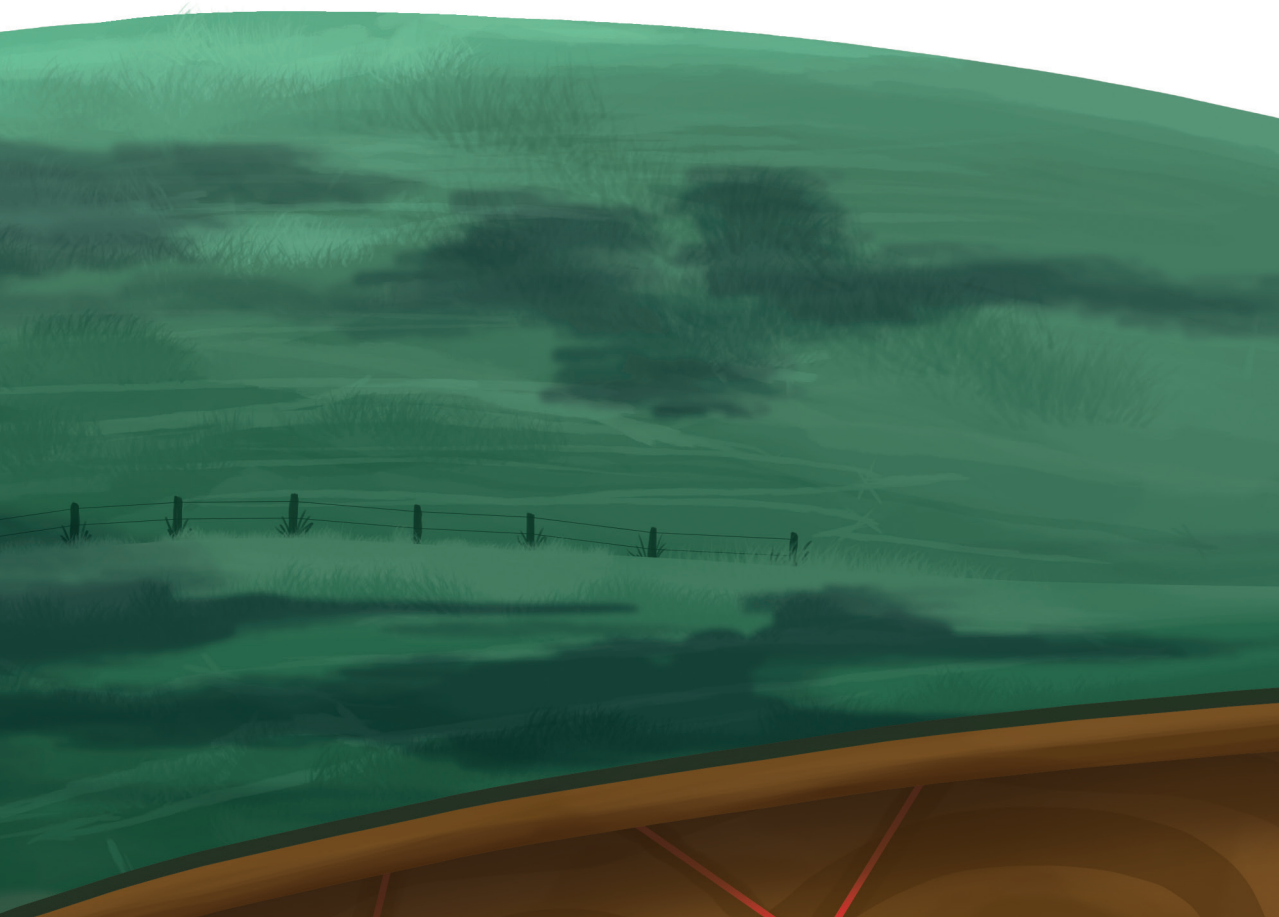
TISKids is a unique study specifically designed to compare two treatment strategies. Two comparable groups are generated through randomization so that each group only differs by the initial induction treatment started. During a 5 year follow-up period, the effects of these two strategies will be compared. Both major patient-related outcomes as well as other important healthcare related outcomes will be addressed aiming to obtain as much information as possible concerning the benefits, risks and costs of both strategies.

Over the recent years and because of increasing literature supporting early IFX use, IFX is being prescribed increasingly sooner after diagnosis. The guidelines for pediatric CD treatment were changed in their recommendations on this topic: They now advocate first-line IFX use for children with active perianal fistulizing disease and state that first-line IFX may also be considered for patients with high-risk of poor outcome.(5) However, the data supporting this recommendation is not conclusive. The benefits and risks of this new strategy are not well studied, nor compared with those of the conventional step-up strategy. Especially the comparative risks and costs of top-down treatment are not well known. This study will thus offer solid answers to these important and urgent clinical questions.

References

1. Levine A, Koletzko S, Turner D, Escher JC, Cucchiara S, de Ridder L, et al. ESPGHAN revised Porto criteria for the diagnosis of inflammatory bowel disease in children and adolescents. J Pediatr Gastroenterol Nutr. 2014;58(6):795-806.
2. Benchimol EI, Fortinsky KJ, Gozdyra P, Van den Heuvel M, Van Limbergen J, Griffiths AM. Epidemiology of pediatric inflammatory bowel disease: a systematic review of international trends. Inflamm Bowel Dis. 2011;17(1):423-39.
3. Van Limbergen J, Russell RK, Drummond HE, Aldhous MC, Round NK, Nimmo ER, et al. Definition of phenotypic characteristics of childhood-onset inflammatory bowel disease. Gastroenterology. 2008;135(4):1114-22.
4. Pigneur B, Seksik P, Viola S, Viala J, Beaugerie L, Girardet JP, et al. Natural history of Crohn's disease: comparison between childhood- and adult-onset disease. Inflamm Bowel Dis. 2010;16(6):953-61.
5. Ruemmele FM, Veres G, Kolho KL, Griffiths A, Levine A, Escher JC, et al. Consensus guidelines of ECCO/ESPGHAN on the medical management of pediatric Crohn's disease. J Crohns Colitis. 2014;8(10):1179-207.
6. Vatn MH. Mucosal healing: impact on the natural course or therapeutic strategies. Digestive diseases (Basel, Switzerland). 2009;27:470-5.
7. Lichtenstein GR, Feagan BG, Cohen RD, Salzberg BA, Diamond RH, Chen DM, et al. Serious infections and mortality in association with therapies for Crohn's disease: TREAT registry. Clin Gastroenterol Hepatol. 2006;4(5):621-30.
8. Lichtenstein GR. Approach to Steroid-Dependent and Steroid-Refractory Crohn's Disease. J Pediatr Gastroenterol Nutr. 2001;33:S27-S35.
9. Markowitz J, Hyams J, Mack D, Leleiko N, Evans J, Kugathasan S, et al. Corticosteroid therapy in the age of infliximab: acute and 1-year outcomes in newly diagnosed children with Crohn's disease. Clin Gastroenterol Hepatol. 2006;4(9):1124-9.
10. Hyams J, Crandall W, Kugathasan S, Griffiths A, Olson A, Johanns J, et al. Induction and maintenance infliximab therapy for the treatment of moderate-to-severe Crohn's disease in children. Gastroenterology. 2007;132(3):863-73.
11. Vande Casteele N, Ferrante M, Van Assche G, Ballet V, Compernolle G, Van Steen K, et al. Trough concentrations of infliximab guide dosing for patients with inflammatory bowel disease. Gastroenterology. 2015;148(7):1320-9 e3.
12. Steenholdt C, Brynskov J, Thomsen OO, Munck LK, Fallingborg J, Christensen LA, et al. Individualised therapy is more cost-effective than dose intensification in patients with Crohn's disease who lose response to anti-TNF treatment: a randomised, controlled trial. Gut. 2014;63(6):919-27.
13. Cozijnsen MA, Escher JC, Griffiths A, Turner D, de Ridder L. Benefits and risks of combining anti-tumor necrosis factor with immunomodulator therapy in pediatric inflammatory bowel disease. Inflamm Bowel Dis. 2015;21(4):951-61.
14. Church PC, Guan J, Walters TD, Frost K, Assa A, Muise AM, et al. Infliximab maintains durable response and facilitates catch-up growth in luminal pediatric Crohn's disease. Inflamm Bowel Dis. 2014;20(7):1177-86.
15. Cozijnsen MA, de Ridder L. Infliximab More Effective in Therapy-Naive Than in Therapy-Refractory Patients. J Pediatr Gastroenterol Nutr. 2015;61(3):e15.

16. Lionetti P, Bronzini F, Salvestrini C, Bascietto C, Canani RB, De Angelis GL, et al. Response to infliximab is related to disease duration in pediatric Crohn's disease. Aliment Pharmacol Ther. 2003;18(4):425-31.
17. Kugathasan S, Werlin SL, Martinez A, Rivera MT, Heikenen JB, Binion DG. Prolonged duration of response to infliximab in early but not late pediatric Crohn's disease. Am J Gastroenterol. 2000;95(11):3189-94.
18. Rutgeerts P, Diamond RH, Bala M, Olson A, Lichtenstein GR, Bao W, et al. Scheduled maintenance treatment with infliximab is superior to episodic treatment for the healing of mucosal ulceration associated with Crohn's disease. Gastrointest Endosc. 2006;63:433-42.
19. D'Haens G, Baert F, van Assche G, Caenepeel P, Vergauwe P, Tuynman H, et al. Early combined immunosuppression or conventional management in patients with newly diagnosed Crohn's disease: an open randomised trial. Lancet. 2008;371(9613):660-7.
20. Fan R, Zhong J, Wang ZT, Li SY, Zhou J, Tang YH. Evaluation of "top-down" treatment of early Crohn's disease by double balloon enteroscopy. World J Gastroenterol. 2014;20(39):14479-87.
21. Lee JS, Lee JH, Lee JH, Lee HJ, Kim MJ, Lee HJ, et al. Efficacy of early treatment with infliximab in pediatric Crohn's disease. World J Gastroenterol. 2010;16(14):1776-81.
22. Lee YM, Kang B, Lee Y, Kim MJ, Choe YH. Infliximab "Top-Down" Strategy is Superior to "Step-Up" in Maintaining Long-Term Remission in the Treatment of Pediatric Crohn Disease. J Pediatr Gastroenterol Nutr. 2015;60(6):737-43.
23. Kim MJ, Lee JS, Lee JH, Kim JY, Choe YH. Infliximab therapy in children with Crohn's disease: a one-year evaluation of efficacy comparing 'top-down' and 'step-up' strategies. Acta Paediatr. 2011;100(3):451-5.
24. Walters TD, Kim MO, Denson LA, Griffiths AM, Dubinsky M, Markowitz J, et al. Increased effectiveness of early therapy with anti-tumor necrosis factor-alpha vs an immunomodulator in children with Crohn's disease. Gastroenterology. 2014;146(2):383-91.
25. Turner D, Griffiths AM, Walters TD, Seah T, Markowitz J, Pfefferkorn M, et al. Mathematical weighting of the pediatric Crohn's disease activity index (PCDAI) and comparison with its other short versions. Inflamm Bowel Dis. 2012;18(1):55-62.
26. Castor Electronic Data Capture, Ciwit BV, Amsterdam, The Netherlands [Internet]. 2016.
27. Daperno M, D'Haens G, Van Assche G, Baert F, Bulois P, Maunoury V, et al. Development and validation of a new, simplified endoscopic activity score for Crohn's disease: the SES-CD. Gastrointest Endosc. 2004;60:505-12.
28. Otley A, Smith C, Nicholas D, Munk M, Avolio J, Sherman PM, et al. The IMPACT questionnaire: a valid measure of health-related quality of life in pediatric inflammatory bowel disease. J Pediatr Gastroenterol Nutr. 2002;35(4):557-63.
29. Loonen HJ, Grootenhuis MA, Last BF, de Haan RJ, Bouquet J, Derkx BH. Measuring quality of life in children with inflammatory bowel disease: the impact-II (NL). Qual Life Res. 2002;11(1):47-56.

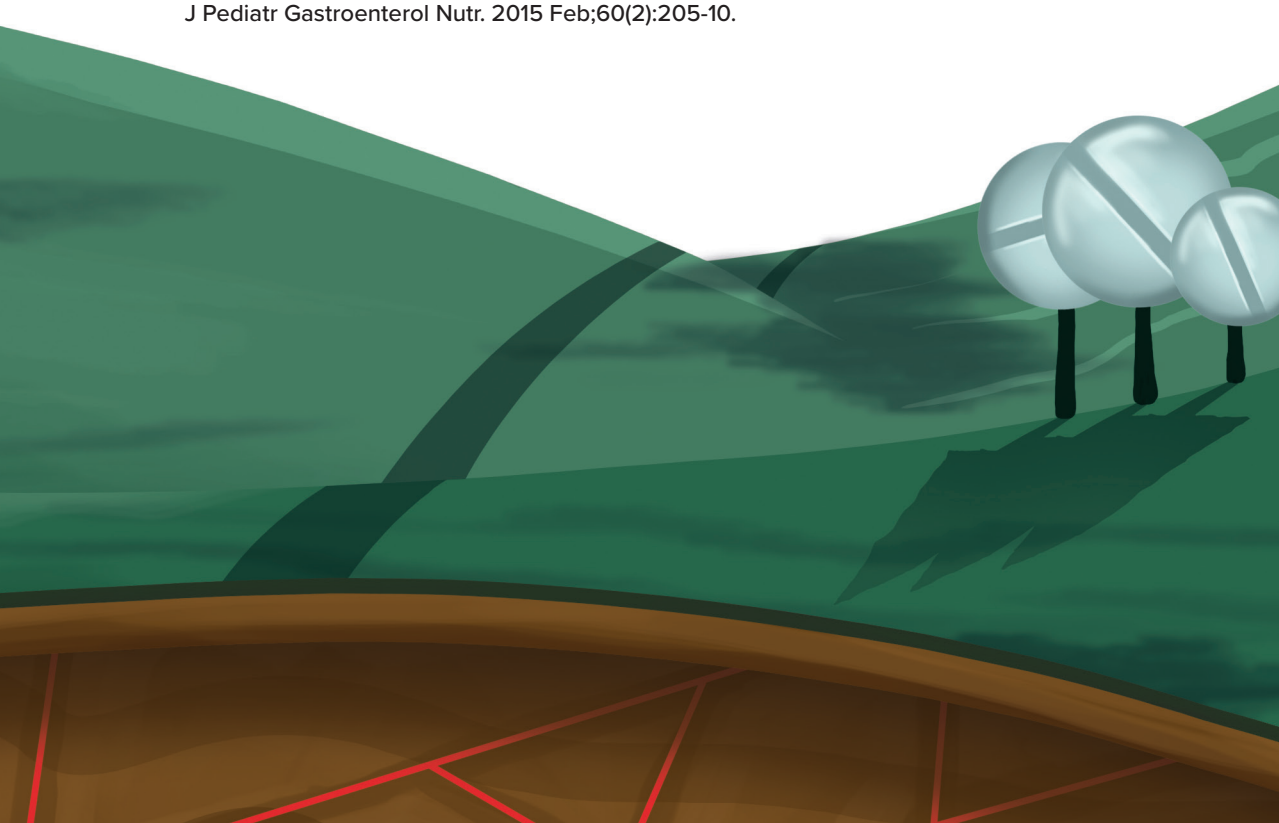


Chapter 5

Adalimumab therapy in children with Crohn's disease previously treated with infliximab

Martinus Cozijnsen, Vera Duif, Freddy Kokke, Angelika Kindermann, Patrick van Rheenen, Tim de Meij, Maaike Schaart, Gerard Damen, Obbe Norbruis, Rolf Pelleboer, Anita Van den Neucker, Herbert van Wering, Thalia Hummel, Johanna Oudshoorn, Johanna Escher, and Lissy de Ridder, on Behalf of the Dutch PIBD Working Group Kids with Crohn and Colitis

J Pediatr Gastroenterol Nutr. 2015 Feb;60(2):205-10.



Abstract

Objective Adalimumab, a humanized anti-tumor necrosis factor antibody, is an effective treatment in adult patients with refractory Crohn's disease (CD). The available literature on its efficacy in children remains limited. We aimed to evaluated its real-world efficacy in pediatric CD patients and to compare its efficacy between infliximab non-responders and patients who lost response to infliximab.

Methods All Dutch CD patients receiving adalimumab before the age of 18 after previous infliximab therapy, were identified. We analyzed longitudinal disease activity, assessed by the mathematically weighted pediatric Crohn's disease activity index (wPCDAI) or the Physician Global Assessment (PGA), and adverse events.

Results Fifty-three CD patients were included. Twelve patients received monotherapy and the others received combination treatment with thiopurines (n=21), methotrexate (n=11), steroids (n=7) or exclusive enteral nutrition (n=2). Median follow-up was 12 months (IQR 5-23). Remission was reached in 34 patients (64%, wPCDAI<12.5 or PGA=0) after a median of 3.3 months, and maintained by 50% for 2 years. Eleven patients (21%) reached response but not remission (decrease in wPCDAI≥17.5 or decrease in PGA). Eighteen patients (34%) failed adalimumab treatment because of non-response (n=4), lost response (n=11) or adverse events (n=3). More infliximab non-responders failed adalimumab treatment than patients who lost response to infliximab (2/3 vs 8/34, HR 18.8, CI 1.1-303.6). Only one patient encountered a serious adverse event, a severe but nonfatal infection.

Conclusions In clinical practice, adalimumab induces remission in two-thirds of children with infliximab refractory CD.

Introduction

Crohn's disease (CD) is a chronic inflammatory bowel disease (IBD) that presents before the age of 20 in 25-30% of its patients.(1) In the Netherlands, the reported incidence of CD in children and adolescents under the age of 18 is 2.1 per 100,000.(2) CD manifests more severely in childhood and adolescence than in adulthood, with growth retardation and delayed puberty, worse malnutrition and a higher risk of complications such as strictures, abscesses or fistulas.(1, 3)

For pediatric CD patients, first-line treatment consists of exclusive enteral nutrition or corticosteroids to induce remission and thiopurines or methotrexate to maintain it. If this first-line of treatment fails, anti-tumor necrosis factor (TNF) antibody therapy is often indicated.(4)

The first anti-TNF antibody that was studied in and registered for CD patients was infliximab, a chimeric (mouse/human) monoclonal antibody. Infliximab both induces and maintains remission in CD patients.(5, 6) Unfortunately, one-third of these patients withdraw from infliximab therapy within three years, and half withdraw within five years.(6, 7) Some withdraw because they never respond to the drug, but most because they lose response.

One known reason for loss of response to infliximab is development of antibodies-to-infliximab (ATI) that neutralize the drug.(8, 9) To reduce such an antibody reaction, adalimumab, a fully humanized anti-TNF monoclonal antibody, was developed and tested for CD treatment.(10, 11) Its efficacy and safety in pediatric patients has been studied in one large prospective study(12) and two retrospective studies(13, 14). The positive results from the prospective study have led to registration of adalimumab for pediatric usage.

In this Dutch nationwide study, we aimed to evaluate the real-world efficacy of adalimumab in pediatric CD patients who previously failed treatment with infliximab. We included both patients who had no response to infliximab as well as those who lost response, and compared adalimumab efficacy between these two groups.

Methods

We performed a nationwide, observational cohort study and invited all Dutch pediatric gastroenterologists that prescribe biologicals to pediatric IBD patients, to participate. Those who agreed were asked to identify all eligible patients. Inclusion criteria were: (1) diagnosed with CD; (2) treated with adalimumab before the age of 18; and (3) treated with infliximab before the start of adalimumab. Exclusion criteria were: (1) conflicting comorbidity (such as auto-immune diseases or other chronic intestinal pathology); (2) recorded bad treatment adherence; and (3) previous participation in the prospective study by Hyams et al.(12)

We collected the following information from patient records: patient characteristics, disease localization and behavior, previous treatment history, longitudinal disease activity from the start of adalimumab treatment and adverse events.

Adalimumab efficacy assessment

In order to assess the efficacy of adalimumab therapy, disease activity was retrospectively assessed by the first or second author using the mathematically weighted pediatric Crohn's disease activity index (wPCDAI)(15) or a 4-stepped Physician Global Assessment (PGA) when the wPCDAI could not be calculated. Disease activity was categorized into 4 categories: remission, mild, moderate, and severe.

Since timing of follow-up visits was unstructured, follow-up visits were sought closest to and pooled at the following time-points: 1 month, 4 months, 8 months and 12 months after the start of treatment and then yearly depending on availability.

Based on the development in disease activity, the effect of adalimumab at each time point was categorized as either clinical response, clinical remission, no response or loss of response. Clinical response was defined as a decrease in the wPCDAI by at least 17.5 points, or a decrease in PGA from either moderate or severe to mild. A wPCDAI below 12.5 or a PGA equaling zero was defined as remission. Loss of response was defined as an increased disease activity to moderate or severe after having reached response or remission.

If adalimumab treatment was discontinued, we documented the reason for discontinuation. In the case of lack of efficacy (non-response, loss of response) or intolerance (allergic reaction or adverse events), we considered these patients as having failed adalimumab. Secondly, we considered patients requiring CD related surgery during follow-up as having failed adalimumab due to loss of efficacy. Follow-up of

these patients stopped when surgery was performed, even when adalimumab therapy continued after. Patients requiring perianal surgery or surgery for reasons other than disease progression were not categorized as having failed adalimumab and follow-up in these patients therefore continued.

Statistical analysis

Data collection and analysis were performed using SPSS version 21. Continuous nonparametric data are presented as median (IQR), parametric data was absent. Categorical data are presented by the number of cases and the proportion of cases (%). Kaplan-Meier analyses and Cox proportional hazard models were used to analyze and visualize adalimumab failure in relation to time. The Fisher exact test was used to compare categorical data between groups. We used 0.05 as the cut-off point for statistical significance.

Ethical considerations

This study was exempted from Institutional Review Board approval as it involved the collection of data generated by routine medical care. The data were collected and recorded by the investigators in such a manner that subjects could not be identified, directly or through identifiers linked to the subjects.

Results

In total 15 Dutch centers participated, i.e. 10 centers of the Dutch pediatric IBD working group (KiCC) and 5 additional centers. Between 2005 and 2013, 59 pediatric CD patients had switched from infliximab to adalimumab of whom 53 met the inclusion and exclusion criteria. Twelve of the 15 centers kept record of the number of CD patients that received IFX during this period: 351 received infliximab of whom 55 switched to adalimumab (median switching rate 13.3% (IQR 9.8-18.1)). The patients characteristics are displayed in Table 1.

Table 1. Patient characteristics

		n(%) or median (IQR)
Total included patients		53
Gender	Female	27 (50.9%)
Age	At diagnosis	11 (8-13)
	At adalimumab commencement	14 (13-16)
Disease location*	Ileocecal (L1)	6 (11.3%)
	Colonic (L2)	11 (20.8%)
	Both ileal and colonic (L3)	36 (67.9%)
Disease behaviour*	Not stricturing nor penetrating (B1)	42 (79.2%)
	Stricturing (B2)	8 (15.1%)
	Penetrating (B3)	1 (1.9%)
	Both penetrating and stricturing (B4)	2 (3.8%)
	History of perianal disease	19 (35.8%)
Growth delay at diagnosis*		20 (37.7%)
Prior treatment	Exclusive enteral nutrition	32 (60.4%)
	Steroids	50 (94.3%)
	Mesalazine	27 (50.9%)
	Purine antimetabolites	52 (98.1%)
	Methotrexate	26 (49.1%)
Infliximab treatment duration (months)		15.7 (10.8-25.8)
Type of infliximab failure	Lost response	34 (64.2%)
	Allergic reaction	11 (20.8%)
	Side effects	5 (9.4%)
	Non-response	3 (5.7%)
ATI presence	Tested	38 (71.7%)
	Positive (>15AE/ml)	21 (55.3%)
History of CD related surgery		12 (22.6%)

*ATI=antibodies to infliximab. *As defined by the Paris Classification(20)*

Previous treatment

Thirty-two patients (60%) had received at least 1 course of exclusive enteral nutrition, 50 (94%) had been treated with steroids, 27 (51%) with mesalazine, 52 (98%) with purine antimetabolites (azathioprine and/or mercaptopurine) and 26 (49%) with methotrexate. The median duration of infliximab treatment was 15.6 months (IQR 10.8-25.8). Reasons to stop with infliximab were non-response (n=3, 6%), loss of response (n=34, 64%), allergic reactions (n=11, 21%) or side effects (n=5, 9%).

In a large subgroup (n= 38, 72%) antibodies to infliximab (ATI) had been tested prior to adalimumab commencement. Twenty-one of those had tested positive (ATI concentration > 15 AE/ml), 16 of whom (76%) had lost response to infliximab, 3 (14%) had developed an allergic reaction and 2 (10%) had suffered side effects. The other 17 patients had tested negative for ATI (2 with non-response, 11 who lost response, 3 with allergic reactions and 1 with side effects).

Twelve patients (23%) had undergone partial or total bowel resection prior to adalimumab commencement, at a median age of 14 years (IQR 11-14), a median of 21 months before the start of adalimumab therapy (IQR 11-33).

Adalimumab treatment

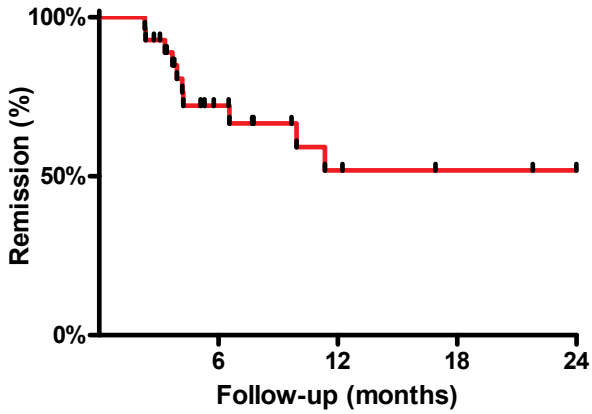
Adalimumab induction regimens differed. Thirty-nine patients (74%) started with a double dosage prior to maintenance treatment, the remainder received the maintenance dosage straight from the start. Initial maintenance dose were based on body weight (20-40 mg for patients less than 40 kg, 40-80 mg for patients above 40 kg). Treatment escalation was needed in 13 patients (25%), performed by either increasing the dose, shortening the dose interval, or both.

At the start of adalimumab 41 patients (77%) were using concomitant CD related medication, including immunomodulators (thiopurines (n=21) or methotrexate (n=11)), steroids (n=7) or exclusive enteral nutrition (n=2).

Response to adalimumab

We followed the children and teenagers in our cohort for a total of 66 patient-years, i.e. for a median of 12 months per patient (IQR 5-23). In 95% of the follow-up visits disease activity was assessed by either the wPCDAI (45%) or the PGA (50%). For the remainder this was not possible. The timing of the follow-up visits differed by a median of 14% (IQR 5-27) from the presented time-points.

At adalimumab commencement, 3 patients were in clinical remission. One patient had switched to adalimumab treatment because of infliximab-related vasculitis, the second had recently undergone an ileocecal resection, and in the third luminal activity was seen with high levels of ATI, despite mild symptoms and normal markers of inflammation. During follow-up remission was reached in 34 patients (64%) after a median of 3.3 months (IQR 1.7-8.3). Ten of those subsequently lost remission after a median of 4.7 months (IQR 2.7-10.3). Survival analysis demonstrates a 50% maintained remission rate at 24 months (Figure 1). Eleven out of the remaining 19 patients did not reach remission but did reach clinical response (Table 2).



Months	0	6	12	18	24
Patients at risk	34	14	7	5	4

Figure 1. Duration of remission

Cumulative hazard curve displaying the duration of remission, i.e. the time from induction of remission untill a first relapse, for those patients reaching remission during follow-up. After 6 months 70% of these patients maintained remission and 50% after 24 months.

Adalimumab failure

During the observation period 18 patients (34%) failed adalimumab therapy, eleven of whom discontinued therapy and seven failed because CD related surgery was performed. None of the patients discontinued therapy because of remission. Six patients had first reached remission but lost response (n=4) or suffered adverse effects (n=2), another 6 patients had reached clinical response but lost response (n=6), and the remaining 6 patients did not respond to adalimumab and failed due to non-response (n=4), adverse effects (n=1) or lost response (n=1) – the latter patient had mild disease activity at baseline, infliximab was stopped because of an allergic reaction, and mild activity was retained with adalimumab for 12 months until a relapse occurred.

Table 2. Response to adalimumab

Follow-up (months)	0	1	4	8	12	24	36	48
Remission	3 (6)	11 (21)	18 (38)	20(57)	16 (53)	9 (47)	4 (67)	1 (100)
Response but no remission	-	20 (38)	8 (17)	6(17)	4 (13)	4 (21)	1 (17)	-
No response	-	17 (32)	7 (15)	3 (9)	2 (7)	1 (5)	-	-
Loss of response	-	-	7 (15)	6(17)	8 (27)	5 (26)	-	-
Missing evaluation	-	5 (9)	7 (15)	-	-	-	1 (17)	-
Number of patients on adalimumab treatment	**53**	**53**	**47**	**35**	**30**	**19**	**6**	**1**
No more follow-up	-	0	0	7	11	20	29	34
Adalimumab failure	-	0	6	11	12	14	18	18

Table displaying the response to adalimumab during follow-up, The results are presented as number (%=N/total of patients on continued therapy).

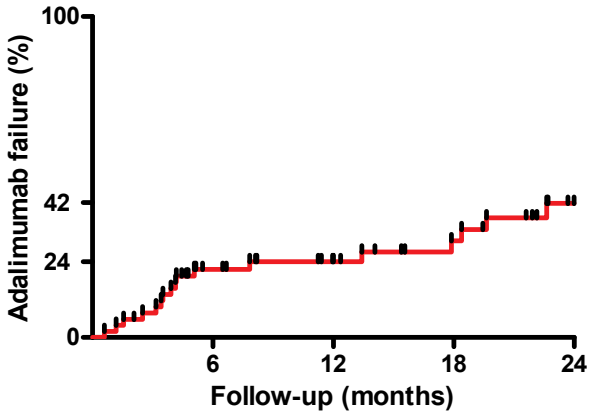
Adalimumab treatment failed or discontinued within a median of 5.3 months (IQR 2.9-18.0), the 4 non-responders discontinued within a median of 3.2 months (IQR 1.4-4.0), the 11 patients who lost response failed within a median of 7.5 months (IQR 3.5-18.4), the 3 patients with adverse effects within a median of 16 months (1.5, 16.1 and 19.6). Adalimumab failure over time is displayed using a cumulative hazard curve in Figure 2: 24% failed within 12 months and 42% within 24 months.

Twelve patients were evaluated for ATA formation at least once during follow-up. In 4 patients ATA were present, 3 patients suffered loss of response and the last suffered adverse effects (fatigue after injections, hair loss, pain and redness at the injection site).

Sub analyses

Adalimumab was less effective in patients that had not responded to infliximab than in those who had lost response to infliximab. Only one of the three patients with non-response to infliximab reached remission during follow-up versus 24 out of 34 patients who had lost response to infliximab (1/3 (33%) vs 24/34 (71%), P=0.24). Furthermore, two of the former patients developed adalimumab failure (non-response and loss of response) compared to 8 of the latter patients (2/3 (67%) vs 8/34 (24%), P=0.17; HR 18.8, CI 1.1-304) (Figure 3).

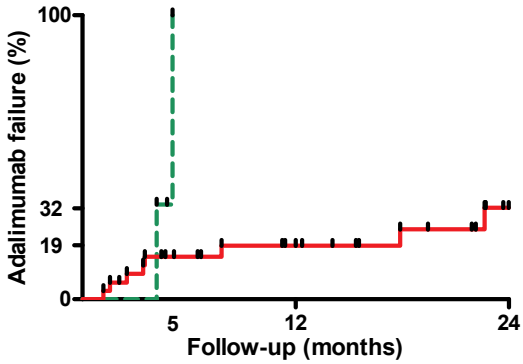
ATI presence had been studied in 38 of 53 patient (72%) and found present in 21. We detected a trend towards a higher remission rate in patients with ATI than in those without ATI at the time of infliximab failure (17/21 (81%) vs 9/17 (53%), P=0.09) and a trend towards a lower failure rate (4/21 (19%) vs 7/17 (41%), P=0.13; HR 0.37, CI 0.11-1.23) (Figure 4). Furthermore, none of the former patients suffered non response to adalimumab compared to 4 of the latter patients (24%). At 12 and 24 months, 14% and 22% of the former patients had failed adalimumab versus 40% and 55% of the latter patients.



Months	0	6	12	18	24
Patients at risk	53	35	27	20	11

Figure 2. Adalimumab failure over time

Cumulative longitudinal adalimumab failure: Within 12 months 24% of the patients failed adalimumab therapy, rising to 42% within 24 months.



Patients at risk	months	0	5	12	24
IFX non-responders		3	0	0	0
IFX loss of responders		34	27	19	6
Hazard ratio		18.8 (1.1-303.6)			

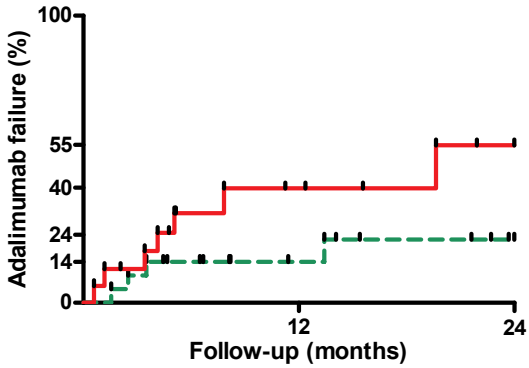
Figure 3. Comparing the risk of adalimumab failure in non-responders vs. patients who lost response to infliximab

Kaplan-Meier analysis displaying adalimumab failure over time separately for patients that had not responded to or had lost response to infliximab. Within 4.7 months, two out of three non-responders failed adalimumab therapy, for the other patient no more follow-up was available. Patients with non-response to infliximab had higher risk for adalimumab failure than patients who had lost response (HR 18.8, CI 1.1-303.6).

No differences in remission or failure rates were found between patients who did and did not receive induction treatment (remission: 26/39 (67%) vs 8/14 (57%), P=0.54; failure: 12/39 (31%) vs 6/14 (43%), P=0.31; HR 0.5, CI 0.16-1.57), nor between patients who did and did not receive concomitant immunomodulators at baseline (remission: 22/32 (69%) vs 12/21 (57%), P=0.28; failure: 9/32 (28%) vs 9/21 (43%), P=0.21; HR 0.7, CI 0.28-1.95).

Adverse events

In 21 patients (40%), a total 37 adverse events were recorded, being related to adalimumab by the treating physician, of which 14 (38%) where infections (Table 3). Only one serious adverse event occurred, a sepsis and meningitis secondary to a sinusitis (1 SAE per 66 patient years(PY), 1.5 SAE/100PY). In three patients adalimumab therapy was stopped due to adverse effects, in one patient because of fatigue, hair loss, and pain and redness at the injection site, in the second because of discomfort after injections and recurring upper respiratory tract infections, and vasculitis associated skin manifestation in the third.



Patients at risk	months	0	12	24
Prior ATI formation		21	11	5
No prior ATI formation		17	6	2
Hazard ratio		0.37 (0.11-1.23)		

Figure 4. Comparing the risk of adalimumab failure between those with vs. without prior ATI formation

ATI=antibodies to infliximab. Kaplan-Meier analysis displaying adalimumab failure over time in relation to prior ATI status. After 12 and 24 months, 14% and 22% of the patients with prior ATI formation failed adalimumab versus 40% and 55% of the patients without ATI formation at the time of infliximab failure (HR 0.37, CI 0.11-1.23).

Table 3. Adverse events

			Events
Severe infections	Sinusitis/sepsis/meningitis		1
Mild infections	Recurring conjunctivitis		3
	Upper respiratory tract infection		3
	Candida	Oral	2
		Peristomal	1
	Vasculitis		1
	Otitis media		1
	Herpes zoster		1
	Vaginal infection		1
Other	Injections painful		5
	Injection site irritation		4
	Eczema or other skin disorders		3
	Discomfort after injection		2
	Hair loss		2
	Arthralgia		2
	Tiredness		2
	Headache		1
	Dizziness		1
	Myalgia		1
Total number of adverse events			37
Number of inflicted of patients			21 (40%)

In total 37 adverse events were reported in 21 patients (40%), of which 38% where infections. Only 1 severe adverse event was reported, a severe infection.

Discussion

In this nationwide, observational study, adalimumab therapy induced remission in two thirds of the infliximab refractory patients, of whom 50% maintained remission up to 2 years. Adalimumab failure occurred in 24% within 1 year and in 42% within 2 years. Only 1 serious adverse event occurred.

The remission rates correspond well with those in previously published retrospective studies by Russell et al and Rosh et al(13, 14), but less with those in the prospective study by Hyams et al(12). The latter authors reported lower remission rates during follow-up than the authors of the retrospective studies did. This discrepancy might be the result of a difference in denominator, as Hyams et al divided the number of patient in remission by the number of included patients, whereas the retrospective studies, including the current study, divided it by the number of patients still receiving therapy. Combining the retrospective data, adalimumab induced remission in 61 to 64% of the infliximab refractory patients within a median of 2.4 to 3.3 months, and after 12 months of treatment 41 to 53% of the patients still receiving adalimumab, were in remission.

Secondly, the adalimumab failure rate in our cohort corresponds well with that in the retrospective studies, and also with that in the prospective study. Overall, adalimumab failure occurred in 15 to 28% of the infliximab refractory patients within the first year and in 20 to 42% within 2 years.

Lastly, the incidence of serious adverse events (SAE) within our cohort (1 SAE within 66 PY, 1.5 SAE/100PY) was lower than that within previously published studies. Within the study of Russell et al four SAE were reported within 72.5 PY (5.5 SAE/100PY) and in the prospective study 63 SAE developed within 152 PY (41 SAE/100PY). The higher incidence in the prospective study, may be the result of the use of more extensive criteria for SAE and more stringent monitoring.

Efficacy in infliximab non-responders vs loss of responders

Within our cohort, we have demonstrated that a small group of patients who had not responded to infliximab (n=3), had higher risk for adalimumab failure than those who had lost response to infliximab. Some patients do not respond well to anti-TNF therapy, while other patients do, which may be the result of a difference in underlying disease mechanism. Different disease mechanisms may require different treatment strategies.

This issue has not been studied previously in pediatric CD patients, only in adult IBD patients. Ho et al studied CD patients and found a trend for lower remission rates and higher failure rates in infliximab non-responders compared to initial responders (percentages and significance not clarified).(16) Garcia-Bosch et al studied ulcerative colitis (UC) patients and found a difference in response rates after 12 weeks of treatment between infliximab prior non responders (2/6, 33%) and initial responders (25/33, 76%, p=0.01).(17) Both studies affirm the difference in efficacy found in our study.

Efficacy in patients with vs without ATI

We found a trend for higher remission rates and lower failure rates in patients with ATI than in those without. A possible explanation for this efficacy difference is this: Patients initially responding to the first anti-TNF agent that are confronted with loss of response resulting from anti-drug antibodies, evade the causative factor for loss of response and regain response by switching to another anti-TNF agent. On the contrary, patients without anti-drug antibodies at the time of anti-TNF treatment failure, fail for another yet unknown reason, which makes regaining response more challenging. Failure due to ATI therefore seems more favorable, since a response can be more easily regained.

This issue has not been studied previously in pediatric CD patients, only in adult IBD patients. In the GAIN trial no difference was seen in remission rates at 4 weeks between CD patients with and without ATI prior to adalimumab (11/50 (22%) vs 19/88 (22%)).(11) West et al reported no relationship between the presence of ATI and response to adalimumab in CD patients, but serum ATI levels were higher in adalimumab non responders than in responders, suggesting a negative influence of high ATI concentrations on adalimumab response.(18) Afif et al found a trend for increased response in ATI positive UC patients versus ATI negative patients (5/8 (63%) vs 1/4 (20%), P≈0.5).(19)

Strengths and limitations

This study is the first nationwide study in pediatric CD patients evaluating adalimumab therapy in clinical practice and is strengthened by its population based design. Because of its observational character it reflects daily practice and its nationwide approach provides the inclusion of a broad spectrum of patients. However, the retrospective design has several limitations.

First, the presented data might not be complete. Although participating centers were asked to identify all eligible patients, some may have been missed. When selectively more or less severely diseased patients were missed, the reported efficacy might differ from the efficacy in the total population. Some non-serious adverse events may also have been missed, such as minor infections treated in primary care practice.

Secondly, the efficacy assessment was suboptimal. We used clinical disease activity indices and not mucosal healing or markers for mucosal healing to evaluate adalimumab efficacy. Furthermore, the wPCDAI could not be calculated at each time point and a PGA was used in the remainder.

Lastly, not all patients were treated in the same way. Patients received different adalimumab induction dosages and different concurrent medication. Decisions concerning their treatment were based on the judgement of the treating physician, as were decisions concerning treatment cessation. Variations in confounders may have biased our outcome data as well as the sub analyses.

Conclusions and generalizability

This study demonstrates that in clinical practice adalimumab is an effective therapy for infliximab refractory pediatric CD patients with only limited side effects. We therefore recommend its usage in these otherwise difficult to treat patients. Well powered, long-term pharmacovigilance studies are needed to further establish the safety of adalimumab therapy in pediatric CD patients, especially regarding late onset adverse events, such as malignancies.

Adalimumab appears to be less effective in the treatment of infliximab non-responders and patients previously failing infliximab without the presence of ATI. To further establish these efficacy differences, they should be confirmed in larger cohorts. Because of its clinical relevance, research should attempt to elucidate possible differences in disease mechanism, so that disease mechanism specific therapy can be given, and costly, ineffective therapy can be avoided.

References

1 Kelsen J, Baldassano RN Inflammatory bowel disease: the difference between children and adults. Inflamm Bowel Dis 2008;14 (Suppl 2):S9-11.

2 van der Zaag-Loonen HJ, Casparie M, Taminiau JA, et al. The incidence of pediatric inflammatory bowel disease in the Netherlands: 1999-2001. J Pediatr Gastroenterol Nutr 2004;38(3):302-07.

3 Vasseur F, Gower-Rousseau C, Vernier-Massouille G, et al. Nutritional status and growth in pediatric Crohn's disease: a population-based study. Am J Gastroenterol 2010;105(8):1893-900.

4 Van Assche G, Dignass A, Reinisch W, et al. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Special situations. J Crohns Colitis 2010;4(1):63-101.

5 Hyams J, Crandall W, Kugathasan S, et al. Induction and maintenance infliximab therapy for the treatment of moderate-to-severe Crohn's disease in children. Gastroenterology 2007;132(3):863-73.

6 De Bie CI, Hummel TZ, Kindermann A, et al. The duration of effect of infliximab maintenance treatment in paediatric Crohn's disease is limited. Aliment Pharmacol Ther 2011;33(2):243-50.

7 Hyams JS, Lerer T, Griffiths A, et al. Long-term outcome of maintenance infliximab therapy in children with Crohn's disease. Inflamm Bowel Dis 2009;15(6):816-22.

8 Rutgeerts P, Van Assche G, Vermeire S Review article: Infliximab therapy for inflammatory bowel disease--seven years on. Aliment Pharmacol Ther 2006;23(4):451-63.

9 Cassinotti A, Travis S Incidence and clinical significance of immunogenicity to infliximab in Crohn's disease: a critical systematic review. Inflamm Bowel Dis 2009;15(8):1264-75.

10 Colombel JF, Sandborn WJ, Rutgeerts P, et al. Adalimumab for maintenance of clinical response and remission in patients with Crohn's disease: the CHARM trial. Gastroenterology 2007;132(1):52-65.

11 Sandborn WJ, Rutgeerts P, Enns R, et al. Adalimumab induction therapy for Crohn disease previously treated with infliximab: a randomised trial. Ann Intern Med 2007;146(12):829-38.

12 Hyams JS, Griffiths A, Markowitz J, et al. Safety and efficacy of adalimumab for moderate to severe Crohn's disease in children. Gastroenterology 2012;143(2):365-74.e2.

13 Rosh JR, Lerer T, Markowitz J, et al. Retrospective Evaluation of the Safety and Effect of Adalimumab Therapy (RESEAT) in pediatric Crohn's disease. Am J Gastroenterol 2009;104(12):3042-9.

14 Russell RK, Wilson ML, Loganathan S, et al. A British Society of Paediatric Gastroenterology, Hepatology and Nutrition survey of the effectiveness and safety of adalimumab in children with inflammatory bowel disease. Aliment Pharmacol Ther 2011;33(8):946-53.

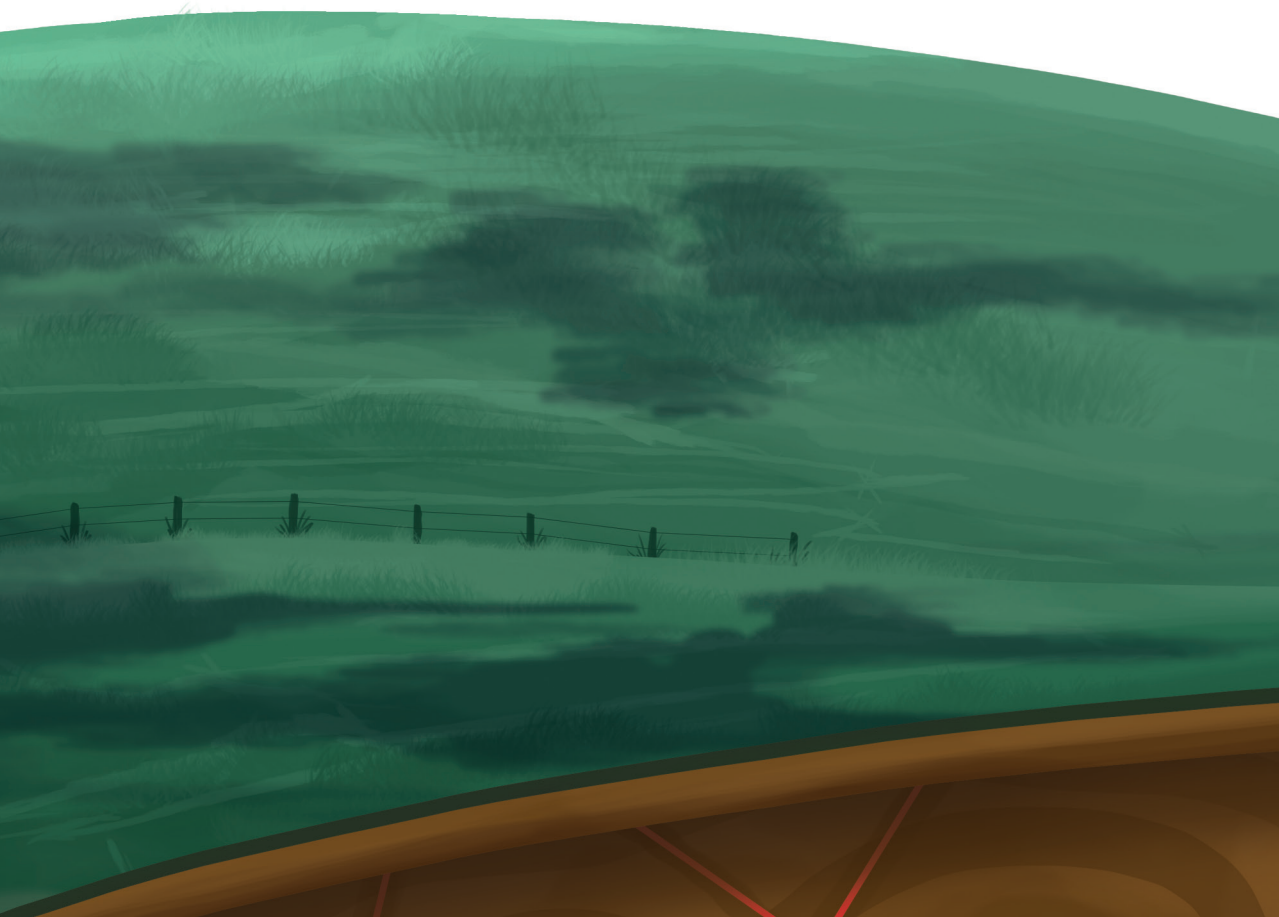
15 Turner D, Griffiths AM, Walters TD, et al. Mathematical weighting of the pediatric Crohn's disease activity index (PCDAI) and comparison with its other short versions. Inflamm Bowel Dis 2012;18(1):55-62.

16 Ho GT, Mowat A, Potts L, et al. Efficacy and complications of adalimumab treatment for medically-refractory Crohn's disease: analysis of nationwide experience in Scotland (2004–2008). Aliment Pharmacol Ther 2009;29(5):527-34.

17 Garcia-Bosch O, Gisbert JP, Canas-Ventura A, et al. Observational study on the efficacy of adalimumab for the treatment of ulcerative colitis and predictors of outcome. J Crohns Colitis 2013;7(9):717-22.

18 West RL, Zelinkova Z, Wolbink GJ, et al. Immunogenicity negatively influences the outcome of adalimumab treatment in Crohn's disease. Aliment Pharmacol Ther 2008;28(9):1122-6.

19 Afif W, Leighton JA, Hanauer SB, et al. Open-label study of adalimumab in patients with ulcerative colitis including those with prior loss of response or intolerance to infliximab. Inflamm Bowel Dis 2009;15(9):1302-7.
20 Levine A, Griffiths A, Markowitz J, et al. Pediatric modification of the Montreal classification for inflammatory bowel disease: the Paris classification. Inflamm Bowel Dis 2011;17(6):1314-21.

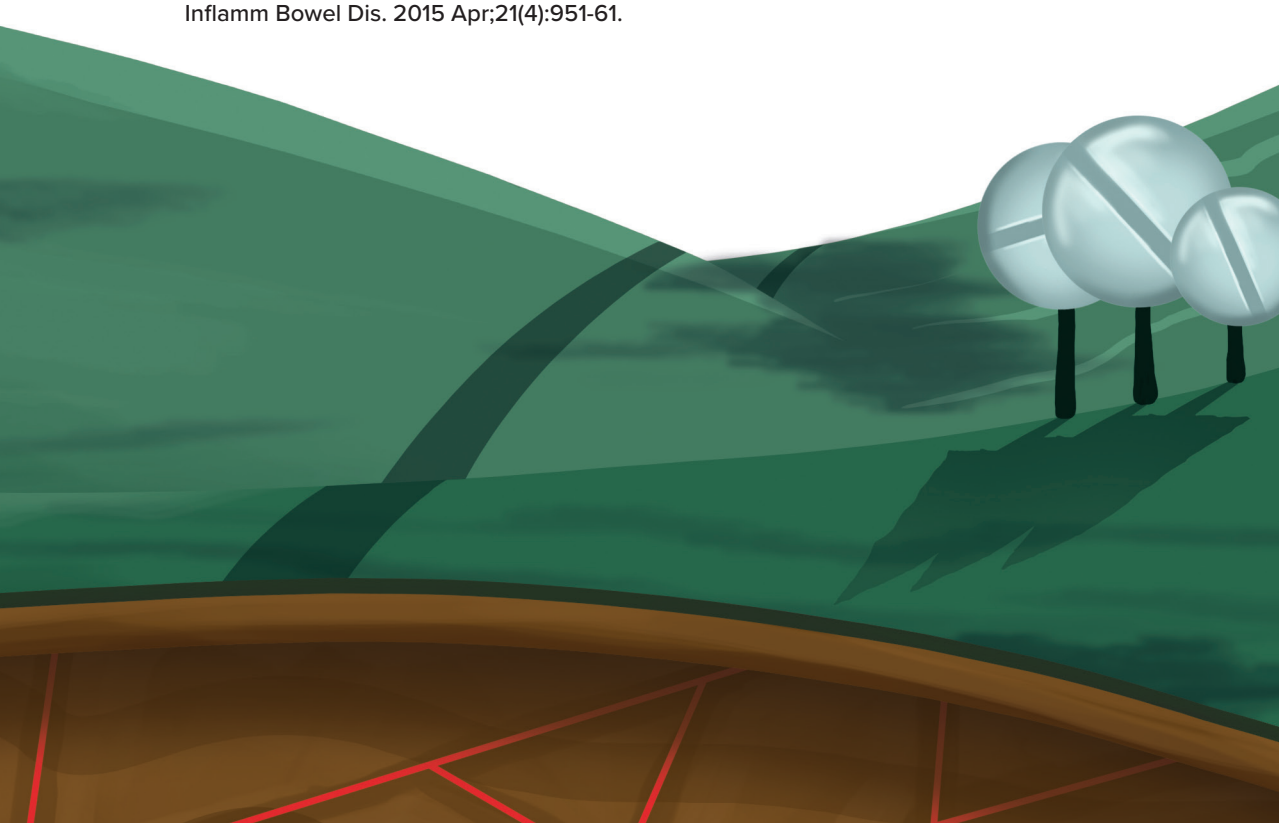


Chapter 6

The benefits and risks of combining anti-tumor necrosis factor with immunomodulator therapy in pediatric inflammatory bowel disease

M.A. Cozijnsen, J.C. Escher, A. Griffiths, D. Turner, L. de Ridder.

Inflamm Bowel Dis. 2015 Apr;21(4):951-61.



Abstract

Since the introduction of anti-tumor necrosis factor (TNF) therapy as treatment of inflammatory bowel disease (IBD), care of pediatric and adult IBD patients has significantly improved. In order to further improve treatment efficacy and durability, multiple trials have compared the efficacy of combination therapy, using anti-TNF therapy combined with an immunomodulator (a thiopurine or methotrexate), with that of anti-TNF monotherapy with contradicting results. The safety of combined therapy has been questioned following several reported cases of hepatosplenic T-cell lymphoma in young IBD patients so treated.. Physicians prescribing anti-TNF therapy to IBD patients are required to weigh the benefits of combined therapy with its risks. To inform physicians treating children with IBD of these benefits and risks, we reviewed studies in pediatric and adult IBD patients comparing efficacy, durability and/or safety of combined therapy with anti-TNF monotherapy.

Introduction

Crohn's disease (CD) and ulcerative colitis (UC) are chronic inflammatory bowel diseases (IBD), which present before the age of 20 years in 25-30% of CD and in 20% of UC patients.(1) In comparison to adult-onset disease, chronic inflammation in childhood onset CD leads often to impairment of linear growth and pubertal development, whereas childhood onset UC is more often extensive and therefore more often associated with acute severe exacerbations.(1, 2) First-line treatment of pediatric CD traditionally consists of exclusive enteral nutrition or corticosteroids to induce remission and immunomodulators (IM), i.e. thiopurines or methotrexate (MTX), to maintain remission – neither thiopurines nor MTX are labelled for the use in pediatric IBD.(3) In pediatric UC, based on disease severity, initial treatment consists of 5-aminosalicylic acid (5-ASA) or corticosteroids followed by 5-ASA maintenance therapy, optionally combined with thiopurines.(4) When these therapies fail, anti-tumor necrosis factor (TNF) antibody therapy is often indicated.

Anti-TNF antibody therapy can induce and maintain remission in both pediatric and adult IBD patients.(5-7) It has become increasingly important in young patients, given the necessity to heal their intestine in a timely fashion so that normal growth can be restored, while growth potential remains. Indeed, the recent ESPGHAN-ECCO guideline on the management of pediatric CD proposes using anti-TNF in high-risk patients from disease onset, such as those with severely impaired growth. Though very efficacious, approximately 10-20% of anti-TNF treated patients annually lose response to anti-TNF therapies.(8-10) This may partly be explained by neutralizing anti-drug antibodies, but in remaining cases its reason is unknown.(8) Researchers hypothesized that combined treatment with an anti-TNF antibody and an IM will increase the efficacy of therapy and reduce the risk of loss of response. (11-14) Particularly in young patients maintaining therapy response is important given their long lives ahead.

Pediatric gastroenterologists are required to weigh the potential benefits against the risk of combination therapy. In case evidence in pediatric IBD is limited, data from adult IBD studies should be extrapolated. This article reviews the literature to date comparing the efficacy and/or safety of combination therapy (anti-TNF with IM therapy) with anti-TNF monotherapy for the treatment of pediatric and adult IBD patients.

Efficacy of combination vs. monotherapy in CD patients

Pediatric CD patients

In pediatric CD patients, only one RCT was specifically designed to compare the efficacy of combination therapy with that of anti-TNF monotherapy (Table 1). The RCT by Kierkus et al (15) included 99 pediatric CD patients who were treated with IFX induction therapy. At week 10, responders were – in non-blinded fashion – randomized to receive either continued combination therapy through week 54 or combination therapy until week 26 and then IFX monotherapy until week 54. At 54 weeks, loss of response rates, defined by increase in Pediatric Crohn's Disease Activity Index, were similar in both groups (2/45 (4%) vs. 2/39 (5%), P≈0.9), similar proportions of patients required an increase or change of therapy (13/45 (29%) vs. 11/39 (28%), P≈0.9), and equal proportions of patients had increased SES CD scores compared to baseline (13/45 (29%) vs. 11/39 (28%), P≈0.9).

Additionally, one post-hoc analysis of a prospective trial and seven retrospective studies compared the efficacy of combination vs. monotherapy in children with CD. Hyams et al (16) performed a post-hoc analysis of the patients included in the RCT IMAgINE 1, which assessed the safety and efficacy of adalimumab in treating children with moderate-to-severe CD. Of the 188 patients treated with ADA, 117 (62%) used concomitant IM at baseline. Similar remission rates were observed at week 26 in patients with and without concomitant IMs (42/117 (35.9%) vs. 21/71 (29.6%), P≈0.4). ADA clearance and the occurrence of anti-drug antibodies were similar in both groups (clearance: 12.4±5.74 mL/h vs 15.2±7.88 mL/h; ATI occurrence: 2/117 (1.7%) vs 4/71 (5.6%), P≈0.2).(17)

Five retrospective pediatric cohort studies, totaling 512 patients, showed no difference in remission or loss of response rates between those treated with combination therapy or monotherapy.(18-22) However, Russell et al (23), in a cohort of 70 CD patients treated with ADA, found a higher remission rate in patients receiving combination therapy (ADA+IM) than in those receiving monotherapy (34/46 (74%) vs. 9/24 (37%), P=0.003).Furthermore, Church et al(24), among a cohort of 195 children with CD treated with IFX, found an increased duration of response in those treated with combination vs. monotherapy (Hazard ratio: 0.25 (CI 0.08-0.76)), both in IM naïve and experienced patients – loss of response was defined in this study as complete loss of responsiveness to IFX infusions, rather than simply a need to increase dose or shorten interval in order to maintain response. It should be emphasized that these retrospective cohorts studies, as well as the aforementioned post-hoc analysis are severely biased due to "confounding-by-indication", i.e. patients with more severe disease may have been more likely to have received IM therapy, and thus even if combination therapy was more effective, it just leveraged the outcome to those of the milder patients on monotherapy.

Up to date, the evidence available within pediatric CD is too limited to conclude whether or not combination therapy is more effective than anti-TNF monotherapy. Although the only RCT did not find a significant difference in efficacy, a clinically significant difference may well exist smaller than the RCT could detect. Moreover, it only compared remission maintenance rates between combination vs. monotherapy treated patients and not remission induction rates. Since the evidence in children is limited, extrapolation from adult literature should be considered.

Adult CD patients

In adult CD patients, three RCTs were specifically designed to compare the efficacy of combination therapy with that of anti-TNF monotherapy. Van Assche et al (14)randomized 80 CD patients with mild or no disease activity after 6 months combination therapy to either continue combination therapy or to switch to IFX monotherapy. After 104 weeks of follow-up, similar clinical relapse rates were seen in both groups (24/40 (60%) vs. 22/40 (55%), P=0.65). Furthermore, no difference was seen in their mucosal healing rates at week 104 (16/25 (64%) vs. 14/23 (61%); P≈0.8). However, higher median IFX trough levels were found in patients continuing IM therapy (median IFX trough 2.87 µg/mL (1.42–4.80) vs. 1.65 µg/mL (0.54–3.53), P=0.0001) and ATIs were detected in a non-significantly lower proportion of patients (2/40 (5%) vs. 5/40 (12.5%), P=0.43).

The placebo-controlled SONIC trial (11) compared the effectiveness and safety of IFX plus placebo, AZA plus placebo and combination therapy with both drugs in 508 adult CD patients with moderate-to-severe disease, naïve to both AZA and IFX. After 26 weeks of therapy, more patients treated with combination therapy (96/169 (57%)) were in corticosteroid-free remission than those treated with IFX (75/169 (44%), P=0.02) or AZA (51/170 (30%)). Furthermore, a trend for increased mucosal healing rates among patients with ulcers present at baseline, was seen in the combination therapy group (47/107 (44%)) compared to the IFX monotherapy group (28/93 (30%), P=0.06). Higher IFX trough levels and less often antibodies-to-IFX (ATIs) were found in the patients receiving combination therapy vs. those receiving monotherapy (median IFX trough: 3.5 vs. 1.6 µg/ml, P<0.001; ATI week 30: 1/116 (0.9%) vs. 15/103 (14.6%), P<0.001).

The COMMIT trial by Feagan et al (13) compared the efficacy and safety of combined treatment with IFX and MTX, with that of IFX and placebo for both the induction and maintenance of remission in adult CD patients. A total of 126 anti-TNF naïve CD patients who had initiated prednisone therapy for active symptoms were included (tapering of prednisone therapy started 7 days after randomization). At 14 weeks and at 50 weeks, no differences were seen in prednisone-free remission rates between the two treatment arms (week 14: 48/63 (76%) vs. 49/63 (78%), P=0.83; week 50: 27/48 (56%) vs. 28/49 (57%); P=0.86). However, again, fewer patients developed ATIs and a trend for higher IFX trough levels was seen in the combination group (ATI: 4% vs. 20%, P=0.01; median IFX trough: 6.4 vs. 3.8 µg/ml, P=0.08).

Table 1. Overview of prospective studies that compared the efficacy of combination therapy to anti-TNF monotherapy in pediatric and adult CD patients

Type	Year	Authors	Patients	N	Treatment	Duration
RCT	2014	Kierkus et al(15)	Pediatric CD, week 10 responders to IFX	84	[1] IFX+IM [2] IFX	54 wks
Post-analysis of RCT	2014	Hyams et al (16) and Eckert et al (17)	Pediatric CD, week 4 responders to ADA	188	[1] ADA+IM [2] ADA	52 wks
RCT	2014	Feagan et al (COMMIT)(13)	Adult CD, receiving prednisone<6wk	126	[1] IFX+MTX [2] IFX+PCB	50 wks
RCT	2010	Colombel et al (SONIC)(11)	Adult CD, moderate-to-severe disease	508	[1] IFX+AZA [2] IFX+PCB; [3] AZA+PCB	26 wks
RCT	2008	Van Assche et al(14)	Adult CD, controlled disease	80	[1] IFX+AZA [2] IFX	104 wks
Post-hoc analysis of 11 RCTs*	2013	Jones et al (27)	Adult CD		[1] Anti-TNF+IM [1a] IFX+IM [1b] ADA+IM [1c] CZP+IM [2] Anti-TNF [2a] IFX [2b] ADA [2c] CZP	
Post-hoc analysis of 18 RCTs*	2014	Kopylov et al (26)	Adult CD		ADA ADA+IM	

Endpoints		Result**	
a)	Loss of clinical response	a)	[1] 2/45 (4%) vs. [2] 2/39 (5%), P≈0.9
b)	Necessity to change therapy	b)	[1] 13/45 (29%) vs. [2] 11/39 (28%), P≈0.9
c)	Increase of SES-CD	c)	[1] 13/45 (29%) vs. [2] 11/39 (28%), P≈0.9
a)	Clinical remission wk 26	a)	[1] 42/117 (36%) vs. [2] 21/71 (30%), P≈0.4
b)	Wk 26 responders who maintained response through wk 52	b)	[1] 9/36 (25%) vs. [2] 13/44 (30%), P≈0.8
a)	CFCR wk 14	a)	[1] 48/63 (76%) vs. [2] 49/63 (78%), P=0.83
b)	Maintained remission wk 50	b)	[1] 27/48 (56%) vs. [2] 28/49 (57%), P=0.86
c)	ATIs presence	c)	[1] 3/63 (4%) vs. [2] 13/63 (20%), **P=0.01**
d)	Median IFX trough levels	d)	[1] 6.35 µg/mL vs. [2] 3.75 µg/ mL, P=0.08
a)	CFCR wk 26	a)	[1] 96/169 (57%) vs. [2] 75/169 (44%), **P=0.02**; [3] 51/170 (30%)
b)	MH wk 26	b)	[1] 47/107 (43.9%) vs. [2] 28/93 (30.1%), P=0.06; [3] 18/109 (16.5%)
c)	ATIs present wk 30	c)	[1] 1/116 (0.9%) vs. [2] 15/103 (14.6%), **P<0.001**
d)	Median IFX trough levels wk 26	d)	[1] 3.5 µg/mL vs. [2] 1.6 µg/mL, **P<0.001**
a)	Treatment failure	a)	[1] 24/40 (60%) vs. [2] 22/40 (55%), P=0.65
b)	MH wk 104	b)	16/25 (64%) vs. [2] 14/23 (61%), P≈0.8
c)	ATIs	c)	[1] 2/40 (5%) vs. [2] 5/40 (13%), P=0.43
d)	Median IFX trough levels wk 8-54	d)	[1] 2.87 µg/mL (1.42-4.80) vs. [2] 1.65 µg/mL (0.54-3.53), **P<0.0001**
a)	Remission 26wks	a)	[1] vs. [2], OR 1.06 (CI 0.83-1.35)
b)	Response induction		[1a] vs. [2a] OR **1.79 (1.06-3.01)**;
c)	Response maintenance		[1b] vs. [2b] OR 0.88 (0.58-1.35);
d)	Partial fistula closure		[1c] vs. [2c] OR 0.93 (0.65-1.34)
e)	Complete fistula closure	b)	[1] vs. [2], OR 1.06 (0.81-1.40)
		c)	[1] vs. [2], OR 1.46 (0.70-3.05)
		d)	[1] vs. [2], OR 1.26 (0.84-1.88)
		e)	[1] vs. [2], OR 1.1 (0.68-1.79)
a)	Remission induction	**a)**	**OR 0.78 (0.64-0.95)**
b)	Response induction	b)	OR 0.75 (0.53-1.04)
c)	Remission wk 52	c)	OR 1.08 (0.79-1.48)
d)	Response wk 52	d)	OR 1.21 (0.74-1.99)

Table 1. Continued

Type	Year	Authors	Patients	N	Treatment	Duration
Post-hoc analysis of 4 IFX RCTs*	2009	Lichtenstein et al (25)	Adult CD, active disease (ACCENT I) or draining fistulas (ACCENT II)	1383	[1] IFX+IM [2] IFX	54 wks
Post-hoc analysis of RCT*	2007	Colombel et al (CHARM) (37)	Adult CD, moderate-to-severe disease	854	[1] ADA+IM [2] ADA	56 wks

N=number; RCT=randomized clinical trial; CD=Crohn's disease; IFX=infliximab; IM=immunomodulator (AZA or 6-mercaptopurine or MTX); MTX=methotrexate; PCB=placebo; AZA=azathioprine; ADA=adalimumab; CZP= certolizumab; wk=week; SES-CD=Simplified endoscopic score for CD; CFCR=Corticosteroid-free clinical remission;

Overall, two RCTs have demonstrated that IFX combined with AZA, but not with MTX, increases remission rates in patients with active disease vs. IFX monotherapy.(11, 13) However, this modest treatment difference was achieved using a fixed IFX dosing schedule, i.e. precisely 5 mg/kg every 8 weeks. Individualization of therapy, with treatment targeted to adequate serum drug concentration, may nullify this efficacy difference. Secondly, although combination therapy did not improve remission maintenance rates nor failure rates in two RCTs, it did increase IFX trough levels and/or lowered the occurrence of ATIs in these trials. (13, 14)

Results of post-hoc analyses of adult anti-TNF RCTs are conflicting. Lichtenstein et al (25) found similar remission rates in combination and monotherapy treated CD patients included in ACCENT 1 and 2 (321 received IFX, of whom 84 used concomitant IMs). However, concomitant IMs did reduce the occurrence of ATIs in ACCENT 2 (ACCENT I: 5/90 (6%) vs. 24/245 (10%), P≈0.2; ACCENT II: 1/42 (2%) vs. 15/83 (18%), P≈0.01), but median IFX concentrations did not differ. Kopylov et al (26), in a post-hoc analysis of ADA RCTs, found lower remission induction rates in mono vs. combination therapy treated patients (300/976 (31%) vs 365/1008 (36%), OR 0.78 (0.64-0.96)), but remission rates at 12 months were similar (152/337 (45%) vs 197/496 (40%), OR 1.08 (0.78-1.48)). Jones et al (27), in a post-hoc analysis of 11 anti-TNF RCTs, found higher remission rates at 6 months using combination therapy with IFX vs. monotherapy (OR 1.79 (1.06-3.01)), but not combination therapy with ADA (OR 0.88 (0.58-1.35)) or certolizumab (OR 0.93 (0.65-1.34)).

Endpoints		Result**	
a)	ACCENT I: clinical response	a)	[1] 27/54 (50%) vs. [2] 69/170 (41%), P≈0.3
b)	ACCENT I: clinical remission	b)	[1] 20/54 (37%) vs. [2] 55/171 (32%), P≈0.5
c)	ACCENT II: fistula response	c)	[1] 12/28 (43%) vs. [2] 30/63 (48%), P≈0.8
d)	ACCENT II: complete fistula response	d)	[1] 9/28 (32%) vs. [2] 24/63 (38%), P≈0.6
e)	ACCENT I: ATI presence	e)	[1] 5/90 (6%) vs. [2] 24/245 (10%), P≈0.2
f)	ACCENT II: ATI presence	f)	[1] 1/42 (2%) vs. [2] 15/83 (18%), **P≈0.01**
g)	Median IFX concentration	g)	No differences between group [1] and [2] in all studies (numbers to comprehensive to disclose)
a)	Remission wk 26	a)	[1] 53/136 (39%) vs. [2] 15/36 (42%), P≈0.8
b)	Remission wk 56	b)	[1] 50/136 (37%) vs. [2] 12/36 (33%), P≈0.8

*ATIs=antibodies-to-infliximab; MH=mucosal healing; OR=odds ratio. * Treatment of the two sub-groups is displayed, not of the randomization arms ** When the P-value is followed by the "≈" symbol, no P-value is provided in the original article; we estimated the P-value using the Fisher Exact Test*

Lastly, six retrospective observational cohort studies compared combination therapy with anti-TNF monotherapy in treating adult CD. Most of these studies compared sustained clinical benefit rates or therapy failure rates of patients with and without concomitant IMs. Some found combination therapy to be more beneficial than monotherapy (28-30) whereas others did not (10, 31, 32).

Although approximately half of the studies show similar response, loss of response or remission rates, it is clear from the vast majority of studies that combined therapy is associated with higher IFX trough levels and decreased ATI rates.(11, 13, 14, 25) These two findings have been consistently associated with better clinical outcome.(33-35) The lack of this finding in some of the aforementioned studies might be explained by a small effect size, or by confounding-by-indication. Moreover, the results of one small (n=5) retrospective study even suggest that concomitant IMs may be used to treat patients who have lost response to anti-TNF agents due to anti-drug antibodies.(36) On the other hand, combination therapy may be less effective in IM refractory that in IM naïve patients.

Fewer studies have compared ADA combination therapy with monotherapy and no RCT has done so.(26-28, 30, 37) Furthermore, only one adult study compared ADA concentrations in patients treated with combination vs. monotherapy, which found similar concentrations.(38)

Efficacy of combination vs. monotherapy in UC patients

Pediatric UC patients

Thus far, the evidence available within pediatric UC is too limited to conclude whether or not combination therapy is more effective than anti-TNF monotherapy. Two studies compared their efficacy in treatment of pediatric UC, but both may suffer from confounding-by-indication bias (Table 2). In the T72 trial by Hyams et al (7), 60 pediatric UC patients with moderate-to-severe disease were treated with IFX and the responders at week 8 (44/60) were randomized to receive IFX either every 8 or 12 weeks. At baseline, 32 patients received concomitant IM therapy while 28 did not. In a post-hoc analysis, no differences in outcomes were observed between the groups (response week 8: 23/32 (72%) vs. 21/28 (75%), P≈0.8; remission week 8: 13/32 (41%) vs. 11/28 (39%), P≈0.9; mucosal healing week 8: 21/32 (67%) vs. 20/28 (71%); P≈0.6; remission week 54: 5/11 (46%) vs. 3/10 (30%), P≈0.5).

In an earlier analysis from the North American pediatric IBD registry, Hyams et al (39) studied 52 pediatric UC patients that had started IFX before the age of 18, of whom 32 (63%) used concomitant IMs at the start of IFX. Although not significant, a clear clinical trend for lower colectomy rate was noted in the combination group at 3 months (13% vs. 38%), at 6 months (25% vs. 38%), at 12 months (26% vs. 50%), and at 24 months (47% vs. 78%).

Adult UC patients

One RCT in adult UC patients was specifically designed to compare combination vs. monotherapy. In the UC-SUCCESS trial, Panaccione et al (12) randomized 239 adult UC patient with moderate-to-severe disease, naïve to anti-TNF therapy, to receive either AZA plus placebo, IFX plus placebo, or a combination of both drugs. At 16 weeks, more patients treated with combination therapy were in remission (31/78 (40%)) than those treated with IFX (17/77 (22%), P=0.017) or AZA (18/76 (24%), but no significant differences were noted in mucosal healing rates (49/78 (63%) vs. 52/77 (55%), P=0.30). Interestingly, IFX alone was not better than AZA. Fewer patients developed ATIs in the combination therapy group than in the IFX monotherapy group (1/31 (3%) vs. 7/37 (19%), P≈0.045); IFX drug levels were not reported. Like in SONIC, a fixed IFX dosing schedule was used.

Lichtenstein et al (25) performed a post-hoc analysis of UC patients included in the IFX trials ACT 1&2(484 received IFX, of whom 227 (47%) received concomitant IM). No differences in clinical response or remission rates were found between patients with and without IM. However, concomitant IMs did reduce the occurrence of ATIs (ACT I: 1/59 (2%) vs. 8/53 (15%), P≈0.01; ACT II: 1/43 (2%) vs. 12/53 (23%), P≈0.005), but IFX levels did not differ, except in ACT II at 30 weeks (higher IFX levels in the combination therapy group).

Four retrospective cohort studies presented analyses comparing combination with monotherapy for the treatment of UC. Armuzzi et al (40) included 126 UC patients treated with IFX of whom 71 also received IMs. More patients treated with combination vs. those treated with monotherapy had steroid-free remission at six and 12 months (63% vs. 40%, P=0.009; 59% vs. 31%, P=0.002). The rate of both steroid-free remission and mucosal healing at 12 months was higher in the former (42% vs. 20%, P=0.008). Additionally, more thiopurine naïve patients treated with combination therapy achieved steroid-free remission at six and 12 months than thiopurine experienced patients (80% vs. 50%, P=0.009; 80% vs. 39%, P<0.001). Hayes et al (41), within a cohort of 85 UC patients receiving IFX, observed an increased duration of IFX therapy at 1 year in combination vs monotherapy treated patients (90% vs 61%, P=0.016). Furthermore, combination therapy resulted in higher IFX levels (20.4 mg/L vs. 10.5 mg/L, P=0.025) and less frequent ATI formation (4.5% vs. 33%, P=0.031). Within a cohort of 109 UC patients with a median follow-up of 46 months, Jeuring et al (42) found higher risk for loss of response in patients treated with monotherapy vs. combination therapy (hazard ratio 2.4 (1.1-5.1)). Garcia-Bosch et al (43), in a very small cohort of 48 UC patients treated with ADA, reported response rates of 74% (26/35) among patients receiving concomitant IM vs. 62% (8/13, P≈0.5) in those treated with ADA alone.

Thus far, increased efficacy has only been demonstrated in adult UC patients treated with IFX plus AZA compared with IFX monotherapy. Like in CD, combination therapy seems to reduce the occurrence of ATIs and increase IFX drug levels, and it may be more efficacious in AZA naïve patients than AZA experienced patients.

Table 2. Overview of studies that compared the efficacy of combination therapy to anti-TNF monotherapy in pediatric and adult UC patients

Type	Year	Authors	Patients	N	Treatment	Duration
Post-hoc analyses of RCT*	2012	Hyams et al (T72)(7)	Pediatric UC, moderate-to-severe disease	60	[1] IFX+IM; [2] IFX	54 wks
Observational	2010	Hyams et al (39)	Pediatric UC	52	[1] IFX+IM; [2] IFX	104 wks
RCT	2014	Panaccione et al (UC-SUCCESS)(12)	Adult UC, moderate-to-severe disease	239	[1] IFX+AZA; [2] IFX+PCB [3] AZA+PCB	16 wks
Post-hoc analysis of 4 IFX RCTs*	2009	Lichtenstein et al (25)	Adult UC, moderate-to-severe disease	1383	[1] IFX+IM; [2] IFX*	30-54 wks
Observational	2013	Armuzzi et al (40)	Adult UC, active disease	126	[1] IFX+IM; [1a] TP naïve [1b] TP exp [2] IFX*	52 wks
Retrospective	2014	Hayes et al	Adult UC	85	[1] IFX+IM [2] IFX	
Observational	2013	Jeuring et al (42)	Adult UC	109	[1] IFX+IM [2] IFX	

*N=number; RCT=randomized clinical trial; UC=ulcerative colitis; IFX=infliximab; IM=immunomodulator (AZA or 6-mercaptopurine or MTX); AZA=azathioprine; MTX=methotrexate; TP=thiopurine; exp=experienced; wk=week; CFCR=Corticosteroid-free clinical remission; ATIs=antibodies-to-infliximab; MH=mucosal healing; HR=hazard ratio. * Treatment of the two groups of the sub analysis, not of the randomization arms. ** When the P-value is followed by the "≈" symbol, no P-value is provided in the original article; we estimated the P-value using Fisher Exact Test*

Endpoints	Result**
a) Clinical response wk 8 b) Clinical remission wk 8 c) Clinical remission wk 54	a) [1] 23/32 (72%) vs. [2] 21/28 (75%), P≈0.8 b) [1] 13/32 (41%) vs. [2] 11/28 (39%), P≈0.9 c) [1] 5/11 (46%) vs. [2] 3/10 (30%), P≈0.5
Colectomy rates at 3, 6, 12 and 24 months	[1] vs. [2]: 13 vs. 38%, 25 vs. 38%, 26 vs. 50%, 47 vs. 78% respectively, P>0.05
a) CFCR wk 16 b) MH wk 16 c) ATIs wk 16	a) [1] 31/78 (40%) vs. [2] 17/77 (22%), **P=0.017**; [3] 18/76 (24%) b) [1] 49/78 (63%) vs. [2] 52/77 (55%), P=0.30; [3] 28/76 (37%) c) [1] 1/31 (3%) vs. [2] 7/37 (19%), **P≈0.045**
a) ACT I: clinical response wk 54; b) ACT I: clinical remission wk 54; c) ACT II: clinical response wk 30 d) ACT II: clinical remission wk 30 e) ACT I: ATI presence f) ACT II: ATI presence g) Median IFX concentration	a) [1] 56/125 (45%) vs. [2] 53/118 (45%), P≈1.0 b) [1] 42/125 (34%) vs. [2] 42/118 (36%), P≈0.7 c) [1] 56/102 (55%) vs. [2] 73/139 (53%), P≈0.8 d) [1] 37/102 (36%) vs. [2] 37/139 (27%), P≈0.1 e) [1] 1/59 (2%) vs. 8/53 (15%), **P≈0.01** f) [1] 1/43 (2%) vs. 12/53 (23%), **P≈0.005** g) Overall median IFX concentrations did not differ, except in ACT II at 30 wk: IFX concentrations were higher in [2] (numbers to comprehensive to disclose)
a) Steroid free remission 26 wks b) Steroid free remission 52 wks c) Steroid free remission & MH 52 wks	a) [1] 63% vs. [2] 40%, **P=0.009**; [1a] 80% vs. [1b] 50%, **P=0.009** b) [1] 59% vs. [2] 31%, **P=0.002**; [1a] 80% vs. [1b] 39%, **P<0.001** c) [1] 42% vs. [2] 20%, **P=0.008**
a) Remission 52 wks b) Continued therapy c) Mean serum IFX (mg/L) d) Detectable ATIs	a) [1] 20/37 (54%) vs. [2] 13/27 (48%), P=0.80 b) [1] 90% at 52 wks vs. 61% at 52 wks, **P=0.016** c) [1] 20.4 ±13.2 vs. [2] 11.2 ±13.5, **P=0.025** d) [1] 1/22 (4.5%) vs. [2] 5/15 (33%), **P=0.031**
Risk for loss of response (HR)	[2] vs. [1]: 2.36 (1.10-5.08)

Safety of combination therapy in IBD patients

Safety in pediatric IBD

Besides the potential benefits of combination therapy, the risks of additional medication should be carefully considered, especially in children who may well have many future treatment years. It is important to note that thiopurines were the traditional IM used in pediatric CD and UC, either as monotherapy or in combination with anti-TNF. Hence more data exist concerning toxicity profile of thiopurines in young IBD patients in comparison to MTX, although its use in pediatric CD has increased in recent years (Table 3).

The DEVELOP registry is designed to study long-term (20 years) safety of IFX and other therapies in pediatric-onset IBD (ClinicalTrials.gov Identifier: NCT00606346). Preliminary data showed that combination therapy resulted in higher malignancy rates in patients receiving combination therapy than in those receiving anti-TNF monotherapy (0.11/100PY (4/3599PY) vs. 0/100PY (0/748PY), $P<0.05$).(45)

Rosh et al (44) performed a post-hoc analysis on the patients included in IMAgINE 1 and its extension trial OLE, which included 192 pediatric CD patients that were treated with ADA for a total of 422 patient-years (PY). More children treated with combination therapy at baseline had serious infections but this did not reach significance (6.2 E/100PY vs. 3.5 E/100PY, $P>0.05$).

Safety in adult IBD

None of the four aforementioned adult RCTs specifically designed to compare combination therapy vs. monotherapy found increased AE rates in the combination therapy group, nor increased serious adverse events (SAEs), infections or serious infections.(11-14) On the contrary, within the SONIC trial (11), *fewer* SAEs occurred in the patients receiving combination therapy (27/179 (15%) vs. 39/163 (24%), $P=0.04$), likely due to reduced infusion reactions (9/179 (5%) vs. 27/163 (17%), $P<0.001$) and better disease control and thus fewer CD associated infections such as abscesses.

The previously mentioned post-hoc analysis by Jones et al (27) showed similar SAE rates across both patients groups. In a sub-group analysis, combination therapy with IFX was associated with *fewer* infusion reactions (OR 0.43 (0.19-0.97)). Infection and serious infection rates were also similar in the aforementioned post-hoc analysis by Lichtenstein et al (25) and an additional post-hoc analysis, which utilized patient data from all industry driven IFX trials in adult IBD patients, found similar malignancy and mortality rates.(45) In contrast, Osterman et al (46) performed a post-hoc analysis on CD patients included in ADA RCTs (CLASSIC I&II, CHARM, GAIN, EXTEND, ADHERE) to assess the risk of non-melanoma skin cancer (NMSC) and other malignancies in combination and monotherapy treated patients. Of the 1594 patients treated with ADA, 694

(44%) used concomitant IMs and these patients had higher risk of NMSC and other malignancies than those on monotherapy (adjusted relative risks: NMSC: 2.82 (1.07-7.44); other malignancies: 3.46 (1.08-11.06)). Similar AE rates were found by the aforementioned post-hoc analysis of ADA RCTs by Kopylov et al, but only few trials had addressed adverse events.(26)

Within the TREAT registry (47), a prospective cohort in which 6,273 CD patients have been enrolled with an average follow-up duration of 5.2 years, 3,420 received IFX treatment, of whom 1,780 also received IM. In total 252 malignancies occurred, slightly more in patients receiving IM or combination therapy, but this did not reach statistical significance (IFX+IM: 119/3,517 (3.4%), OR 3.33 (0.46-24); IFX mono: 5/247 (2.0%), OR 1.96 (0.23-17); IM mono: 102/2,413 (4.2), OR4.19 (0.58-30)). Using multivariate Cox regression analysis, neither combination therapy nor IFX monotherapy were associated with time to malignancy (HR=1.22 (0.81-1.86), P=0.34 and HR=0.59 (0.28-1.22), P=0.16).

Two other case-control studies were published comparing the risk of malignancy in combination vs. monotherapy. Within a cancer registry, Herrinton et al (48) analyzed the risk of lymphoma in IBD patients compared to non IBD patients. A total of 16,023 IBD patients with 89,064 recorded patient years (PY), both anti-TNF use, thiopurine use, and treatment with both drugs resulted in increased risk of lymphoma, the latter resulted in the highest risk (SIR: anti-TNF: 5.2 (3.5-6.8); thiopurine: 1.4 (1.2-1.7); combination 6.6 (4.4-8.8)). Anti-TNF monotherapy was associated with malignancy more often than with thiopurine monotherapy but it is noteworthy that all patients treated with anti-TNF monotherapy had previously received thiopurine treatment.

Deepak et al (49) performed a case-control study to assess the risk of T-cell non-Hodgkin lymphomas (NHLs) and hepatosplenic T-cell lymphoma (HSTCL) in IBD patients using anti-TNF therapy (with or without thiopurines) compared to other IBD therapies. The American Food and Drug Administration (FDA) adverse events database and Medline were searched to identify patients. A total of 45 IBD patients with NHL were identified. Compared to other therapies, anti-TNF combined with thiopurines resulted in higher risk of T-cell NHL (OR 4.98–354.09; P<0.0001), whereas anti-TNF monotherapy did not result in higher risk (OR 0.13–10.61; P=1.00). The same was true for the sub-analysis of the risk for HSTCL (IFX+IM vs. control: OR 2.99–993.04; P<0.0001; IFX vs. control: OR 0.02–15.70; P=1.00). Both the risk of NHL and HSTCL was also increased in thiopurines monotherapy (NHL: OR 8.32–945.38; P<0.0001; HSTCL: OR 6.90–3045.2; P<0.0001)

Taken together, it seems that most evidence from children and adults indicate a minor but significant increase in malignancy rate using combination therapy with thiopurines compared with monotherapy. In contrast, no consistent findings indicate increased AE and SAE rates with combination therapy, including infections or serious infections.

Table 3. Overview of studies that compared the safety of combination therapy with anti-TNF monotherapy in pediatric and adult IBD patients

Type	Year	Authors	Patients	N	Treatment	Duration
Post-hoc analysis of RCT*	2014	Rosh et al(44)	Pediatric CD	192	[1] ADA+IM [2] ADA	1-5 years
Observational	2013	Colletti et al (DEVELOP)(45)	Pediatric CD&UC	4343	[1] Anti-TNF+IM [2] Anti-TNF` [3] IM	
RCT	2014	Colombel et al (SONIC)(11)	Adult CD	508	[1] IFX+AZA; [2] IFX+PCB; [3] AZA+PCB	26 wks
RCT	2014	Panaccione et al (UC SUCCESS)(12)	Adult UC	239	[1] IFX+AZA; [2] IFX+PCB; [3] AZA+PCB	8 wks
RCT	2010	Feagan et al (COMMIT)(13)	Adult CD	126	[1] IFX+MTX; [2] IFX+PCB	50 wks
RCT	2008	Van Assche et al(14)	Adult CD	80	[1] IFX+AZA; [2] IFX	104 wks
Post-hoc analysis of 11 RCTs*	2013	Jones et al (27)	Adult CD		[1] Anti-TNF+IM [1a] IFX+IM [2] Anti-TNF [2a] IFX	
Post-hoc analysis of 4 IFX RCTs*	2009	Lichtenstein et al (25)	Adult CD&UC	1383	[1] IFX+IM [2] IFX	30-54 wks
Post-hoc analysis of 5 IFX RCTs*	2012	Lichtenstein et al (46)	Adult CD&UC		[1] IFX+IM [2] IFX [3] IM	30-54 wks

Endpoints	Results**
Serious infections	[1] 6.2/100PY vs. [2] 3.5/100PY, P>0.05
Malignancy	[1] 4/3599PY=0.11/100PY vs. [2] 0/748PY=0/100PY, **P<0.05**,[3] 3/2411PY=0.12/100PY
a) AE b) SAE c) Infections d) Serious infections e) Infusion reactions	a) [1] 161/179 (90%) vs. [2] 145/163 (89%), P=1; [3] 144/161 (89%) b) [1] 27/179 (15%) vs. [2] 39/163 (24%), **P=0.04**; [3] 43/161 (27%) c) [1] 75/179 (42%) vs. [2] 75/163 (46.0), P=0.45; [3] 73/161 (45.3) d) [1] 7/179 (4%) vs. [2] 8/163 (5%), P=0.61; [3] 9/161 (6%) e) [1] 9/179 (5%) vs. [2] 27/163 (17%), **P<0.001**; [3] 9/161 (6%)
a) AE b) SAE c) Infusion reactions	a) [1] 30/80 (38%) vs. [2] 26/78 (33%), P≈0.6; [3] 41/79 (52%) b) [1] 3/80 (4%) vs. [2] 2/78 (3%), P≈0.6; [3] 6/79 (8%) c) [1] 0/80 (0%) vs. [2] 0/78 (0%), P=1; [3] 1/79 (1%)
a) AE b) SAE c) Infusion reactions	a) A list of most common AEs***, P>0.05 b) [1] 6/63 (10%) vs. [2] 2/63 (3%), P≈0.1 c) [1] 1/63 (2%); [2] 3/63 (5%), P≈0.6
a) AE b) SAE c) Infections d) Infusion reaction	a) [1] 24/40 (60%) vs. [2] 25/40 (63%), P≈0.8 b) [1] 3/40 (8%) vs. [2] 3/40 (8%), P≈1.0 c) [1] 12/40 (30%) vs. [2] 10/40 (25%), P≈0.6 d) [1] 3/40 (8%) vs. 2/40 (5%), P≈1.0
a) SAE (OR) b) Infusion reaction (OR)	a) [1] vs. [2] 1.11 (0.56-2.20) b) [1a] vs. [2a] **0.43 (0.19-0.97)**
a) Infections b) Serious infections	a) [1] 166/376 (44%) vs. [2] 281/631 (45%), P≈ 0.9 b) [1] 14/376 (4%) vs. [2] 20/631 (3%), P≈ 0.6
a) Malignancy b) Mortality	a) [1] 1/300PY=0.33/100PY (0.01-1.86) vs. [2] 1/303PY=0.33/100PY (0.01-1.84) vs [3] 2/170PY=1.17/100PY (0.14-4.24) b) [1] 3/1020PY=0.29/100PY (0.06-0.86) vs. [2] 2/1256PY=0.16/100PY (0.02-0.58)

Table 3. Continued

Type	Year	Authors	Patients	N	Treatment	Duration
Post-hoc analysis of 6 ADA RCTs*	2014	Osterman et al (47)	Adult CD	1594	[1] ADA+IM; [2] ADA	4-204 wks
Observational	2014	Lichtenstein et al (TREAT) (48)	Adult CD	6273	[1] IFX+IM [2] IFX [3] IM	
Case-control study	2011	Herrinton et al (49)	Adult CD&UC	16023	[1] Anti-TNF+TP [2] Anti-TNF [3] TP	
Case-control study	2013	Deepak et al	Adult CD&UC	45	[1] IFX+TP; [2] IFX [3] TP [4] Control drugs	

*N=number; RCT=randomized clinical trial; CD=Crohn's disease; UC=ulcerative colitis; ADA=adalimumab; IM=immunomodulator (AZA or 6-mercaptopurine or MTX); IFX=infliximab; AZA=azathioprine; PCB=placebo; MTX=methotrexate; TP=thiopurines (AZA/6MP); wks=weeks; AE=adverse event; SAE=serious adverse event; PY=patient-years; SIRR=standardized incidence risk ratio, i.e. risk as compared to a control population; OR=odds ratio. * Treatment of the two groups of the sub analysis, not of the randomization arms. ** When the P-value is followed by the "≈" symbol, no P-value is provided in the original article; we calculated an estimated P-value using Fisher Exact Test. *** No overall percentage of AEs in each group is given, only a list of the most common AEs in both groups and their between group difference*

Endpoints	Results**
a) Non melanoma skin cancer b) Other malignancies	a) 11/1401=0.8/100PY vs. [2] 4/1649=0.2/100PY, **RR: 2.82 (1.07-7.44)** b) 14/1401=1.0/100PY vs. [2] 6/1649=0.4/100PY, **RR: 3.46 (1.08-11.06)**
a) Malignancy rates b) Malignancy risk (HR)	a) [1] 119/3,517 (3.4%), OR 3.33 (0.46-24) vs. [2] 5/247 (2.0%), OR 1.96 (0.23-17) vs [3] 102/2,413 (4.2), OR4.19 (0.58-30) b) [1] 1.22 (0.81-1.86), P=0.34 and [2] 0.59 (0.28-1.22), P=0.16 and [3] 1.43 (0.92-2.21), P=0.11
Lymphoma risk	[1] 1/678PY, **SIRR 6.6 (4.4-8.8)** [2] 1/774PY, **SIRR 5.2 (3.5-6.8)** [3] 4/8723PY, **SIRR 1.4 (1.2-1.7)**
a) Non hodgkin lymphoma/other events b) Hepato-splenic T-cell lymphoma/other events	a) [1] 36/12 vs. [2] 6/71 vs. [3] 1/14 vs. [4] 1/14; [1] vs. [4] **OR=4.98–354.09**, [2] vs. [4] OR=0.13–10.61, [3] vs. [4] **OR=8.32-945.38** b) [1] 23/12 vs. [2] 1/71 vs. [3] 17/3 vs. [4] 0/14, [1] vs. [4] **OR=2.99–993.04**, [2] vs. [4] OR=0.02–15.70, [3] vs. [4] **OR=6.90-3045.2**

Conclusions and future perspectives

Although almost all studies in pediatric IBD patients did not find increased benefit for combination vs. monotherapy, the available evidence in children is scarce. Several adult trials have shown higher treatment efficacy in patients receiving IFX combination therapy, especially for induction of remission. However, the treatment benefit is at most modest, and might be overcome by optimization of IFX therapy dosing. Although IM naïve may benefit more than IM experienced patients, concomitant IMs have demonstrated to increase IFX levels and reduce immunogenicity rates regardless of past IM use. The reduction of immunogenicity rates may lead to a benefit in terms of increased durability of responsiveness to therapy , which is particularly important for young patients given their long lives ahead. Combination therapy does, however, seem to increase the risk of malignancy. Although no more safety issues were identified in the reviewed trials, the use of combination therapy will expose patients to the individual toxicities of both drugs, next to potential risks due to the combination of drugs.

In a joint consensus guideline of the European Crohn's and Colitis organization (ECCO) and the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) on the medical management of pediatric Crohn's disease (3), the authors concluded that there is insufficient evidence to define the risk/benefit ratio for combining anti-TNF with IM therapy. The authors did note that combination therapy in the first six months of anti-TNF therapy may be associated with a lower rate of antibodies development and loss of response, but that this benefit should be weighed against the eventually increased lymphoma risk with thiopurines. The use of concomitant low dose MTX may be safer but it is much less evidence-based, and not supported by the COMMIT trial. In the pediatric UC guideline of the same organizations (4) the authors concluded that there is no good evidence to support combining IFX with thiopurines in children with thiopurines refractory UC and that the balance of safety vs. benefits of combination treatment needs to be fully explained. It is noteworthy that several newer studies have published on this topic after the UC guidelines.

Although clearly more research is needed on this topic, the current evidence is sufficient to make some recommendations. The use of combination therapy in clinical practice may be indicated in patients having high risk of serious disease related complications, such as growth retardation, formation of strictures or fistulas, or need for surgery. For instance, significant pan enteric disease, which is not amenable to surgery and is associated with increased long term complications, might indicate combination therapy in the beginning. The timing of stepping down to monotherapy should be individualized. Using combination therapy for a short duration (e.g. 6 months) at the start of anti-TNF

treatment may reduce the occurrence of ATIs and reduce loss of response rates while minimizing adverse effects. On the other hand, as more and more evidence associates thiopurine use with increase malignancy rates, MTX may be used in combination with anti-TNF therapy instead at least in boys with CD (due to the teratogenicity risk of MTX in teenage girls). However the benefits of combined therapy with MTX have been less studied and proven.

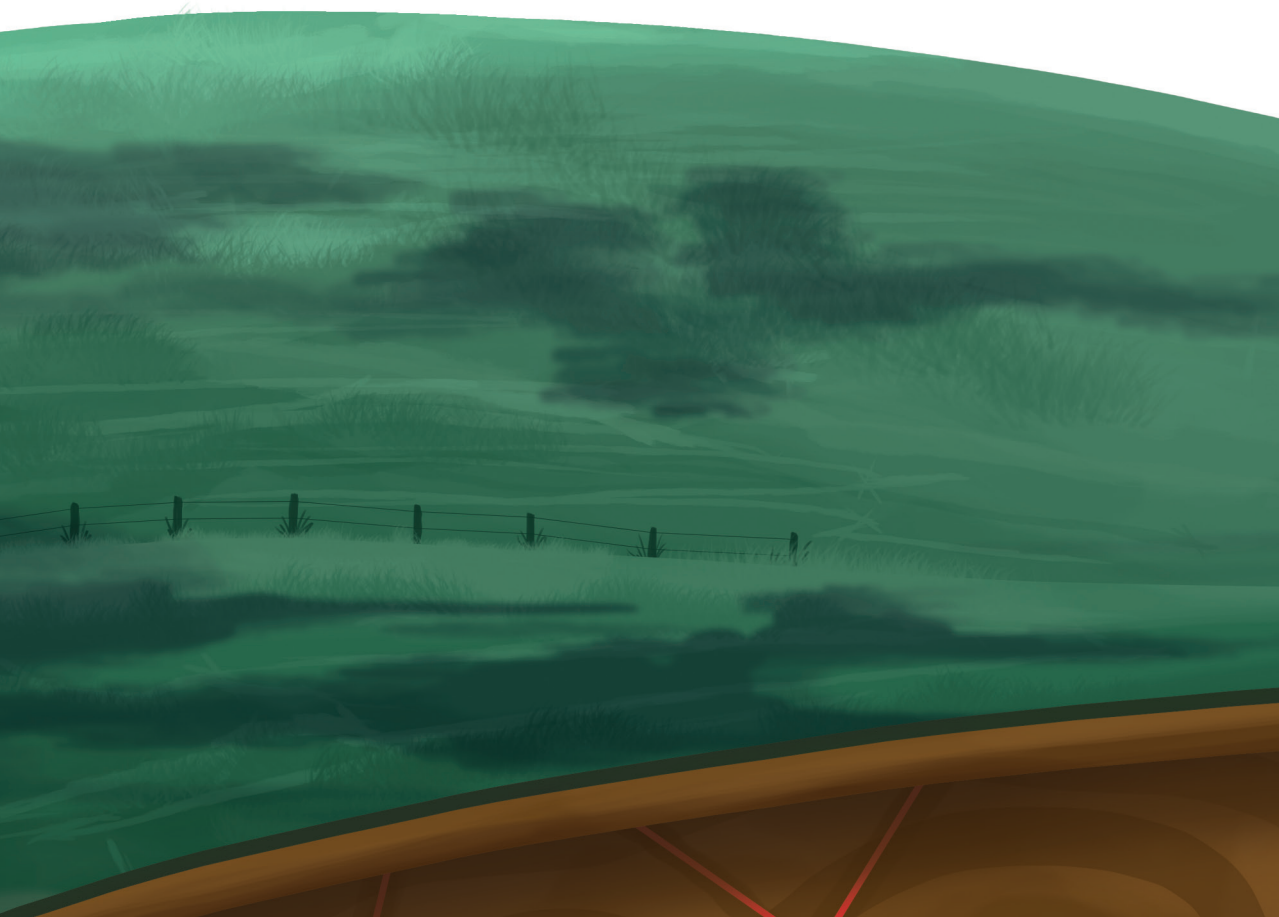
A personalized strategy, aiming at balancing the risk and benefit given individual phenotype and predictors, is likely the preferred strategy until more level 1 evidence is available in children. Regardless of the recommendations, families should always be involved in the decision making while providing a balanced view of the pros and cons of each alternative.

References

1. Kelsen J, Baldassano RN. Inflammatory bowel disease: the difference between children and adults. Inflamm Bowel Dis. 2008;14 S9-11
2. Vasseur F, Gower-Rousseau C, Vernier-Massouille G, et al. Nutritional status and growth in pediatric Crohn's disease: a population-based study. Am J Gastroenterol. 2010;105:1893-1900
3. Ruemmele FM, Veres G, Kolho KL, et al. Consensus guidelines of ECCO/ESPGHAN on the medical management of pediatric Crohn's disease. J Crohns Colitis. 2014
4. Turner D, Levine A, Escher JC, et al. Management of pediatric ulcerative colitis: joint ECCO and ESPGHAN evidence-based consensus guidelines. J Pediatr Gastroenterol Nutr. 2012;55:340-361
5. Hyams J, Crandall W, Kugathasan S, et al. Induction and maintenance infliximab therapy for the treatment of moderate-to-severe Crohn's disease in children. Gastroenterology. 2007;132:863-873
6. Hyams JS, Griffiths A, Markowitz J, et al. Safety and efficacy of adalimumab for moderate to severe Crohn's disease in children. Gastroenterology. 2012;143:365-374.e362
7. Hyams J, Damaraju L, Blank M, et al. Induction and maintenance therapy with infliximab for children with moderate to severe ulcerative colitis. Clin Gastroenterol Hepatol. 2012;10:391-399 e391
8. De Bie CI, Hummel TZ, Kindermann A, et al. The duration of effect of infliximab maintenance treatment in paediatric Crohn's disease is limited. Aliment Pharmacol Ther. 2011;33:243-250
9. Hyams JS, Lerer T, Griffiths A, et al. Long-term outcome of maintenance infliximab therapy in children with Crohn's disease. Inflamm Bowel Dis. 2009;15:816-822
10. Schnitzler F, Fidder H, Ferrante M, et al. Long-term outcome of treatment with infliximab in 614 patients with Crohn's disease: results from a single-centre cohort. Gut. 2009;58:492-500
11. Colombel JF, Sandborn WJ, Reinisch W, et al. Infliximab, Azathioprine, or Combination Therapy for Crohn's Disease. N Engl J Med. 2010;362:1383-1395
12. Panaccione R, Ghosh S, Middleton S, et al. Combination therapy with infliximab and azathioprine is superior to monotherapy with either agent in ulcerative colitis. Gastroenterology. 2014;146:392-400 e393
13. Feagan BG, McDonald JW, Panaccione R, et al. Methotrexate in combination with infliximab is no more effective than infliximab alone in patients with Crohn's disease. Gastroenterology. 2014;146:681-688 e681
14. Van Assche G, Magdelaine-Beuzelin C, D'Haens G, et al. Withdrawal of immunosuppression in Crohn's disease treated with scheduled infliximab maintenance: a randomized trial. Gastroenterology. 2008;134:1861-1868
15. Kierkus J, Iwanczyk B, Wegner A, et al. Efficacy infliximab with immunomodulator and infliximab alone of maintenance therapy in children with Crohn's disease multicenter randomized study. 8th Congress of ECCO, P525 2013
16. Hyams JS, Ruemmele F, Colletti RB, et al. Impact of concomitant immunosuppressant use on adalimumab efficacy in children with moderately to severely active crohn's disease: Results from imagine 1. Gastroenterology. 2014;146:S-214
17. Eckert D, Mensing S, Sharma S, et al. Pharmacokinetics of adalimumab in pediatric patients with moderate to severe crohn's disease. Gastroenterology. 2013;144:S228
18. Rosh JR, Lerer T, Markowitz J, et al. Retrospective Evaluation of the Safety and Effect of Adalimumab Therapy (RESEAT) in pediatric Crohn's disease. Am J Gastroenterol. 2009;104:3042-3049
19. Assa A, Hartman C, Weiss B, et al. Long-term outcome of tumor necrosis factor alpha antagonist's treatment in pediatric Crohn's disease. J Crohns Colitis. 2013;7:369-376

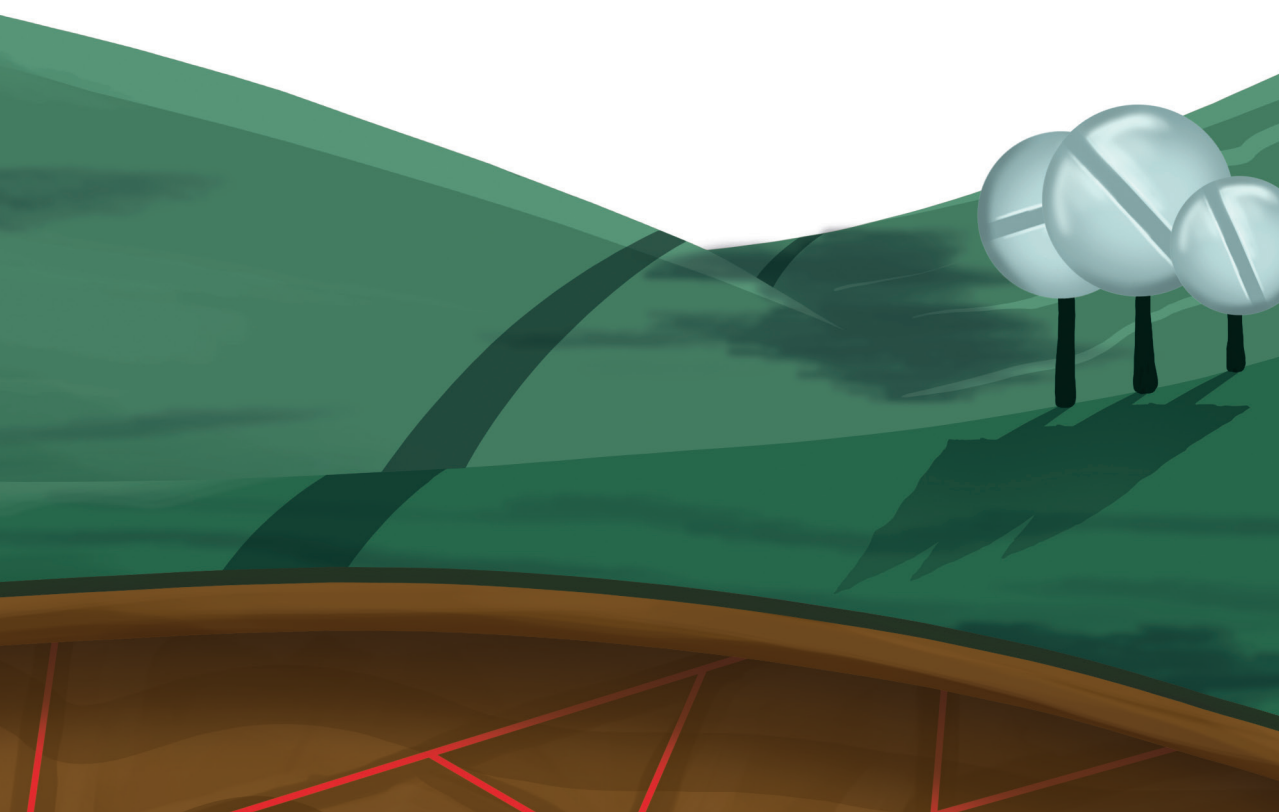
20. Gouldthorpe O, Catto-Smith AG, Alex G, et al. Loss of response to long-term infliximab therapy in children with Crohn's disease. Pharmaceuticals. 2013;6:1322-1334
21. Vahabnezhad E, Rabizadeh S, Dubinsky MC. A 10-year, single tertiary care center experience on the durability of infliximab in pediatric inflammatory bowel disease. Inflamm Bowel Dis. 2014;20:606-613
22. Nuti F, Viola F, Civitelli F, et al. Biological therapy in a pediatric crohn disease population at a referral center. J Pediatr Gastroenterol Nutr. 2014;58:582-587
23. Russell RK, Wilson ML, Loganathan S, et al. A British Society of Paediatric Gastroenterology, Hepatology and Nutrition survey of the effectiveness and safety of adalimumab in children with inflammatory bowel disease. Aliment Pharmacol Ther. 2011;33:946-953
24. Church PC, Guan J, Walters TD, et al. Infliximab maintains durable response and facilitates catch-up growth in luminal pediatric Crohn's disease. Inflamm Bowel Dis. 2014;20:1177-1186
25. Lichtenstein GR, Diamond RH, Wagner CL, et al. Clinical trial: benefits and risks of immunomodulators and maintenance infliximab for IBD-subgroup analyses across four randomized trials. Aliment Pharmacol Ther. 2009;30:210-226
26. Kopylov U, Al-Taweel T, Yaghoobi M, et al. Adalimumab monotherapy versus combination therapy with immunomodulators in patients with Crohn's disease: A systematic review and meta-analysis. J Crohns Colitis. 2014
27. Jones J, Kaplan G, Peyrin-Biroulet L, et al. Impact of concomitant immunomodulator treatment on efficacy and safety of anti-TNF therapy in Crohn's disease: a meta-analysis of placebo controlled trials with individual patient-level data. 21st UEGWEEK, OP053; 2013
28. Peters CP, Eshuis EJ, Toxopeus FM, et al. Adalimumab for Crohn's disease: Long-term sustained benefit in a population-based cohort of 438 patients. J Crohns Colitis. 2014
29. Eshuis EJ, Peters CP, van Bodegraven AA, et al. Ten years of infliximab for Crohn's disease: outcome in 469 patients from 2 tertiary referral centers. Inflamm Bowel Dis. 2013;19:1622-1630
30. Reenaers C, Louis E, Belaiche J, et al. Does co-treatment with immunosuppressors improve outcome in patients with Crohn's disease treated with adalimumab? Aliment Pharmacol Ther. 2012;36:1040-1048
31. Sokol H, Seksik P, Carrat F, et al. Usefulness of co-treatment with immunomodulators in patients with inflammatory bowel disease treated with scheduled infliximab maintenance therapy. Gut. 2010;59:1363-1368
32. Sprakes MB, Ford AC, Warren L, et al. Efficacy, tolerability, and predictors of response to infliximab therapy for Crohn's disease: a large single centre experience. J Crohns Colitis. 2012;6:143-153
33. Levesque BG, Greenberg GR, Zou G, et al. A prospective cohort study to determine the relationship between serum infliximab concentration and efficacy in patients with luminal Crohn's disease. Aliment Pharmacol Ther. 2014;39:1126-1135
34. Cornillie F, Hanauer SB, Diamond RH, et al. Postinduction serum infliximab trough level and decrease of C-reactive protein level are associated with durable sustained response to infliximab: a retrospective analysis of the ACCENT I trial. Gut. 2014
35. Paul S, Del Tedesco E, Marotte H, et al. Therapeutic drug monitoring of infliximab and mucosal healing in inflammatory bowel disease: a prospective study. Inflamm Bowel Dis. 2013;19:2568-2576
36. Ben-Horin S, Waterman M, Kopylov U, et al. Addition of an immunomodulator to infliximab therapy eliminates antidrug antibodies in serum and restores clinical response of patients with inflammatory bowel disease. Clin Gastroenterol Hepatol. 2013;11:444-447

37. Colombel JF, Sandborn WJ, Rutgeerts P, et al. Adalimumab for maintenance of clinical response and remission in patients with Crohn's disease: the CHARM trial. Gastroenterology. 2007;132:52-65
38. Awni WM, Eckert D, Sharma S, et al. Pharmacokinetics of adalimumab in adult patients with moderately to severely active ulcerative colitis. Gastroenterology. 2013;144:S229
39. Hyams JS, Lerer T, Griffiths A, et al. Outcome following infliximab therapy in children with ulcerative colitis. Am J Gastroenterol. 2010;105:1430-1436
40. Armuzzi A, Pugliese D, Danese S, et al. Infliximab in steroid-dependent ulcerative colitis: effectiveness and predictors of clinical and endoscopic remission. Inflamm Bowel Dis. 2013;19:1065-1072
41. Hayes MJ, Stein AC, Sakuraba A. Comparison of efficacy, pharmacokinetics, and immunogenicity between infliximab mono- versus combination therapy in ulcerative colitis. J Gastroenterol Hepatol. 2014;29:1177-1185
42. Jeuring S, Van Den Heuvel T, Romberg-Camps M, et al. Concomitant use of immunomodulators increases long term response to infliximab in ulcerative colitis-a population-based IBD-SL cohort study. Gastroenterology. 2013;144:S431
43. Garcia-Bosch O, Gisbert JP, Canas-Ventura A, et al. Observational study on the efficacy of adalimumab for the treatment of ulcerative colitis and predictors of outcome. J Crohns Colitis. 2013;7:717-722
44. Rosh J, Ruemmele F, Dubinsky M, et al. Long-term safety of adalimumab in paediatric patients with Crohn's disease. 9th Congress of ECCO, P426; 2014
45. Lichtenstein GR, Rutgeerts P, Sandborn WJ, et al. A pooled analysis of infections, malignancy, and mortality in infliximab- and immunomodulator-treated adult patients with inflammatory bowel disease. Am J Gastroenterol. 2012;107:1051-1063
46. Osterman MT, Sandborn WJ, Colombel JF, et al. Increased risk of malignancy with adalimumab combination therapy, compared with monotherapy, for Crohn's disease. Gastroenterology. 2014;146:941-949
47. Lichtenstein GR, Feagan BG, Cohen RD, et al. Drug therapies and the risk of malignancy in crohn's disease: Results from the TREAT(trademark) registry. Am J Gastroenterol. 2014;109:212-223
48. Herrinton LJ, Liu L, Weng X, et al. Role of thiopurine and anti-TNF therapy in lymphoma in inflammatory bowel disease. Am J Gastroenterol. 2011;106:2146-2153
49. Deepak P, Sifuentes H, Sherid M, et al. T-cell non-Hodgkin's lymphomas reported to the FDA AERS with tumor necrosis factor-alpha (TNF-alpha) inhibitors: results of the REFURBISH study. Am J Gastroenterol. 2013;108:99-105



Chapter 7

General discussion



General discussion

The primary aim of this thesis was to compare the efficacy and safety of top-down and step-up infliximab treatment strategies within pediatric IBD. Additional aims were to develop a novel index that correlates with mucosal inflammation, to study differences in the immune responses upon infliximab or prednisolone treatment, to evaluate the real-world efficacy of adalimumab and to compare the benefits and risks of combining anti-TNF and immunomodulator therapy with anti-TNF monotherapy.

In this chapter the results of this thesis are put into perspective and recommendations for further research are given.

Current scientific data suggest superiority of top-down infliximab treatment

While writing this discussion, the Top-down Infliximab Study in Kids with Crohn's disease (TISKids) study is ongoing (**Chapter 3**) and results at one year follow-up are submitted to a medical scientific journal undergoing peer review. At 52 weeks, the sustained clinical remission rate was higher in the top-down group (19/46 [41%]) than in the step-up group (6/48 [12%], p=0.002). We thus concluded, based on one year follow-up, that top-down infliximab treatment is superior to step-up treatment in inducing and maintaining clinical remission in children with moderate-to-severe CD. In addition, the results from the analysis of patients treated in the Infliximab Top-down Study in Kids with Crohn's diseases (ITSKids) – the randomized controlled trial (RCT) that preceded TISKids, but was stopped prematurely because of a low inclusion rate - suggest a superior short-term effectiveness of top-down treatment: at week 10, patients treated top-down had better clinical outcomes and inflammatory proteins were lower in RNA of blood leukocytes and in serum (Th1 related, neutrophil related, and tissue remodeling proteins – see **Chapter 4**). Besides TISKids and ITSKids, the other available evidence on the relative effectiveness of top-down infliximab treatment (as compared to step-up treatment) also suggests higher effectiveness of top-down treatment: two prospective studies revealed higher clinical and endoscopic remission rates[1,2] and retrospective studies in pediatric CD patients revealed lower relapse rates[3–6] and higher remission rates.[7] We conclude that the results of the TISKids study advocate change of treatment guidelines and to make top-down infliximab treatment the standard treatment strategy in children with moderate-to-severe CD.

Besides short-term effectiveness, it is speculated that starting CD treatment with infliximab from diagnosis may result in better long-term treatment outcomes. Firstly, it may prevent disease complications (e.g. strictures, fistulas, extra intestinal manifestations) and need for surgery. We designed TISKids with a follow-up of 5 years in order to also assess long-term outcomes such as complication rates and need for surgery. Secondly, starting with infliximab from diagnosis may reduce a subsequent need for infliximab maintenance treatment and risk of disease relapses. We test this hypothesis in TISKids by stopping infliximab after the 5th infusion in patients that achieved clinical disease remission –the down part of the top-down strategy. Results at the longer term (up to 5 years) will follow after study completion.

Besides effectiveness, treatment choices should be based on the safety of the available treatment options. As stated in the General Introduction, step-up treatment has several disadvantages: prednisolone treatment has considerable side effects and only rarely induces endoscopic remission[8–10] and EEN necessitates a complete refrain from normal food for a 6-8 weeks which is unpleasant and hard to comply with. Moreover, even after

successful induction of remission, 28% of pediatric CD patients with moderate-to-severe disease activity have shown to require infliximab treatment in the first year after diagnosis.[11] For these patients, the step-up strategy delays the initiation of effective treatment at the cost of side effects while increasing the risk of CD progression and complications.

Infliximab is well tolerated by most patients, but may contribute to serious adverse effects such as acute and delayed infusion reactions, serious infections and opportunistic infections.[8,12–15] In the TISKids study after 52 weeks of follow-up, the number of patients with an adverse event was not significantly different between both treatment groups: 95 adverse events were reported in 22/50 (44%) top-down treated patients and in 28/47 (60%) step-up treated patients (p=0.125).

More uncertainty remains for the risks of malignancies and mortality, which are more rare but serious adverse events. Recent evidence of a large long-term observational registry (DEVELOP) found an increased risk of malignancy for past use of combination treatment with anti-TNF and a thiopurine, while there still is ongoing debate whether the use of a thiopurine alone induces increased risk in pediatric IBD patients.[16] By pooling patients with past use of a thiopurine alone and past use of both a thiopurine and anti-TNF treatment, the investigators found an increased risk for patients that had used a thiopurine (with or without anti-TNF). Authors concluded that infliximab treatment does not increase risk of malignancy while thiopurine treatment does. However, their conclusion is questionable, because in the originally defined patient groups – before pooling of the groups – only past use of both a thiopurine and anti-TNF treatment associated with risk of malignancy, and past use of a thiopurine alone did not. Furthermore the study was sponsored by a pharmaceutical company with a financial interest in infliximab. Findings of a recent prospective multinational observational study, indicate current thiopurine use may be a risk factor for development of lymphomas in pediatric-onset IBD patients, and not infliximab.[17] In summary, evidence suggests that the risk of malignancy seems most increased when using both infliximab and a thiopurine, less increased when using only a thiopurine, and not increased when using only infliximab treatment.

TISKids is designed to also compare the safety of the top-down and step-up treatment strategies both at short term (one year follow-up) and at longer term (5 years of follow-up). TISKids does not have enough statistical power to compare the risk of rare adverse events between treating top-down and step-up.

Endoscopic and symptomatic remission are primary treatment goals

Endoscopic remission should be one of the primary goals for CD treatment, together with symptomatic remission. Literature clearly demonstrates less disease relapses and reduced complication rates when endoscopic remission is achieved.[8,18–20] The combination of both endoscopic and symptomatic remission is essential. Treating toward symptomatic remission only would leave the possibility open for subclinical, lingering inflammation, while treating toward endoscopic remission neglects the symptoms hindering patients. Experts of the Pediatric Inflammatory Bowel Disease Network (PIBDnet) and the pediatric committee of European Crohn's and Colitis Organization (ECCO) agree that clinical trials including pediatric CD patients should use mucosal inflammation as endpoint of treatment effectiveness.[21,22]

Besides in clinical trials, assessment of endoscopic remission should have an important place in routine clinical outcome measurement since it currently is the best measure of disease activity. One step further would be to make endoscopic remission the target of CD treatment. Currently, the treatment guideline – currently being revised - does not recommend to make endoscopic remission the target of treatment.[8] It states that the benefits and risks of intensifying treatment when patients are in clinical remission but not in endoscopic remission are still under debate. Recently, an open-label RCT demonstrated in adult CD patients that using high fecal calprotectin (≥250µg) and C-reactive protein (CRP; ≥5µg/L) versus only clinical disease activity (CDAI≥150) and steroid use as criteria for treatment intensification with anti-TNF treatment, improves the rate of endoscopic remission at one year follow-up.[25] Still an RCT is needed in pediatric CD to demonstrate the relative benefits and risks of treating towards endoscopic remission versus current treatment target in pediatric CD (relieve symptoms, optimize growth, and improve quality of life while minimizing drug toxicity).[8]

Although endoscopic evaluation is the best way to assess endoscopic remission, it is invasive, costly and poses potential risks, including the requirement of anesthesia in children and bowel preparation.[26] Therefore, noninvasive measures of endoscopic remission are desirable for tight monitoring of CD patients. This is why we developed the MINI index (**Chapter 2**). The MINI index identifies children with endoscopic remission with high sensitivity and specificity based on noninvasive parameters. Thereby it provides a means to tightly monitor endoscopic remission, in addition to using endoscopic evaluations. PIBDnet experts propose to use the MINI index as outcome measure in clinical trials in addition to endoscopy or when endoscopy is not feasible.[21] Currently, the accuracy of the MINI index is tested in adult CD patients, so that in the future one measure can be used regardless of patients' age.

It is hypothesized that achieving histological (microscopic) remission may have additional value over endoscopic (macroscopic) CD remission alone.[27] In theory, the less signs of inflammation, the deeper the disease remission, the longer the duration until a relapse of inflammation. This theory might turn out to be true for CD, but remains to be demonstrated. A first step would be to demonstrate association between histological CD activity scores and clinical outcome. This could be tested on patients included in the TISKids trial, as mucosal biopsies were taken at baseline and – in a subgroup of the patients – again at week 10 and 52. However, several difficulties can be expected. Firstly, the accuracy of mucosal biopsies to detect full histologic remission might be low without any detectable macroscopically evident inflammation to guide biopsy taking, and given the patchiness of CD inflammation and the small part of the mucosal surface that can be assessed by taking biopsies. Secondly, only a portion of patients will achieve endoscopic remission and an even smaller portion both histologic and endoscopic remission. This can limit the statistical power when making the comparison.

Translational medicine requires interdisciplinary collaboration

Within the field of CD, the most important research questions concern the disease pathogenesis, the immunological responses to the various CD treatments, the underlying immune responses that result in endoscopic remission and biological markers that can predict endoscopic remission or treatment response in a broader sense. Further insight on these topics can significantly improve CD treatment. Translational research is pivotal to provide this insight.

The critical factor for successful translational medicine is effective interdisciplinary collaboration. According to the European Society for Translational Medicine (EUSTM), translational medicine is created through a collaboration between the three main pillars benchside, bedside and community.[28] The benchside pillar stands for laboratory discoveries translated into practical clinical applications that benefit patients, the bedside pillar for returning clinical findings to research labs to redefine or create new hypothesis-driven research, and the community pillar for healthy populations, patients and medical practitioners that can provide valuable input to translational research. This interdisciplinary collaboration is challenging since in general, the two disciplines – basic research and clinical research – tend to use different study designs, but also differ in many other aspects such as language, culture, interests, research tools and resources. Furthermore, in most universities and research institutes, basic research and clinical research are conducted in separate departments.

ITSKids and TISKids were designed to study differences in the immune responses of newly diagnosed pediatric CD patients to infliximab, prednisolone and/or exclusive enteral nutrition treatment. Both clinical and basic researchers actively worked together on designing an optimal yet feasible trial within the financial budget. Our pilot analyses revealed several genes in blood leukocytes and serum proteins associated with infliximab treatment initiation and with endoscopic remission (**Chapter 4**). Performing these analyses within the larger TISKids cohort will further substantiate these results by the increase in statistical power. Furthermore, it will allow for subgroup analyses, such as comparison between patients that did and did not achieve endoscopic remission within the different treatment groups, and thereby lead to a deeper understanding of the immune responses that drive mucosal CD inflammation.

Successful completion of an investigator-initiated RCT in pediatric CD is challenging

ITSKids and TISKids are investigator-initiated RCTs largely sponsored by the Netherlands Organization for Health Research and Development (ZonMw). ITSKids, the first of the two trials, was seized prematurely due to a low inclusion speed. An important reason for this low inclusion speed was the inadequacy of research funds to cover the costs of trial medication (especially infliximab at a cost of approximately €500.000 [50 Top-down patients, 5 infusions per patient, 2,5 vials per infusion, approximately €800 per vial in 2013]). Most of the funds were spent on PhD salary and other organizational costs. Because infliximab was not paid for in ITSKids when given top-down, many hospitals in the Netherlands and internationally, while initially supportive, in reality could not commit to the study. Only with sponsorship of a pharmaceutical company that provided the required infliximab biosimilar vials free of charge, the project could continue as TISKids with an adequate inclusion speed. Notably, the investigators remained in the lead in every aspect of the trial – the pharmaceutical company had/has no role in the study design, data collection, statistical analysis, interpretation, or writing of the report.

The other challenge we faced, as stated above, was including the required number of patients within a reasonable timeframe. This is a challenge for most RCTs that include pediatric CD patients, as the risk of developing CD during childhood is limited. Getting a reasonable inclusion rate required us to collaborate with multiple hospitals in multiple nations, which complicated trial management. Multicenter research could be facilitated by creating networks of researchers that are properly organized and staffed to efficiently carry out multicenter trials.

Routine registration of treatment outcomes would facilitate observational research

Two additional aims of this thesis focused on assessing benefits and risks of treatment given in routine clinical practice: to evaluate the real-world effectiveness of adalimumab and to compare the benefits and risks of combining anti-TNF and immunomodulator therapy with anti-TNF monotherapy. We used two different approaches to answer these research questions (a retrospective, observational case series and a literature review).

- We evaluated the real world effectiveness and safety of adalimumab therapy for pediatric CD in an observational case series (**Chapter 5**). Because of its observational character, it reflects daily practice and includes a broad spectrum of patients. However, the retrospective design had several limitations. We missed information on endoscopic remission in the patients treated with adalimumab. A score for mucosal inflammation – such as the SESCD – after endoscopic examination was not routinely registered in clinical practice. Furthermore, for part of the patients and timepoints, we had missing data. And thirdly, not all patients were treated in the same way with respect to dosing and concomitant medication, and also the reasons to initiate adalimumab varied.
- We compared the benefits and risks of combining anti-TNF and immunomodulator therapy with anti-TNF monotherapy using a literature review (**Chapter 6**). Unfortunately, there were not many high quality publications to review that make this comparison in pediatric CD and ulcerative colitis (UC) patients. Because of this lack of information, we partly based our conclusions on results of trials performed in adult patients.

If physicians would register treatment outcomes more systematically and completely as part of routine clinical practice, these research questions could have been answered more easily and reliably with observational research. Also, physicians and hospitals would need to register the data uniformly so that data of multiple centers can be analyzed combinedly. Routinely and uniformly registered data could be collected in a national (or international) patient registry. This would make observational research less time consuming and costly. For research purposes, the larger the registry the better (for statistical power) and the more information the better (more information allows conclusions on more diverse topics).

The biggest challenge for registries is getting the required structural finances to sustain their activities. Besides research funds, potential additional funding sources include the government, healthcare payers and for profit companies active in healthcare (such as pharmaceutical companies).

- Both government and healthcare payers are more inclined to contribute financially to registries if all hospitals in the nation participate in the registry. Scientific associations, such as pediatric IBD working group (KICC) of the Dutch Association of Pediatrics (NVK), the Initiative on Crohn and Colitis (ICC) and the IBD working group of the Dutch Association of Gastrointestinal Liver Physicians (NVMDL), can facilitate national collaboration in registries.
- Additionally, both government, healthcare payers and pharmaceutical companies are more inclined to contribute if the registry provides the information they need or desire. For government and healthcare payers this includes healthcare costs and outcomes. For pharmaceutical companies this includes information they (may) need for post-authorization safety studies (PASS) and post-authorization efficacy studies (PAES) required by EMA.

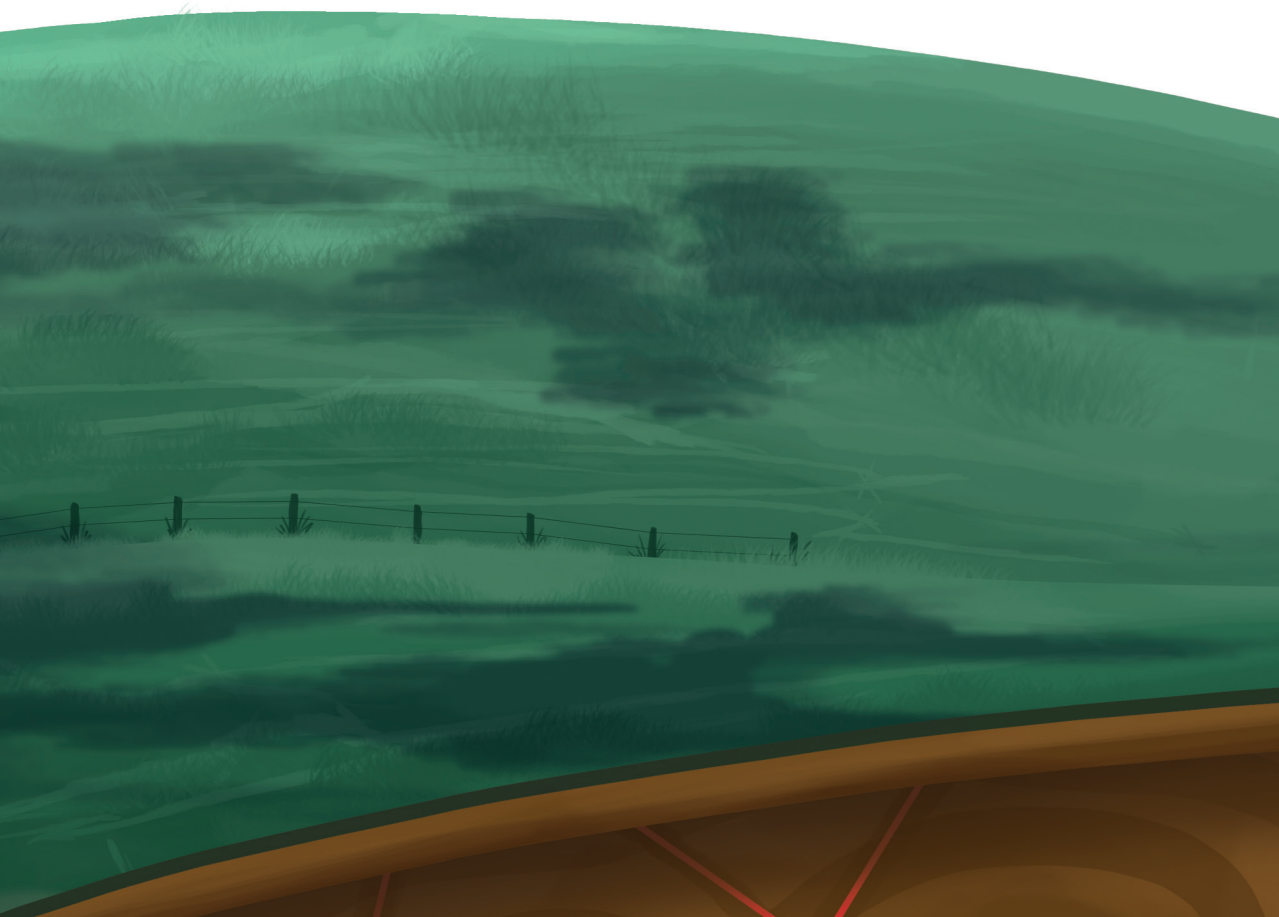
Currently, multiple registries exist, such as the IBDream registry (adult IBD patients), the international ImproveCareNow Registry (both adult and pediatric IBD patients), and the international PIBD-NET Inception Cohort and Safety Registry (pediatric IBD patients). These registries were either founded recently and/or their data was not available for us to use in this thesis.

For pediatric CD patients, routine, systematic and uniform registration of treatment outcomes is even more important than for adult CD patients, because most phase 3 clinical trials for innovative medicines only include adults while pediatric phase 3 trials at best are performed with an average delay of 10 years. Registries could provide high quality data on the real-world effectiveness and safety of these medicines in pediatric patients.

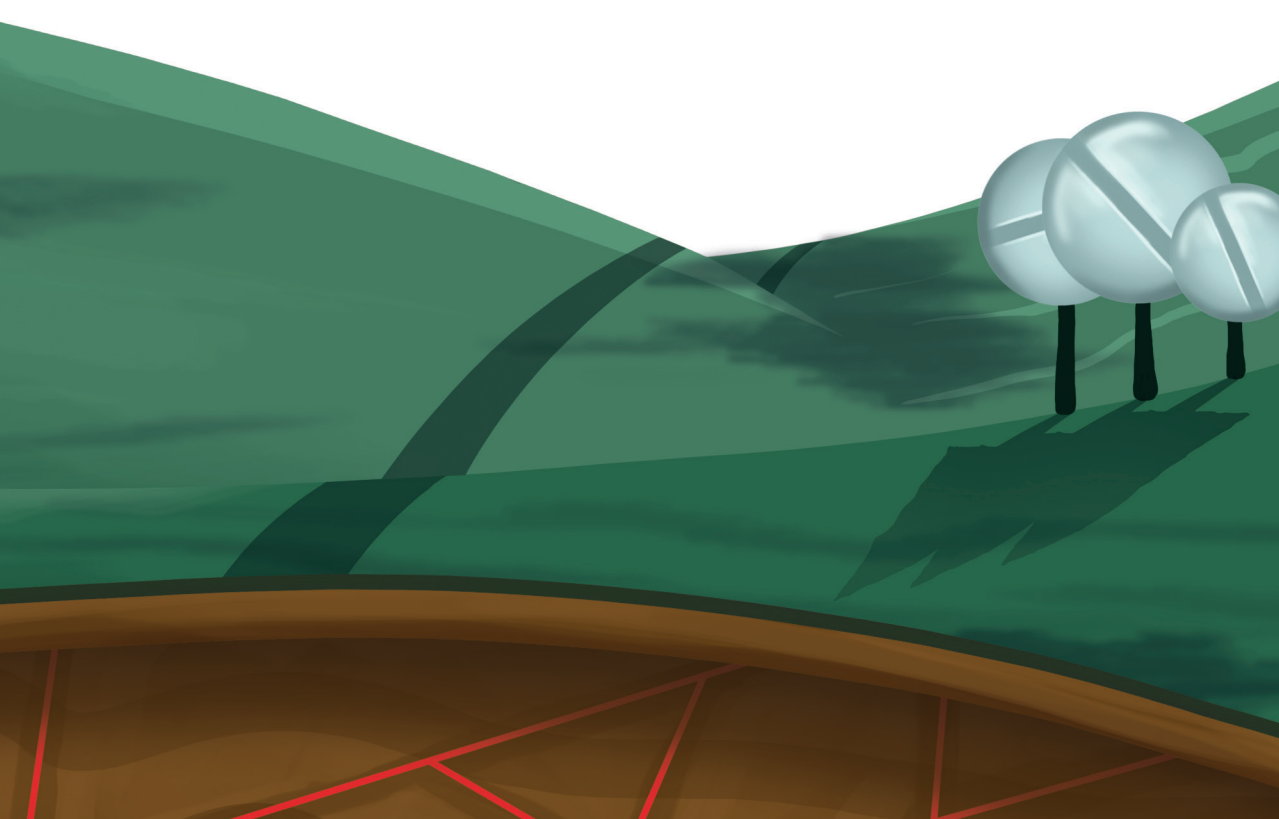
References

1. D'Haens G, Baert F, van Assche G, et al. Early combined immunosuppression or conventional management in patients with newly diagnosed Crohn's disease: an open randomised trial. Lancet 2008;371:660-667.
2. Fan R, Zhong J, Wang ZT, et al. Evaluation of "top-down" treatment of early Crohn's disease by double balloon enteroscopy. World J Gastroenterol 2014;20:14479-14487.
3. Lee JS, Lee JH, Lee JH, et al. Efficacy of early treatment with infliximab in pediatric Crohn's disease. World J Gastroenterol 2010;16:1776-1781.
4. Cozijnsen MA, de Ridder L. Infliximab More Effective in Therapy-Naive Than in Therapy-Refractory Patients. J Pediatr Gastroenterol Nutr 2015;61:e15.
5. Lee YM, Kang B, Lee Y, et al. Infliximab "Top-Down" Strategy is Superior to "Step-Up" in Maintaining Long-Term Remission in the Treatment of Pediatric Crohn Disease. J Pediatr Gastroenterol Nutr 2015;60:737-743.
6. Kim MJ, Lee JS, Lee JH, et al. Infliximab therapy in children with Crohn's disease: a one-year evaluation of efficacy comparing "top-down" and "step-up" strategies. Acta Paediatr 2011;100:451-455.
7. Walters TD, Kim MO, Denson LA, et al. Increased effectiveness of early therapy with anti-tumor necrosis factor-alpha vs an immunomodulator in children with Crohn's disease. Gastroenterology 2014;146:383-391.
8. Ruemmele FM, Veres G, Kolho KL, et al. Consensus guidelines of ECCO/ESPGHAN on the medical management of pediatric Crohn's disease. J Crohns Colitis 2014;8:1179-1207.
9. Lichtenstein GR, Feagan BG, Cohen RD, et al. Serious infections and mortality in association with therapies for Crohn's disease: TREAT registry. Clin Gastroenterol Hepatol 2006;4:621-630.
10. Lichtenstein GR. Approach to Steroid-Dependent and Steroid-Refractory Crohn's Disease. J Pediatr Gastroenterol Nutr 2001;33:S27-S35.
11. Markowitz J, Hyams J, Mack D, et al. Corticosteroid therapy in the age of infliximab: acute and 1-year outcomes in newly diagnosed children with Crohn's disease. Clin Gastroenterol Hepatol 2006;4:1124-1129.
12. Hyams J, Crandall W, Kugathasan S, et al. Induction and maintenance infliximab therapy for the treatment of moderate-to-severe Crohn's disease in children. Gastroenterology 2007;132:863-873.
13. Hyams JS, Griffiths A, Markowitz J, et al. Safety and efficacy of adalimumab for moderate to severe Crohn's disease in children. Gastroenterology 2012;143:365-74.e2.
14. Rahier JF, Ben-Horin S, Chowers Y, et al. European evidence-based Consensus on the prevention, diagnosis and management of opportunistic infections in inflammatory bowel disease. J Crohns Colitis 2009;3:47-91.
15. Veereman-Wauters G, de Ridder L, Veres G, et al. Risk of infection and prevention in pediatric patients with IBD: ESPGHAN IBD Porto Group commentary. J Pediatr Gastroenterol Nutr 2012;54:830-837.
16. Hyams JS, Dubinsky MC, Baldassano RN, et al. Infliximab Is Not Associated With Increased Risk of Malignancy or Hemophagocytic Lymphohistiocytosis in Pediatric Patients With Inflammatory Bowel Disease. Gastroenterology 2017;152:1901-1914.e3.
17. Joosse ME, Aardoom MA, Kemos P, et al. Malignancy and mortality in paediatric-onset inflammatory bowel disease: a 3-year prospective, multinational study from the paediatric IBD Porto group of ESPGHAN. Aliment Pharmacol Ther 2018;48:523-537.

18. Kierkus J, Dadalski M, Szymanska E, et al. The impact of infliximab induction therapy on mucosal healing and clinical remission in Polish pediatric patients with moderate-to-severe Crohn's disease. Eur J Gastroenterol Hepatol 2012;24:495-500.
19. Vatn MH. Mucosal healing: impact on the natural course or therapeutic strategies. Dig Dis Basel Switz 2009;27:470-475.
20. Peyrin-Biroulet L, Ferrante M, Magro F, et al. Results from the 2nd Scientific Workshop of the ECCO. I: Impact of mucosal healing on the course of inflammatory bowel disease. J Crohns Colitis 2011;5:477-483.
21. Turner D, Griffiths AM, Wilson D, et al. Designing clinical trials in paediatric inflammatory bowel diseases: a PIBDnet commentary. Gut 2020;69:32-41.
22. Ruemmele FM, Hyams JS, Otley A, et al. Outcome measures for clinical trials in paediatric IBD: an evidence-based, expert-driven practical statement paper of the paediatric ECCO committee. Gut May 2014.
23. European Medicines Agency - Committee for Medicinal Products for Human Use (CHMP). Guideline on the development of new medicinal products for the treatment of Crohn's Disease. June 2018.
24. Williet N, Sandborn WJ, Peyrin-Biroulet L. Patient-reported outcomes as primary end points in clinical trials of inflammatory bowel disease. Clin Gastroenterol Hepatol 2014;12:1246-1256.
25. Colombel J-F, Panaccione R, Bossuyt P, et al. Effect of tight control management on Crohn's disease (CALM): a multicentre, randomised, controlled phase 3 trial. Lancet Lond Engl 2018;390:2779-2789.
26. Schoepfer AM, Beglinger C, Straumann A, et al. Fecal calprotectin correlates more closely with the Simple Endoscopic Score for Crohn's disease (SES-CD) than CRP, blood leukocytes, and the CDAI. Am J Gastroenterol 2010;105:162-169.
27. Pai RK, Geboes K. Disease activity and mucosal healing in inflammatory bowel disease: a new role for histopathology? Virchows Arch Int J Pathol 2018;472:99-110.
28. Cohrs RJ, Martin T, Ghahramani P, et al. Translational Medicine definition by the European Society for Translational Medicine. Eur J Mol Clin Med 2014;2:86-88.



Addenda



Summary

The primary aim of this thesis was to compare the efficacy and safety of the experimental top-down treatment strategy with the conventional step-up treatment strategy in children and adolescents with Crohn's disease (CD). In the top down infliximab treatment strategy, patients start with infliximab – a highly effective treatment option – instead of reserving it for those that don't respond to other treatment options, which is the conventional step-up treatment strategy. Additional aims were to develop a novel index that correlates with mucosal CD inflammation, to study differences in the immune responses upon infliximab or prednisolone treatment, to evaluate the real-world efficacy of adalimumab and to compare the benefits and risks of combining anti-tumor necrosis factor (TNF) and immunomodulator therapy with treating only with anti-TNF.

In **Chapter 2**, we report the accuracy of a novel mucosal inflammation, non-invasive (MINI) index that strongly correlates with mucosal inflammation and that can accurately assess endoscopic remission. The MINI index was generated on a large prospective cohort of pediatric Crohn's disease (CD) patients and was validated on three independent prospective cohorts. A cutoff of <8 best balanced sensitivity and specificity in reflecting endoscopic remission. A cutoff of <6 had a higher positive predictive value (PPV) to reflect endoscopic remission (86%) and ≥8 was most accurate to diagnose mucosal inflammation (PPV 90%). The added benefit of the MINI index over measurement of fecal calprotectin alone was small but significant, especially for patients with concentrations of fecal calprotectin from 100 to 599 mg/g. The MINI index can be used both in clinical practice for tight monitoring of mucosal inflammation and facilitating appropriate selection of children for ileocolonoscopy, and serve as an outcome measure in clinical trials instead of, or in addition to ileocolonoscopy to increase feasibility and enrollment rates.

Chapter 3 describes the study protocol of the international multicenter open-label randomized controlled trial (RCT) – Top-down Infliximab Study in Kids with CD (TISKids) – we set up to compare the efficacy and safety of two treatment strategies: the top-down infliximab treatment strategy and the conventional step-up treatment strategy. The chapter describes the study design and the outcome parameters that are measured. While writing this thesis, the study is ongoing. In November 2018 we've included the last patient and after an initial follow-up of 1 year, we'll be able to assess and report on the primary and secondary study outcomes. Currently, the manuscript with the results at one year follow-up is submitted for publication. The study will continue for a total follow-up duration of 5 years.

Chapter 4 describes the results of the analyses of the immune responses of patients treated in the international multicenter open-label RCT Infliximab Top-down Study in Kids with CD (ITSKids). We aimed to study the differences in the immune responses of newly diagnosed pediatric CD patients to infliximab and prednisolone treatment, as the underlying immune mechanisms by which infliximab treatment restores homeostasis are largely unknown. We demonstrated that infliximab treatment reduces systemic and intestinal CD inflammation more effectively than prednisolone treatment does. We identified three dominant pathways that associate with infliximab treatment and with endoscopic remission: Firstly, infliximab treatment homogeneously reduces the expression and concentration of Th1 related genes and proteins. Secondly, infliximab treatment reduces the expression of S100 calcium binding proteins and the concentrations of several neutrophil chemo attractants. Thirdly, infliximab treatment reduces the transcription and concentration of proteins involved in tissue remodeling. Furthermore, we identified these pathways in analyses of patient blood rather than in analyses of mucosal biopsies, thereby proving it possible to use patients' blood to monitor immune responses to infliximab treatment.

Chapter 5 describes the results of a nationwide, observational case series in which we assessed the safety and effectiveness of adalimumab therapy in pediatric CD patients that previously failed infliximab treatment in a real-world setting. Adalimumab induced remission in two-thirds of the infliximab refractory patients, of whom 50% maintained remission up to two years. Adalimumab failure occurred in 24% within 1 year and in 42% within two years. Only one serious adverse event occurred. The results corresponded well with literature. Additionally, within our cohort, we demonstrated that primary non-responders to infliximab had a higher risk for adalimumab failure than those who had lost response to infliximab. Furthermore, we found a trend for a relatively higher remission rate and lower failure rate in patients who had developed antibodies toward infliximab, compared to those without.

Chapter 6 is a review of scientific literature in which we compare the benefits and risks of combining anti-TNF treatment with immunomodulator therapy based on published evidence. Although almost all studies in pediatric patients with inflammatory bowel disease did not find increased benefit for combination versus monotherapy, the available evidence in children is scarce. Several adult trials have shown higher treatment efficacy in patients receiving infliximab combination therapy, especially for induction of remission. However, the treatment benefit is modest and might be overcome by optimization of infliximab therapy dosing. Moreover, combination therapy does seem to increase the risk of malignancy. Thus, we concluded that, although evidence of increased effectiveness is lacking in pediatric CD, based on adult CD literature, it is likely that combination therapy is more effective at the cost of increased risk of adverse effects.

Samenvatting

De ziekte van Crohn (ZvC) is een chronische darmontsteking die kan voorkomen in het hele spijsverteringskanaal (van 'mond tot kont'). Meestal is de ontsteking gelokaliseerd in het laatste gedeelte van de dunne darm (het terminale ileum), in de dikke darm of zowel in de dunne als dikke darm. De ontsteking kan resulteren in vernauwingen van de darm en in de vorming van fistels (niet-natuurlijk kanalen tussen twee lichaamsholten of tussen een lichaamsholte en de huid). In een klein deel van de patiënten kunnen ook ontstekingen buiten het spijsverteringskanaal voorkomen. Aangezien de precieze oorzaak van de ziekte niet bekend is, is de darmontsteking momenteel niet te genezen en daarom chronisch van aard. Behandeling van de ZvC kan de ontsteking onderdrukken, maar de ontsteking kan na verloop van tijd weer terugkomen.

Door de ontsteking kunnen patiënten last krijgen van maag-darm klachten (buikpijn, diarree, bloedige ontlasting), vermoeidheid, gewichtsverlies en – bij kinderen – een verminderde lengtegroei. De ontsteking kan resulteren in verhoogde concentraties CRP (naar het Engelse *C-reactive protein*) in het bloed van patiënten en een verhoogde bezinkingssnelheid van rode bloedcellen (erythrocyten). In de ontlasting van patiënten kunnen verhoogde concentraties van het ontstekingseiwit calprotectine gevonden worden. De diagnose ZvC wordt definitief gesteld als de arts tijdens een kijkoperatie (endoscopie) de kenmerkende ontsteking van de ZvC heeft gezien en de patholoog in biopten (kleine hapjes darmweefsel genomen tijdens kijkonderzoek) van de darm eveneens de kenmerkende ontstekingsreactie heeft gezien.

Een ziekte die lijkt op de ZvC is colitis ulcerosa. Samen vormen zij de twee meest voorkomende vormen van chronische darmontsteking, ook wel IBD genoemd naar het Engelse *Inflammatory Bowel Disease*. Colitis ulcerosa verschilt van de ZvC doordat bij colitis ulcerosa alleen de dikke darm ontstoken is en er geen vernauwingen van de darm en fistelvorming voorkomen. Kinderartsen in Nederland behandelen jaarlijks ongeveer drieduizend IBD patiënten.

In mijn promotieonderzoek vergeleek ik de effectiviteit en veiligheid van twee behandelstrategieën voor kinderen met de ZvC: de experimentele top-down infliximab behandelstrategie en de conventionele step-up behandelstrategie. In de top-down infliximab behandelstrategie starten patiënten met de ZvC direct na diagnose met infliximab behandeling, terwijl het nu gebruikelijk is om infliximab te reserveren voor patiënten die niet (langer) reageren op andere behandelingsopties - de conventionele step-up behandelstrategie.

Daarnaast had mijn promotietraject als doelstellingen om

- een nieuwe ziekte score te ontwikkelen die goed correleert met de ontsteking van de darmwand,
- om de verschillen in immuunrespons te bestuderen tussen behandeling met infliximab en prednisolon in kinderen met de ZvC,
- om de effectiviteit van adalimumab bij de behandeling van kinderen met de ZvC in de klinische praktijk te evalueren en
- om de voordelen en risico's van het gecombineerd behandelen met een anti-tumornecrosefactor (TNF) en een immunomodulator te vergelijken met behandelen met alleen anti-TNF.

De volgende alinea's vatten de hoofdstukken van mijn proefschrift samen.

In **hoofdstuk 2** beschrijven we een nieuwe, niet-invasieve ziektescore – de MINI-index – die sterk correleert met de ontsteking in de darmwand van patiënten met de ZvC. Deze index kan nauwkeurig aangeven in welke patiënten de darmwand hersteld is en in welke patiënten (nog) niet. We hebben de MINI-index opgesteld op basis van een groot prospectief cohort van kinderen met de ZvC en gevalideerd op drie onafhankelijke prospectieve cohorten. De toegevoegde waarde van de MINI-index ten opzichte van alleen een meting van fecaal calprotectine – een biomarker voor ontsteking in de darmwand – is klein maar significant. Vooral voor patiënten met concentraties van fecaal calprotectine tussen 100 en 599 mg/g draagt de MINI-index diagnostisch bij. Artsen kunnen de MINI-index in de klinische praktijk gebruiken voor het monitoren van de mate van ontsteking van de darmwand en het selecteren van patiënten voor nader darmonderzoek met ileocolonoscopie. Daarnaast kunnen onderzoekers de MINI-index gebruiken in klinische onderzoeken voor het meten van uitkomsten van zorg voor kinderen met de ZvC in plaats van, of als aanvulling op ileocolonoscopie. Omdat het scoren van de MINI-index minder belastend is voor patiënten dan ileocolonoscopie, vormt deze uitkomstmaat een minder grote drempel tot deelname aan klinisch onderzoek.

Hoofdstuk 3 beschrijft het TISKids onderzoek – *Top-down Infliximab Study in Kids with Crohn's disease*. Dit is een internationaal multicenter open-label gerandomiseerd onderzoek waarin we de effectiviteit en veiligheid van twee behandelstrategieën met elkaar vergelijken: de top-down infliximab behandelstrategie en de conventionele step-up behandelstrategie. We verwachten namelijk dat door infliximab direct na de diagnose voor te schrijven, er een hogere kans is op herstel van de darmwand. Dit kan resulteren in minder opvlammingen van de ziekte, minder complicaties (zoals darmvernauwingen en fistels), minder ziekenhuisopnames en minder noodzaak tot operatieve verwijdering van delen van de darm. Het hoofdstuk beschrijft de opzet van het onderzoek en de uitkomsten

die we meten. Het onderzoek loopt momenteel nog. In november 2018 is de laatste patiënt geïncludeerd en in november 2019 voltooide de laatste patiënt het eerste follow-up jaar. De resultaten over het eerste jaar follow-up jaar zijn inmiddels geanalyseerd en beschreven in een conceptartikel dat is ingediend bij een wetenschappelijk tijdschrift. De studie zal doorlopen tot alle patiënten 5 jaar gevolgd zijn, zodat we ook de effecten van de twee behandelstrategieën op lange termijn kunnen vergelijken.

Hoofdstuk 4 beschrijft de resultaten van een analyse van de immuunresponsen van patiënten die werden behandeld in het ITSKids onderzoek - *Infliximab Top-down Study in Kids with Crohn's disease* – de voorloper van de TISKids studie. Het doel van deze analyse was om de verschillen in de immuunresponsen op behandeling met infliximab en prednisolon te bestuderen in het bloed van nieuw gediagnosticeerde kinderen met de ZvC. Er was namelijk weinig bekend over de wijze waarop infliximab de darmwand van kinderen met de ZvC herstelt en welke immuunresponsen hieraan ten grondslag liggen. In dit hoofdstuk tonen we aan dat infliximab zowel de systemische ontsteking in het bloed als de ontsteking in de darmwand sterker vermindert dan prednisolon. We associëren drie dominante immuunresponsen met infliximab behandeling en herstel van de darmwand. Ten eerste vermindert infliximab de expressie en concentratie van T-helpercellen type 1 (Th1)-gerelateerde genen en eiwitten. Daarnaast vermindert infliximab de expressie van S100 calciumbindende eiwitten en concentraties van chemokinen die neutrofielen rekruteren naar plekken van ontsteking. Ten derde vermindert infliximab de expressie en concentratie van eiwitten die betrokken zijn bij remodellering van weefsel. We tonen in dit hoofdstuk ook aan dat, naast biopten uit de darmwand, bloed gebruikt kan worden voor onderzoek naar de immuunresponsen bij patiënten met de ZvC. Het afnemen van bloed is minder belastend voor patiënten dan het afnemen van darmbiopten, wat toekomstig onderzoek naar immuunresponsen kan vergemakkelijken.

Hoofdstuk 5 beschrijft de resultaten van een landelijk, observationeel onderzoek, waarin we de veiligheid en effectiviteit evalueren van adalimumab behandeling bij kinderen met de ZvC die niet (langer) reageren op infliximab behandeling. Adalimumab – dat gericht is tegen hetzelfde ontstekingseiwit als infliximab (Tumornecrosefactor, TNF) – bracht tweederde van deze patiënten in remissie. Na twee jaar behandeling waren de helft van deze patiënten nog steeds in remissie. In een kwart van de patiënten die initieel goed reageerden op adalimumab, verloor adalimumab zijn werking binnen één jaar na start van de behandeling en bij 42% van de patiënten binnen twee jaar. In de onderzochte patiëntenpopulatie trad slechts één ernstige bijwerking op. Deze resultaten komen overeen met eerder gepubliceerde onderzoeken. Daarnaast tonen we in dit onderzoek aan dat het succes van adalimumab afhangt van hoe patiënten reageerden op infliximab (wanneer zij dit eerder voorgeschreven kregen): adalimumab is minder kansrijk als infliximab nooit succesvol is

geweest en meer kansrijk als infliximab aanvankelijk de ZvC in remissie bracht, maar na verloop van tijd zijn werkzaamheid verloor. Ook waren er (niet-significante) aanwijzingen dat adalimumab effectiever is als het eerdere verlies van respons op infliximab gepaard ging met de vorming van antilichamen tegen infliximab, dan wanneer dit hiermee niet gepaard ging.

Hoofdstuk 6 vat de wetenschappelijke literatuur samen die de effectiviteit en veiligheid van het combineren van anti-TNF-behandeling met een immunomodulator vergelijkt met die van anti-TNF monotherapie. Er zijn weinig onderzoeken gepubliceerd die deze vergelijking maken in kinderen met de ZvC of colitis ulcerosa (CU) – een andere chronische darmontsteking. De onderzoeken die gepubliceerd zijn vonden geen hogere effectiviteit voor combinatietherapie, maar hun gezamenlijke bewijslast is onvoldoende voor betrouwbare conclusies. Onder volwassen patiënten met de ZvC of CU toonden verschillende onderzoeken wél aan dat combinatietherapie een grotere kans biedt op remissie dan anti-TNF monotherapie. Het verschil is echter bescheiden en zou mogelijk ook bereikt kunnen worden door optimalisatie van de dosering van anti-TNF behandeling. Bovendien lijkt combinatietherapie het risico op maligniteiten te vergroten. We concluderen dat combinatietherapie waarschijnlijk effectiever is voor kinderen met de ZvC of CU dan anti-TNF monotherapie, maar ook een hoger risico geeft op bijwerkingen. Dit concluderen we op basis van onderzoeken bij volwassenen, aangezien er weinig onderzoek is gedaan naar kinderen.

Co-authors' affiliations

Name	Affiliation
Byung-Ho Choe	Kyungpook National University Children's Hospital, School of Medicine, Kyungpook National University, Daegu, Korea
Yon Ho Choe	Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea
Gerard Damen	Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands
Vera Duif	Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands
Johanna C. Escher	Erasmus Medical Center-Sophia Children's Hospital, Rotterdam, The Netherland
Anne Griffiths	Hospital for Sick Children, Toronto, Canada
Thalia Hummel	Medisch Spectrum Twente, Enschede, The Netherlands
Jeffrey Hyams	Connecticut Children's Medical Center, Hartford, CT, USA
Maria M.E. Jongsma	Erasmus Medical Center-Sophia Children's Hospital, Rotterdam, The Netherland
Ben Kang	Kyungpook National University Children's Hospital, School of Medicine, Kyungpook National University, Daegu, Korea
Angelika Kindermann	Emma Children's Hospital-Academic Medical Center, Amsterdam, The Netherlands
Freddy Kokke	Wilhelmina Children's Hospital-University Medical Center, Utrecht, The Netherlands
Sibylle Koletzko	Dr. von Hauner Children's Hospital, LMU, Munich, Germany
Javier Martín-de-Carpi	Hospital Sant Joan de Déu, Barcelona, Spain
Tim de Meij	VU University Medical Center, Amsterdam, The Netherlands
Anita Van den Neucker	Maastricht University Medical Center, Maastricht, The Netherlands
Obbe Norbruis	Isala Hospital, Zwolle, The Netherlands
Johanna Oudshoorn	Gelre Hospitals, Apeldoorn, The Netherlands
Rolf Pelleboer	Catharina Hospital, Eindhoven, The Netherlands
Merel van Pieterson	Erasmus Medical Center-Sophia Children's Hospital, Rotterdam, The Netherland
Patrick van Rheenen	Beatrix Children's Hospital-University Medical Center Groningen, Groningen, The Netherlands
Lissy de Ridder	Erasmus Medical Center-Sophia Children's Hospital, Rotterdam, The Netherland

Name	Affiliation
Frank M. Ruemmele	Université Paris Descartes – Sorbonne Paris Cité, APHP- Hôpital Necker Enfants Malades, Service de Gastroentérologie, Paris, France
Richard K. Russell	Royal Hospital for Children, Glasgow, Scotland
Janneke N. Samsom	Erasmus University Medical Centre -Sophia Children's Hospital, Rotterdam, The Netherlands
Maaike Schaart	Willem-Alexander Kinderziekenhuis-Leiden University Medical Center, Leiden, The Netherlands
Assaf Ben Shoham	Clalit Health Services, Jerusalem, Israel
Dan Turner	Shaare Zedek Medical Center, The Hebrew University of Jerusalem, Israel
Thomas Walters	Hospital for Sick Children, Toronto, Canada
Herbert van Wering	Amphia Hospital, Breda, The Netherlands

About the author

Martinus Arend (Maarten) Cozijnsen was born on the 12th of January in Apeldoorn, the Netherlands.

He completed his secondary school education at the Gymnasium Apeldoorn in the summer of 2004. Afterwards, he started with his medical training at the medical faculty of the Vrije Universiteit (VU) in Amsterdam. He graduated his 'doctoraal examen' in 2010 and finished his internships in 2012 after which he obtained his medical degree.

In February 2013 he started with his PhD program at the department Pediatric Gastroenterology of the Erasmus Medical Center-Sophia Children's Hospital in Rotterdam, under the supervision of Prof. dr. Johanna Escher and Dr. Lissy de Ridder of the department of pediatric gastroenterology and Dr Janneke Samsom of the Laboratory of Pediatrics. His PhD program focused on improving the treatment of children and adolescents with Crohn's disease. He facilitated the start and was project manager of the investigator-initiated international multicenter randomized controlled trial ITSKids, financed by a research grand of The Netherlands Organization for Health Research and Development (ZonMw). Due to a low inclusion rate, he and his research team terminated ITSKids and initiated TISKids with additional support of Pfizer and Crocokids. Results of the primary endpoint are expected to be published in 2020.

Besides starting and managing prospective trials, Maarten initiated and published a variety of research initiatives: retrospective case studies, (systematic) literature reviews, translational research, and developed and validated a clinical disease index. In 2017 he received the UEG National Scholar Award for best abstract for his abstract regarding the development and validation of the MINI-index. Between 2014 and 2016, Maarten was board member of the Young Initiative of Crohn and Colitis (Y-ICC), a national network for young scientists and physicians focusing on inflammatory bowel diseases.

From July 2017 to June 2020, Maarten worked as a strategy consultant in healthcare at SiRM – Strategies in Regulated Markets – with a focus on medicines, medical specialist care and acute care. From June 2020, he works at Dutch Hospital Data as project leader to provide information products to hospital managers, healthcare workers and regulators.

List of publications

Included in this thesis

Cozijnsen MA, Swagemakers S, Raatgeep HC, Simons-Oosterhuis Y, de Ridder L, Samsom JN. Infliximab has more impact than prednisolone on leukocyte RNA expression and serum inflammatory protein concentrations in peripheral blood of therapy naïve pediatric Crohn's disease patients. Preparing for publication

Cozijnsen MA, Ben Shoham A, Kang B, Choe BH, Choe YH, Jongsma MME, Russell RK, Ruemmele FM, Escher JC, de Ridder L, Koletzko S, Martín-de-Carpi J, Hyams J, Walters T, Griffiths A, Turner D. Development and Validation of the Mucosal Inflammation Noninvasive Index For Pediatric Crohn's Disease. Clin Gastroenterol Hepatol. 2020 Jan;18(1):133-140.

Cozijnsen MA, Samsom JN, de Ridder L. Anti-Tumour Necrosis Factor Therapy for Pediatric Crohn's Disease: Improved Benefits Through Treatment Optimisation, Deeper Understanding of Its Risks, and Reduced Costs due to Biosimilar Availability. Pediatr Drugs. 2018 Feb;20(1):19-28

Cozijnsen MA, van Pieterson M, Samsom JN, Escher JC, de Ridder L. Top-down Infliximab Study in Kids with Crohn's disease (TISKids): an international multicentre randomised controlled trial. BMJ Open Gastroenterol. 2016 Dec 22;3(1):e000123

Cozijnsen MA, Escher JC, Griffiths A, Turner D, de Ridder L.Benefits and risks of combining anti-tumor necrosis factor with immunomodulator therapy in pediatric inflammatory bowel disease. Inflamm Bowel Dis. 2015 Apr;21(4):951-61

Cozijnsen MA, Duif V, Kokke F, Kindermann A, van Rheenen P, de Meij T, et al. Schaart M, Damen G, Norbruis O, Pelleboer R, Van den Neucker A, van Wering H, Hummel T, Oudshoorn J, Escher J, de Ridder L; Dutch PIBD Working Group Kids with Crohn and Colitis. Adalimumab therapy in children with Crohn disease previously treated with infliximab.J Pediatr Gastroenterol Nutr. 2015 Feb;60(2):205-10

Not included in this thesis

Cozijnsen MA, Turner D. Optimizing fecal calprotectin accuracy - more than one way to skin that cat! Clin Gastroenterol Hepatol. 2019 Jun 20 [Epub ahead of print]

Cozijnsen L, van der Zaag-Loonen HJ, **Cozijnsen MA**, Braam RL, Heijmen RH, Bouma BJ, Mulder BJM. Differences at surgery between patients with bicuspid and tricuspid aortic valves. Neth Heart J. 2019 Feb;27(2):93-99

Offringa M, Newton R, **Cozijnsen MA**, Nevitt SJ. Prophylactic drug management for febrile seizures in children. Cochrane Database Syst Rev. 2017 Feb 22;2:CD003031

Cozijnsen L, van der Zaag-Loonen HJ, **Cozijnsen MA**, Braam RL, Heijmen RH, Mulder BJ. Knowledge of native valve anatomy is essential in follow-up of patients after aortic valve replacement. Int J Cardiol. 2016 Dec 15;225:172-176

Cozijnsen MA, de Ridder L. Infliximab More Effective in Therapy-Naïve Than in Therapy-Refractory Patients. J Pediatr Gastroenterol Nutr. 2015 Sep;61(3):e15

Cozijnsen MA, Cozijnsen L, Maas AC, Bakker-de Boo M, Bouma BJ. A ventricular septal defect with a giant appendiform aneurysm of the membranous septum. Neth Heart J. 2013 Mar;21(3):152-4

PhD portfolio

Summary of PhD training and activities	
Name PhD student: M.A. Cozijnsen	PhD period: 2013-2017
Erasmus MC department: Pediatrics, division gastroenterology	Promotor: Prof. dr. J.C. Escher
Research school: Molecular Medicine Postgraduate School (MolMed)	Co-promotor: Dr L. de Ridder and dr. J. Samsom

General academic skills courses	**Year**	**Workload (ECTS)**
Postdoc Career Development Initiative: Employability outside academia	2016	1
University Medical Center Utrecht / Julius Institute: Cochrane course systematic review	2015	0,5
Integrity in scientific research	2014	0,3
Biomedical English writing	2014	3
Systematic literature search and endnote	2014	1
Basiscursus regelgeving en organisatie voor klinisch onderzoekers' (BROK)	2013	1
MolMed - Basis introduction course on SPSS	2013	1
CPO mini course	2013	0,3
Specific research skills courses		
Partek course on microarray and next generation sequencing	2015	0,7
Ingenuity pathway analysis course	2015	0,5
Infection & immunity summer course	2013	4
National conferences		
Initiative on Crohn and Colitis (ICC) day 2016 "Life Cycle IBD"	2016	0,3
Symposium "IBD Binnenste Buiten"	2015	0,3
ICC day 2014 "Leven in de darm & leven met de darm"	2014	0,3
Sophia Research Days - Across borders (poster)	2014	1

General academic skills courses	Year	Workload (ECTS)
Vena - De arts en wetenschapper als ondernemer: Koersen op “waardevolle” innovaties	2014	0,3
Dutch Society for Immunology - Anniversary Congress 2014	2014	0,3
Symposium Kids with Crohn’s and colitis (oral presentation)	2014	1
Young-ICC symposium	2013-2016	0,3
5th Symposium & Master Classes on Mucosal Immunology	2013	0,5
International conferences		
United European Gastroenterology (UEG) Week (oral presentation)	2017	1
European Crohn’s and Colitis Organization (ECCO) congress Amsterdam	2016	1
Pediatric IBD congress	2015	1
ECCO congress Barcelona	2015	1
Third international symposium on PIBD, Rotterdam	2014	1
European Society for Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) congress Jerusalem (oral presentation)	2014	1
ECCO congress Kopenhagen (poster)	2014	1
Clinical Exchange Program Sponsored by MSD	2013	0,3
ESPGHAN Young Investigators Research Forum (oral presentation)		
Other		
Board member Young-ICC	2014	3
Weakly meeting laboratory of pediatrics	2013-2017	2
Journal club laboratories of pediatrics and hematology	2014-2015	1
Pharmacology research meetings	2013	1
Awards		
UEG National Scholar Award	2017	
Total		**31,9**