Optimization and De-Escalation of Biologicals in Patients with Crohn's Disease

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COLOPHON

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Chapter 1

General introduction

Crohn's disease [CD] is a chronic inflammatory and intermitting immune-mediated disease of the gastrointestinal tract. Together with ulcerative colitis [UC], it encompasses the term ''inflammatory bowel disease [IBD]'' which is characterized by alternating periods of remission and recurrent periods of inflammation.[1]

Inflammation is typically asymmetrical, segmental and transmural and starts with superficial aphthous ulcers that may develop into deep extensive ulcers that could lead to disease complications, including intestinal strictures and penetrating complications [abscesses and fistulas].[2-4] Perianal fistulizing CD [pCD] is the most common fistula and is a major problem as up to half of the patients are affected by these fistulas leading to considerable morbidity and decreased quality of life [5].

During the course of CD, which may begin in young adulthood, the inflammatory process is primarily localized in the terminal ileum and colon, however CD can affect any segment of the gastrointestinal tract. Furthermore, CD may cause extraintestinal manifestations of the joints, the eyes, the skin, or the liver. Symptoms commonly include abdominal pain, chronic diarrhea, weight loss and fatigue.

Even though knowledge regarding the etiology of CD has increased significantly over the past decades, the disease pathogenesis is still not fully understood. It is believed that dysregulated immune responses result from a complex interplay between environmental factors, altered gut microbiota, immune responses and genetic susceptibility leading to a very heterogeneous clinical presentation and treatment response between patients.[6-9]

Since CD is incurable, the therapeutic goal is to achieve quiescent disease ['remission'] and to reduce the risk of relapse to optimize quality of life.[10] Currently, multiple treatment options with different mechanisms of action are available including corticosteroids [prednisolone, budesonide], immunomodulators [thiopurines and methotrexate], biologicals [anti-tumour necrosis factor (TNF), ustekinumab and vedolizumab] and small molecules compounds [JAK-STAT inhibitors]. Different treatment options are available for induction and maintenance of remission in patients with UC, however are not registered for patients with CD including mesalazine [aminosalycilates] and tofacitinib and filgotinib [JAK-STAT inhibitors].

Biologicals

Biologicals are antibodies produced by biological rather than chemical processes. The era of biologicals started with the approval of anti- TNF therapy in 1998. Anti-TNF therapy, including infliximab and adalimumab, have the most extensive history of evidence regarding effectiveness and safety, are administered systemically, either intravenously or subcutaneously, and are often the least expensive due to biosimilars [generic anti-TNF treatments].[11] Anti-TNF antibodies have multiple mechanisms of action including the neutralization of TNFα, which play a major key role in inflammation.[12-15] TNFα, a pro-inflammatory cytokine, is directly involved in the pathogenesis of CD as it is highly expressed in the intestinal mucosa of patients with CD. Working mechanisms of anti-TNF therapy include neutralizing both membrane-bound and soluble cytokine TNFα and inducing intestinal T-cell lymphocyte apoptosis.[12, 13] Although anti-TNF has shown to be efficient in both inducing and maintaining remission leading to a decrease in both hospitalization and surgery in a considerable proportion of patients, anti-TNF agents are withdrawn due to primary or secondary non-response or adverse events.[16, 17] Therefore, a subgroup of patients do not benefit from this treatment leading to the switch of an alternative biological therapy with a different mechanism of action.[18, 19]

New therapeutic treatment options were developed since 2014 including vedolizumab [an intravenous anti-integrin] followed by ustekinumab [UST, a subcutaneous anti-interleukin] in 2016.[20-25] UST is a fully human monoclonal antibody targeting the p40 subunit of interleukin-12 and interleukin-23 and prevents interaction with the cell surface receptor and further cytokine activation.[23] Previous literature reported, however, superior efficacy for ustekinumab as compared to vedolizumab in patients refractory to anti-TNF therapy.[26, 27] Recently, new drugs are developed as treatment option including upadacitinib [JAK-STAT inhibitor] and ozanimod [sphingosine-1-phosphate (S1P) modulators]. Moreover, more pipeline drugs are being developed with different mechanisms of action including risankizumab [IL-23 inhibitors] which will be soon available for both CD and UC.[28] However, despite the expanding range of treatment options, a majority of patients develop complications and require a surgical resection during their course of disease.[29, 30]

Intestinal resection

An intestinal resection is an important treatment modality which is performed in approximately 25% of patients within 10 years following CD diagnosis.[31] Despite the need for surgery has declined over time, almost half of the patients with CD will require intestinal resection during the disease course.[32-34] The most common intestinal resection options include small bowel resection, ileocecal or ileocolonic resection [ICR] and colectomy. Patients with symptoms or signs of active inflammation are generally first treated with medical treatment as suggested in the European Crohn's and Colitis Organisation [ECCO] guidelines whereas surgery is considered in patients with localized ileocecal CD and stricturing disease.[35] In addition, patients who are intolerant or refractory to medical treatment, or have complications including fistulizing or obstructive disease, surgery is the preferred strategy as well.

Postoperative recurrence

Resection of the diseased segment may not be curative since a majority of patients will develop postoperative recurrence which is commonly defined as clinical, endoscopic and surgical recurrence.[36] Postoperative recurrence is common as up to 80% of patients will develop endoscopically detected recurrence and up to 25% will have clinical recurrence within one year whereas surgical recurrence rates are up to 35% within 10 years following primary ICR.[34, 36-40] Regarding the prevention of postoperative recurrence, ECCO guidelines recommend initiation of postoperative prophylactic therapy immediately following ICR in patients at high risk based on clinical risk stratifications, whereas American guidelines suggest to initiate prophylactic treatment following ICR with the exception of patients at low risk for recurrence.[41, 42] Regarding postoperative recurrence, previous literature reported overall advantage of anti-TNF therapy as compared to other therapies on the short-term.[43-47] However, the long-term prognosis of patients receiving prophylactic therapy as compared to patients without prophylaxis is unknown.

Colonoscopy is considered the golden standard in the diagnosis of postoperative recurrence and therefore used for the surveillance of disease activity following ICR. The Rutgeerts' score, to assess postoperative lesions on the neoterminal ileum and on the ileocolonic anastomosis following ICR, stratifies patients into 4 categories according to the severity of the endoscopic lesions. It is, however, suggested that anastomotic ulcers have an ischemic

etiology and therefore may be less predictive of progressive CD, which led to the development of the modified Rutgeerts' score [mRS]. This mRS divide the Rutgeerts' score i2 into two different categories, making a difference between anastomotic lesions [i2a] and more than 5 lesions in the neoterminal ileum [i2b]. Although the Rutgeerts' score is widely used in clinical practice and research to assess postoperative endoscopic recurrence, the use of the mRS is still debated as the association with long-term prognosis is unknown. Furthermore, the prognosis of anastomotic lesions versus lesions in the neoterminal ileum is still a matter of debate and it is predictive value for long-term outcomes remains unclear.

De-escalation strategies of anti-TNF therapy

The issue whether anti-TNF therapy can be discontinued in patients in long term remission remains an unanswered dilemma. Concerns related to anti-TNF discontinuation include risk of relapse, timing of stopping treatment when patients are in stable remission on therapy, and possible loss of response following retreatment whereas long-term treatment may lead to side effects, possibly increased risk of malignancy and chronic fatigue and work productivity loss.[48, 49] In addition, even despite the introduction of biosimilars, long-term treatment with anti-TNF therapy leads to significant healthcare costs.[50, 51]

In patients with luminal disease, it is recommended to discontinue anti-TNF therapy under strict conditions based on clinical [no symptoms], biochemical [low levels of fecal calprotectin (FC) or C-protective protein (CRP)] and endoscopic remission [no inflammation during colonoscopy].[52] In addition, discontinuation may be considered in patients at low risk of relapse. However, in clinical practice discontinuation of anti-TNF therapy remains a difficult decision since predictors of the risk of a relapse after discontinuation can be insufficiently weighed on individual patient level.[52] To bypass this dilemma, personalized clinical decision making is warranted based on the prediction of the risk of relapse following discontinuation of anti-TNF therapy for the individual patient. Up to date, only one prediction model has been available, however with a moderate discriminate ability. Therefore, a tool for stratification of relapse risk of CD following anti-TNF cessation is highly needed.

Similarly, the risk of relapse following anti-TNF cessation in patients with pCD is debated. Available studies reported inconsistent results on the relapse rates following anti-TNF discontinuation in patients with pCD. Some previous studies showed that pCD was associated

with an increased risk of relapse as compared to luminal CD. Other studies did not report a difference between both phenotypes.[53-56] Important drawbacks for interpretation of the available literature includes varying endpoints, small sample size and combined analysis of perianal and other [entero-enteric] fistulas. To further assess a strategy of anti-TNF discontinuation, more data on the comparison of cessation of anti-TNF therapy with continuation of therapy are required.

Although deep remission in CD patients seems associated with a decrease in hospitalization and CD-related surgery, better quality of life and health-care cost savings, its impact has been evaluated in discontinuation studies and remains controversial.[57-59] Previous study showed an association between deep remission and a reduced risk of relapse after infliximab discontinuation whereas another study reported no difference in relapse over time between patients in deep remission and patients in both clinical or endoscopic remission.[58-60] This could be explained by the fact that no standardized or validated definition of deep remission has been available leading to different definitions of deep remission which has been used in order to reduce the risk of relapse in discontinuation studies.[58, 60-62] In addition, it is debated whether mucosal healing [absence of mucosal ulcerations] is an essential component of deep remission given the presence of residual endoscopic activity including erythema or erosions in some patients which suggest partial mucosal healing. Importantly, previous studies have shown that up to 30% of patients with CD will still relapse while considered to be in deep remission with low fecal calprotectin and mucosal healing.[60, 63] These findings highlights the importance of additional factors, rather than deep remission, to identify CD patients likely to relapse.

Since the underlying pathophysiology of CD relapse is poorly understood and highlights the difficulty of predicting the risk of relapse in CD patients who are in remission, more accurate biomarkers, including histologic markers are essential to identify patients who are less likely to relapse. Prediction of a relapse based on the mucosal immunological landscape may contribute to the individual patient decision whether stop or not to stop anti-TNF therapy.

Aim and outline of this thesis

The aim of this thesis is to assess different optimization strategies of biological therapy in patients with CD, including escalation- and de-escalation and postoperative strategies.

Part I Optimization strategies during Ustekinumab therapy

Since UST has shown to be an effective alternative therapy, response evaluation following UST induction therapy is important for decisions on maintenance therapy. Timely and adequate identification of non-responders as well as early or late responders is essential to guide clinical decision making and could avoid either under treatment or overtreatment. Although endoscopic response evaluation is the golden standard, non-invasive response evaluation might be preferred. FC has proven to be an accurate, accessible and non-invasive biomarker reflecting mucosal healing. However, whether FC could predict endoscopic response in patients with CD exposed to UST remain unknown. Therefore, in **Chapter 2,** we aimed to assess the potential of FC levels to predict endoscopic response.

Although UST has shown to be effective, loss of response is not uncommon especially in anti-TNF refractory patients. Therefore, optimizing strategies are important following secondary loss of response to UST, notably in a refractory population who have failed multiple classes of biologicals. In **Chapter 3** we aimed to evaluate the effectiveness and safety of a new suggested optimization strategy including a second dose of intravenous UST following secondary loss of response.

Part II postoperative optimization strategies

Since postoperative recurrence is common, abundant data on anti-TNF agents for the prevention of postoperative recurrence of CD have been published. Contrary, data regarding treatment of postoperative recurrence with anti-TNF therapy are scarce. In addition, medication use prior to ICR is not taken into account in current international guidelines on management strategies for postoperative CD. Therefore, in **Chapter 4**, we aimed to assess the effectiveness of retreatment of anti-TNF therapy in patients with postoperative recurrence. In current literature, most studies have reported on 1-year endoscopic outcomes and defined endoscopic recurrence as either mild or severe endoscopic recurrence. The severity of endoscopic recurrence is associated with recurrence of symptoms as well as the need for a re-resection. An evaluation of severe endoscopic recurrence as an endpoint would,

therefore, be of added value. Since data regarding long-term endoscopic outcomes are limited and the effect of postoperative prophylactic therapy on severe endoscopic and surgical recurrence following ICR is unclear, we aimed, in **Chapter 5**, to evaluate the effectiveness of postoperative prophylactic therapy on long-term severe endoscopic and surgical recurrence in patients with CD following ICR.

In **Chapter 6**, we investigated the prognostic value of the mRS on long-term outcomes since this prognostic value of the mRS is unclear in postoperative CD patients. The aim was to assess the prognostic value of the mRS, per index score, with correction for known clinical risk factors to predict the risk of a re-resection and progression to severe endoscopic recurrence on the long-term following ICR.

Part III de-escalation strategies of anti-TNF therapy

In routine practice, anti-TNF discontinuation is still debated due to the uncertainty of the risk of relapse in the individual CD patient. Therefore, a personalized treatment approach, including a prediction model for cessation of anti-TNF therapy will benefit the individual CD patient. A stratification tool to identify patients into those likely to suffer from a relapse and those less at risk would allow rational treatment choices. Identification of patient at low risk for relapse would allow clinician to consider cessation of therapy for the individual patient. Recently a prediction model was developed based on a large individual patient data meta-analysis. In **Chapter 7**, we aimed to validate and update the previously developed prediction model.

Further evaluation of the prognostic performance and update of the model with biochemical and histological data was necessary to improve our ability to adequately select patients for cessation of anti-TNF therapy. Therefore, in **Chapter 8**, a stepped wedge center randomized non-inferiority trail was designed to provide prospective data for further updating the prediction model with biochemical, endoscopic and histologic and new serological data. In addition, a cost-effectiveness analysis will be performed of the new strategy of anti-TNF cessation based on the prediction model. In the previously developed prediction model patients with pCD were excluded. For this specific subgroup of CD patients the risk of relapse following anti-TNF discontinuation in patients in remission is still debated. In **Chapter 9**,

we performed a meta-analysis of individual participant data [IPD] and aimed to assess the risk of relapse after anti-TNF therapy cessation in patients with pCD in remission.

Although multiple factors are associated with the risk of relapse following anti-TNF cessation in order to guide the clinician to decide whether anti-TNF can safely be discontinued, it is still hard to discriminate between patients at low or high risk of relapse and relapse is common. Better stratification of patients into those likely to relapse and those less at risk for relapse would allow rational treatment choices. In **Chapter 10**, we aimed to investigate whether ultra-deep immunophenotyping, through nanostring analysis, allows for identification of patients likely to relapse following anti-TNF cessation. To this end, mucosal biopsies, obtained just before the discontinuation of anti-TNF therapy, were contrasted between patients who did not show relapse and who experienced disease relapse. The biopsies were used for deep immunoprofiling by measuring the expression of 772 immunologically-relevant genes using sequence-specific mRNA probes to directly detect gene expression.

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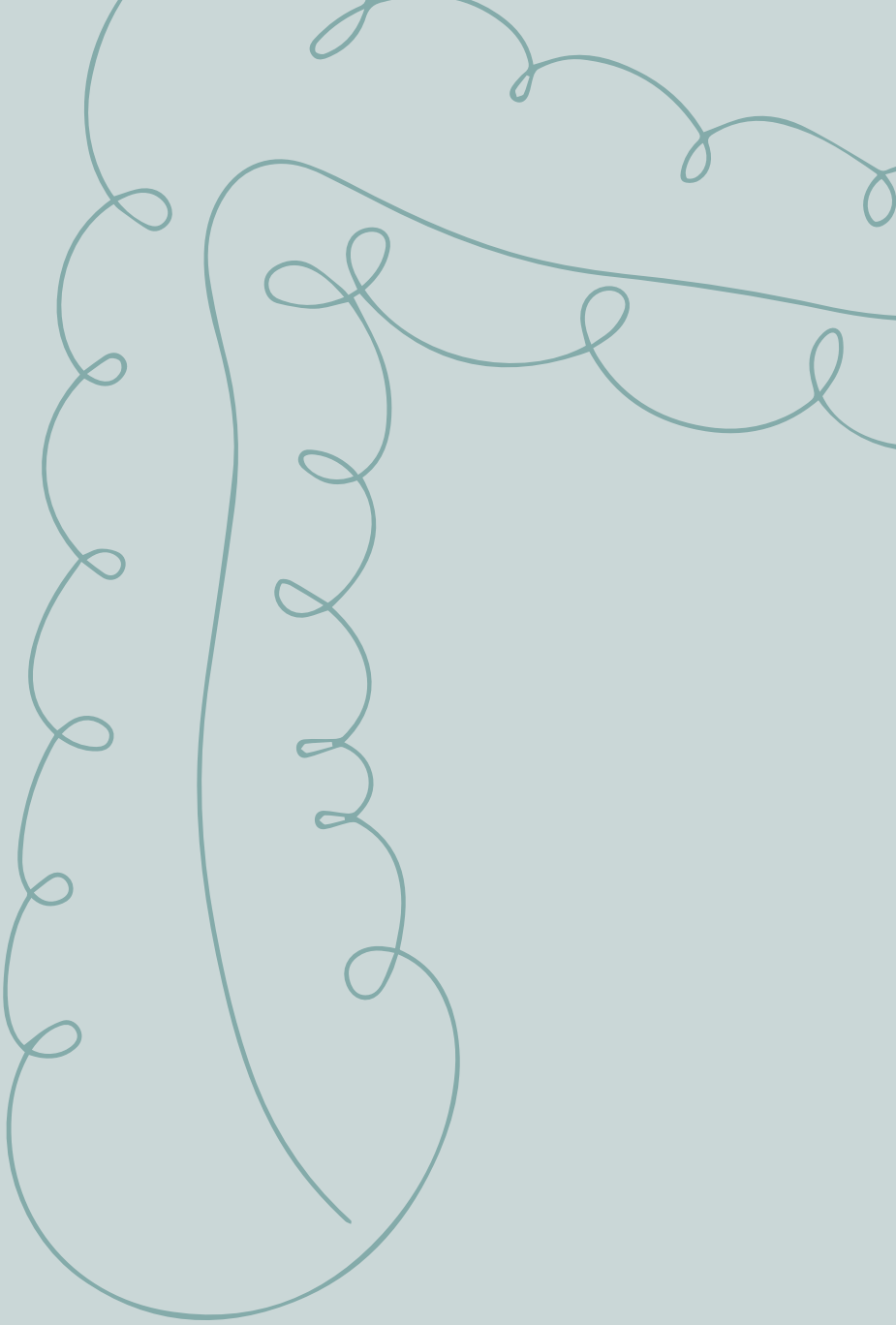

Part I

Optimization strategies during Ustekinumab therapy

Chapter 2

Early fecal calprotectin levels at week 8 may guide therapeutic decisions on Ustekinumab therapy in patients with Crohn's disease

Chapter 3

Re-induction with Intravenous Ustekinumab after Secondary Loss of Response is a Valid Optimization Strategy in Crohn's Disease


2

Chapter 2

Early fecal calprotectin levels at week 8 may guide therapeutic decisions on Ustekinumab therapy in patients with Crohn's disease

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Abstract

Background Response evaluation after induction therapy with ustekinumab (UST) in Crohn's disease (CD) is important for decisions on maintenance therapy. We aimed to assess the potential of fecal calprotectin (FC) levels to predict endoscopic response at week 16.

Methods CD patients with FC >100 µg/g and endoscopic active disease (SES-CD >2, Rutgeerts' score ≥i2) at initiation of UST therapy were enrolled. FC was determined at weeks 0, 2, 4, 8 and 16 and patients underwent a colonoscopy at week 16. The primary outcome was an endoscopic response at week 16 (SES-CD score ≥50% decrease or a decrease of ≥1 points in Rutgeerts' score). The optimal cut-off levels of FC and change in FC to predict endoscopic response were determined using ROC statistics.

Results 59 CD patients were included. Endoscopic response was observed in 21/59 (36%) patients. The diagnostic accuracy for FC levels at week 8 to predict endoscopic response at week 16 showed a predictive value of 0.71. A decrease in FC levels ≥500 µg/g between baseline at week 8 indicates endoscopic response (PPV = 89%), whereas absence of any decrease indicates endoscopic non-response after induction (NPV = 81%).

Conclusions Continuation of UST therapy without endoscopic response evaluation may be considered in patients with a decrease in FC levels of ≥500 µg/g at week 8. The decision on continuation of UST therapy or therapy optimization needs reconsideration in patients without a decrease of FC level. In all other patients, endoscopic response evaluation of induction therapy remains essential for therapeutic decisions.

Introduction

Ustekinumab (UST) is a humanized monoclonal antibody targeting the p40 subunit of the IBD-associated cytokines IL12 and IL23. UST is an effective therapy for Crohn's disease (CD), according to the registration trials and data in real world cohort studies [1,2]. UST may result in rapid symptom improvement in Crohn's disease (CD) patients with observed steroid-free clinical remission in up to 31% of patients at week 12, however, a later clinical response at week 24 has been observed in an additional 7-14% [1,3,4]. Data at these time points are unavailable for endoscopic response. A delayed endoscopic response has been reported after the induction phase, with endoscopic response rates of 21-24% at week 24 increasing to 33-55% at week 52 [5-7].

Therefore, the ideal method and timing of UST response evaluation remains unknown. More objective, adequate and timely identification of early or late responders as well as non-responders is highly important to guide clinical decision making and to avoid unnecessary treatment in non-responders, delayed optimization of treatment in partial responders or unnecessary discontinuation of therapy in delayed responders.

Inadequately timing of response evaluation regarding the effect of UST might lead to either under or over treatment in a substantial proportion of patients. Although endoscopic response evaluation plays an essential role in the management and treatment of CD and is recommended by international guidelines after the start of new medical therapies to identify mucosal improvement, non-invasive response evaluation is preferred due to the disadvantages of endoscopic response evaluation including invasiveness and costs [8,9].

Fecal calprotectin (FC) is an well-studied inflammatory biomarker to guide diagnostic and therapeutic decisions, due to its stability, assay reproducibility and low costs [10]. FC levels

have proven high specificity and sensitivity for endoscopic disease activity and is an accessible, non-invasive, and accurate biomarker reflecting intestinal mucosal improvement and would be ideal to evaluate response [11-13]. To what extent FC levels can predict endoscopic response or histologic induction of remission in CD patients exposed to UST is as yet unknown. In this prospective cohort study, we aimed to assess the potential of early FC levels after UST induction to predict endoscopic response and histologic remission.

Material and methods

Study design and population

A single-center, prospective cohort study was conducted at the Erasmus University Medical Center (Rotterdam, the Netherlands) between December 2016 and December 2019. Consecutive CD patients aged 18 years and older, with both biochemical (FC >100 μg/g) and endoscopic active disease (SES-CD > 2 or Rutgeerts' score ≥ i2) were considered eligible for inclusion.

Ustekinumab therapy

UST therapy was started at the discretion of the treating physician. The initial intravenous infusion with UST at baseline was weight-based, according to label (≈6 mg/kg; 260mg <55kg, 390mg between 55kg and 85kg, 520mg >85kg). The first subcutaneous dose (90 mg) was administered after 8 weeks. Patients with a confirmed endoscopic response at week 16 continued UST maintenance therapy, receiving a dose of 90 mg subcutaneously every 8 or 12 weeks, at the discretion of the treating physician. Concomitant medication (corticosteroids and immunomodulators) during UST treatment was allowed. After UST induction, systemic corticosteroids were tapered to zero at a rate of 5 mg per 1 to 2 weeks, and budesonide was

tapered at a rate of 3 mg every 2 to 6 weeks. If tapering failed, the lowest effective dose of corticosteroid was re-introduced at the discretion of the treating physician.

Data collection

Baseline demographic characteristics were collected, including gender, age, smoking status, disease characteristics according to the Montreal classification [14], history of IBD related surgery and treatment history. The clinical disease activity score (the Harvey Bradshaw Index, HBI [15]) was evaluated at baseline, week 8 and week 16. Serum samples were taken at baseline, week 8 and week 16 and included C-reactive protein (CRP), leukocytes, platelets, hemoglobin and albumin. Serum samples were collected and stored at week 16. UST serum levels were measured at week 16 by ELISA, according to the manufacturer's protocol (LISA Tracker, Theradiag®).

FC levels were determined at baseline, week 2, week 4, week 8 and week 16 using the QuantOn cal (QoC) FC home test (Preventis, Germany) or a quantitative enzyme-linked immunosorbent assay (ELISA) (Bühlmann Laboratories AG, Schönenbuch, Switzerland). All patients were offered the QoC FC home test. If the patient was unable to use the FC home test (due to various reasons), they were offered the ELISA laboratory tests.

Endoscopy was performed at week 16. Endoscopic inflammation was determined using the simple endoscopic score (SES-CD) and the Rutgeerts' score for patients after ileocolonic resection **(Supplementary Table 1)**. During endoscopy, ileal and segmental colonic [ascending, transverse, and descending colon as well as from the sigmoid and rectum] biopsies were collected; whenever possible from inflamed and non-inflamed mucosa. Biopsies were formalin fixed and paraffin embedded for assessment of histological inflammation by the GHAS score [78] by two expert gastrointestinal pathologists (MD and LO).

Outcomes and definitions

The primary outcome was endoscopic response at week 16 after start of UST therapy, defined as ≥50% decrease in SES-CD score, a decrease of 1 or more points in the Rutgeerts' score or a decrease of ≥ 1 point on the four-grade scale mentioned in the **Supplementary Table 1** as judged by the endoscopist in patients with an ileostomy, ileoanal pouch anastomosis or ileorectal anastomosis. Secondary outcomes included endoscopic and histologic remission at week 16 and clinical response and remission at week 16. Clinical response was defined as a decrease of ≥3 points in HBI score as compared to baseline. Clinical remission was defined as a HBI score ≤4. Biochemical remission was defined as a FC< 250µg/g and CRP<10 mg/L. Endoscopic remission was defined as SES-CD ≤ 2, a Rutgeerts score of i0 or i1, or as "no endoscopic disease activity" on the four-grade scale mentioned in **Supplementary Table 1**. Histological remission was defined as GHAS ≤ 4 points, and severe disease activity was defined as GHAS ≥ 10 points **(Supplementary Table 2).**

Statistical analyses

Normally distributed data are presented as mean ± standard deviation (SD) and continuous data with a skewed distribution as median and the first and third quartile (Q1-Q3). Categorical data are presented as numbers and percentages. Chi-square tests and Wilcoxon Rank Sum tests were used to evaluate differences between endoscopic responders and non-responders and for patients being in histologic remission or not, for categorical and continuous non-normally distributed variables, respectively. The optimal (the best discriminatory performance and clinically relevant) cut-off levels of FC and relative change in FC to predict endoscopic response were determined using receiver operating characteristic (ROC) statistics. Area under the curve [AUC], sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of FC to predict endoscopic response and

histologic remission were calculated by cross-tabulation. A two-sided P-value of <0.05 was considered statistically significant. All statistical analyses were performed using IBM SPS Statistics version 25.0 (IBM Corp. Released 2013, IBM Corp, Armon, NY).

Ethical considerations

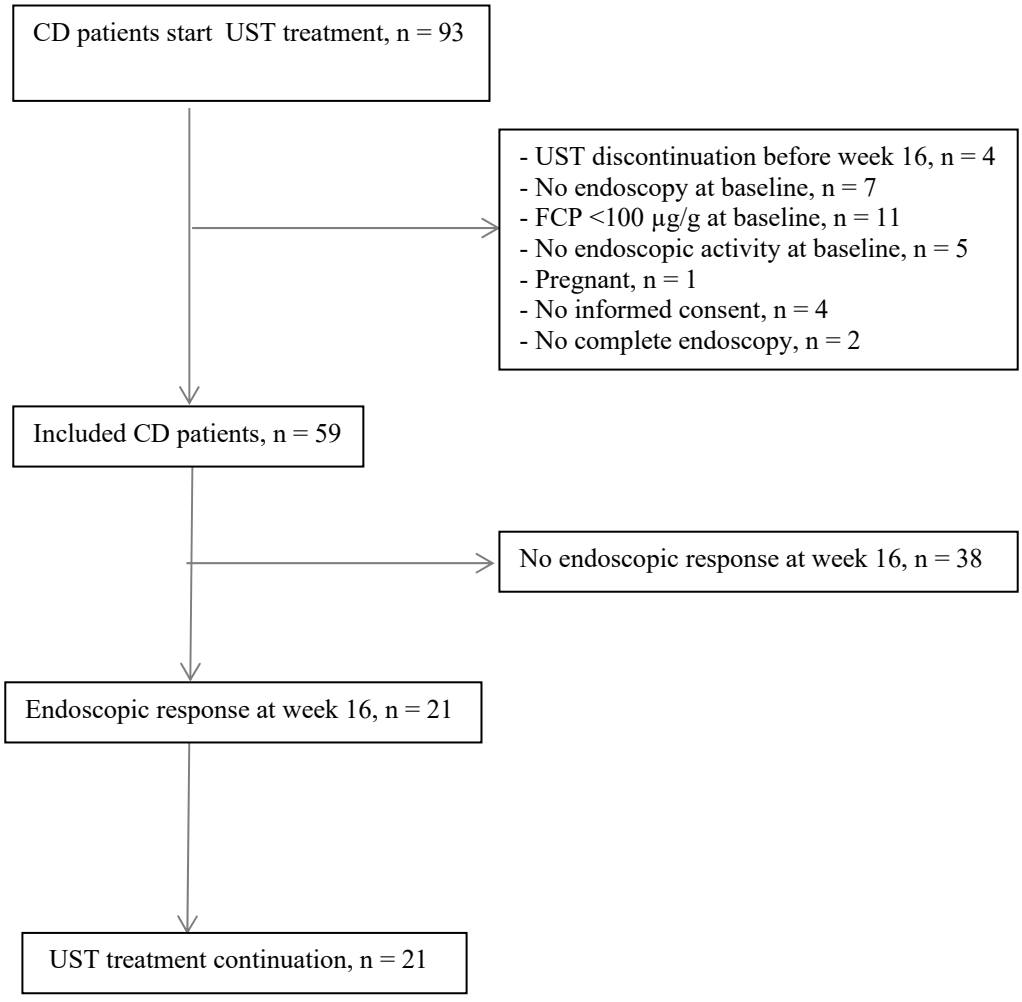
This study protocol was reviewed and approved by the Medical Ethics Committee (METC) Rotterdam (MEC 2004-168 2012), The Netherlands. Informed consent was obtained from all individual participants included in the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Results

A total of 93 patients started UST during the study period, of whom 59 patients met the inclusion criteria and were enrolled **(Figure 1)**. The majority of the included patients were female (64%) with a median age of 38 years (interquartile range [IQR] 26 – 52) **(Table 1)**. UST was initiated after a median disease duration of 12.8 years (IQR 8.1 – 18.1). All patients were exposed to anti-TNF therapy prior the start of UST, of whom a majority (88%) previously failed two or more anti-TNF agents. In total, 52/59 (88%) patients were anti-TNF refractory defined as primary clinical failure of secondary loss of clinical response. In addition, 30 (51%) patients had been exposed to vedolizumab, of whom 27/30 (90%) had refractory disease to vedolizumab. A total of 42/59 (72%) patients, received concomitant immunosuppressive medication. In 34/59 (58%) patients, UST was combined with

corticosteroid induction therapy (13 prednisone; 21 budesonide) and 8/59 (14%) patients were on concomitant immunomodulator therapy (thiopurines n = 3, tioguanine n = 4, tacrolimus n = 1) during UST induction. Corticosteroids were tapered in 19/34 (56%) patients at week 16.

Figure 1. Flow chart of patients.



CD; Crohn's disease, UST; ustekinumab, n; number of patients

Table 1. Baseline patient characteristics

Patients characteristics		**n = 59**
Age*	Median (IQR)	38 (26 – 52)
Sex, female	n (%)	38 (64)
Smoking	n (%)	13 (22)
Disease duration, years	Median (IQR)	12.8 (8.1 – 18.1)
Age at diagnose		
A1 <16 yr	n (%)	16 (27)
A2 17 – 39 yr	n (%)	39 (66)
A3 ≥ 40 yr	n (%)	4 (7)
*Disease location**		
L1 ileal	n (%)	8 (14)
L2 colonic	n (%)	6 (10)
L3 ileocolonic	n (%)	45 (76)
+ L4 upper GI disease	n (%)	7 (12)
*Disease behaviour**		
B1 non stricturing, non-penetrating	n (%)	24 (40)
B2 stricturing	n (%)	28 (48)
B3 penetrating	n (%)	7 (12)
+Perianal disease	n (%)	13 (22)
Prior intestinal resections	n (%)	37 (63)
Prior Anti-TNFα exposure		
naive	n (%)	0 (0)
1	n (%)	7 (12)
≥2	n (%)	55 (88)
Prior vedolizumab exposure	n (%)	30 (51)
Disease activity		
Harvey Bradshaw Index, n = 52	Median (IQR)	9.5 (5.0- 13.0)
C- Reactive Protein, mg/L, n = 52	Median (IQR)	5.9 (1.5 - 37.5)
Faecal calprotectin, µg/g, n = 52	Median (IQR)	604 (331 – 1297)
Simple Endoscopic Score for CD, n = 23	Median (IQR)	11 (9 – 16)
Rutgeers Score, n = 30	Median (IQR)	3 (2 – 4)
Concomitant medication		
Corticosteroids	n (%)	34 (58)
Immunomodulators	n (%)	8 (14)

*At inclusion

N; numbers of patients, CD Crohn's disease, L; location, B; behavior, E; extent, TNFα; tumor necrosis factor alpha

Response to UST induction

The median HBI score at baseline was 9 (IQR 5 – 13) which significantly decreased to 6 (IQR 4 – 10, $p < 0.001$) at week 8 after intravenous administration of UST therapy. A slight increase of the HBI score to 7 (IQR 5 – 11) was observed at week 16, however the HBI remained significantly lower compared with baseline ($p = 0.016$) **(Figure 2).**

Clinical response 8 weeks after initiation of UST therapy was observed in 28/59 (48%) patients, and in 26/59 (44%) at week 16. Clinical remission was observed in 20/59 (34%) and 12/59 (20%) patients at week 8 and 16, respectively.

Median FC significantly decreased from 611 µg/g at baseline (IQR 335 – 1297) to 417 µg/g at week 2 (IQR 181 – 1092, $p = 0.008$). Thereafter, no significant further decrease was observed at week 4 (441 µg/g, IQR 189 – 1279, $p = 0.076$). At week 8, FC decreased significantly further to 370 µg/g (IQR 137 – 1278, $p = 0.012$ versus baseline). However, median FC increased again by week 16, approaching baseline (645 µg/g, IQR 213 – 1466, $p = 0.793$ versus baseline) **(Figure 3)**.

Endoscopic response at week 16 was observed in 21/59 (36%) patients. Endoscopic remission at week 16 was observed in only 7/59 (12%) patients. Patients on concomitant immunomodulators at baseline showed significant higher endoscopic response rates at week 16 as compared with patients without concomitant immunosuppressive therapy at baseline (45% vs 19%; $p = 0.048$). No significant differences were observed between patients with or without concomitant immunomodulators for clinical response and for clinical, biochemical, endoscopic and histologic remission. No significant differences were observed between patients with UST as second- or third-line therapy with regard to endoscopic response (34% vs 37%, $p = 0.861$) or patients with a history of surgery (38% vs 32, $p = 0.641$).

With regard to histology, biopsies were available in 41/59 (69%) patients. In patients with histologic assessment, median GHAS-index at week 16 was 7 [IQR 4 – 8]. Histologic remission was observed in only 12/41 (29%) patients.

Correlation between early FC levels and response to UST

In endoscopic responders, median FC decreased significantly from 841 µg/g (IQR 364 – 1220) at baseline to 277 µg/g (IQR 142 – 1220) at week 2 (p = 0.049), increased to 425 µg/g (143 – 1224) at week 4 (p=0.102), and decreased again significantly down to 170 µg/g (IQR 66 – 717) at week 8 (p = 0.001 as compared to baseline). At week 16, FC increased again to 349 µg (IQR 97 – 1268, p = 0.279 as compared to baseline) (**Figure 4**). In contrast, in endoscopic non-responders, FC levels did not show a significant decrease in subsequent serial FC levels at all predefined time points as compared to baseline **(Figure 4)**.

A statistical difference in median FC was observed between endoscopic responders and non-responders at week 8 only (170 µg/g [IQR 66 – 717] vs 487 µg/g [IQR 227 – 1759], p = 0.010). The FC in endoscopic responders decreased with Δ671 µg/g (80%) as compared with Δ117 µg/g (19%) in non-responders from baseline to week 8 (p = 0.001).

No statistical difference was observed between endoscopic responders and non-responders at baseline ([841.7 µg/g (IQR 364 – 1220] vs 604 µg/g [IQR 304 – 1430], p = 0.818), week 2 ([277 µg/g (IQR 142 – 1220] vs 488 µg/g [IQR 228 – 988], p = 0.555), week 4 ([425 µg/g (IQR 143 – 1224] vs 441 µg/g [IQR 217 – 1628], p = 0.472) and week 16 (349 µg/g (IQR 96 – 1269] vs 771 µg/g [IQR 303 – 1800], p = 0.193).

Figure 2. Serial median HBI during UST induction.

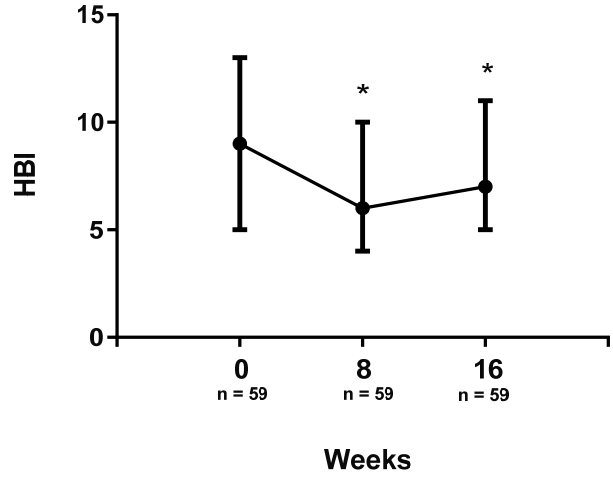
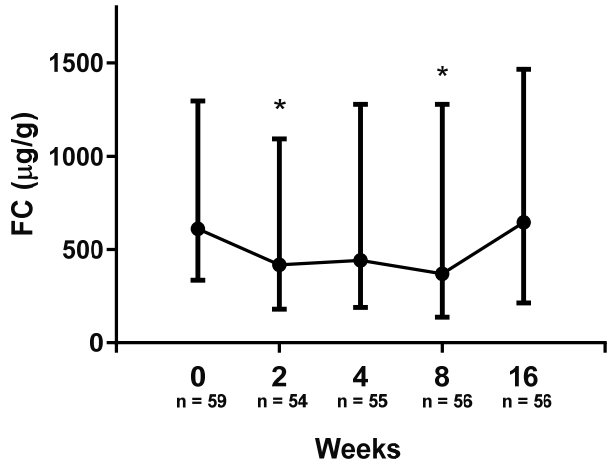


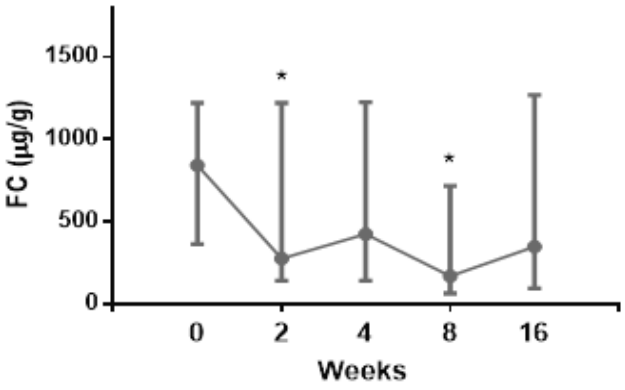
Figure 3. Serial fecal calprotectin during UST induction.



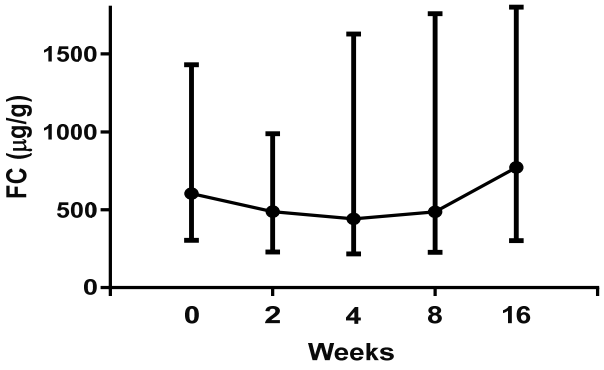
* Significant difference as compared to baseline

Figure 4. Serial fecal calprotectin during UST induction, specified for patients with and without endoscopic response.

A. Endoscopic responders



B. Endoscopic non-responders



* Significant difference as compared to baseline

Due to the low proportion of patients in endoscopic remission, no statistically relevant analyses could be performed to identify a correlation with FC levels. Similarly, no correlation with FC levels could be determined due to the low proportion of patients with histological remission.

Optimal FC levels to predict endoscopic response and endoscopic and histologic remission

After UST induction, FC levels at week 8 has a diagnostic accuracy to predict endoscopic response at week 16 with a corresponding AUC of 0.71 (95% CI 0.57 – 0.85, p = 0.01). However, no optimal cut-off value could be defined to identify endoscopic responders at week 16. FC ≤250 µg/g at week 8, showed a sensitivity of 55% (95% CI 31.5% – 76.9%), specificity of 75% (95% CI 57.8% – 87.9%), positive predictive value (PPV) of 55% (95% CI 38.0% – 70.9%), and negative predictive value (NPV) of 75% (95% CI 64.1% – 83.5%). Similarly, no diagnostic accuracy was demonstrated for FC levels to predict endoscopic remission.

An absolute decrease in FC levels, from baseline to week 8, showed an AUC of 0.72 (95% CI 0.58 – 0.86, p = 0.006). Within the patient subgroup of 47/59 (80%) patients with minimal FC level of 250 ug/g, an absolute decrease of ≥250 µg/g at week 8 resulted in a sensitivity of 65% (95% CI 40.8 – 84.6), specificity of 78% (95% CI 60.9 – 89.9), PPV of 62% (95% CI 44.9 – 76.4), and a NPV of 80% (95% CI 68.2 – 88.2). Within the patient subgroup of 38/59 (64%) patients with minimal FC level of 500 ug/g, a FC cut-off of ≥500 µg/g decrease at week 8 was associated with a sensitivity of 73% (95% CI 57.2 – 85.0), specificity of 67% (95% CI 34.9 – 90.1), PPV of 89% (95% CI 77.9 – 94.8), and NPV of 40% (95% CI 26.3 – 55.5).

Absence of a decrease in in FC levels (defined as a sustained or increased FC level) from baseline to week 8 predicted endoscopic response at week 16 with a sensitivity of 80% (95% CI 56.3 – 94.3), specificity of 47% (95% CI 30.4 – 64.5), PPV of 46% (95% CI 36.6 – 55.2), and a NPV of 81% (95% CI 62.4 – 91.6).

Due to the low proportion of patients in both endoscopic and histologic remission at week 16, no statistically relevant analyses could be performed to identify an FC cut-off for endoscopic or histologic remission.

Discussion

In this prospective cohort, with a high percentage of CD patients exposed to both anti-TNF and vedolizumab, an absolute decrease of ≥500 µg/g between baseline and week 8 was significantly associated with endoscopic response at week 16, whereas the absence of a decrease of FC level at week 8 was associated with the absence of endoscopic response. Therefore, FC measurement at week 8 may guide therapeutic decisions on UST continuation in CD. In patients with a FC decrease of ≥500 µg/g from baseline to week 8, endoscopic response is likely. In these patients, continuation of UST therapy without endoscopic response evaluation may be considered. In patients without a decrease in FC levels, endoscopic response is unlikely. In these patients, the decision on continuation of UST therapy needs to be reconsidered based on an individual patient's level including waiting for a delayed response and postpone endoscopy, dose optimization or stop UST therapy and switch to another class of drugs. Endoscopic response evaluation remains essential in patients with a FC decrease less than 500 µg/g for further therapeutic decisions.

Although FC has proven a high sensitivity and specificity for endoscopic disease activity, published literature reports a widely spread of cut-off values of FC predicting endoscopic remission [57 - 274 µg/g] [11, 17-20]. Similarly, we could not identify an optimal FC cut-off value for endoscopic response to UST in this study. An explanation for the absence of a clear cut-off value for FC to predict endoscopic response as observed in this study could be the inclusion of therapy refractory patients, who were exposed to UST as a third line biological reflecting a more difficult to treat cohort following multiple biologic failures. Failure to multiple classes of biologics results in lower effectiveness of UST[20, 22]. Possibly, these patients suffer from a very heterogeneous disease reflecting a more severe transmural inflammation of CD. It may be hypothesized that the FC levels in our cohort decrease insufficiently due to this highly selected group of refractory patients. A larger cohort with a less therapy refractory population may be of interest to develop a decision making algorithm for the evaluation of the effect of UST induction therapy.

Our cohort showed low endoscopic response and endoscopic remission rates (i.e. 36% and 10%) at week 16, which is in line with a previous study which reported endoscopic response and remission rates of 21% and 7% at weeks 24 (95% and 67% exposed to anti-TNF and vedolizumab) [6]. In addition to the therapy refractory population in this study that might explain the relatively low endoscopic response rate at week 16, a delayed response to UST induction may be of influence as well [23]. This delay may in part be related to the mechanisms by which UST downregulates CD-related inflammation. IL-12/IL-23 blockade, induced by UST, is followed by modulation of T-cell differentiation leading to a subsequently decreased Th1 and Th17 pro-inflammatory cytokine production. It is conceivable that the anti-inflammatory effects initiation through such immune modulation takes longer to manifest compared for instance to the treatment with anti-TNF therapy which directly targets

inflammatory effectors resulting in a rapid improvement [24]. This hypothesis is supported by the fact that the proportion of patients achieving clinical response increases over time. In the ICC registry, 38% of patients receiving UST achieved steroid-free clinical remission at week 24 compared to only 24% at week 12 [1]. In addition, another study reported on 33 patients who did not achieve clinical response at week 12, however did achieve clinical response at 24 weeks [7]. In addition, the predictive value of early FC levels on later endoscopic time points may depend on the timing of endoscopic evaluation. The predictive value of FC was evaluated in the post-hoc analysis from the IM-UNITI trial, and showed that a week 6 FC value of <250 ug/g is an adequate predictor of endoscopic remission (defined as a SES-CD score <3) at week 52 [AUC 0.709] [25]. FC performed better as predictor as compared to clinical remission (CDAI <150) or clinical response (CDAI reduction of ≥100 points), CRP <5 mg/L and UST drug levels at week 6.

In this study, an increase in both HBI score and FC level was observed between week 8 and week 16. A prospective open-label cohort study reported similar outcomes regarding early serial FC measurements with a significant decrease in FC levels after intravenous induction and as well an increase of FC levels beyond week 8 and even further up to week 24 [4]. Low serum levels of UST, possibly due to a too low intravenous induction or subcutaneous dosage during maintenance therapy or the relative long interval between induction and maintenance therapy could be an explanation for the increased FC levels beyond week 8 and the low endoscopic response rates at week 16 [26,27]. Patients rapidly lose their initial response possibly due to the underlying pharmacokinetics mechanisms, referring to the ‘’intestinal sink’’ phenomenon which previously has been demonstrated for anti-TNF therapy [28], and which may be involved in a declined response to UST. It might therefore be suggested that CD patients may need higher levels of UST to achieve the required exposure of UST to neutralize

tissue inflammation and to induce endoscopic response. More studies on pharmacokinetics mechanisms of UST are needed to elucidate this issue.

In this current study, the clinical remission rate [30%] was in line with available data from large Dutch and Belgium real word cohorts reporting clinical remission rates of 23% [week 12] and 31% [week 16] [1, 6]. In contrast, a large Italian cohort reported a higher clinical remission rate [64%], possibly due to higher rate of patients on concomitant corticosteroids and higher threshold of HBI score for definition of clinical remission[29]. In this latter cohort an association was observed between FC>200 µg/g at 2 months is associated with the absence of clinical remission at 3 months. In this study, this observation was not confirmed, since no correlation between FC level at the evaluated time points and clinical remission at week 16 was observed [correlation coefficient -0.011, $p = 0.42$].

Major strengths of this study are its prospective design with clinical, biochemical, and endoscopic assessments. Furthermore, the serial early FC measurements do depict the course of the disease clearly. However, a few limitations need to be considered. Firstly, several factors can affect FC levels, including infections, bowel movements, storage temperature and blood admixture, and could therefore potentially influence the outcomes. In addition, FC levels could also be elevated due to other gastrointestinal diseases, reactive oxygen species and in drug-induced enteropathy. Due to its real-world design in a tertiary referral center, these results do not reflect daily clinical practice, as our cohort reflects a refractory group of patients with severe disease who failed several biologics prior UST initiation and for whom further treatment strategies are still undiscovered. Lastly, given the fact that previous studies demonstrated additional responders to UST at week 24 (instead of week 16), it would be of interest to consider the association between FC levels and endoscopic evaluation at later time points.

In conclusion, the prediction of endoscopic response to UST induction in CD patients based on early FC measurements remains challenging. FC levels at week 8 may guide therapeutic decisions on UST continuation in CD. Continuation of UST therapy, without endoscopic response evaluation, may be considered in patients with a decrease in FC levels of ≥500 μg/g. The decision on continuation of UST therapy needs reconsideration at this time point in patients without a decrease of FC level. In all other patients, endoscopic response evaluation remains essential for therapeutic decisions.

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3

Chapter 3

Re-induction with Intravenous Ustekinumab after Secondary Loss of Response is a Valid Optimization Strategy in Crohn's Disease

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Abstract

Background and Aim Re-induction with intravenous ustekinumab (UST) after secondary loss of response in Crohn's disease (CD) is a relatively new strategy to regain efficacy. This real-world cohort study aimed to evaluate its effectiveness and safety.

Methods CD patients with loss of response after initial response to UST and treated with a second intravenous dose of UST were included. Clinical, biochemical and endoscopic data were collected. Primary outcome was drug survival. Secondary effectiveness outcomes included clinical remission, primary non-response and adverse events.

Results In total, 31 CD patients were included after re-induction with intravenous UST. All patients had failed prior biologic therapy, i.e. 77% were exposed to two or more anti-tumour necrosis factor (TNF) agents and 65% were exposed to vedolizumab prior to initiation of UST treatment. Median treatment duration between initial treatment and re-induction with intravenous UST was 11.1 months [interquartile range 6.9 – 19.5]. UST therapy after a second dose of intravenous UST was maintained in 74% and 71% of the patients at week 20 and 52. Clinical remission rates after re-induction at week 8, 20 and 52 were 37%, 56% and 45%, respectively. Non-response occurred in 16% of the patients. Adverse events were reported in 4 patients.

Conclusions Re-induction with intravenous UST after secondary loss of response results in continuation of UST treatment for at least one year in almost three-quarters of patients and in clinical remission in half of patients after one year. Therefore, UST re-induction may be considered an important rescue treatment option in patients with refractory CD.

Introduction

Ustekinumab (UST) is a biological treatment option for Crohn's disease (CD) targeting the p40 subunit of cytokines interleukin-12 and interleukin-23. UST is prescribed in CD in a weight-based intravenous induction dose followed by subcutaneous maintenance therapy of 90 mg every 8 or 12 weeks [1,2]. In the registration trials, patients with a response to induction treatment were randomized for the maintenance treatment. However, only 57% of the anti-TNF naïve population (UNITI-I) and 39% of the anti-TNF refractory population (UNITI-II) were in clinical remission after 52 weeks of treatment indicating a substantial secondary loss of response after primary response to treatment, especially for anti-TNF refractory CD patients [3]. In addition, observational real-world cohorts (up to 100% anti-TNF refractory) have reported a secondary loss of response rate of 27% and 34% after approximately 52 weeks [4,5]. Therefore, optimizing strategies are needed after secondary loss of response to UST, which are of paramount importance to patients who have failed on several treatment options.

Management of patients with secondary loss of response to UST may include UST optimization with either intensification of UST subcutaneous dosing or reintroduction of intravenous UST. However, data regarding these strategies are scarce. To date, few real-world studies have reported the use of intravenous reintroduction [4, 6-10]. However, only three have assessed the effectiveness and safety of a second dose of intravenous UST during maintenance treatment. These studies were, however, hampered by small sample sizes, short-term follow up, undefined follow up time after re-induction or varying endpoints.

To further study the strategy of re-induction with intravenous UST, this real-world cohort study aimed to evaluate the effectiveness and safety of the re-induction with intravenous UST after secondary loss of response.

Methods

Study design and patients selection

A multicenter cohort study was conducted in CD patients treated with a second intravenous dose of UST between January 2017 and July 2019. Patients were included in 4 academic and 1 teaching hospitals, affiliated with the *Initiative on Crohn and Colitis Registry* (ICC Registry) [6]. CD patients were considered eligible if they received a single intravenous weight-based dose of UST as induction therapy followed by at least one subcutaneous maintenance injection of 90 mg UST as initial UST therapy before exposure to a second dose of intravenous UST.

Selected patients initially experienced a primary response to UST (defined as clinical, biochemical and/or endoscopic improvement as documented in the medical records) and subsequently developed a secondary loss of response (defined as primary response to initial induction therapy which was not maintained). Active disease based on clinical, biochemical and/or endoscopic findings has led to the re-induction with intravenous UST. Patients included in the study of Kopylov et al. [10] (by Radboud UMC and Maastricht UMC) were excluded from this study cohort.

Outcomes and definitions

The primary outcome of this study was drug survival at week 20 and 52 of UST maintenance therapy after the second dose of intravenous UST. Patients who maintained UST therapy

were considered to have had a response or remission to a second dose of intravenous UST. Continuation and shift to maintenance therapy was at the discretion of the treating physician and based on clinical, biochemical and/or endoscopic improvement. Secondary outcomes included proportion of patients in clinical remission [Harvey-Bradshaw Index (HBI) ≤ 4 points] at week 20 and 52, non-response and adverse events. Non-response was defined as no or insufficient response to the second intravenous dose of UST within 8 weeks from re-induction with UST leading to discontinuation of UST therapy due to CD. Patients who discontinued UST treatment because of primary non-response, secondary loss of response or adverse event were considered as a treatment failure. Reasons for discontinuation were at the discretion of the treating physician and was based on clinical, biochemical and/or endoscopic disease activity. Follow-up time was determined based on the date of the second intravenous infusion with UST until week 52. Patients who were lost to follow-up were considered censored cases.

Data collection

Electronic patient records were reviewed. Baseline was defined as the date of the intravenous re-induction with UST. Recorded baseline characteristics (including date of birth, gender, smoking status), disease-specific information (including age and weight at start UST treatment, Montreal Classification [11], disease duration, initial UST treatment dose and scheme, UST start and stop date, prior CD intestinal resections, prior perianal surgery, CD medical treatment history as well concomitant medication use, main indication for initiating of UST (luminal disease, perianal disease, postoperative prevention), induction dose and maintenance interval, number and interval of subcutaneous injections before a second intravenous UST dose, duration of maintenance treatment before re-induction with an intravenous UST dose and main reason for the second dose of UST, biochemical markers

(including C-reactive protein (CRP), haemoglobin, thrombocytes, leukocytes, faecal calprotectin (FC)), endoscopic data (including disease activity, ulcerations, segment of inflammation) and clinical data (including disease activity based on HBI [12]) regarding effectiveness of re-induction with intravenous UST were obtained.

During follow up, data were obtained at week 8, 20, 52 after intravenous re-induction and included HBI, biochemical and endoscopic findings as previously described. In case UST was ceased, the reason for discontinuation was noted. If baseline endoscopy at time of re-induction with intravenous UST was performed within 12 weeks before start of the re-induction, endoscopic findings were included.

Disease activity

Clinical remission was defined as HBI-score ≤4. Biochemical remission was defined as a faecal calprotectin level of ≤250 µg/g and/or CRP concentration of ≤4 mg/L. Endoscopic remission was defined as the absence of ulcerations as assessed by the endoscopist.

Statistical analyses

Descriptive statistics were used for baseline characteristics. Continuous data were presented as median with interquartile range (IQR) or as mean ± standard deviations (SD) according to the distribution. Categorical data were presented as number and percentages. Variables were subsequently compared using Mann-Whitney test for continuous parameters and chi-squared or Fisher's exact test for categorical parameters. Kaplan-Meier survival analysis was used to assess the cumulative drug survival after intravenous re-induction with UST. All data analyses were performed using IBM SPSS Statistics for Windows, version 25.0. [IBM].

Ethical consideration

The ICC Registry was reviewed and approved by the Committee on Research Involving Human Subjects at the Radboudumc (institutional review board: 4076). Written informed consent was obtained from each patient included in the study. The study protocol is conform to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a prior approval by the institution's human research committee.

Results

Baseline characteristics

In total, 166 CD patients were included in the ICC registry in the participating centres during study period. Of these CD patients, 31 patients [female, 65%; median age 37 [IQR 30 - 48]] received a second dose of intravenous UST after a median disease duration of 10.4 years [IQR 6.7 – 16.6]. The clinical and demographics baseline characteristics are shown in **Table 1.** All patients [n = 31] were exposed to at least one anti-TNF agent and twenty four patients [77%] had previously failed two or more anti-TNF agents, respectively. In addition to anti-TNF therapy, twenty patients [65%] were exposed to vedolizumab before initiating UST treatment. The majority of patients started intravenous UST as monotherapy [65%]. At baseline, two patients [7%] received concomitant immunomodulators [thiopurine, methotrexate], 9 patients [29%] received systemic corticosteroids [prednisone], and one patient [3%] received tacrolimus.

Subsequent UST subcutaneous dosing was started at interval 1q12w, 1q8w, 1q7w and 1q4w in 4 [13%], 24 [77%], 2 [7%] and in 1 patient [3%], respectively. In total, 10 patients [32%] were already optimized by shortening the interval prior a second dose of intravenous UST

[1q12w to 1q8w, n =5; 1q12w to 1q7w, n = 1; 1q8w to 1q7w, n = 1; 1q12w to 1q4w, n = 1; 1q8w to 1q4w, n =2].

Re-induction dose and subsequent maintenance therapy

Median treatment duration was 11.1 months [IQR 6.9 – 19.5] between initial UST treatment and re-induction with intravenous UST. At time of intravenous re-induction with UST, all patients had either clinical [median HBI: 8], biochemical [median FCP level; 413 µg/g, median CRP concentration; 12.5 mg/L] or endoscopic disease activity. A total of 5 [16%], 17 [55%] and 9 patients [29%] received a re-induction dose of 260mg, 390mg and 520mg, respectively. After the re-induction therapy, 27 patients [87%] received a consecutive subcutaneous injection of 90mg UST.

Seventeen [61%] and ten patients [32%] started subsequent UST maintenance therapy on a 1q8w and 1q4w interval, respectively. Four patients did not receive maintenance therapy due to non-response to a second intravenous UST dose.

Drug survival

Of the patients who received a second dose of intravenous UST at baseline, 21 [74%] and 20 [71%] patients were still on maintenance UST therapy at week 20 and 52 [**figure 1**], with Kaplan-Meier estimate of drug survival after intravenous re-induction with UST [**Figure 2**]. Two patients [6%] were lost during follow-up due to referral to another hospital.

Table 1. Baseline characteristics.

Patients characteristics		**n = 31**
Age, years[a]	Median [IQR]	37 [30 – 48]
Sex, male	N [%]	11 [35.5]
Smoker, yes	N [%]	6 [19.4]
Age (Montreal classification)		
≤16 years	N [%]	4 [13.0]
17 – 40 years	N [%]	24 [77.3]
>40 years	N [%]	3 [9.7]
Disease location (Montreal classification)		
Ileum (L1)	N [%]	5 [16.1]
Colon (L2)	N [%]	9 [29.0]
Ileocolonic (L3)	N [%]	17 [54.8]
Disease, behavior (Montreal classification)		
Inflammatory disease (B1)	N [%]	15 [48.4]
Stricturing (B2)	N [%]	10 [32.3]
Penetrating (B3)	N [%]	6 [19.4]
Perianal disease	N [%]	9 [29.0]
Extraintestinal manifestations[b]	N [%]	3 [9.1]
Prior surgery, intestinal resection	N [%]	20 [64.5]
Prior surgery, perianal	N [%]	9 [27.3]
Concomitant treatment at baseline[a]		
Immunomodulators (AZA, 6-MP, MTX, 6-TGN)	N [%]	2 [6.5]
Corticosteroids	N [%]	9 [29.0]
Tacrolimus	N [%]	1 [3.2]
Prior anti-TNF therapy		
≥ 1	N [%]	33 [100.0]
≥ 2	N [%]	23 [74.2]
3	N [%]	2 [6.5]
Prior Vedolizumab	N [%]	20 [64.5]
Hospitalization[a]	N [%]	5 [16.0]

[a]At second intravenous UST dose

[b]Including erythema nodosum and arthralgia

UST, Ustekinumab; AZA, azathioprine; 6-MP, 6-mercaptopurine; MTX, methotrexate; 6-TGN, 6-tioguanine; CRP, C-reactive protein; FCP, Fecal calprotectin;

After induction with a second dose of intravenous UST, seventeen patients [61%] started UST maintenance therapy on a 8 week [q8w] interval and ten patients [32%] on a 4 week [q4w] interval. Of the patients on a q4w interval, 40% [n = 4/10] discontinued UST treatment after a median treatment duration of 3.9 months [IQR 2.04 – 4.24], whereas 6% [n = 1/17] on a q8w interval discontinued UST treatment after 6.9 months (p = 0.047).

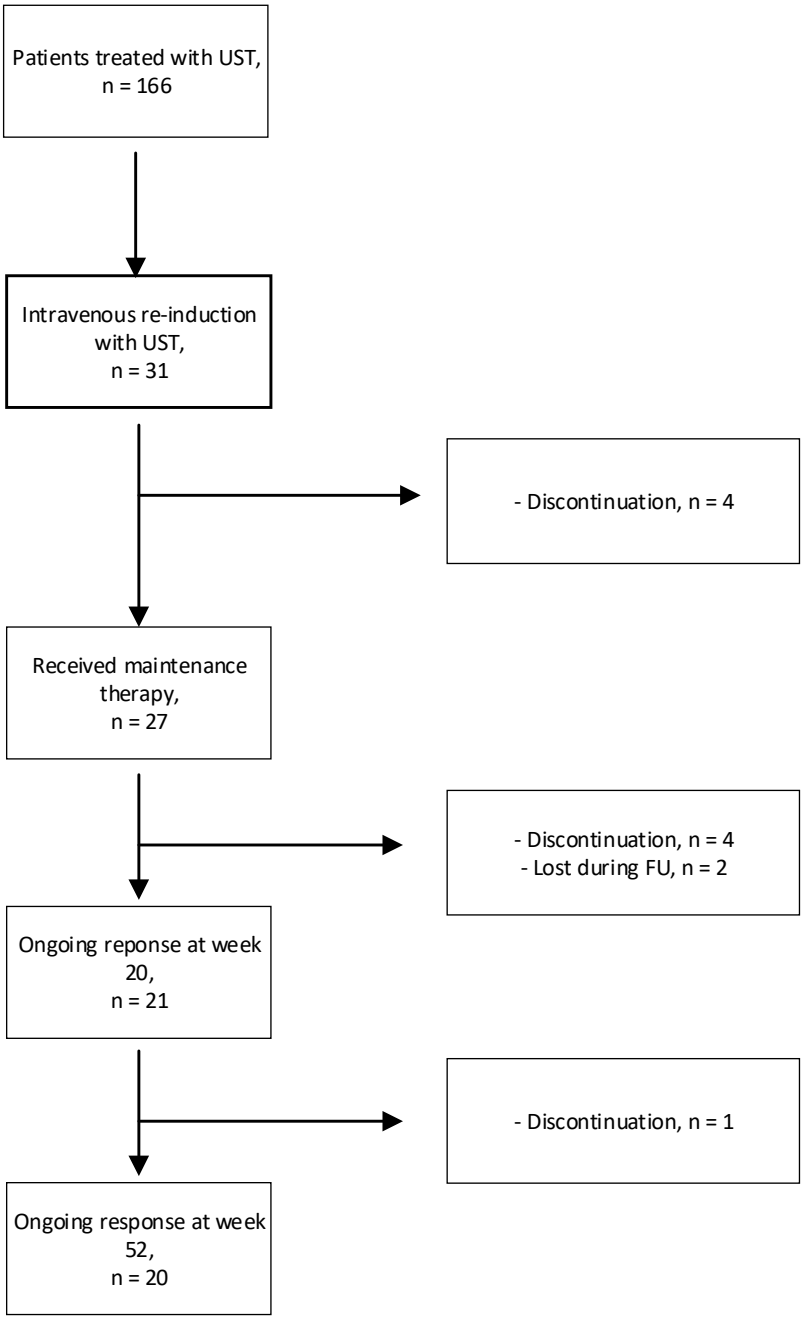
Clinical remission

The proportion of patients in clinical remission at week 8, 20 and 52 was 37% [n = 11/30], 56% [n = 15/27] and 45% [n = 13/29], respectively, for the total study cohort. At baseline, nine patients were in clinical remission [HBI≤4], however received intravenous UST re-induction for biochemical disease activity. One patients was in both clinical and biochemical remission, however received a second dose of intravenous UST due to severe extraintestinal manifestations [erythema nodosum]. In a sub-analysis in which the patients in clinical remission at baseline [n = 9] were excluded, the proportion of clinical remission at week 8, 20 and 52 was 23%, 44% and 40%, respectively.

Endoscopic evaluation was performed in 67% of the patients [n = 6/9] at the time of discontinuation, which showed endoscopic disease activity with ulcerations in four patients [67%] and only mild inflammation in the other two patients.

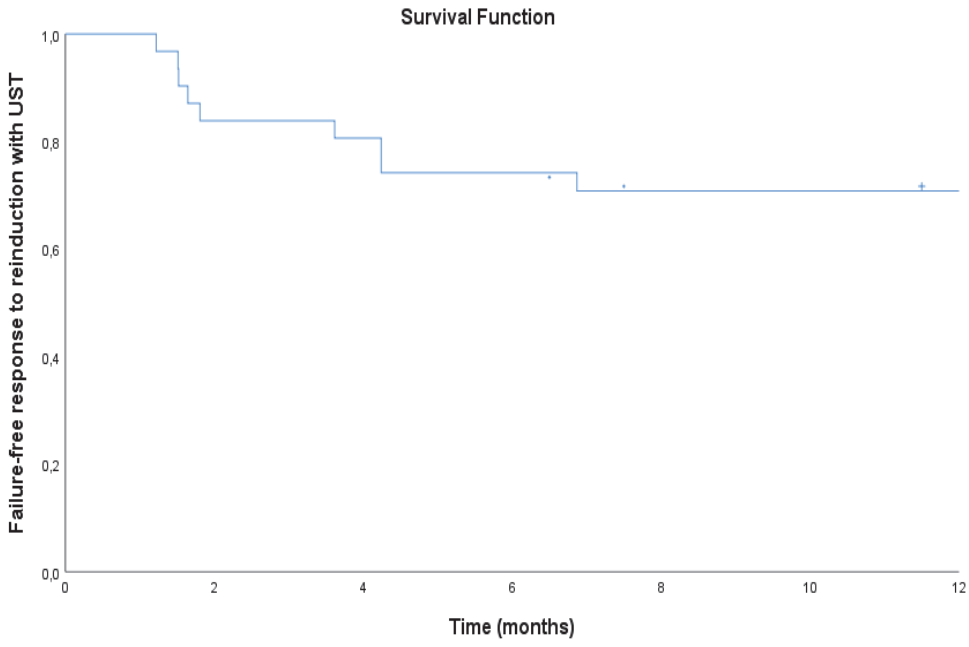
No statistical difference in weight was observed between patients who continued UST after reinduction [median weight 70kg (IQR 56.5 – 103.2)] as compared with patients who discontinued UST [median weight 71kg (IQR 65.5 – 97)] [p = 0.634].

Figure 1. Flow chart of patient cohort.



N, number of patients; UST, ustekinumab; FU, follow up

Figure 2. Kaplan-Meier analysis of cumulative probability of failure-free response to re-induction with UST



Number at risk

31	**26**	**25**	**23**	**20**	**20**	**20**

Discontinuation of UST treatment

Nine of 31 patients [29%] discontinued UST treatment after receiving a second dose of intravenous UST within one year [figure 1]. Median treatment duration until cessation was 1.8 months [IQR 1.5 – 4.2] after re-induction. Reasons for discontinuation of the UST therapy were non-response [n = 5, 56%], loss of response [n = 3, 33%], or adverse event [n = 1, 11%]. Of the patients with non-response to a second dose of intravenous UST, four patients discontinued UST therapy before subcutaneous maintenance therapy and one patient discontinued before week 8 [interval 1q4w].

Adverse event

In total, six patients required hospitalization during UST treatment due to a ileus [n = 4] or severe active disease [n = 2]. In one of these patients, with severe active disease, UST treatment was discontinued after segmental jejunal resection.

During follow up, one malignancy [melanoma] was reported which required surgical resection. Three possibly related adverse events were encountered after the second iv UST dose, including a lower urinary tract infection, intestinal bacterial overgrowth, and pruritus during follow up. No deaths were reported.

Discussion

Re-induction with intravenous UST is a relatively new treatment optimization strategy in CD. This real-world cohort study concerns an anti-TNF refractory CD population with secondary loss of response to UST, and shows that UST therapy, after a second dose of intravenous UST, was maintained in approximately three-quarters of the patients. In addition, a second dose of intravenous UST recaptures initial response in almost half of these patients after one year follow-up. Therefore, re-induction with an intravenous UST dose may be an important rescue treatment option in patients with refractory CD.

A clinical benefit of UST re-induction has been reported previously in approximately up to 50% of patients, however, most data in current available literature are limited to reports on short term-follow-up. In the present study cohort, clinical remission rates after a second dose of intravenous UST were confirmed and follow-up was extended, with response rates of 56% and 45% at week 20 and 52. A recently published multicenter study from Spain [n = 53] evaluated the short-term efficacy of intravenous re-induction and reported a clinical

remission rate of 49% and 43% at week 8 and 16, respectively [13]. In addition, an American, single center prospective cohort [n = 13] reported a significant decrease in HBI after intravenous re-induction [14]. Two other real-world cohort studies from the Netherlands [n = 7] [6] and Israel [n = 30] [10] reported comparable overall response rate of 43% and 50%. Although both studies reported a beneficial effect in a considerable proportion of patients, the response rates vary, which could be explained by the small study populations, short follow-up and varying endpoints. In contrast to these publications, in a study from the United States [n = 18] [7], 83% of the patients achieved clinical response. Nonetheless, this study involved a relatively young population [median age 20.9 years] and a relatively high proportion of patients [67%] received a dose-optimization [either q6w or q4w interval] as maintenance therapy after the second dose of intravenous UST, which could explain the response rate of over 80%. In comparison, in the present study only one-third of patients received dose-optimization [all at a q4w interval] as maintenance therapy. In this subgroup receiving q4w dose-optimization, only 30% of the patients were in clinical remission after 1 year.

The data from our relatively small study population suggest a lower rate of clinical remission in the q4w interval subgroup as compared to the q8w subgroup. It may be possible that the q4w subgroup has dismal disease characteristics as compared to the q8w subgroup, which have directed the treating physicians to shorten the interval of the UST maintenance treatment. However, since we cannot substantiate this hypothesis with clinical data, misinterpretation of the subgroup analysis due to small sample size cannot be refuted. Recognizing that stratification of this small cohort may limit conclusions that can be drawn, further study of larger cohorts to assess the combination of re-induction with intravenous UST followed by subcutaneous UST at q4w interval is required.

Secondary loss of response during UST treatment in CD may be related to low levels of UST. The low anti-UST-antibodies incidence during UST treatment indicates that secondary loss of response is not mainly driven by immunogenicity [15,16]. A more plausible explanation is the need for high peak levels. Several studies have provided evidence on the relevance of UST peak levels. High remission rates of maintenance placebo arms after intravenous UST induction [36% at week 44, UNITI-trial [2], 43% at week 22, the CERTIFI Study Group [14]] indicate that a high peak-dose of UST has a long duration of action and leads to long-term remission in over one third of CD patients. The long duration of efficacy after intravenous UST could be explained due to its long serum half-life of approximately three weeks and an additional period in which drug effects remain [14]. In line with these observations, a previous study reported significant associations between peak concentrations and both endoscopic and biochemical remission at week 24 [15]. These results suggest that the peak level of UST may be associated with response to UST rather than through levels. After intravenous infusion, the median UST concentration is significantly higher [5,7,16,17] when compared to the steady state concentration during maintenance treatment [14,16].

The need for high UST peak levels could be explained by the underlying mechanism of the distribution of the UST volume. Higher serum concentration of the pro-inflammatory cytokines IL-12 and -23 are seen in patients with active disease [18]. This might lead to increased binding of UST to these cytokines due to a higher target concentration resulting in a higher volume of distribution and a (too) low trough concentration. As a result, second dose of intravenous UST after secondary loss of response might be needed for a subset of patients in order to obtain a peak concentration not reached with more frequent subcutaneous maintenance dosing, for a maximal response of the UST treatment, especially in therapy-refractory patients. Due to a lack of encouraging data for the ideal therapeutic window of

UST, further real-world pharmacokinetics trials are warranted to assess the value of therapeutic peak levels of UST treatment in both induction and maintenance therapy. Therapeutic drug monitoring based on UST peak concentration could help to identify a subpopulation of CD patients that merely benefit from high peak levels, and require pulsed intravenous UST maintenance therapy.

Despite promising results of a second dose of intravenous UST after secondary loss of response, not all patients responded in our cohort study. The underlying biological drivers of the disease in these patients contribute to the response to therapy, such as a change in the underlying inflammatory response. More research is needed to identify these biological drivers in non-responders to re-induction.

To investigate the optimal strategy of UST optimization, a prospective, randomized, clinical trial is warranted to provide more data on predictors and efficacy. The POWER-study (ClinicalTrials.gov Identifier: NCT03782376), is an ongoing prospective randomized placebo-controlled study which includes patients after secondary loss of response to UST maintenance to evaluate the efficacy and safety of the re-induction with intravenous UST. Patients are randomized between intravenous re-induction with UST versus continuing with regular subcutaneous q8w 90 mg UST administration. Results of this study are eagerly awaited. In addition, a randomized study comparing shortening of the subcutaneous UST interval versus re-induction with intravenous UST to recapture response would also be of great interest to fill this knowledge gap.

This study provides informative findings regarding a scarcely studied topic among UST optimization strategy in CD with assessment of outcomes through 52 weeks. Nevertheless, a few limitations of this study need to be considered. The main limitation is the small sample

size which affects the generalizability of the findings and did not allow to identify predictors of response. Second, due to its retrospective design we should be extra-cognizant of selection bias. To bypass this bias, patients with interval shortening could serve as control group, both for the actual intervention as well as for possible biases of physicians electing UST re-induction versus those favouring an interval-shortening approach. Furthermore, data on both peak and trough levels were not systematically assessed in our study and, therefore, clinical outcomes could not be correlated with drug levels. Although the relatively small numbers, this multicenter study sheds additional light on the role of re-induction with a second dose of intravenous UST for refractory CD patients with secondary loss of response to the initial UST treatment.

In conclusion, re-induction with an intravenous UST dose after secondary loss of response results in therapy continuation for a minimum of one year in approximately three quarters of the patients and in clinical remission in half of the patients with refractory CD. Therefore, UST re-induction may be considered an important rescue treatment option.

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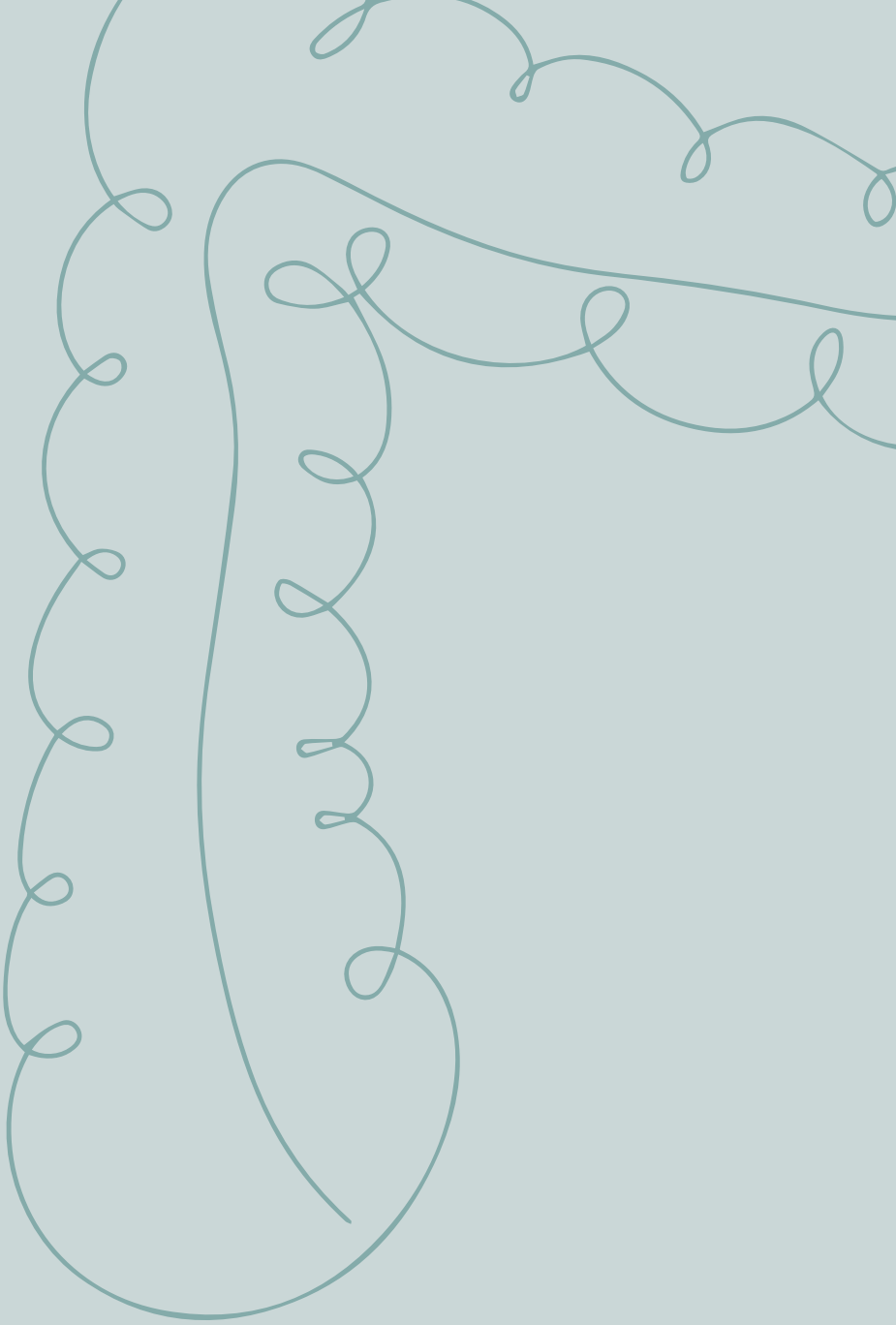

Part II

Postoperative optimization strategies

Chapter 4

Retreatment with anti-tumour necrosis factor therapy in combination with an immunomodulator for recurrence of Crohn's disease after ileocecal resection results in prolonged continuation as compared to anti-TNFα monotherapy

Chapter 5

Postoperative prophylaxis prevents surgical and severe endoscopic recurrence after primary ileocecal resection in CD patients

Chapter 6

Prognostic value of the modified Rutgeerts' score for long-term outcomes after primary ileocecal resection in Crohn's disease


4

Chapter 4

Retreatment with anti-TNFα therapy in combination with an immunomodulator for recurrence of Crohn's disease after ileocecal resection results in prolonged continuation as compared to anti-TNFα monotherapy

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Abstract

Background A considerable proportion of Crohn's disease patients that undergo ileocecal resection (ICR) have failed anti-tumour necrosis factor (TNF) therapy preoperatively. This study aimed to assess the effectiveness of retreatment of anti-TNF therapy in patients with postoperative recurrence.

Methods A real-world cohort study was performed in CD patients who underwent primary ICR after anti-TNF therapy failure, and who were retreated with anti-TNF therapy for postoperative symptomatic CD. Primary outcome was treatment failure defined as the need for (re)introduction of corticosteroids, immunosuppressants or biologicals or the need for re-resection. Sub-analyses were performed on nature of preoperative anti-TNF failure (primary non-response, secondary loss of response, intolerance), indication for ICR (refractory, stricturing, penetrating disease), combination therapy with immunomodulators, retreatment with the same anti-TNF agent and preoperative exposure to 1 vs >1 anti-TNF agents.

Results In total, 66 of 364 patients were retreated with anti-TNF therapy following ICR. Cumulative rates of treatment failure at 1 and 2 years were 28% and 47%. Treatment failure rate at 2 years was significantly lower in patients receiving combination therapy as compared to anti-TNF monotherapy (30% vs. 49%, P=0.02). No difference in treatment failure was found with regards to the nature of preoperative anti-TNF failure (P=0.76), indication for ICR (P=0.88) switch of anti-TNF agent (P=0.55) agent, and preoperative exposure to 1 vs. >1 anti-TNF agents (P=0.88).

Conclusion Retreatment with anti-TNF therapy for postoperative CD recurrence after primary ICR is a valid strategy after preoperative failure. Combination therapy is associated with a lower rate of treatment failure.

Introduction

Although the need for surgery has decreased over time, up to 40% of patients with Crohn's disease (CD) will require an intestinal resection during the disease course [1]. Postoperative recurrence is common, since up to 25% of patients will develop clinical recurrence and up to 80% will develop endoscopic recurrence within one year [2-6].

A majority of CD patients have been exposed to anti-tumour necrosis factor (TNF) therapy prior to ileocecal resection (ICR). On the one hand previous failure of anti-TNF therapy is associated with failure of a second attempt [7-8], while on the other hand early CD lesions after intestinal resection may comprise a new opportunity for response to anti-TNF therapy. This hypothesis is substantiated by the observation of distinct characteristics of the immune infiltrate in the neo-terminal ileum of CD lesions after ileocecal resection, as compared to longstanding ileitis [9].

In contrast to abundant data on anti-TNF agents for the prevention of postoperative recurrence of CD, data regarding treatment of postoperative recurrence with anti-TNF therapy are scarce. In addition, medication use prior to ICR is not taken into account in current international guidelines on management strategies for postoperative CD [10,11]. To date, only three real-world studies have assessed the effect of anti-TNF therapy as treatment for postoperative recurrence. However, these studies comprised mostly anti-TNF naïve patients [12-14]. In addition, only one study has investigated the effect of anti-TNF treatment for postoperative recurrence in paediatric CD patients who were refractory to anti-TNF therapy preoperatively [15]. Therefore, the effect of retreatment with anti-TNF therapy after resection of the affected bowel region in previously anti-TNF refractory adult CD patients is unknown.

This study aimed to assess the effectiveness of retreatment with anti-TNF therapy for postoperative recurrence in CD patients who failed anti-TNF preoperatively.

Methods

Study design and patients

A retrospective, multicenter study was conducted in CD patients who underwent primary ICR for the indication of CD between January 2000 and January 2020. Eligible patients were identified from local hospital pathology databases of the participating centers, including 4 teaching and 6 academic hospitals. Patients aged 16 years and older, who were exposed to anti-TNF therapy preoperatively, were considered eligible. Patients who were retreated with anti-TNF therapy (infliximab or adalimumab) as the first treatment choice for postoperative clinical recurrence were included. Postoperative clinical recurrence was defined as symptomatic CD after ICR necessitating initiation of medical treatment. In case corticosteroids or 5-aminosalicylates (5-ASA) were the first treatment choice for postoperative clinical recurrence followed by anti-TNF therapy (within 3 months in case of corticosteroid use), patients were also included. Exclusion criteria included primary postoperative prophylaxis with an anti-TNF agent.

Outcome and definitions

The primary endpoint was treatment failure, defined as the (re)introduction of treatment (including 5-ASA, corticosteroid, immunosuppressants or other biologics) for symptomatic disease or the need for re-resection at 1 and 2 years following ICR. These patients were considered to have an inadequate response to a second exposure of anti-TNF therapy. Sub-

analyses were performed on the nature of preoperative anti-TNF failure, indication for ICR, combination therapy with immunomodulators vs anti-TNF monotherapy, retreatment with the same or a different anti-TNF agent postoperatively and preoperative exposure to 1 vs >1 anti-TNF agents. Preoperative anti-TNF therapy failure types were defined as primary non-response (absent or insufficient improvement of clinical, biochemical or endoscopic inflammation after anti-TNF therapy), secondary loss of response (primary response to initial therapy which was not maintained) or intolerance (discontinuation of anti-TNF therapy due to side effects). Indications for ICR included refractory disease (non-stricturing and non-penetrating disease), stricturing disease or penetrating disease. Additionally, the association of the interval between anti-TNF initiation and treatment failure within 1 or 2 years was assessed.

Data collection

Electronic patient records were retrospectively reviewed. Data were collected until re-resection, loss to follow-up or death. Baseline characteristics (including age, sex and smoking history), disease characteristics (including Montreal classification [16], disease duration, medical treatment history, concomitant treatment), biochemical markers (anti-TNF antibodies and trough levels within 12 months prior to ICR), time between ICR and retreatment of anti-TNF and postoperative endoscopic and radiologic data were obtained. If an endoscopy was performed within 16 weeks before start of retreatment or treatment failure, endoscopic findings were included. Endoscopic disease activity was defined as Rutgeerts classification ≥i2a [5] and radiological disease activity was defined as active inflammation on abdominal ultrasound, CT or MRI as assessed by a local radiologist. In case of anti-TNF re-treatment failure, pharmacologic data (anti-TNF antibodies and trough levels) were collected.

Statistical analysis

Continuous variables were reported as median and interquartile range [IQR]. Categorical variables were reported as frequencies and percentages. The time to event was defined as the time between anti-TNF initiation and treatment failure. Regarding patients who did not have treatment failure, the observation was censored at the time of maximal follow-up, loss to follow-up or death. A Pearson's *r* test was performed to assess whether there was a point-biserial correlation between time to start anti-TNF treatment and treatment failure within 1 and 2 years. The observed cumulative incidence of treatment failure following ICR was calculated using the Kaplan-Meier Method. Sub-analyses on the nature of preoperative anti-TNF failure, combination therapy with immunomodulators vs. anti-TNF monotherapy, and retreatment with the same anti-TNF agent postoperatively were compared using the Log Rank test. All data analyses were performed using IBM SPSS Statistics for Windows, version 25.0.

Ethics approval

This study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its protocol was assessed and approved by the Medical Ethical Research Committee of the Erasmus University Medical Centre on the 10th of November 2017.

Results

Baseline characteristics

A total of 364 patients who underwent an ICR and received anti-TNF therapy prior to surgery were identified. Of these patients, 159/364 (44%) patients experienced postoperative clinical recurrence, of whom 66/159 (42%) patients received reintroduction of anti-TNF as treatment and were included in the study population **(figure 1).**

The majority of the included patients were female (62%) with a median age of 35 years (IQR 26 –50) **(table 1)**. Median disease duration until ICR was 4.7 years (IQR 1.8 – 9.3). Indication for ICR was refractory disease (non-stricturing and non-penetrating disease) in 26/66 (39%) patients, stricturing disease in 28/66 (42%) patients or penetrating disease in 12/66 (18%) patients. Median total postoperative follow-up was 6.3 years (IQR 4.3 – 8.3). In total, 47/66 (71%) patients were exposed to one anti-TNF agent (47% infliximab, 24% adalimumab) and 19/66 (29%) patients were exposed to both anti-TNF agents alternately prior to ICR. After ICR, 22/66 (33%) patients received prophylactic immunomodulatory therapy (3/22 (14%) methotrexate, 19/22 (86%) thiopurines) postoperatively.

Preoperative anti-TNF failure

Regarding anti-TNF failure prior to ICR, secondary loss of response was the reason in the majority of patients (n = 45/66, 68%), whereas 12/66 (18%) had primary non-response and 7/66 (11%) discontinued anti-TNF treatment due to intolerance to anti-TNF therapy **(table 1)**. At time of cessation of anti-TNF therapy preoperatively, the median serum level of adalimumab was 4.2 μg/ml (IQR 2.98 – 6.63, n = 6) and for infliximab 4.4 μg/ml (IQR 2.43 – 9.20, n = 6). Antibodies to anti-TNF therapy were reported in only a few patients at the

time of discontinuation prior to ICR for adalimumab (12 x 10-9g/L, IQR 12 – 4303, n = 4) and infliximab (12 x 10-9g/L, IQR 12 – 12, n = 3).

Postoperative recurrence

Regarding postoperative clinical recurrence, 31/66 (47%) patients were treated with infliximab and 35/66 (53%) were treated with adalimumab. Thirty-seven out of 66 (56%) patients received the same anti-TNF agent preoperatively and postoperatively, and 29/66 (44%) were treated with a different agent. Of these patients, 7/66 (11%) received prednisone primarily, followed by anti-TNF therapy. Median time between ICR and treatment with anti-TNF therapy following postoperative clinical recurrence was 9.4 months (IQR 6.5 – 18.4). In total, 44/66 (67%) patients started anti-TNF as monotherapy whereas 22/66 (33%) received concomitant immunomodulators.

Regarding concomitant immunomodulators, 18/22 patients continued postoperative prophylactic immunomodulatory therapy at time of reintroduction of anti-TNF therapy and 4/22 patients started a concomitant immunomodulator in combination with anti-TNF therapy.

Endoscopic evaluation was performed in 32/66 (49%) of the patients at time of postoperative clinical recurrence, of whom 25/32 patient (78%) had a Rutgeerts score ≥i2a (i2 = 13 [40%], i3 = 9 [27%], i4 = 3 [9%] i0 = 5 [16%], i1 = 2 [6%]).

Postoperative treatment failure

During total follow-up, treatment failure after retreatment with anti-TNF therapy was observed in 44/66 (67%) of whom 3/44 (7%) underwent a re-resection. Median time to treatment failure was 1.2 years (IQR 0.6 – 4.2). Kaplan-Meier estimates of the treatment failure rates were 28% and 44% after one and two years, respectively (**figure 2**).

Table 1. Baseline characteristics.

Patients characteristics		**n = 66**
Age, years[b]	Median [IQR]	34 [24 – 48]
Sex, female	N [%]	41 [62]
Smoker, yes[a]	N [%]	27 [41]
Age at diagnosis		
- ≤16 years	N [%]	13 [20]
- 17 – 40 years	N [%]	37 [56]
- >40 years	N [%]	16 [24]
Disease location at ICR		
- Ileum (L1)	N [%]	42 [64]
- Colon (L2)	N [%]	0 [0]
- Ileocolonic (L3)	N [%]	24 [36]
Disease, behaviour at ICR		
- Non stricturing, non-penetrating (B1)	N [%]	22 [33]
- Stricturing (B2)	N [%]	27 [41]
- Penetrating (B3)	N [%]	17 [26]
- Perianal disease (p)	N [%]	8 [12]
Preoperative anti-TNF therapy		
- Infliximab	N [%]	31 [47]
- Adalimumab	N [%]	16 [24]
- Both	N [%]	19 [29]
Preoperatively ustekinumab	N [%]	-
Preoperatively vedolizumab	N [%]	1 [2]
Reason anti-TNF therapy was withdrawn		
- Primary non-response	N [%]	12 [18]
- Secondary loss of response	N [%]	45 [68]
- Intolerance	N [%]	7 [11]
- Missing	N [%]	2 [3]

[a]At time of ICR; [b]At time of retreatment; ICR, ileocecal resection; TNF, tumor necrosis factor

One (2%) and two (5%) patients underwent a re-resection within one and two years, respectively. Endoscopy data after anti-TNF re-introduction were available for 19/44 patients (43%) at time of treatment failure of whom fifteen patients (79%) had endoscopic disease activity (Rutgeerts score ≥ 2a). No correlation was observed between time of start anti-TNF treatment for postoperative clinical recurrence and treatment failure ($p = 0.790$).

At time of treatment failure, the median serum level of adalimumab was 6.0 μg/ml (range 0.03 – 12, n = 2) and infliximab was 3.0 μg/ml (IQR 0.30 – 12, n = 11). Antibodies to anti-TNF therapy were detected at time of discontinuation in 3 patients treated with adalimumab (35 x 10-9g/L, IQR 35 – 150) and in 7 patients treated with infliximab (12 x 10-9g/L, IQR 12 – 280) of whom 7 (70%) and 3 (30%) patients received mono- and combination therapy, respectively.

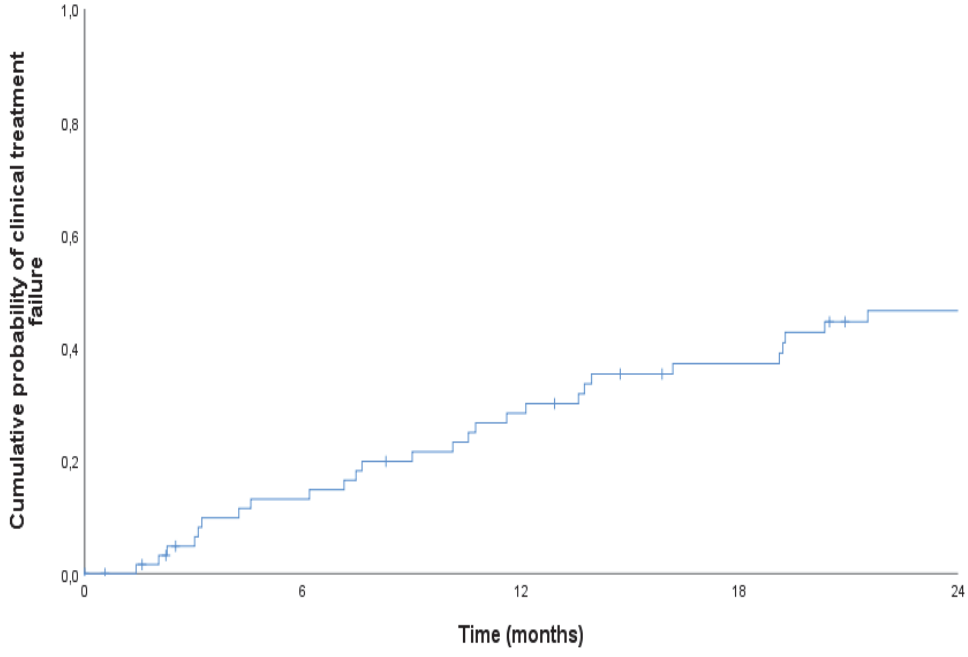
Regarding patients with primary non-response, secondary loss of response and intolerance to anti-TNF therapy, the cumulative rate of treatment failure at 1 year was 25%, 23% and 57% (Log-Rank, p = 0.102), in patients with primary non-response, secondary loss of response and intolerance to anti-TNF therapy, respectively. After two years, the cumulative rate of treatment failure was 25%, 45% and 71% (Log-Rank, p = 0.760).
In patients with refractory, stricturing or penetrating disease as indication for ICR, the 1-year cumulative rate of treatment failure was 31%, 26% and 17% (Log-Rank, p = 0.996), respectively. After 2 years, the cumulative rates of treatment failure were 50%, 41% and 28% (Log-Rank, p = 0.880), respectively.

With regard to combination therapy with immunomodulators, the cumulative rates of treatment failure after 1 year were 9% and 32% in patients receiving combination therapy with an immunomodulator as compared to patients who were exposed to anti-TNF monotherapy (Log-Rank, p = 0.004). After 2 years, the cumulative rate of treatment failure was 30% and 49% (p = 0.016, **figure 3**).Time to treatment failure was 2.1 years (IQR 1.2 – 4.0) in patients receiving combination therapy as compared to 1.1 years (IQR 0.4 – 3.1) in patients exposed to anti-TNF monotherapy.

Regarding retreatment with the same anti-TNF agent postoperatively, the cumulative rates of treatment failure at 1 year was 30% in patients retreated with the same agent as compared with 21% in patients who were switched to another agent postoperatively (Log-Rank, p = 0.349). After 2 years, the cumulative rates of treatment failure were 36% and 51% (Log-Rank, p = 0.548).

Regarding preoperative exposure to 1 vs >1 anti-TNF agents, the cumulative rates of treatment failure at 1 year was 26% in patients exposed to only one anti-TNF agent preoperatively as compared with 33% in patients who were switched to another agent preoperatively (Log-Rank, p = 0.797). After 2 years, the cumulative rates of treatment failure were 42% and 63% (Log-Rank, p = 0.884).

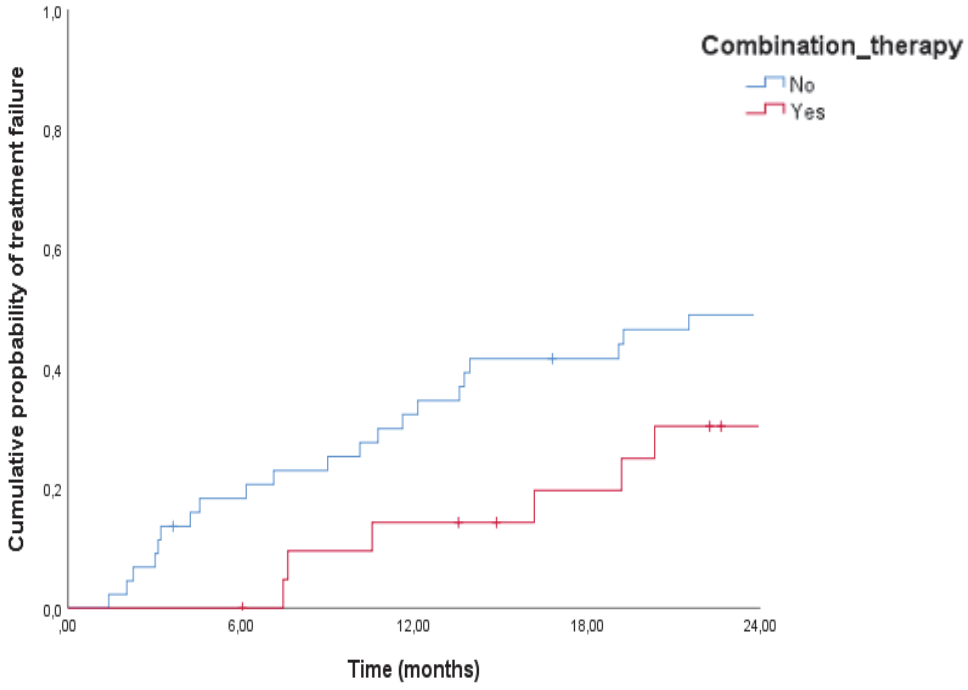
Figure 2. Kaplan Meier analysis of treatment failure in CD patients in whom anti-TNF was restarted due to clinical recurrence.



At risk

66	52	42	34	27

Figure 3. Kaplan Meier analysis of treatment failure in CD patients who were retreated with anti-TNF therapy after diagnosis of postoperative clinical recurrence for the subgroups combination therapy with immunomodulators vs monotherapy.



At risk combination therapy

22	22	18	15	11

At risk monotherapy

44	35	29	24	21

Discussion

Recurrence of symptomatic CD after a primary ICR occurs in over 40% of patients who have been exposed to anti-TNF therapy preoperatively. Especially in case of a therapy refractory disease course preoperatively, an important clinical question in postoperative CD management is whether treatment, rather than prevention, of postoperative recurrence can be effectively managed with a second exposure to anti-TNF therapy. This real-world cohort study showed that retreatment with anti-TNF therapy for postoperative CD recurrence after primary ICR is continued in more than half of patients after two years. Anti-TNF therapy in combination with an immunomodulator results in continuation of therapy in a significantly higher proportion of patients, i.e. approximately two-third of the patients. Therefore, retreatment with anti-TNF therapy especially in combination with an immunomodulator may be an effective strategy for postoperative clinical recurrence of CD in patients treated with an anti-TNF agent postoperatively.

In current available literature, three studies, including an Italian study (n = 13), a Japanese study (n = 8) and a Spanish study (n = 179) investigated the impact of diagnosis and treatment with anti-TNF therapy on postoperative endoscopic recurrence after surgery and have shown beneficial effect of anti-TNF therapy for postoperative recurrence varied from 61% to 75% depending on the timing of endoscopic evaluation. Importantly, all studies reported a low overall percentage of patients who failed anti-TNF preoperative, respectively 3/13 (23%), 2/8 (25%) and 53/179 (30%) [12-14]. Only one previous study, with a pediatric cohort of patients who failed anti-TNF preoperatively despite adequate serum trough levels (pharmacodynamics failure), investigated the effectiveness of retreatment with anti-TNF after ICR. Children treated with adalimumab prior to surgery and retreated with this anti-TNF agent had a similar rate of clinical remission after 12 months compared with those who

had not received anti-TNF therapy prior to surgery [15]. This suggests that paediatric CD patients who failed anti-TNF therapy and underwent ICR can be retreated with the same agent for postoperative recurrence with a high success rate similar to that of anti-TNF naïve patients [15]. Our study confirms the beneficial effect of retreatment with anti-TNF therapy in adults for postoperative clinical recurrence of CD. Unfortunately, the retrospective study design did not allow for the differentiation of pharmacokinetic, immunogenic or pharmacodynamic failure of anti-TNF therapy. Future studies are required to assess the predictive value of preoperative anti-TNF trough levels for the postoperative success of retreatment.

A possible explanation for the high success rate of retreatment could be the distinct mucosal profiles of cytokines which are produced during different stages of CD. Macroscopically unaffected neo-terminal ileum contains elevated levels of TNF. However, these TNF levels are not increased in mucosal biopsies of the terminal ileum of CD patients with pre-operative longstanding ileitis (taken from the resection specimens) despite histopathological confirmed inflammation [9]. This difference in anti-TNF production might reflect a functional change in immunological pathways activated during the stage of disease especially in patients undergoing ICR. This change in cytokine expression could support the choice of anti-TNF therapy as treatment strategy for postoperative recurrence.

Subgroup analysis showed that treatment was more effective in patients receiving anti-TNF in combination with an immunomodulator compared to patients receiving anti-TNF monotherapy. This observation is in line with previous data which suggest that immunomodulators may need to be started or continued in CD patients upon initiation of anti-TNF therapy, based on the presumption that immunosuppressive therapy is expected to

substantially improve efficacy, increase serum drug concentrations, and reduce immunogenicity [17-20].

To the best of our knowledge, this is the first study which reports the effectiveness of retreatment with anti-TNF therapy in adult CD patients who preoperatively failed anti-TNF therapy. A strength of the current study is the long follow-up period. Secondly, in this study all patients were treated in both academic and teaching centers, which increases its generalizability to a wider CD population. However, some limitations need to be taken into consideration. First, no standard endoscopic evaluation was performed at the start of anti-TNF. Therefore, endoscopic recurrence was not taken into account and no correlation between anti-TNF treatment failure and endoscopic lesions could be established. Secondly, the retrospective character of the study, resulting in the absence of a preoperative anti-TNF naïve control group. In addition, the small sample size did not allow to identify predictors of treatment failure. Another limitation is the lack of data regarding adverse events and tolerability of the treatment, including prolonged combination of anti-TNF and immunosuppressants. Although anti-TNF levels were collected, trough levels were not routinely assessed in our cohort. Therefore, we could not exemplify the exact reason for treatment failure and no conclusion can be drawn. Even with these limitations, this multicenter study sheds additional light on the role of retreatment with anti-TNF therapy for postoperative recurrence in CD patients.

In conclusion, retreatment with anti-TNF therapy for postoperative recurrence is a valid treatment option, since half of the CD patients remain in remission two years after retreatment. Combination therapy with an immunomodulator is associated lower treatment failure rates.

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Chapter 5

Postoperative prophylaxis prevents surgical and severe endoscopic recurrence after primary ileocecal resection in Crohn's Disease patients

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On behalf of the Dutch Initiative on Crohn and Colitis (ICC)

Submitted

Abstract

Background Prophylaxis following ileocecal resection (ICR) is recommended in patients with Crohn's disease (CD), particularly in patients at increased risk of recurrence. This study aimed to evaluate the effect of prophylaxis on long-term prognosis.

Methods A retrospective cohort study was performed in patients with CD who underwent a primary ICR. Patients were divided into two groups: prophylaxis (<12 weeks following ICR) versus no prophylaxis. Outcomes were surgical recurrence and severe endoscopic recurrence (modified Rutgeerts score ≥ i3). Inverse probability of treatment weighting (IPTW) method was used to adjust for confounding and selection bias. Survival and association between prophylaxis and both outcomes were assessed with Kaplan-Meier analyses and Cox proportional hazard models.

Results 811 patients underwent an ICR [median follow-up 5.8 years (IQR 2.5–10.7)]. Prophylaxis was initiated in 37% of the patients. Cumulative rates of surgical and endoscopic recurrence at 1, 2, 5 and 10 years were significantly lower in patients with prophylaxis versus no prophylaxis [1%, 3%, 9% and 19%, vs. 3%, 4%, 11% and 23%, $p < 0.05$] and [4%, 8%, 15% and 27% vs. 10%, 16%, 25% and 40%, $p < 0.01$]. IPTW analysis showed a lower risk of surgical and severe endoscopic recurrence in patients treated with prophylaxis [aOR 0.52; 95% CI 0.33–0.82; aOR 0.53; 95% CI 0.35–0.81]. Prophylaxis was identified as protective factor for surgical [aHR 0.67, 95% CI 0.45–0.99] and severe endoscopic recurrence [aHR 0.54, 95% CI 0.37–0.78].

Conclusion Prophylaxis following primary ICR in patients with CD may be effective to prevent long-term complications including surgical and severe endoscopic recurrence.

Introduction

Intestinal surgery is an important treatment modality in patients with Crohn's disease [CD].[1] However, resection of the affected segment may not be curative as patients with CD often develop postoperative recurrence. Among patients who have undergone ileocecal resection [ICR], endoscopic recurrence rates have been reported up to 70% within one year after surgery.[2]

Postoperative recurrence may be more severe and more rapid in high risk patients.[3, 4] Therefore, European guidelines recommend prophylactic medication after intestinal resection in patients at high risk of recurrence based on clinical risk stratification. The American guidelines suggest to initiate early prophylactic treatment in all patients and to reserve no prophylaxis only for patients at low risk.[5, 6]

In current available literature, a beneficial effect of prophylactic medication with immunomodulators, anti-tumour necrosis factor [TNF] therapy or newer biologicals has been reported for the prevention of postoperative recurrence.[7-10] However, most studies focused on 1-year endoscopic outcomes and defined endoscopic recurrence at a strict cut-off of Rutgeerts' score ≥ i2. The severity of endoscopic recurrence is associated with recurrence of symptoms as well as the need for a re-resection.[11] An evaluation of severe endoscopic recurrence as endpoint would, therefore, be of added value. In addition, data on the benefit of prophylactic medication to prevent long-term severe endoscopic recurrence would be highly relevant since follow-up in available studies is limited to approximately 1-3 years.[12, 13] Furthermore, the effect of prophylactic medication on surgical recurrence is unknown.

Therefore, this study aims to evaluate the long-term effectiveness of postoperative prophylactic medication on surgical and severe endoscopic recurrence following primary ICR in patients with CD.

Methods

Study design and population

A retrospective multicentre study was performed in CD patients who underwent primary ICR for the indication of CD. Consecutive CD patients following primary ICR were identified from local hospital pathology databases of the participating centres, [six academic and four teaching centres] between January 2000 and November 2020. Patients aged ≥ 16 years with ileal disease with or without colon involvement, and who underwent a primary ICR for the indication of CD were included. Exclusion criteria included prior intestinal resection, missing data on prophylactic medication, and the absence of follow-up data.

Patients were divided into two groups according to the postoperative treatment strategy they received following ICR. Patients were included in the prophylaxis group if they received medication [immunomodulators (thiopurines and methotrexate), anti-TNF therapy (infliximab and adalimumab), biologicals other than anti-TNF therapy (ustekinumab, vedolizumab) and combination therapy (immunomodulator in combination with an anti-TNF agent)] for the prevention of postoperative recurrence within 12 weeks following ICR. Monotherapy with 5-aminosalicylates [5-ASA] or corticosteroids was not considered prophylactic medication. The ‘no prophylaxis group’ comprised patients who did not receive prophylactic medication following ICR. The initiation or optimization of medical treatment [i.e. (re)introduction of corticosteroids, immunomodulators or biologicals] for symptomatic

disease, drug intolerance or optimization driven by endoscopy during follow-up was recorded.

Outcomes and definitions

The primary outcome of this study was surgical recurrence defined as a re-resection for CD (including small bowel and colon resections) during follow-up. Surgical recurrence within 3 months from primary ICR was considered as a re-resection due to postoperative complications and not considered as surgical recurrence. Secondary outcome was severe endoscopic recurrence (modified Rutgeerts score ≥ i3) during follow-up.

Data collection

Data were retrospectively extracted from electronic patient records including: baseline characteristics [sex, age, smoking history], disease-specific characteristics [disease duration and Montreal classification], medical treatment history and type of postoperative prophylaxis were collected. The modified Rutgeerts' score was graded separately for each ileocolonoscopy during follow-up by four trained physicians [SBH, JA, EB and JS] based on available photos and/or the endoscopy report for all patients. Follow-up data were collected until death, loss to follow-up or until last available visit.

Statistical analysis

Continuous variables were described by the median and interquartile range [IQR]. Categorical variables were described by frequency and percentages. Comparisons between groups were performed using the Mann-Whitney U test for continuous variables and the χ^2 test for categorical variables. Statistical significance was set at $p < 0.05$.

Inverse probability of treatment weighting (IPTW) method was chosen to retain all included patients in the estimation of the treatment effects and preservation of statistical power.[14]

Propensity scores were calculated, with the use of a multiple logistic regression model, in which treatment assignment (prophylaxis versus no prophylaxis) was regressed based on the following covariates/potential confounding factors: age at diagnosis, disease location and behaviour at time of surgery (according to the Montreal classification), active smoking, preoperative exposure to a biological and perianal disease at time of surgery. Analysis using IPTW are referred to as weighted analyses, whilst analyses in the unweighted cohort are referred to as unadjusted analyses. Weighted analyses were displayed with adjusted odds ratios (aOR) and the corresponding 95% credible intervals (CI).

Survival probabilities for both outcomes at 1, 2, 5 and 10 years following ICR were determined using the Kaplan-Meier estimator and compared between patients with prophylaxis and patients without prophylaxis medication using log-rank tests. The time to event was defined as the time between primary ICR and surgical or severe endoscopic recurrence, respectively. Patients who did not experience the respective event(s) were censored at the end of follow-up. Associations between the two outcomes and potential risk factors were investigated using multivariable proportional hazards models. Models were fitted in the Bayesian framework which allowed us to simultaneously impute missing values in covariates.[15] The models included a random effect for the study centre to take potential correlation into account between patients treated in the same hospital. Results from the Bayesian proportional hazards models are presented as hazard ratios and corresponding 95%. The exact time of severe endoscopic recurrence is unobserved and only known to be in the interval between the last endoscopy at which no severe endoscopic recurrence was found and the endoscopy at which severe endoscopic recurrence was diagnosed, i.e., it is interval censored. We used the midpoint of the interval as event time in our primary analysis for severe endoscopic recurrence. The exact time of severe endoscopic recurrence is unobserved and only known to be in the interval between the last endoscopy at which no severe

endoscopic recurrence was found and the endoscopy at which severe endoscopic recurrence was diagnosed, i.e., it is interval censored. Limited by the available software, it was not possible to appropriately take into account both the interval censoring and the missing values in covariates. Preliminary analyses using the subset of completely observed covariates showed that using the mid-point of the interval as event time instead of the time of the endoscopy at which severe endoscopic recurrence was observed resulted in hazard ratios and expected survival probabilities similar to the corresponding results from a model that explicitly treated the response as interval censored. We, therefore, use this midpoint of the interval as event time in our primary analysis for severe endoscopic recurrence and performed sensitivity analyses by repeating the analysis using the time of the endoscopy at which severe endoscopic recurrence was diagnosed.. In addition, we performed sensitivity analyses by restricting the data to patients who had a follow-up endoscopy within one year following primary ICR as well as by considering different types of prophylactic therapies [thiopurines, anti-TNF therapy and combination therapy]. Since the number of events among patients with endoscopy within one year was insufficient to obtain reasonably precise parameter estimates for the full set of covariates, we used ridge-regression to penalize the regression coefficients. In the sensitivity analyses considering different types of prophylactic therapy, patients with "other" therapy were excluded due to the small group size. Analyses were performed in R version 4.1.1 (2021-08-10) (R Core Team 2021) with the help of the package **JointAI** (version 1.0.4).[16]

Approval

This study was assessed and approved by the Medical Ethical Research Committee of the Erasmus University Medical Centre Rotterdam on the 10th of November 2017 [MEC-2017-1151]. This study was performed in accordance with the declaration of Helsinki.

Results

Baseline characteristics

In total, 822 patients who underwent an ICR for the indication of CD were identified. In nine patients, prophylactic medication was initiated after 12 weeks, and in two patients data on prophylaxis were missing. These patients were excluded from further analysis. Of the remaining 811 patients, 297/811 [37%] received postoperative prophylactic medication and were included in the prophylaxis group, and 514/811 [63%] patients were included in the no prophylaxis group. The median duration of postoperative follow-up after ICR was 5.8 years [IQR 2.5 – 10.7]. Patients in the prophylaxis group more frequently had perianal fistulizing disease at time of surgery [p = 0.008] whereas patients in the no prophylaxis group, were significantly more females [p = 0.001], active smokers [p = 0.001] and older patients at time of ICR [p = 0.001] **[Table 1]**. Montreal classification at index surgery did not differ between the prophylaxis group and no prophylaxis group [p = 0.077; p = 0.489] **[Table 1]**.

Prophylaxis group

In total, 181/297 [61%] patients received an immunomodulator [167/181 (92%) thiopurines, 14/181 (8%) methotrexate], 58/297 [20%] anti-TNF, 50/297 [16%] anti-TNF agent in combination with an immunomodulator, and 8/297 [3%] other biologicals [5/297 (1%) ustekinumab, 3/297 (1%) vedolizumab] following ICR **[Table 1].**

During total follow-up, the need for therapy optimization was observed in 150/297 [51%] patients after a median interval of 17 months [IQR 7.8 – 37.9] following ICR. Of these patients, 47/150 [31%] received corticosteroids, 1/150 [1%] 5-ASA, 42/150 [28%] immunosupressants [7/42 (17%) methotrexate, 35/42 (83%) thiopurines], 53/150 [35%] anti-TNF therapy [29/53 (55%) adalimumab, 24/53 (45%) infliximab], 3/150 [3%] ustekinumab and 4/150 [2%] vedolizumab. Reason for therapy optimization included symptomatic disease

in 125/145 [86%] patients, drug intolerance in 15/145 [10%] and unknown reason in 5/145 [4%].

No prophylaxis group

Medical treatment for postoperative recurrence was initiated in 364/514 [71%] patients after a median follow-up of 15 months [IQR 7.2 – 46.5]. In 255/364 [71%] patients, medical treatment was started due to clinical recurrence and 109/364 [29%] patients due to endoscopic recurrence with a corresponding modified Rutgeerts' score of i1 (6/109 [6%]), i2a (17/109 [16%]), i2b (41/109 [38%]), i34 (29/109 [27%]) or i4 (16/109 [15%]). The received treatment received for postoperative recurrence comprised corticosteroids in 125/364 [34%], mesalazine in 15/364 [4%], immunomodulators in 128/364 [35%] [thiopurines 111/128 (87%) , methotrexate 17/128 (13%)], and biologicals in 95/364 [26%] patients [anti-TNF therapy 79/95 (83%), vedolizumab 9/95 (10%), ustekinumab 7/95 (7%)].

Surgical recurrence

In total, 124/811 [15%] patients underwent a re-resection during follow up. Median time to surgical recurrence was 4.4 years [IQR 1.8 – 7.7]. The overall cumulative incidence of postoperative surgical recurrence in the total cohort was 2%, 1 year following primary ICR, and increased to 4%, 10% and 22%, after 2, 5 and 10 years following primary ICR. Surgical recurrence occurred in 32/297 [11%] patients in the prophylaxis group and in 92/514 [18%] patients with no prophylaxis [p = 0.007]. The cumulative risk of surgical recurrence at 1, 2, 5 and 10 years was significantly lower in patients with prophylaxis as compared to patients without prophylaxis; 1%, 3%, 9% and 18% vs. 3%, 4%, 11% and 23% [log-rank, $p = 0.005$, $p = 0.07$, $p = 0.01$ and $p = 0.034$] **[Figure 1].**

Table 1. Baseline characteristics

Patients characteristics		**Total cohort n = 811**	**Prophylaxis n = 297**	**No prophylaxis n = 514**	***P-value***
Female	N [%]	502 [61]	161 [54]	341 [66]	0.001
Active smoking, n = 769	N [%]	283 [35]	85 [29]	198 [39]	0.003
Disease duration, years	Median [IQR]	3.1 [0.7 – 8.0]	3.8 [1.3 – 8.4]	5.5 [0.45 – 7.5]	0.001
Age at surgery, years	Median [IQR]	32 [24.1 – 45.1]	28.9 [22.7 – 39.0]	34.4 [26.6 – 48.0]	0.001
Disease location[*]					0.077
L1 Terminal ileum	N [%]	516 [64]	177 [60]	339 [66]	-
L2 Colon	N [%]	-	-	-	-
L3 Ileocolonic	N [%]	295 [36]	120 [40]	175 [34]	-
Disease, behaviour[*]					0.489
B1 Non-stricturing, non-penetrating	N [%]	176 [22]	62 [21]	114 [22]	-
B2 Stricturing	N [%]	496 [49]	140 [47]	256 [50]	-
B3 Penetrating	N [%]	239 [30]	95 [32]	144 [28]	-
Perianal disease[*]	N [%]	94 [12]	45 [15]	49 [10]	0.008
Preoperatively treatment for IBD					
5-ASA	N [%]	323 [40]	111 [38]	212 [41]	0.503
Anti-TNF therapy	N [%]	356 [44]	181 [61]	175 [34]	0.001
Thiopurines (AZA/TG/MP)	N [%]	474 [55]	240 [81]	234 [46]	0.001
Methotrexate	N [%]	125 [15]	48 [16]	77 [15]	0.677

Table 1. continued

Prophylactic strategy					-
None	N [%]	514 [63]	-	514 [63]	
Methotrexate	N [%]	14 [2]	14 [5]	-	
Thiopurines	N [%]	167 [21]	167 [56]	-	
Anti-TNF therapy	N [%]	58 [7]	58 [20]	-	
Ustekinumab	N [%]	5 [1]	5 [2]	-	
Vedolizumab	N [%]	3 [1]	3 [1]	-	
Combination therapy**	N [%]	50 [6]	50 [16]	-	

* at time of ICR

** anti-TNF agent and immunomodulator

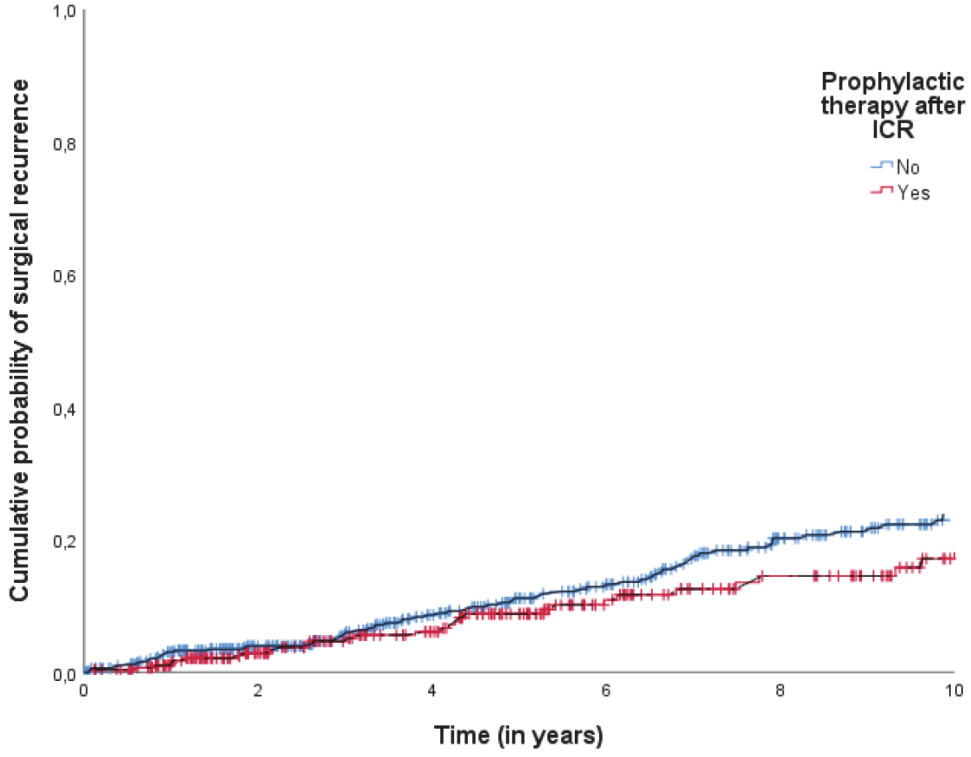
Propensity-scored weighted analysis showed that patients treated with prophylaxis were less likely to experience surgical recurrence [aOR 0.52; 95% CI 0.33 – 0.82] as compared to patients with no prophylaxis. Median time to surgical recurrence did not differ between the prophylaxis group and the no-prophylaxis group [4.1 years (IQR 1.9 – 6.6) vs. 5.3 year (IQR 2.8 – 8.9), p = 0.137].

The vast majority of patients with re-resection in the prophylaxis group (26/32 [81%]), had also received optimization of medical therapy, with a median interval between optimization and re-resection of 34 months [IQR 12.2 – 55.9].

Severe endoscopic recurrence

In total, 1787 colonoscopies were performed during follow-up in 692/811 [85%] patients. Median time to first colonoscopy following ICR was 9.3 months [IQR 6.0 – 25.0] with an index modified Rutgeerts' score of i0 (200/661 [30%]), i1 (116/661 [17%]), i2a (104/661 [16%]), i2b (133/661 [20%]), i3 (63/661 [10%]) or i4 (45/661 [7%]) [missing data on modified Rutgeerts score in 31/692, 5%]. During follow-up, severe endoscopic recurrence was diagnosed in 200/692 [29%] patients during follow up. Median time to severe endoscopic recurrence was 2.5 years [IQR 0.8 – 6.5]. The overall cumulative rates of postoperative severe endoscopic recurrence were 8%, 14%, 22% and 36%, at 1, 2, 5 and 10 years following ICR. During follow-up, 670/1787 [35%] colonoscopies were performed in the prophylaxis group and 1170/1787 [65%] in the no-prophylaxis group. In the prophylaxis group, 447/514 [87%] patients underwent a colonoscopy as compared to 245/297 [82%] patients in the no prophylaxis group [p = 0.083]. Severe endoscopic recurrence was observed in 49/245 [20%] patients with prophylaxis and in 151/447 [34%] patients without prophylaxis following ICR [p < 0.001].

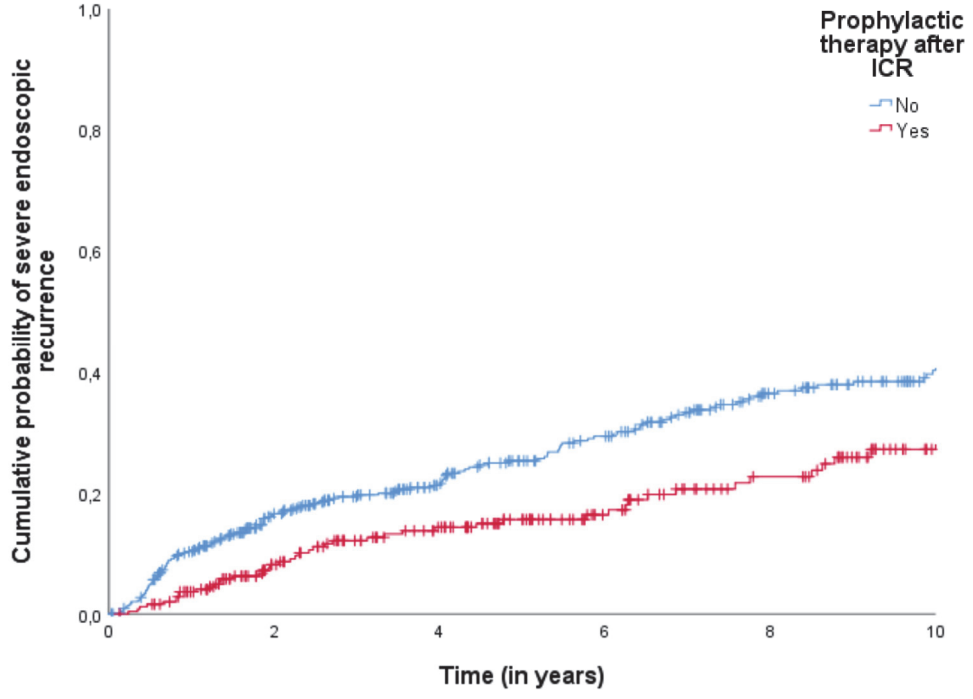
Figure 1. Kaplan-Meier analysis of postoperative surgical recurrence in CD patients after ICR according to prophylactic medication



The cumulative rates of severe endoscopic recurrence at 1, 2, 5 and 10 years following ICR were significantly lower in patients with prophylaxis as compared to no prophylaxis; 4%, 8%, 15% and 27% vs. 10%, 16%, 25% and 40%, respectively [log-rank, $p < 0.001$, $p < 0.001$, $p < 0.001$ and $p = 0.001$; **Figure 2].** The median time to severe endoscopic recurrence did not differ between those originally assigned to prophylaxis compared to those without prophylaxis [2.6 years (IQR 1.3 – 6.7) vs. 2.5 years (IQR 0.7 – 6.4), $p = 0.418$].

A small majority of patients with severe endoscopic recurrence in the prophylaxis group had received optimization of medical therapy, [26/49 patients, 53%]. In these patients, the median interval between optimization and severe endoscopic recurrence was 22.7 [IQR 12.2 – 63.6] months.

Figure 2. Kaplan-Meier analysis of postoperative severe endoscopic recurrence in CD patients after ICR according to prophylactic medication.



Risk factors associated with surgical recurrence and severe endoscopic recurrence

Multivariable analyses showed a benefit of prophylaxis for the prevention of surgical recurrence [adjusted hazard ratio (aHR) 0.67; 95% CI 0.5 – 0.9], whereas active smoking [aHR 1.68; 95% CI 1.2 – 2.4], preoperative exposure to biologicals [aHR 1.77; 95% CI 1.2 – 2.6] and ileocolic disease [aHR 1.45; 95% CI 1.0 – 2.1] were identified as risk factors for surgical recurrence [**Table 2]**. Prophylaxis [aHR 0.54; 95% CI 0.4 – 0.8] and penetrating disease [aHR 0.54; 95% CI 0.4 – 0.9] were identified as protective factor for severe postoperative endoscopic recurrence. Contrarily, active smoking was identified as risk factor with severe endoscopic recurrence [aHR 1.4; 95% CI 1.1 – 1.9].

In the sensitivity analyses in patients who underwent a colonoscopy within one year following ICR [n = 408 (50%), prophylaxis group 133/297 (45%), no prophylaxis group 275/514 (54%); $p = 0.017$], no association between prophyxis medication and postoperative recurrence was observed. In multivariable analyses, no factors were significantly associated with surgical recurrence. A positive microscopic resection margin [aHR 1.7; 95% CI 1.1 – 2.9] and penetrating disease [aHR 0.44; 95% CI 0.22 – 0.84, **Supplementary Table 1**] were significantly associated with severe endoscopic recurrence.

In addition, among patients who received postoperative prophylaxis, thiopurines and combination therapy were identified as protective factors for surgical [aHR 0.51; 95% CI 0.31 – 0.83 and aHR 0.09; 95% CI 0.00 – 0.60] and severe postoperative recurrence [aHR 0.54; 95% CI 0.35 – 0.82 and aHR 0.06; 95% CI 0.00 – 0.35].

Table 2. Multivariable Cox proportional hazard models for surgical and severe endoscopic recurrence

	Surgical recurrence			Severe endoscopic recurrence		
Variable	**aHR**	**2.5%**	**97.5%**	**aHR**	**2.5%**	**97.5%**
Prophylactic medication	**0.7**	**0.5**	**0.9**	**0.5**	**0.4**	**0.8**
Age at diagnosis	0.9	0.9	1.0	1.0	1.0	1.0
Ileocolic disease at surgery (Montreal classification L3, Reference: ileum)	**1.5**	**1.0**	**2.1**	1.3	0.9	1.8
Stenotic disease at surgery (Montreal classification B2, reference: non-stricturing, non-penetrating)	1.2	0.8	2.0	0.8	0.6	1.2
Penetrating disease (Montreal classification B3, reference: non-stricturing, non-penetrating)	0.8	0.5	1.3	**0.6**	**0.4**	**0.9**
Active smoking	**1.7**	**1.2**	**2.4**	**1.4**	**1.0**	**1.9**
Preoperative exposure to biologicals	**1.8**	**1.2**	**2.6**	1.4	1.0	1.1
Perianal fistula	1.3	0.7	2.1	1.0	0.6	1.6
Positive resection margin	1.1	0.7	1.8	1.3	0.9	1.9

aHR = adjusted hazard ratio

Discussion

In this large multicentre retrospective cohort study with long-term real-world follow-up, surgical and severe endoscopic recurrence rates were significantly lower in patients with CD who received postoperative prophylaxis as compared to no prophylaxis following primary ICR. To adjust for differences in baseline patient characteristics, IPTW was used to prevent to adjust for confounding and selection bias. IPTW analyses showed that patients treated with postoperative prophylaxis were less likely to experience surgical and severe endoscopic recurrence. In addition, postoperative prophylactic medication was identified as a protective factor for both surgical and severe endoscopic recurrence following ICR in multivariable analysis.

In our study, initiation of direct prophylaxis following ICR seems more effective in the prevention of long-term surgical and severe endoscopic recurrence as compared to treatment initiation after clinical or endoscopic recurrence is diagnosed. Possible strategies for the postoperative management of patients with CD after ICR include prophylaxis in all patients, prophylaxis based on risk stratification, early endoscopy (or other imaging) guided therapy or no prophylactic treatment.[5, 6, 17] The optimal strategy to prevent long-term postoperative recurrence is yet unclear. The landmark POCER trial showed the superiority of early endoscopic evaluation at 6 months and therapy optimization based on endoscopic findings. In this trial, postoperative prophylaxis was prescribed according to clinical risk stratification.[18] The risk stratification strategy to start prophylaxis seems a valid strategy to avoid overtreatment of a substantial proportion of patients, although this strategy inevitably results in under treatment of patients considered at low risk.[4] The retrospective design of our study does not allow to fully correct for the indication of prophylaxis as decided by the treating physician. In addition, a direct comparison with a risk stratification strategy or

endoscopy guided therapy is not possible in this study, since risk stratification and performance of an early ileocolonoscopy were not common practice during the study period. Sensitivity analyses on the subgroup of patients who underwent a first ileocolonoscopy within one year after primary ICR showed no association between prophylaxis and surgical or severe endoscopic recurrence. These outcomes can be explained by the lower number of cases in these analyses, which has resulted in wide confidence intervals. In addition, we used ridge-regression to penalize the regression coefficients, which will force the coefficients towards the null hypothesis (i.e. no association). Therefore, further prospective studies need to be awaited including the SOPRANO-CD study in which patients with CD undergoing an ICR will be randomized to prophylaxis or endoscopy-guided therapy (NCT05169593).

The benefit of postoperative prophylaxis for patients with CD after primary ICR, as observed in this study, is in line with literature on early treatment of CD. Early treatment with anti-TNF agents during the disease course increases the probability of achieving deep remission which is associated with an improved prognosis with regard to complications or risk of surgery.[19] Similarly, other CD medication trials showed better outcomes in patients with short CD duration as compared with a less robust response in those patients with longer disease duration.[20, 21] An explanation for the reduced medication response in patients with longer CD disease duration has yet to be found, but may reflect irreversible vascular changes, structural bowel damage or possibly an altered cytokine profile and microbiome due to longstanding chronic inflammation. It is therefore hypothesized that the initiation of treatment, immediately following ICR, might lead to better long-term prognosis as compared to waiting for disease recurrence. Since patients who undergo ICR, may be considered in the ‘’deepest’’ remission after removing the entirety of the affected segment. This hypothesis is substantiated by the observation of a previous study reporting differences in cytokine

expression in pre-operative and post-operative mucosal samples of CD patients who underwent ICR as compared to longstanding ileitis.[22] This difference in mucosal cytokines production might reflect a functional change in immunological pathways possibly leading to an altered response to medical treatment postoperatively.[22]

Risk stratification guides clinicians to identify patients who may benefit from prophylaxis following ICR. In this study, active smoking at surgery was identified as risk factor for surgical and severe endoscopic recurrence, as previously demonstrated in available literature, which underlines the importance of smoking cessation following ICR.[3, 18, 23] In addition, preoperative exposure to biologicals and ileocolic disease were associated with surgical recurrence. Contrary, penetrating behaviour at surgery was found to be protective factor for the development severe endoscopic recurrence. This latter finding is in line with previous literature reporting a significant association with a lower risk of postoperative endoscopic and clinical recurrence.[24] An early ICR in less therapy refractory patients may explain this outcome. These patients are more likely to undergo a reduced timing before surgery in combination with postoperative prophylaxis suggesting that upfront surgery followed by postoperative prophylaxis is associated with reduced recurrence risk for these patients.[24]

Our study showed that thiopurines as prophylaxis are protective for postoperative recurrence, whereas anti-TNF therapy was not associated. This contradicts recent evidence which supports the superiority of anti-TNF agents as prophylaxis, as compared to thiopurines, for the prevention of clinical, endoscopic and severe endoscopic postoperative recurrence.[25] Therefore, these data need to be interpret with caution. A plausible explanation is the higher prevalence of low risk patients [patients without the presence of risk factors for recurrence as stated by the ECCO guidelines[5]] in the thiopurine subgroup, as compared to the anti-TNF subgroup [44% vs 36%, $p = 0.03$], leading to a more beneficial outcome of this prophylactic

strategy. In addition, in the anti-TNF subgroup, significantly more patients were preoperatively exposed to anti-TNF therapy, as compared to the thiopurines subgroup [95% vs 40%, $p < 0.001$].With regard to the best therapeutic strategy, the data on vedolizumab in a postoperative setting are promising [REPREVIO, eudraCT Number 2015-000555-24].[26] New individualized treatment choices, for instance based on therapy response prior to ICR or characterization of the inflammatory infiltrate in the resection specimen, may be promising to initiate the most beneficial therapy on individual basis.[27]

To the best of our knowledge, this is the first real-world study evaluating the long-term effectiveness of postoperative prophylaxis on surgical and severe postoperative endoscopic recurrence in patients who underwent a primary ICR. In addition, we have included patients from both academic and teaching hospitals which increases the generalizability of the findings. Furthermore, we used propensity scores were to correct for potential confounding and selection bias. Despite these strengths, a few limitations need to be considered. First, an ileocolonoscopy was not performed at specific time points, which could lead to confounding by indication. To investigate the potential impact of this limitation, we performed additional analyses taking into account the interval censoring using the time of the endoscopy at which severe endoscopic recurrence was diagnosed, and obtained consistent results. Finally, this cohort enclosed patients over a long period, probably leading to differences in treatment strategies over time. This may have influenced the outcomes of this study. In addition, as our study concerns a wide time period, several changes of postoperative management including improved medication strategies, access to endoscopy and development of strict and non-invasive monitoring may have influenced the outcomes. Therefore, we have performed a sensitivity analyses with patients who undergone an ileocolonoscopy within one year. This study design did not allow to correct for all these potential confounding factors.

In conclusion, in our cohort, patients treated with postoperative prophylaxis experienced significantly lower rates of surgical and severe endoscopic recurrence up to 10 years following primary ICR, as compared to patients not treated with prophylaxis. In addition, postoperative prophylaxis was identified as independent protective factor for both surgical and severe endoscopic recurrence in both weighted and multivariable analysis. Further studies are required to identify the optimal postoperative treatment strategy and identify patients that will benefit most from the start of prophylaxis shortly after ICR.

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6

Chapter 6

Prognostic value of the modified Rutgeerts' score for long-term outcomes after primary ileocecal resection in Crohn's disease

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Abstract

Introduction The prognostic value of the modified Rutgeerts score (mRS) in patients with Crohn's disease (CD) needs to be further elucidated. This study assessed the prognostic value of the mRS for long-term outcomes after primary ileocecal resection (ICR) in patients with CD.

Methods CD patients after primary ICR with an available mRS at first postoperative ileocolonoscopy (index mRS) were retrospectively included. Primary outcome was surgical recurrence. Secondary outcomes were clinical recurrence and progression to severe endoscopic recurrence (≥i3). Cox proportional hazard models were used to assess the association between index mRS and outcomes.

Results 652 patients were included (mean follow-up: 6.4 years, SD: 4.6). Surgical recurrence rates were 7.7%, 5.3%, 12.9%, 19.1%, 28.8%, 47.8% for index mRS i0, i1, i2a, i2b, i3 and i4. Clinical recurrence occurred in 42.2% (i0), 53.7%(i1), 58.5% (i2a), 80.2% (i2b), 79.4% (i3) and 95.3% (i4). Progression to severe endoscopic recurrence occurred in 21.1% (i0), 33.9% (i1), 26.8% (i2a) and 33.3% (i2b). An index mRS of i2b (adjusted hazard ratio [aHR] 3.0; 1.5–5.6), i3 (aHR 4.0 ;2.0–7.9) and i4 (aHR 8.0; 4.0-16.0) were associated with surgical recurrence. An index mRS of i1 (aHR 1.7; 1.2–2.4), i2a (aHR 1.7; 1.2–2.4), i2b (aHR 4.4; 3.2–6.0), i3 (aHR 3.6; 2.5–5.2) and i4 (aHR 7.3; 4.8–10.9) were associated with clinical recurrence. An index mRS of i1 (aHR 2.0;1.1–3.7) or i2b (aHR 2.5;1.4–4.6) were associated with progression to severe endoscopic recurrence.

Discussion The increasing mRS corresponds closely with the risk for surgical and clinical recurrence. An index mRS ≥ i2b is associated with surgical recurrence, an index mRS ≥ i1 is associated with clinical recurrence and i1 or i2b with progression to severe endoscopic recurrence. These results support tight monitoring of disease activity and treatment optimization in patients with ileal lesions, and a more conservative management in patients with anastomotic lesions.

Introduction

Patients with Crohn's disease (CD) are still at considerable risk for an intestinal resection although the risk has declined over the past decades.[1] An intestinal resection is an important treatment modality which is performed in approximately 25% of patients within 10 years after CD diagnosis.[2] An ileocecal resection (ICR) is the most common surgical procedure in CD.[3] Despite an intestinal resection may induce disease remission and provide relief of CD symptoms, [4, 5] surgery is not curative and recurrence at the ileocolic anastomosis and/or in the neoterminal ileum is common.[6]

Ileocolonoscopy is considered the golden standard for the diagnosis of postoperative recurrence in patients with CD.[7] The Rutgeerts' score (RS) was developed as endoscopic scoring system to assess the severity of recurrence of inflammation at the ileocolic anastomosis and in the neoterminal ileum. The original RS stratifies the endoscopic severity into five groups (i0 – i4).[8] High indices of the RS (≥ i2) are associated with higher risk for clinical recurrence and re-resection as compared to lower RS (i0-i1).[9] However, the prognostic value per index score of the RS is unknown.

The modified Rutgeerts score (mRS) was proposed to differentiate i2 into lesions confined to the anastomosis (i2a) versus lesions in the neoterminal ileum (i2b), and is currently used to assess the severity of postoperative endoscopic recurrence.[10] The nature of anastomotic lesions (i2a) is unknown and may be related to a post-ischemic surgical phenomenon or related to staples, instead of CD recurrence.[11] Several studies have reported conflicting clinical outcomes of anastomotic lesions on several measures of postoperative recurrence (clinical-, surgical recurrence and/or progression to [severe] endoscopic recurrence).[12-18] In a recently published individual participant data meta-analysis, no difference was observed

between i2a and i2b lesions for clinical recurrence and/or a surgical re- intervention.[19] However, no adjustment for known risk factors was conducted for the latter outcome. In addition, progression to severe endoscopic recurrence was not assessed. Therefore, the initiation or optimization of medication after an endoscopic diagnosis of ulcerations at the ileocolic anastomosis remains a matter of debate.

In this cohort study, we assessed the prognostic value of the mRS (per index score), after correction for known clinical risk factors, to predict the risk for surgical and clinical recurrence and progression to severe endoscopic recurrence after primary ICR in patients with CD.

Methods

Participants and study design

Consecutive patients, who underwent a primary ICR for the indication of CD between 2000 – 2019, were identified from a multicenter, retrospective database from six academic and four teaching hospitals in the Netherlands. All patients with CD (I) ≥ 16 years, (II) who underwent ICR with restoration of the intestinal continuity and (III) who had ≥ 1 postoperative ileocolonoscopy assessed with the use of the mRS, were included. Exclusion criteria were a permanent stoma, a re-resection before the first postoperative endoscopic assessment, prior intestinal resections, other indications for ICR (e.g., gastro-intestinal malignancy) and/or absence of follow-up data.

Outcomes

The primary outcome of this study was surgical recurrence (i.e. re-resection of the small bowel and/or colon) for CD recurrence during follow-up. Surgical recurrence within 3

months from primary ICR was considered as a re-resection due to postoperative complications and not considered as surgical recurrence. The secondary outcomes were (I) clinical recurrence defined as CD-related complaints with subsequent endoscopic recurrence (mRS ≥ i2b), surgical recurrence, radiologic recurrence (assessed by a local radiologist on ultrasonography, computed tomography or magnetic resonance imaging) and/or therapeutic optimization (i.e., initiation of corticosteroids, immunomodulators or biologicals for symptomatic disease) and (II) progression to severe endoscopic recurrence (mRS ≥ i3) in patients with an index mRS i0 – i2b.

Data collection

Baseline and clinical data were retrieved from the individual medical charts including demographics, surgical and disease characteristics and prior medical treatment. The date of index ileocolonoscopy (i.e., first operative ileocolonoscopy) was set as start of the follow-up and time at risk of this study. The mRS at the first postoperative ileocolonoscopy (i.e., index mRS) was used to assess the outcomes. The mRS was graded separately by four trained physicians (SB, JA, EB and JS) based on available photos and/or the endoscopy report for all patients. Follow-up time was defined as the interval between the index ileocolonoscopy (t0) and time to event. Patients were censored in case of the event was not observed (i.e., end of follow-up or lost to follow-up).

Statistical analyses

Descriptive statistical analysis (frequency, percentage, mean, standard deviation [SD], median and interquartile range [IQR]) was used to describe the research sample. Categorical variables were quoted as the number and percentage. Continuous variables were tested for normality using the Shapiro-Wilk test. Normal distributed variables were presented as mean and SD, whilst non-normal distributed variables were presented as median and IQR. Kaplan-

Meier curves, with log-rank test for significance, were used to describe and compare survival probabilities between individual mRS.

Associations between index mRS score and known clinical risk factors (according to the current guidelines), and the three time-to-event outcomes (surgical and clinical recurrence, and progression to severe endoscopic recurrence) were investigated using Cox proportional hazards models.[7, 20, 21] The following variables were included for multivariable analysis: age at diagnosis, penetrating disease at time of surgery (according to the Montreal classification), maintenance therapy during follow-up (i.e., continuation of postoperative prophylactic medication or start of medication within six weeks following index ileocolonoscopy with an anti-tumour necrosis factor agent [TNF] and/or an immunomodulator), time to index ileocolonoscopy.[7, 20, 21] The models included a random effect for the study center to take potential correlation into account between patients treated in the same hospital.

Since severe endoscopic recurrence is not observed directly and only known to lie within the interval between the first ileocolonoscopy at which it was not yet present and the last ileocolonoscopy at which it was diagnosed, sensitivity analysis with interval censoring for severe endoscopic recurrence was performed. Analyses were performed in R version 4.1.3 (R Core Team 2022) with the help of the packages icenReg (version 2.0.15) and survival.[22]

Ethics

This study was performed in accordance with the declaration of Helsinki and approved by the Medical Ethical Research Committee of the Erasmus University Medical Centre Rotterdam (MEC-2017-1151).

Results

Baseline characteristics

A total of 652 patients with CD who underwent a primary ICR were included. The majority of patients was female (62.9%) with a mean age of 35.6 years (SD: 13.8) and a median disease duration of 3.1 years (IQR: 0.8 – 8.2) at time of ICR (**Table 1**). Disease localization was restricted to the ileum in 63.8% (n=418) of patients and 36.2% (n=236) of patients had ileocolic disease at ICR. Following primary ICR, postoperative prophylactic treatment was initiated in 36.7% (n=239) of the patients, and concerned immunomodulator monotherapy (61.1%, n=146), anti-TNF monotherapy agent (21.8%, n=52), combination therapy (immunomodulator and anti-TNF agent) (14.6%, n=35), ustekinumab (2.1%, n=5) and vedolizumab (0.4%, n=1).

Index ileocolonoscopy was performed at a median of 8.7 months (IQR: 5.9 – 23.9) following primary ICR. The mean follow-up period after index ileocolonoscopy was 6.4 years (SD: 4.6). The index mRS comprised i0 in 195 patients (29.9%), i1 in 113 patients (17.3%), i2a in 101 patients (15.5%), i2b in 131 patients (20.1%), i3 in 66 patients (10.1%) and i4 in 46 patients (7.0%). Following the index ileocolonoscopy, maintenance therapy was initiated, within six weeks following ileocolonoscopy, in 14.4%, 14.2%, 30.7%, 43.5%, 50.0%, and 58.7% of the patients with i0, i1, i2a, i2b, i3 and i4.

Index modified Rutgeerts score and surgical recurrence

Overall surgical recurrence rate was 15.3% (n=100) after a mean time to re-resection of 2.3 years (IQR: 0.6 – 4.5). During follow-up, surgical recurrence occurred in 7.7%, 5.3%, 12.9%, 19.1%, 28.8%, 47.8% in the patients with i0, i1, i2a, i2b, i3 and i4 (**Figure 1**). Surgical recurrence rates were not significantly higher in patients with an index mRS of i2b as compared to patients with an index mRS of i2a (28.8% vs. 19.1%) (**log-rank test, p=0.16**).

Table 1. Baseline characteristics

Patients characteristics	Outcomes
Female sex, n (%)	410 (62.9)
Age at diagnosis, mean (SD)	30.1 (13.8)
Montreal classification (age), n (%)	
A1: <17 years	86 (13.2)
A2: 17 – 40 years	423 (64.9)
A3: > 40 years	143 (21.9)
Montreal classification (location of disease) at surgery, n (%)	
L1: Ileal	416 (63.8)
L3: Ileocolic	236 (36.2)
Montreal classification (behaviour of disease) at surgery, n (%)	
B1: Non-stricturing, non-penetrating	149 (22.9)
B2: Stricturing	323 (49.5)
B3: Penetrating	180 (27.6)
Perianal disease at time of surgery, n (%)	83 (12.7)
Active smoking at time of surgery, n (%)	231 (35.4)
Medication exposure prior to ICR, n (%)	
Corticosteroids	536 (82.2)
Immunomodulator	433 (66.4)
Anti-TNF agent	294 (45.1)
Ustekinumab	7 (1.1)
Vedolizumab	14 (2.1)
Time between diagnosis and ICR (in years), median (IQR)	3.1 (0.8 – 8.2)
Age at surgery, mean (SD)	35.6 (13.8)
Postoperative prophylactic treatment, n (%)	239 (36.7)
Immunomodulator monotherapy	146 (61.1)
Anti-TNF monotherapy	52 (21.8)
Combination therapy (immunomodulator and anti-TNF agent)	35 (14.6)
Ustekinumab	5 (2.1)
Vedolizumab	1 (0.4)
Time between ICR and index ileocolonoscopy (in months), median (IQR)	8.7 (5.9 – 23.9)
Rutgeerts score at index ileocolonoscopy, n (%)	
i0	195 (29.9)
i1	113 (17.3)
i2a	101 (15.5)
i2b	131 (20.1)
i3	66 (10.1)
i4	46 (7.0)

IBD = inflammatory bowel disease. IQR = interquartile range. SD = standard deviation; mRS = modified Rutgeerts' score; ICR= primary ileocecal resection

Index modified Rutgeerts score and clinical recurrence

626 patients (96.0%) were eligible for the analysis on clinical recurrence. Clinical recurrence occurred in 63.1% (n=412) of the patients and was reported in 42.2%, 53.7%, 58.5%, 80.2%, 79.4% and 95.3% in the patients with i0, i1, i2a, i2b, i3 and i4 (**Figure 2**). Clinical recurrence rates were significantly higher in patients with an index mRS of i2b as compared to patients with an index mRS of i2a (80.2% vs. 58.5%) (**log-rank test, p<0.001**).

Index modified Rutgeerts score and progression to severe endoscopic recurrence

During follow-up, 55.9% of the patients (n=304)(57.4% i0, 53.6% i1, 53.9% i2a, 57.3% i2b) with an index mRS i0 – i2b underwent >1 postoperative ileocolonoscopy. In this subset of patients, progression to severe endoscopic recurrence (i3-i4) was reported in 27.7% (n=84). Progression to severe endoscopic recurrence rates occurred in 21.1% (i0), 33.9% (i1), 26.8% (i2a) and 33.3% (i2b) (**Figure 3**). Severe endoscopic recurrence rates were not significantly higher in patients with an index mRS of i2b as compared to patients with an index mRS of i2a (33.3% vs. 26.8%) (**log-rank test, p = 0.47**).

Figure 1. Kaplan Meier curve of surgical recurrence-free survival (n = 652)

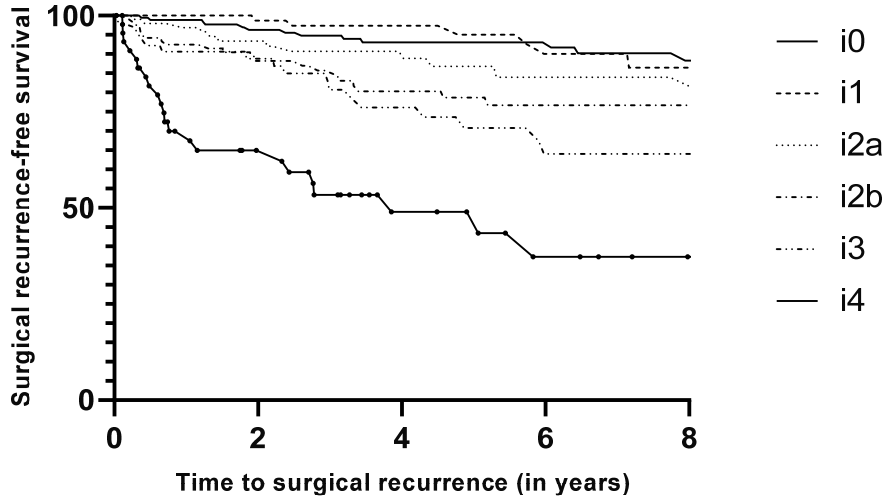


Figure 2. Kaplan Meier curve of clinical recurrence-free survival (n = 626)

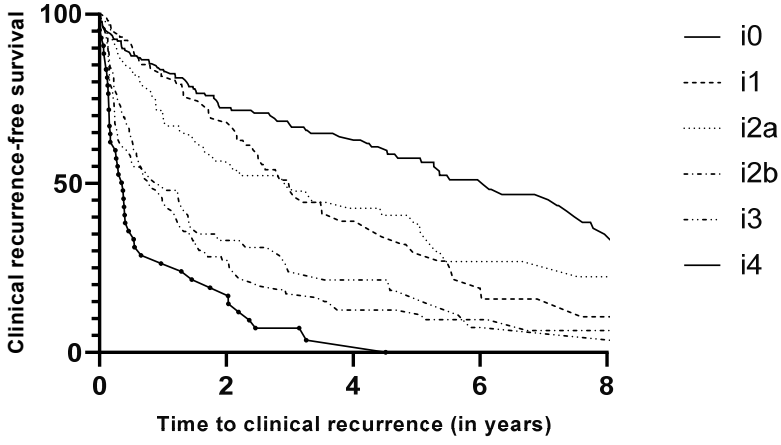
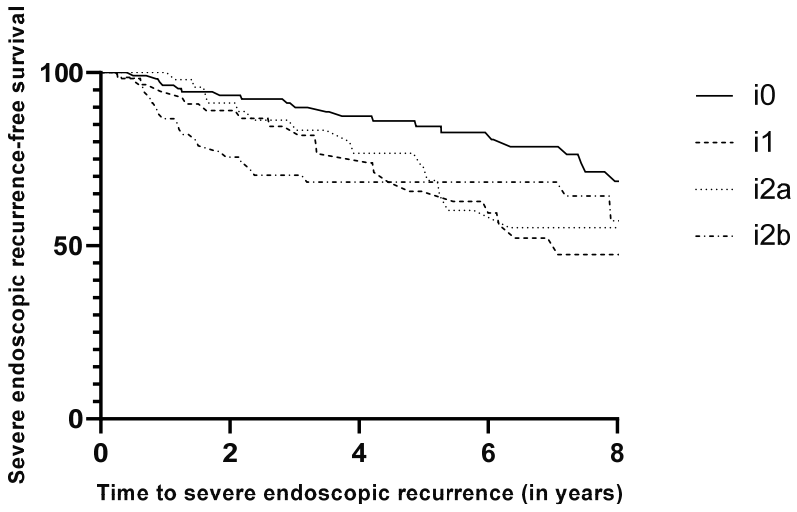


Figure 3. Kaplan Meier curve of severe endoscopic recurrence-free survival (n = 304)



Association of the modified Rutgeerts score with outcomes

After adjusting for the included clinical risk factors, an index mRS of i1 (adjusted hazard ratio [aHR] 0.7; 95% confidence interval [CI] 0.3 – 1.9]) and anastomotic lesions (i2a)(aHR 1.7; 95% CI 0.8 – 3.5) were not associated with surgical recurrence in multivariable analysis (**Table 2**). An index mRS of i2b (aHR 2.9; 95% CI 1.5–5.6), i3 (aHR 4.0; 95% CI 2.0–7.9) and i4 (aHR 8.0; 95% CI 4.0 – 16.0) were independently associated with surgical recurrence during follow-up. An increased time to index ileocolonoscopy was associated with surgical recurrence (aHR 1.1; 95% CI 1.0 – 1.2). No other associations were reported.

An index mRS of i1 (aHR 1.7; 95% CI 1.2–2.4), i2a (aHR 1.7; 95% CI 1.2–2.4), i2b (aHR 4.4; 95% CI 3.2–6.0), i3 (aHR 3.6; 95% CI 2.5–5.2) and i4 (aHR 7.3; 95% CI 4.8–10.9) were associated with clinical recurrence. Furthermore, active smoking at surgery (aHR 1.4; 95% CI 1.1–1.7) and maintenance therapy with an immunomodulator (aHR 0.6; 95% CI 0.5–0.7) were associated with clinical recurrence.

Concerning progression to severe endoscopic recurrence, an index mRS of i2a was not associated with progression to severe endoscopic recurrence (aHR 1.9; 95% CI 0.9 – 3.7). An index mRS of i1 and i2b was independently associated with progression to severe endoscopic recurrence (aHR 2.0; 95%-CI 1.1 – 3.7 [i1]) (aHR 2.5; 95%-CI 1.4 – 4.6 [i2b]). No clinical risk factors were associated with progression to severe endoscopic recurrence.

After interval censoring, sensitivity analysis showed no association of anastomotic lesions (i2a) with progression to severe endoscopic recurrence (aHR 1.8; 95% CI 0.9 – 3.8) (**Supplementary Table 1**). In line with the earlier findings, an association for an index mRS of i2b, on progression to severe endoscopic recurrence, was observed in multivariable analysis (aHR 2.1; 95%-CI 1.1 – 4.1).

Table 2. Multivariable Cox proportional hazard models

Variables	Surgical recurrence HR (95% CI)	Clinical recurrence HR (95% CI)	Severe endoscopic recurrence HR (95% CI)
Age at diagnosis	1.0 (0.9 – 1.0)	1.0 (0.9 – 1.0)	1.0 (0.9 – 1.0)
Active smoking	1.4 (0.9 – 2.1)	1.4 (1.1 – 1.7)	1.5 (0.9 – 2.3)
Disease behaviour at time of surgery (Montreal classification)			
Non-stricturing, non-penetrating disease	REF	REF	REF
Stricturing disease	1.4 (0.8 – 2.3)	1.0 (0.7 – 1.2)	0.9 (0.5 – 1.5)
Penetrating disease	0.9 (0.5 – 1.8)	0.9 (0.7 – 1.3)	0.8 (0.4 – 1.5)
Maintenance therapy after index ileocolonoscopy[*]			
None	REF	REF	REF
Immunmodulator	0.7 (0.4 – 1.1)	0.6 (0.5 – 0.7)	0.8 (0.5 – 1.3)
Anti-TNF monotherapy/combination therapy[¥]	1.1 (0.6 – 1.9)	1.0 (0.7 – 1.3)	1.1 (0.6 – 2.1)
Time to index ileocolonoscopy (in months)	1.1 (1.0 – 1.2)	1.0 (0.9 – 1.1)	1.0 (0.9 – 1.1)
Index modified Rutgeerts score			
i0	REF	REF	REF
i1	0.7 (0.3 – 1.9)	1.7 (1.2 – 2.4)	2.0 (1.1 – 3.7)
i2a	1.7 (0.8 – 3.5)	1.7 (1.2 – 2.4)	1.9 (0.9 – 3.7)
i2b	2.9 (1.5 – 5.6)	4.4 (3.2 – 6.0)	2.5 (1.4 – 4.6)
i3	4.0 (2.0 – 7.9)	3.6 (2.5 – 5.2)	-
i4	8.0 (4.0 – 16.0)	7.3 (4.8 – 10.9)	-

[*] Defined as the continuation of postoperative prophylactic medication or start of medication within six weeks following index ileocolonoscopy with an anti-tumour necrosis factor agent [TNF] and/or an immunomodulator

[¥] Combination therapy comprises therapy with an immunomodulator and an anti-TNF agent.

HR = hazard ratio; 95% CI = 95% confidence interval; REF = reference; TNF = tumour necrosis factor.

Discussion

In this study, the increasing mRS corresponds closely with the risk for surgical and clinical recurrence in patients with CD following a primary ICR, but not with the risk for progression to severe endoscopic recurrence. In multivariable analysis, anastomotic lesions (i2a) were not associated with a re-resection, in contrast to an index mRS ≥ i2b. Similarly, anastomotic lesions were not associated with severe endoscopic recurrence, in contrast to mild lesions in the neoterminal ileum (index mRS of i1 or i2b). An index mRS ≥i1 is associated with clinical recurrence. Tight monitoring to timely optimize medication seems indicated in patients with inflammation in the ileum (index mRS of i1 and ≥ i2b) in order to prevent progression to severe endoscopic recurrence and/or surgical recurrence. In patients with inflammation confined to the anastomosis, a more conservative approach seems appropriate.

Current American and European guidelines recommend escalation or initiation of medication in patients with a RS ≥i2.[20, 21] Refinement of these recommendations into mRS ≥ i2b seems indicated based on the findings of this study as well as previous observations on long-term outcome of anastomotic lesions.[14, 17, 18] The more indolent disease course in patients with anastomotic lesions as compared to ileal inflammation with regard to progression to severe endoscopic lesions has also been shown in two retrospective multicenter studies.[14, 17] In addition, Hammoudi *et al.* reported a shorter clinical recurrence-free survival in patients with ileal lesions at index ileocolonoscopy as compared to patients with lesions confined to the anastomosis.[18] These findings are in line with our results showing that an index mRS of i1 is associated with both clinical recurrence and progression to severe endoscopic recurrence, whereas an index mRS of i2a is merely associated with clinical recurrence. These outcomes may be explained by a distinct pathological mechanism of anastomotic lesions as compared

to ileal lesions, in which the role of ischemia is debated.[18, 23] A recent published meta-analysis with individual patient data reported no difference was observed between i2a and i2b lesions on the outcomes of clinical recurrence and/or a surgical re-intervention.[19] However, the analyses for a surgical re-intervention were not corrected for known risk factors associated with recurrence. In this study, after adjusting for known clinical risk factors, an index mRS ≥ i2b was found to be independently associated with surgical recurrence and progression to severe endoscopic recurrence which supports the recommendation to consider therapy optimization in patients with an index mRS of ≥ i2b following primary ICR**.**

Despite the lack of a statistically significant association between anastomotic lesions and surgical recurrence and progression to severe endoscopic recurrence, the risks for both outcomes were still as high as 12.7% and 26.8% during follow-up. Further research to identify risk factors and/or biomarkers for postoperative recurrence is warranted in order to appropriately manage patients with anastomotic lesions. The need for more accurate biomarkers seems underscored by the lack of association between clinical risk factors, except for active smoking and maintenance therapy with an immunomodulator with clinical recurrence, and long-term outcomes in multivariable analysis in this study.

Recently, a new endoscopic scoring system has been proposed in which endoscopic scoring should be adapted to the anastomotic technique.[24] The (m)RS has been developed for the assessment of an end-to-end anastomosis. In the modern era, wide lumen stapled side-to-end or side-to-side anastomosis have been preferred over the end-to-end anastomosis in order to prevent anastomotic leakage, fecal stasis and stenosis of the anastomosis. When the (m)RS is applied to endoscopically assess these anastomotic techniques, anatomic locations such as the ileal blind loop and ileal body are disregarded.[24, 25] Prospective analysis of inflammation at these locations and subsequent refinement of the endoscopic score is awaited.

Our study is the first to assess the predictive value of the mRS on long-term outcomes in postoperative CD patients. Despite the consideration of objective outcome measures in a large population (from both academic and non-academic hospitals) of patients who underwent a primary ICR with long-term follow-up, limitations of this study need to be taken into consideration. First, as the indication of subsequent ileocolonoscopies could not be assessed, due to the retrospective design, confounding by indication may be present. Secondly, as our study concerns a wide time period, several changes of postoperative management may have influenced the outcomes including improved access to endoscopy, development of strict and non-invasive monitoring and medication strategies. This study design did not allow to correct for all these potential confounding factors. With regards to the changes in the postoperative endoscopic strategy, a substantial number of patients (40%) did not undergo an index ileocolonoscopy within 1 year postoperatively which is recommended by the current guidelines.[7, 20, 21] To adjust for potential confounding, we have included time to index ileocolonoscopy in the multivariable analysis. Finally, perianal fistulizing disease, plexitis and/or granulomas in the resection specimen are considered risk factors for postoperative recurrence in current guidelines.[7, 20, 21] Due to the restriction of number of variables that could be included in multivariable analysis, the findings are not corrected for the presence of perianal fistulas. In addition, standardized data on the presence of plexitis and/or granulomas in the resection specimen were unavailable in the pathology reports.

In conclusion, the increasing mRS at index ileocolonoscopy corresponds closely with the risk for surgical and clinical recurrence after primary ICR. Anastomotic lesions (i2a) are not associated with surgical recurrence and progression to severe endoscopic recurrence, in contrast to lesions in the neoterminal ileum (≥ i2b). An index mRS ≥i1 is associated with

clinical recurrence. In addition, i1 lesions are associated with progression to severe endoscopic recurrence. These results support conservative management and no need for escalation of therapy in patients with anastomotic lesions and tight monitoring of disease activity and treatment optimization in patients with ileal lesions.

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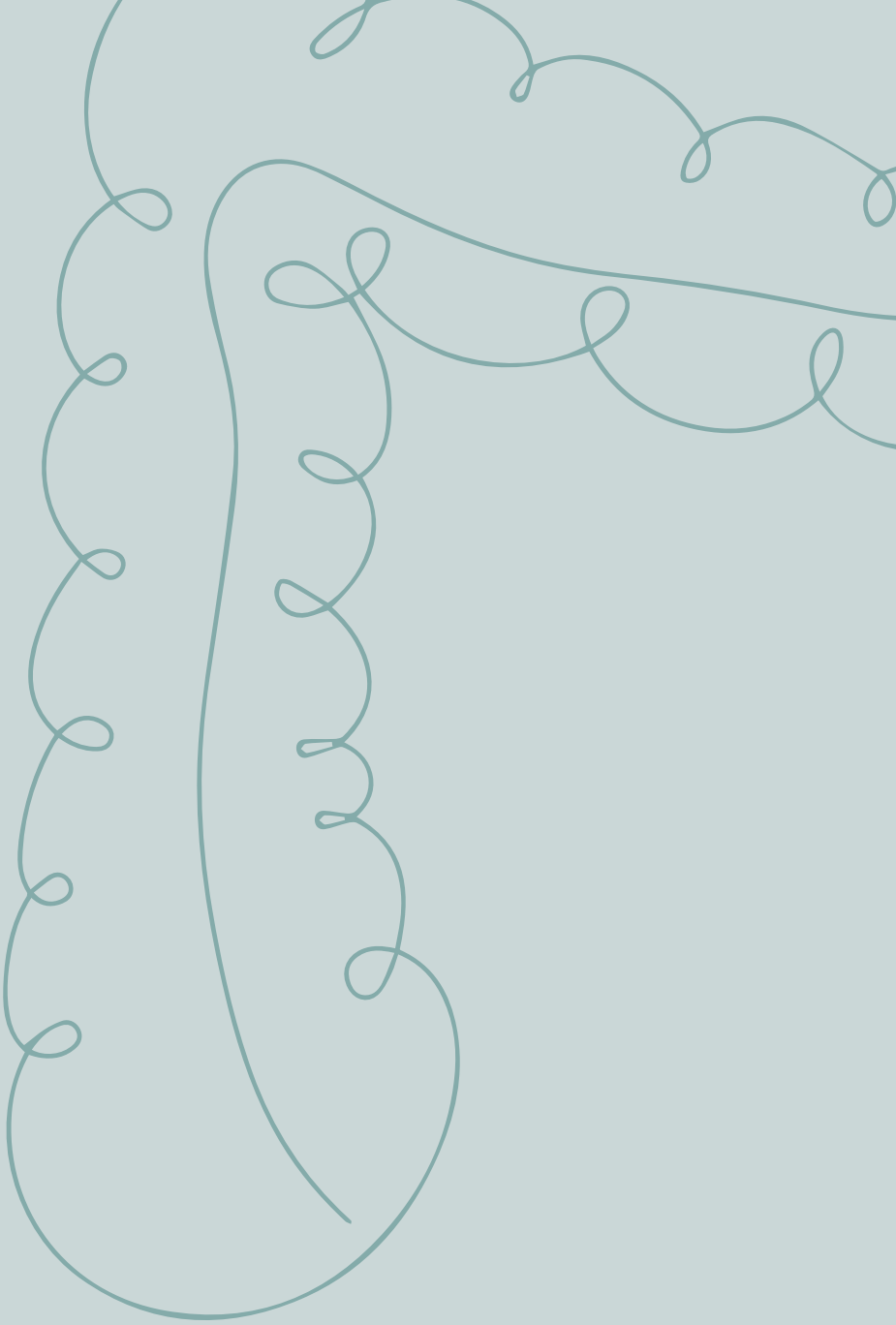

Part III

De-escalation strategies

Chapter 7

Prediction model to safely CEASE anti-TNF therapy in Crohn's disease: Validation of a predictive diagnostic tool for cessation of anti-TNF therapy in CD in a Dutch cohort

Chapter 8

Diagnostic tool to Safely CEASE Anti-TNF Therapy in Crohn's Disease: Centre-Specific Stepped Wedge Randomized Controlled Trial

Chapter 9

Cessation of anti-Tumour Necrosis factor Therapy in Patients with Perianal Fistulizing Crohn's Disease: Individual Patient Data Meta-Analysis of 315 patients from 11 studies

Chapter 10

The predictive value of immunoprofiling for relapse in Crohn's Disease patients after cessation of anti-TNF therapy

Chapter 7

Validation and update of a prediction model for risk of relapse after cessation of anti-TNF treatment in Crohn's disease

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Abstract

Background Anti-TNF therapy is effective for the treatment of Crohn's disease. Cessation may be considered in patients with low risk of relapse. We aimed to externally validate and update our previously developed prediction model to estimate the risk of relapse after cessation of anti-TNF therapy.

Methods We performed a retrospective cohort study in seventeen Dutch hospitals. Crohn's disease patients in clinical, biochemical or endoscopic remission were included after anti-TNF cessation. Primary outcome was a relapse necessitating treatment. Discrimination and calibration of the previously developed model were assessed. After external validation, the model was updated. The performance of the updated prediction model was assessed in internal-external validation and by using decision curve analysis.

Results 486 patients were included with a median follow-up of 1.7 years. Relapse rates were 35% and 54% after one and two years. At external validation, the discriminative ability of the prediction model was equal to that found at development of the model (c-statistic 0.58 (95% CI 0.54-0.62)), though the model was not well-calibrated on our cohort (calibration slope: 0.52 (0.28 – 0.76). After an update, a c-statistic of 0.60 (0.58 - 0.63) and calibration slope of 0.89 (0.69 – 1.09) were reported in internal-external validation.

Conclusions Our previously developed and updated prediction model for the risk of relapse after cessation of anti-TNF in Crohn's disease shows reasonable performance. The use of the model may support clinical decision making to optimize patient selection in whom anti-TNF can be withdrawn. Clinical validation is ongoing in a prospective randomized trial.

Introduction

Anti-tumour necrosis factor (anti-TNF) therapy is frequently prescribed as induction and maintenance treatment in moderate to severe Crohn's disease (CD)[1-3]. A majority of CD patients receive long-term anti-TNF therapy to maintain remission. However, exposure to anti-TNF therapy is associated with significant disadvantages, including side effects such as infections, an increased risk of malignancy [4]-6, chronic fatigue [7-9], work-productivity loss [8,9] and significant healthcare costs [10,11].

In daily practice, cessation of anti-TNF therapy in CD patients in remission is still debated. Anti-TNF therapy is infrequently withdrawn mainly due to the uncertainty of the risk of relapse in the individual CD patient [12,13]. A more personalized treatment approach, including a prediction model for anti-TNF cessation will benefit the individual CD patient and the healthcare system at large. Therefore, a stratification tool to identify patients who can safely cease anti-TNF therapy can be clinically useful.

Until recently, the model developed in the STORI trial by GETAID has been the only available prediction model with a reported predictive power (concordance statistic, c-statistic) of 0.71 in the original article [14]. However, external validation of the STORI model in an individual patient data meta-analysis (IPD-MA) by our group (CEASE phase 0) on 14 cohorts (n = 1317), showed a less robust discriminative ability, with a c-statistic of 0.51 [15]. Based on this IPD-MA, a prediction model was developed to safely cease anti-TNF therapy with a reported c-statistic of 0.58 in internal-external validation. After an update of the prediction model with faecal calprotectin, an improved c-statistic of 0.63 was reported in a subgroup analysis.

In the current study (CEASE phase I), we aimed to validate and update the previously developed prediction models in a large independent Dutch CD cohort.

Material and Methods

Study design

We performed a multicentre, retrospective cohort study (CEASE phase 1 cohort), in seventeen hospitals in the Netherlands (five academic and twelve general teaching hospitals). CD patients who discontinued anti-TNF therapy between January 2000 and August 2019 were included in this study. CD patients were identified either from medical records through a search in the electronic patient database or the available medical lists from the hospital pharmacy using the keywords 'Crohn's disease', 'anti-TNF therapy', 'infliximab' and 'adalimumab'.

Patients were included between July 2019 and January 2020. Included patients received anti-TNF therapy (adalimumab or infliximab) ≥6 months for the primary indication of luminal CD. Included patients had to be in remission at the moment of discontinuation of anti-TNF and concomitant treatment with immunomodulatory therapy was allowed. Remission was defined as either clinical, biochemical or endoscopic remission. Due to the infrequent availability of standardized tools to quantify disease activity (i.e. Harvey-Bradshaw Index or Crohn's Disease Activity Index), clinical remission was defined as the absence of symptoms based on the global assessment and documentation of the treating physician. Biochemical remission was defined as the absence of biochemical markers of inflammation (CRP < 5 mg/l and FC <250 μg/g). Endoscopic remission was defined as the absence of macroscopic inflammation (erosions or ulcerations), based on the findings in the endoscopy report.

Patients were excluded if they ceased anti-TNF therapy primarily due to other reasons (e.g. infections, side effects), or if a top-down strategy was applied where patients received anti-TNF therapy less than 6 months.

Sample size

For external validation, at least 100 events are required to reliably estimate the performance of a prediction model [16]. We assumed that a minimum of 20% of the included patients would relapse within the follow-up time. Therefore, to include at least 100 events (relapses) the estimated sample size was 500 patients for the full cohort. Based on the model performance in phase 0, the required sample size for a full re-estimation of the phase 0 FC model was calculated as well [16]. To obtain a shrinkage factor of 0.85, an estimated sample of 487 patients was needed. This sample size also satisfies the second and third criterion outlined by Riley et al.: a small difference between apparent and adjusted Nagelkerke R^2 (<0.05); and a precise estimate of overall risk (95% CI width < 0.1). Hence, a sample size of 500 patients was expected to provide sufficient statistical power for external validation and a model update.

Endpoints

The primary endpoint was the proportion of documented relapses, defined as a relapse of luminal disease activity or the occurrence of (new) CD complications (i.e. extra-intestinal manifestations (EIM), [perianal] fistula and/or abscess) that necessitated introduction of additional treatment including biologicals, corticosteroids, immune-suppressants or surgery. A *clinical* relapse was defined as the presence of symptoms such as abdominal pain, diarrhoea, perianal fistulas or the presence of EIM (e.g. arthritis, uveitis, erythema nodosum, pyoderma gangrenosum). A *biochemical* relapse was defined as CRP ≥5 mg/l and/or FC ≥250 μg/g. An *endoscopic* relapse was defined as the presence of macroscopic inflammation at endoscopy (i.e. erosions and/or ulcerations), as interpreted by the endoscopist. The secondary

endpoint was the sustained effect of retreatment with the same or other anti-TNF agent. The sustained clinical benefit of retreatment with anti-TNF therapy was considered successful if patients were still treated with this agent twelve months after their relapse.

Data collection

Electronic patient records were retrospectively reviewed. Information on patient characteristics, disease-specific information, biochemical markers and endoscopic data were obtained, at moment of stopping anti-TNF therapy (baseline) and at relapse. At baseline, biochemical markers and endoscopic appearance were recorded if they were obtained one year before, or two months after discontinuation of anti-TNF therapy.

Previously collected data

In addition to the data collected in CEASE phase 1, we used data from phase 0 of the CEASE project in some analyses. We refer to the CEASE phase 0 cohorts as *development cohorts*, since the model was initially developed within these cohorts. A detailed report of study- and patient characteristics of the CEASE phase 0 cohorts was provided[17], in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement[18]. The cohort of phase 0 was based on several international cohorts, including one Dutch cohort[19]. There were minor differences in the in- and exclusion criteria between phase 0 and phase 1, including the duration of anti-TNF exposure (12 months vs 6 months, respectively). We included the same IPD that Pauwels et al. used in the development of the phase 0 model [15], with the addition of patients between 13 and 15 years old, resulting in a total number of 1330 IPD from the phase 0 cohorts in our analyses.

The *validation cohort* refers to the cohort described in this manuscript, phase 1 of the CEASE project, based on patients from Dutch hospitals. We externally validated the previously

developed model on the validation (phase 1) cohort only. After external validation, the development (phase 0) and validation (phase 1) cohorts were combined to perform the model update.

Previously developed model

The previously developed model (phase 0 model) is a Cox regression model that includes the following predictors*:* younger age at diagnosis (HR=1.5 for A1 vs A2), age at cessation (HR=1.2 per 10 years younger), upper gastrointestinal tract involvement (HR=1.3 for L4 vs non-L4), clinical symptoms at cessation (HR=2.2), smoking (HR=1.4), longer disease duration (HR=1.07 per 5 years), no concomitant immunosuppressant's (HR=1.4), second line anti-TNF (HR=1.3), adalimumab (HR=1.22 for adalimumab vs infliximab) and C-reactive protein (HR=1.04 per doubling) (**table 3**).

In a subgroup analysis, faecal calprotectin (FC) was added to the model as a predictor (phase 0 FC model). This improved the discriminative ability (c-statistic 0.63) (**table 3**). Low FC levels were associated with a favourable outcome after anti-TNF therapy cessation, which is in line with available literature [14,20].

Statistical Analysis

Reporting on this study was done according to the TRIPOD statement [21]. Statistical analyses were performed using IBM SPSS Statistics version 26.0 and R version 4.0.3 [22]. Descriptive statistics were provided with frequencies and percentages for qualitative variables and medians and interquartile ranges (IQRs) for quantitative variables. We assumed missing values were missing at random and imputed missing values using the mice algorithm [23]. Kaplan-Meier analysis was used to quantify the crude risk of relapse after cessation of anti-TNF therapy.

External validation of the previously developed model

To evaluate differences in case-mix between the development (CEASE phase 0) and validation (CEASE phase 1) samples, we compared the distributions of predicted 1-year relapse risk in the respective samples [24]. All predictions were calculated using the exact formulae of the previously developed models. The previous models were developed in a meta-analysis stratified by cohort, meaning that each development (CEASE phase 0) cohort had its own baseline hazard estimate. One phase 0 cohort consisted of Dutch patients, so we assumed the baseline hazard of this cohort in our predictions for the validation cohort.

The discrimination and calibration of the previously developed models with and without FC were assessed in the validation cohort. The discriminative ability of the models was quantified using Harrell's c-statistic [25]. The c-statistic ranges from 0 to 1, where 0.5 indicates that the prediction model is equivalent to a coin flip, while a value of 1 indicates perfect discrimination. Calibration was assessed graphically using calibration plots, which were characterized by the calibration slope and calibration-in-the-large. In our calibration plots, the validation cohort is divided into five groups defined by predicted event rate quintiles. The observed event rates in these groups are plotted against the predicted event rates, which ideally should lie on the 45-degree line. The calibration-in-the-large compares the average predicted risk to the average observed risk and is equal to zero in case of perfect agreement. The calibration slope measures whether predictor effects are on average correct and should ideally be equal to one.

Model update

After the external validation of the previously developed models, a model update was performed. In this update, *faecal calprotectin* was included in the model, the continuous version of *age at diagnosis* replaced the Montreal A classification, and *disease duration* and

clinical remission were removed (due to linear dependence on *age* and *age at diagnosis* and an extremely small number of patients without clinical remission in the phase 1 cohort, respectively). The model was then refitted in an IPD-MA on the combined cohorts of CEASE phase 0 and CEASE phase 1. To check the validity of combining the data, we statistically tested for differences in effects between phase 0 and phase 1 cohorts using a model with interaction terms. In addition, we performed cross-validation, where the updated model was fitted on the phase 1 cohort and validated on the phase 0 cohorts, and vice versa.

Validation of the updated model

The resulting updated prediction model (*phase 1 model*) was validated using internal-external validation. Internal-external validation is a procedure where every cohort is left out once, so that a model can be developed on the remaining cohorts and validated on the cohort that was left out [26]. In these validations, the discriminative ability was assessed using the c-statistic and calibration was quantified using calibration-in-the-large and calibration slope. A pooled c-statistic, calibration-in-the-large and calibration slope were estimated with a random effects model. Heterogeneity across studies in performance measures was quantified by the I^2 statistic [27]. 95% confidence intervals of the pooled performance measures were calculated.

Decision curve analysis was used to assess the clinical usefulness of the updated prediction model [28,29]. In a decision curve analysis, the ability of a prediction model to select patients for ceasing treatment is compared to the default strategies of continuing or stopping anti-TNF treatment in all patients. The net benefit of using a prediction model for patient selection is calculated by summing the benefits (correctly identifying patients who would relapse within 1 year) and subtracting the harms (continuing treatment in patients who would not relapse), using a weighting factor related to the corresponding threshold probability. The weighting factor expresses the number of patients one is willing to continue anti-TNF treatment, to

correctly identify one patient who would relapse within 1 year. The net benefit of using the CEASE phase 1 model was investigated for a range of clinically relevant threshold probabilities.

Implementation of the updated model

Finally, we constructed a prognostic tool as a web-interface to present a user-friendly version of the updated CEASE model to predict the 1-year risk of relapse after cessation of anti-TNF treatment, which will become available upon publication of this manuscript.

Ethical approval and patient consent

The ethical committee of the Amsterdam UMC approved this study (reference number W19_100#19.130). The IRB waived the need for an informed consent procedure. Instead, patients were actively informed about the study and were given the right to 'opt-out'. At the Medical Centre Leeuwarden a written informed consent procedure was performed.

Results

Baseline characteristics

In total, 7226 CD patients who met the search criteria were screened for eligibility. Of these patients, 486 were included in the final analysis (**Supplementary data; Appendix A**). Median follow-up time after cessation of anti-TNF therapy was 1.7 years (IQR 0.8 – 3.1). Baseline characteristics are shown in **Table 1**. A total of 129 patients (27%) were previously exposed to adalimumab or infliximab. 132 patients (27%) underwent prior intestinal resection, of which the majority were ileocolonic resections (n = 99; 75%). After cessation of anti-TNF therapy, concomitant therapy was maintained in 176 patients (36%; thiopurines, n = 153, methotrexate, n = 23).

A colonoscopy report was available in 192 patients (39%), with procedures performed at a median time of 0.6 months before cessation of anti-TNF therapy (IQR 0.2 – 2.4 months). Absence of any endoscopic inflammation was documented in 90% (n = 172). In the other 20 patients (10%), mild disease was observed with signs of erosions in 10 (50%) and/or ulcerations in five patients (25%), which was contained to the ileum in 45% (n = 9). Despite mild disease activity on endoscopy, these patients were included as they were in either clinical and/or biochemical remission.

Table 1. Baseline characteristics (n = 486)

Factor	**N (%) or median (IQR)**
Follow-up time, years	1.7 (0.8 – 3.1)
Age, years	37.9 (29.1 – 50.3)
Female	309 (64)
Active smoker (n = 326)	94 (29)
Disease duration, years	9.1 (4.7 – 17.1)
Montreal classification (n = 374)	
Age at diagnosis	
A1 ≤ 16 years	45 (12)
A2 17-40 years	273 (73)
A3 > 40 years	56 (12)
Location	
L1 Terminal ileum	96 (26)
L2 Colon	90 (24)
L3 Ileocolonic	185 (49)
+ L4 Upper GI	28 (7)

Table 1. Continued

Factor	N (%) or median (IQR)
Prior intestinal resection	132 (27)
Previously treated with anti-TNF	129 (27)
Anti-TNF used	
Adalimumab	237 (49)
Infliximab	249 (51)
Schedule adalimumab	
Every other week	203 (86)
Every week	14 (6)
Interval lengthened	20 (8)
Schedule infliximab	
Standard (8 weeks)	219 (88)
Intensified (6 weeks)	13 (5)
Other	17 (7)
Duration anti-TNF therapy, years	4.1 (2.0 – 6.6)
Concomitant medication continued after cessation of anti-TNF	
Thiopurine	153 (31)
Methotrexate	23 (5)
Biochemical (within 1 year before, or 1 month after stop anti-TNF)	
Haemoglobin, mmol/L (n = 431)	8.6 (8.0 - 9.1)
Leukocytes, * 10^9/L (n = 422)	7.3 (6.0 – 9.1)
Thrombocytes, * 10^3/mm^3 (n = 414)	268 (227 – 320)
Albumin, g/L (n = 213)	40 (37 – 44)
CRP, mg/L (n = 408)	3 (1 – 5)
Calprotectin, mg/kg (n = 249)	43 (16 – 136)
Trough level, µg/ml (n = 147)	3.2 (1.0-6.4)

N = number of patients, CRP = C-reactive protein

Relapse

In total, 277 patients (57%) experienced a relapse after cessation of anti-TNF therapy (including clinical relapse n = 220 [79%], biochemical relapse n = 130 [47%], endoscopic relapse n = 118 [43%], relapse confirmed by imaging other than colonoscopy n = 31 [11%]), with median time to relapse of 0.8 years (IQR 0.4 – 1.7, **Table 2**). Relapse rates were 35% (31%-39%) and 54% (49%-59%) after one and two years, respectively (**Figure 1**).

In 20 patients (7%), recurrence of perianal disease was the indication to restart treatment. However, we did not observe the development of *new* perianal fistula after cessation of anti-TNF. Seven patients (3%) required reintroduction of anti-TNF therapy due to EIM.

In 31 patients (11%), the diagnosis of a relapse and reintroduction of therapy was not supported by objective measures of inflammation (either biochemical analysis, endoscopy or imaging) and was solely based on patients' symptoms.

Figure 1. Kaplan Meier Survival curve

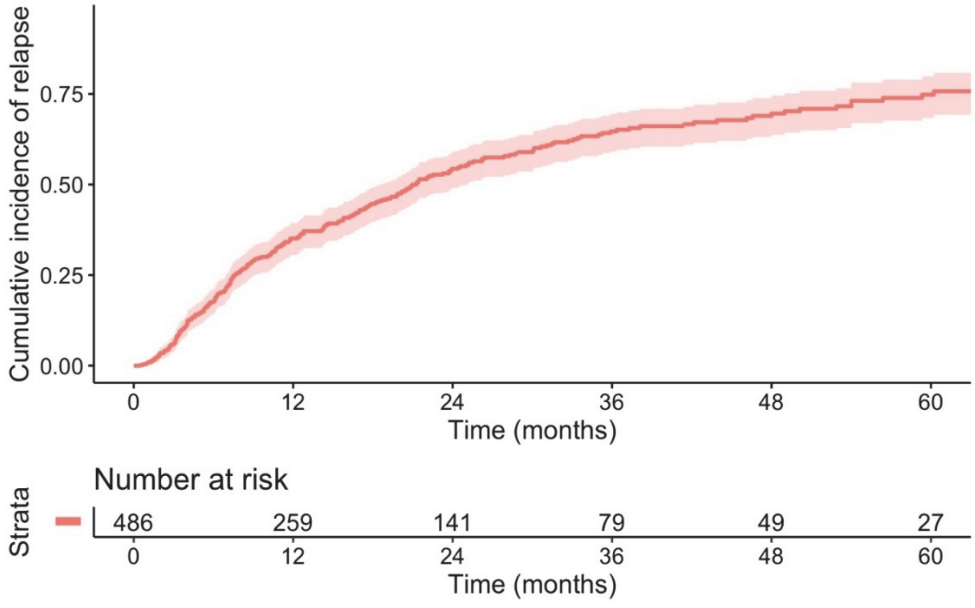


Table 2. Relapse characteristics (n = 277)

Factor	N (%) or median (IQR)
Time until relapse, years	0.80 (0.41 – 1.69)
Relapse within 1 year	160 (58)
Relapse within 2 years	228 (82)
Type of relapse	
Clinical	220 (79)
Biochemical	130 (47)
Endoscopic	118 (43)
Perianal disease	20 (7)
Extra-intestinal manifestations	7 (3)
Type of reintroduced therapy	
Antibiotics	6 (2)
Aminosalicylates	11 (4)
Thiopurines	62 (22)
Methotrexate	9 (3)
Steroids	96 (35)
Biological	174 (63)
Surgery	12 (4)
Need for hospitalization	42 (15)
Type of reintroduced biological after relapse (n = 174)	
Adalimumab	98 (56)
Infliximab	64 (37)
Vedolizumab	9 (5)
Ustekinumab	3 (2)
Effect reintroduction anti-TNF (n = 129)	
Response/remission	104 (81)
Stopped due to non-response/side-effects	25 (19)
Retreatment successful with the same anti-TNF	
Adalimumab (n = 61)	50 (82)
Infliximab (n = 47)	40 (85)

Treatment after relapse

174 patients (63%) were treated with a biologic agent after they experienced a relapse, most of whom restarted anti-TNF therapy (n = 162, 93%). 98 patients (56%) started adalimumab and 64 patients (37%) infliximab, of whom 15% received combination therapy with azathioprine (n = 20) or methotrexate (n = 4). 133 patients (82.1%) restarted the same anti-TNF treatment that was ceased before (infliximab; n = 60, adalimumab; n = 73). In the remaining twelve patients, vedolizumab (n = 9, 5%) and ustekinumab (n = 3, 2%) were started.

Median follow-up after relapse was 1.8 years (IQR 0.8 – 3.3). Seventeen patients (10%) experienced primary non-response or loss of response after reintroduction of anti-TNF, and eight patients (5%) ceased their anti-TNF due to side effects. 104 patients (81%) achieved either clinical response or remission after anti-TNF was restarted. Retreatment with the same anti-TNF agent was effective (exposure >12 months) in 85% and 82% for infliximab and adalimumab, respectively. 33 patients did not have a sufficient follow up period to conclude whether anti-TNF was effective after reintroduction. However, these patients were still receiving this treatment at the end of the follow-up period (median follow-up 0.5 years, IQR 0.2 – 0.8), suggesting they responded sufficiently.

External validation of the previously developed model (CEASE phase 0 model)

Comparability of the development and validation cohorts

We refer to Pauwels et al. [17] for details on the baseline characteristics in the development (CEASE phase 0) cohorts. For the previously developed model without FC, the median predicted 1-year risk of relapse was 0.31 [IQR 0.23 – 0.41] in the development sample compared to 0.30 [IQR 0.24 – 0.37] in the validation sample. The model with FC generated a median predicted 1-year risk of 0.35 [IQR 0.26, 0.48] in its development sample and 0.27

[IQR 0.21-0.39] in the validation sample. The distributions of predicted 1-year relapse risk were reasonably similar across development and validation samples (**Supplementary data; Appendix B**).

Performance of the previously developed model in the validation cohort

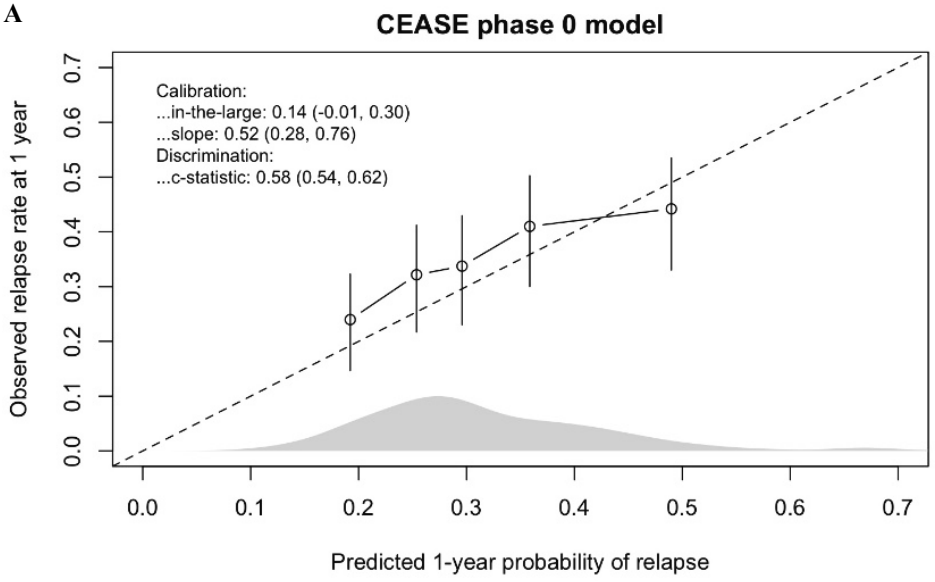
At external validation, the c-statistic of the previously developed model without FC was 0.58 (95% CI 0.54-0.62) (**Figure 2a**). The previously developed model with FC had similar performance (c = 0.57 (95%CI 0.53-0.61)) (**Figure 2b**). There was reasonable agreement between the predicted and observed relapse rates, though both phase 0 models under predicted risk for low-risk patients and over predicted risk for high-risk patients in our data. On average, the predictor effects were too strong, as indicated by calibration slopes below one (calibration slopes were 0.52 (95% CI 0.28-0.76), and 0.31 (95% CI 0.13-0.49) for the phase 0 model and the phase 0 FC model, respectively). The calibration-in-the-large (0.14 (95% CI-0.01-0.30) and 0.19 (95% CI -0.19-0.57)) shows that the average predicted risk of both models was below the average observed risk.

Model update

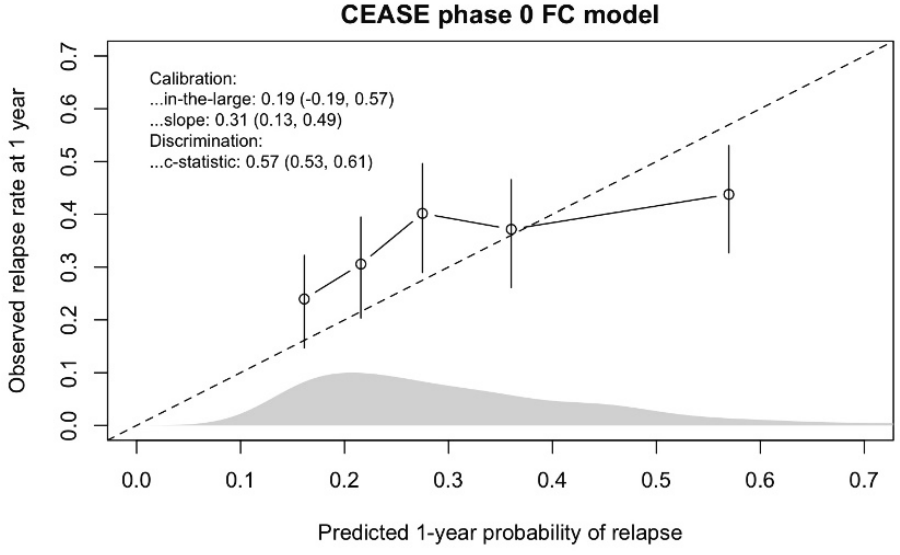
For our model update, data from the 486 patients in the validation (phase 1) cohort was combined with the data from the development (phase 0) cohorts, amounting to a total of 1816 patients. The prognostic model that resulted from our model update is shown in **Table 3**. The model formula is available as Supplementary data (**Appendix C**). Statistical interaction-by-phase tests revealed no statistically significant differences in predictor effects between phase 0 and phase 1 (**Table 3**). Cross-validation showed a comparable performance of the models in both datasets (**Supplementary data; Appendix D**). Both findings suggest that it was reasonable to combine the datasets for a model update.

Figure 2a and 2b. (A) Calibration plot Phase 0 model. (B) Calibration plot Phase 0 model with fecal calprotectin

A



B



Validation of the updated model

At internal-external validation (**Figure 3a**), we obtained a pooled c-statistic of 0.60 (0.58, 0.63), with I^2 estimated at 0%, suggesting no between-cohort heterogeneity in discriminative ability of the model. The calibration slope was estimated at 0.89 ((0.69, 1.09), I^2=0), only slightly below 1 (**Figure 3b**). Calibration-in-the large was estimated at 0.02 (-0.22, 0.27) with an I^2 of 80%, reflecting substantial differences in baseline risk (**Figure 3c**). Decision curve analysis (**Figure 4**) showed that the use of the updated model as a decision tool yields a higher net benefit than default strategies for threshold probabilities over 20%.

Table 3: Hazard ratios and associated 95% confidence intervals of the CEASE prognostic models.

Predictor	Previously developed model (phase 0) HR (95% CI)	Previously developed model with FC (phase 0) HR (95% CI)	Updated model (phase 1) HR (95% CI)
Age, per 10 years	0.86 (0.75, 1.00)	0.90 (0.71, 1.14)	0.94 (0.86, 1.03)
Smoking, yes vs no	1.39 (1.15, 1.67)	1.52 (1.10, 2.08)	1.31 (1.11, 1.53)
Age at diagnosis, A2 vs A1	0.69 (0.53, 0.90)	0.46 (0.30, 0.72)	
Age at diagnosis, A3 vs A1	0.71 (0.40, 1.25)	0.74 (0.29, 1.92)	
Age at diagnosis, per 5 years			0.94 (0.90, 1.00)
L4 Upper GI, yes vs no	1.32 (0.96, 1.79)	1.62 (0.98, 2.70)	1.15 (0.89, 1.49)
Disease duration, every 5 years	1.07 (0.98, 1.17)	1.02 (0.90, 1.16)	
Immunosuppressant, yes vs no	0.70 (0.58, 0.85)	0.87 (0.61, 1.23)	0.68 (0.58, 0.79)
Type of anti-TNF used, IFX vs ADA	0.82 (0.67, 1.01)	0.96 (0.66, 1.41)	0.87 (0.74, 1.03)
Second-line anti-TNF, yes vs no	1.32 (1.01, 1.69)	1.72 (1.09, 2.70)	1.13 (0.92, 1.39)
Clinical remission, yes vs no	0.45 (0.25, 0.83)	0.31 (0.16, 0.58)	
CRP, per doubling, *mg/L*	1.04 (1.00, 1.08)	1.00 (0.94, 1.08)	1.02 (0.98, 1.07)
FC, per doubling, *µg/g*		1.13 (1.02, 1.27)	1.10 (1.05, 1.16)
Apparent c-statistic	0.59	0.63	0.61
Internal-external validation pooled c-statistic	0.58 (0.55, 0.61)†	0.63 (0.59, 0.67)‡	0.60 (0.58, 0.63)§

HR = Hazard ratio, GI = gastrointestinal, ADA = adalimumab, IFX = infliximab, CRP = C-reactive protein, FCP = Faecal Calprotectin

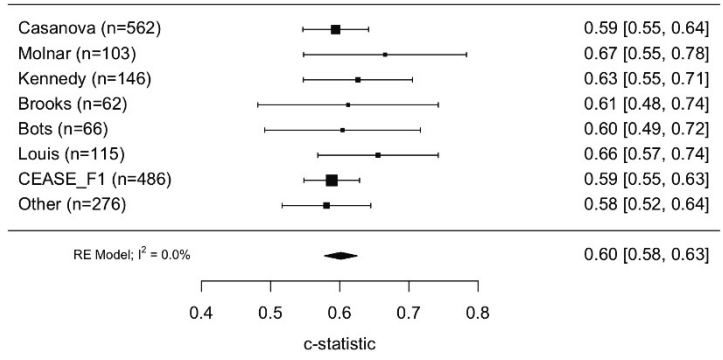
† On the phase 0 data

‡ On a subset of the phase 0 data

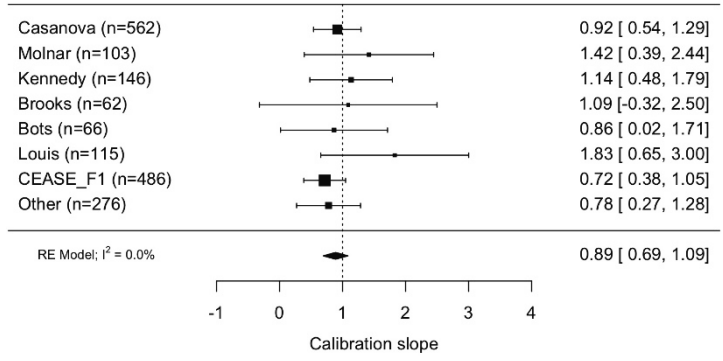
§ On the combined phase 0 and phase 1 data

Figure 3. (**A**) Internal-external validation CEASE Phase 1 model (predictive performance). (**B**) Internal-external validation CEASE Phase 1 model (calibration-in-the-large). (**C**) Internal-external validation CEASE Phase 1 model (calibration slope).

A



B



C

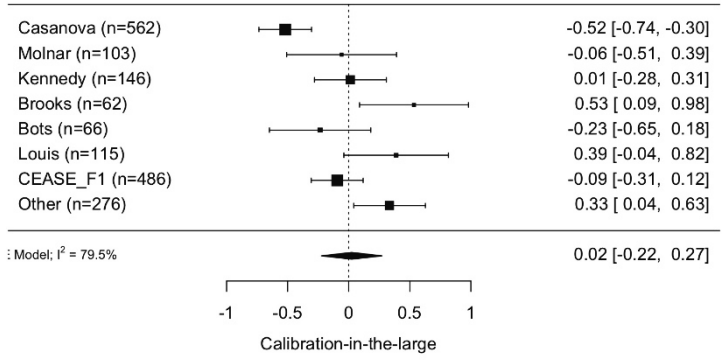
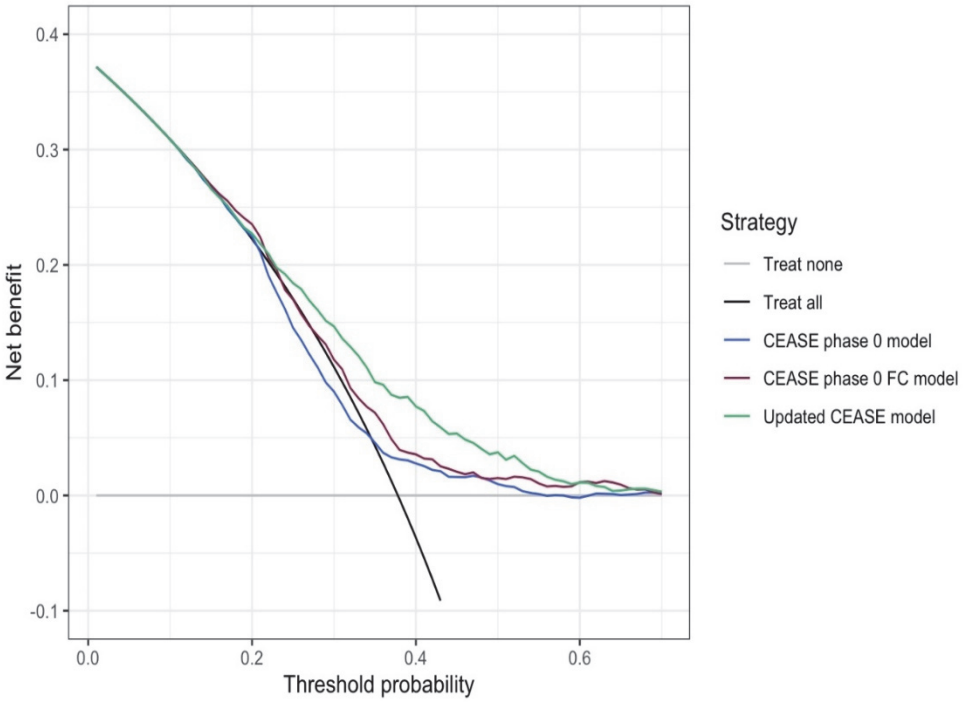


Figure 4. Decision curve analysis



Discussion

A valid tool for patient identification to safely cease anti-TNF therapy is highly desirable. Recently, we published a prediction model with modest discriminative ability, based on an IPD-MA of 14 studies [17]. In this cohort, our previously developed phase 0 prediction model was externally validated and updated to estimate the risk of relapse in individual patients after cessation of anti-TNF therapy with a reasonable discriminative ability.

The initial phase 0 model was not well-calibrated on the validation cohort: the predictions were too low for low-risk patients and too high for high-risk patients. Overall, the predictor

effects were too strong for the phase 1 data. This could possibly be explained by, but may not be limited to, the difference in distribution of baseline characteristics between the phase 0 cohorts and the phase 1 cohort, e.g., concomitant immunotherapy, 63% vs. 36%, and endoscopic remission, 44% vs 82%. Secondly, the collected data of the initial model enclosed a different period compared to the data of the validation cohort, leading to potential differences in treatment strategies. Thirdly, in the phase 0 model with faecal calprotectin, 'clinical remission' was significantly associated with sustained remission after cessation of anti-TNF (HR 0.30; 95% CI 0.16 - 0.57). In the validation cohort, only 12 patients (2%) were not in clinical remission (this subgroup of patients demonstrated baseline remission as indicated by either biochemical or endoscopic remission prior to cessation of anti-TNF therapy). Furthermore, 'clinical remission' was based on the medical records instead of validated questionnaires which were mostly used in the cohorts of phase 0. Therefore, this variable could not be used to accurately predict the risk of relapse in phase 1.

Although the updated model still showed moderate discriminative performance, the performance was stable across cohorts. In other fields, including oncology and fertility research, prediction models with similar c-statistics varying between 0.58 and 0.64 are frequently used as a guide for making decisions [30-42]. Despite this seemingly moderate c-statistic, these models may still have added value for decision making in daily practice. In addition, we demonstrated that our model may be useful as a prognostic tool for individualized decision making in clinical practice across a wide range of thresholds (0.2-0.7). This threshold expresses how the benefit of treatment, i.e. the prevention of relapse, is weighed against the harm of treatment, i.e. treating non-relapsing patients unnecessarily. If a clinician saw no harm at all in treating patients unnecessarily, it would be most beneficial to keep all patients on treatment. If, however, there is harm in treating patients unnecessarily,

such that the clinician is willing to treat at most five patients to prevent relapse in one of them, then the use of our updated model showed increased net benefit compared to keeping all patients on treatment. The model is most useful if the decision threshold is around 33%, implying a benefit to harm ratio around two (67/33) and a willingness to treat around two patients to prevent one relapse. In addition to the willingness to cease anti-TNF therapy by the treating physician, the patient's decision is equally important. The prediction model might aid patients as well in the process of shared decision making.

Our updated model may support a better cessation strategy compared to current international IBD guidelines which state that anti-TNF cessation is recommended only in patients in long-standing stable deep remission (clinical, biochemical and endoscopic) [43]. Another important indication for using the prediction model could be that it not only supports the decision of anti-TNF cessation in low-risk patients, but it will also avoid unjustifiable anti-TNF cessation in a subgroup of patients with a high-risk of relapse. In addition, a stimulating thought for using this prediction model is the knowledge regarding the successful retreatment rates with anti-TNF therapy after relapse. Our cohort reported high success rate of 81% which is in line with available literature [14,44,45].

This external cohort reported a one-year relapse rate of 32%, which is in line with available literature (26% - 44%) [14,17,44-47]. The differences between cohorts could be partly explained by heterogeneity in the definitions of 'remission' and 'relapse'. Louis et al. [14] included patients who were in steroid-free remission and in others, discontinuation was attempted in patients who were in clinical remission [44,46]. In the study by Bots et al. [47], as well as in our study, patients who were in either clinical, biochemical, or endoscopic remission were included. Moreover, relapse was defined as clinical symptoms [14] or as disease activity leading to a therapeutic intervention [17,18,23,24]. This implicates the endpoint to be largely subjective,

as it is only based on the judgement of the treating gastroenterologist (risking non-inflamed patients to be designated as having a relapse) and not based on objective evidence such as biomarkers or endoscopy to confirm active inflammation.

Although the prediction models have been reasonably validated, some limitations need to be discussed. As we collected patient data retrospectively from electronic patient databases, the assessment of 'clinical remission' could have been interpreted differently by the treating physicians. In addition, due to its retrospective character, missing data on biochemical markers and endoscopic procedures was inevitable. Moreover, anti-TNF serum concentrations were particularly difficult to obtain, as this was not routinely measured in many patients. Due to this, the level of anti-TNF serum concentration could not be identified as a significant predictor for relapse, which has previously been reported in the literature [20,47]. In our prospective follow-up study, anti-TNF serum concentration will be measured at baseline.

Our study accentuates the difficulty of predicting the risk of relapse in CD patients who cease anti-TNF therapy in daily clinical practice as the underlying pathophysiology of relapse is poorly understood. Identification of new biomarkers for a better discrimination between high- and low-risk patients is necessary. Further research is warranted to update the prediction model, including biochemical, serological, histologic and/or genetic markers. Previous studies reported on mucosal cytokines and serological markers which might be associated with the risk of relapse, as normalization of IL-17a expression and mucosal TNF predicts long term remission after anti-TNF discontinuation [48]. In addition, a recently published study reported on protein biomarkers and metabolomics markers which were associated with relapse [49], while other studies discovered potential biomarker candidates associated with the risk of short- and long-term relapse after discontinuation of

infliximab[49,59]. More quantified research into such predictors is warranted to further update and strengthen our prediction model.

We have used the updated prediction model to create a prognostic tool which will be publicly available as a user-friendly web-interface on Evidencio. However, further evaluation of the prognostic performance of the model is necessary before it can be used in daily practice. To do this, we have initiated a multicentre (200 patients from nineteen centres), center-specific stepped wedge randomized controlled trial (RCT) (Netherlands Trial Register: NL8891). In addition, this RCT will provide prospective data for further updating the prediction model with biomarkers, histological and endoscopic data, as well as insight in the cost-effectiveness of the new strategy of stopping anti-TNF therapy based on the prediction model.

In conclusion, our previously developed prediction model to safely cease anti-TNF therapy has been validated and updated in this external Dutch multicentre CD cohort. After validation and update, the model showed reasonable discriminative performance and improved calibration. A further update of the model with biochemical and histological data is necessary to improve our ability to adequately select patients for cessation of anti-TNF therapy. We will further improve this prediction model through a large national RCT to assess whether this updated prediction model leads to a better selection of patients for anti-TNF cessation as compared to daily practice.

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Chapter 8

Diagnostic tool to safely CEASE anti-TNF therapy in Crohn's disease: a stepped wedge cluster randomized trial

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Submitted

Abstract

Introduction Anti-TNF agents are effective for induction and maintenance treatment of Crohn's disease. Long-term treatment may have disadvantages, such as possible adverse events, interference with daily activities and high costs. Guidelines on when to stop anti-TNF therapy are lacking. Therefore, we have previously developed and validated the CEASE tool to stratify patients in high- or low-risk of relapse after cessation of anti-TNF agents. In the current study, we aim to assess the clinical impact of the tool and validate its prognostic performance.

Methods & Analysis The CEASE trial is a stepped wedge center-randomized non-inferiority trial. Nineteen centers will initially continue to offer anti-TNF treatment to patients with a low risk of relapse. These patients form the control group. The participating centers will be randomly allocated to one of three study arms, which determines when they implement the CEASE anti-TNF stop strategy: after 6, 12 or 18 months. Patients who stop their anti-TNF therapy under this new strategy will serve as the intervention group. At least 114 patients in the control group as well as 114 in the intervention group will be included. Inclusion criteria are patients with Crohn's disease with anti-TNF treatment exposure ≥ 12 months, who have a low risk of relapse (≤33.3%) according to the CEASE tool, and a Harvey Bradshaw Index < 5. The primary endpoint is a CD relapse, which is defined as a Harvey Bradshaw Index ≥ 5 with either fecal calprotectin ≥ 250 (both at two measurements) or ulcerations on endoscopy, or extra-intestinal manifestations which require therapeutic intervention. The proportions of relapse at 1 year will be compared between the control and intervention groups. Secondary objectives are prognostic performance of the tool, cost-effectiveness, adverse outcomes and disease-related quality of life.

Background

Since its introduction over 20 years ago, anti-tumour necrosis factor (anti-TNF) therapy has become a pivotal treatment for moderate to severe Crohn's disease (CD) and is effective for inducing and maintaining remission [1-3]. If remission is achieved, patients are usually treated for many years with this agent without concrete endpoint. However, long-term exposure to anti-TNF treatment may lead to side effects, an increased risk of malignancy [4], work-productivity loss and chronic fatigue [5, 6]. Furthermore, despite the introduction of biosimilars, long-term treatment with anti-TNF results in significant healthcare costs, as increasing numbers of patients are being treated with this agent [7, 8].

Previous research shows a relatively consistent relapse risk of 26-44% one year after cessation of anti-TNF therapy [9-14]. In clinical practice, however, it is still debated when to consider cessation of therapy, as the risk of relapse in the individual patient is unclear. Previous studies have tried to assess risk factors to identify patients with an increased risk of relapse, but this has not yet been translated into a useful and validated clinical tool [9]. A more personalized treatment approach by using such tool may aid patients and physicians in their process of shared decision making.

The CEASE project has been designed to develop, validate and implement a diagnostic tool to predict the risk of relapse after cessation of anti-TNF in CD patients. In *phase 0* of the project, the prediction model was developed in an individual patient data meta-analysis on 14 international cohorts (n = 1317) with a predictive power (concordance statistic, c-statistic) of 0.63 [10]. The CEASE tool was externally validated and updated in *phase 1* of the project based on a retrospective cohort study in 17 centers in The Netherlands (n = 486)[15]. After an update of the prediction model, a c-statistic of 0.60 (0.58 - 0.63) and calibration slope of 0.89

(0.69 – 1.09) were reported in internal-external validation (**table 1**). Before implementation in daily practice, however, the clinical impact of the model has yet to be established.

In *phase 2* of the CEASE project we aim to assess the clinical impact of using the tool to cease treatment in CD patients with a low risk of relapse. Secondly, we aim to prospectively assess the prognostic performance of the previously developed, upgraded and validated CEASE tool. In this manuscript, we discuss our objectives, study design as well as potential drawbacks.

Objectives

- To assess the clinical impact of the diagnostic tool by comparing the proportions at 1 year of CD relapse in the control and intervention groups. A relapse of CD is defined as a clinical relapse (Harvey-Bradshaw Index (HBI) ≥ 5) on two consecutive measurements, with two weeks between both measurements, necessitating medical or surgical intervention in combination with one of following criteria:
  - Biochemical relapse; fecal calprotectin (FC) ≥ 250 µg/g on two consecutive measurements, with two weeks between both measurements, or;
  - Endoscopic relapse; ulcerations on endoscopy.

  CD complications include the following:
  - Active fistula
  - Perianal abscess
  - Extra-intestinal manifestations, including but not limited to pyoderma gangrenosum, erythema nodosum, arthritis, uveitis.
- To assess the prognostic performance of the CEASE tool to predict CD relapse or complications in patients in stable disease remission one year after cessation of anti-TNF therapy.

- To compare the time to relapse between the control and intervention group at the end of follow-up.
- To compare the sustained low-risk for relapse according to the CEASE-tool between the control and the intervention group at the end of follow up.
- To compare disease-related and general quality of life between the control and intervention group every 3 months until the end of study, based on the Inflammatory Bowel Disease Specific Quality of Life (IBD-Q) and EQ-5D-5L, respectively.
- To compare disease activity by the HBI and patient reported outcome (PRO-2) between the control and intervention group every 3 months until the end of follow-up.
- To assess the cost-effectiveness and cost-utility of ceasing anti-TNF therapy during 12 months of follow-up on average with treatment continuation as its best alternative.
- To compare the rates of (serious) adverse events that are (possibly) related to continuation or cessation of anti-TNF therapy between control and intervention group, respectively.
- To identify additional prognostic factors that are associated with a higher or lower relapse risk (i.e. biochemical, endoscopic and histological factors).

Table 1. CEASE diagnostic tool.

predictor	HR
Age (per 10 years)	0.94 (0.86, 1.03)
Smoking = Yes	1.31 (1.11, 1.53)
Age_diagnosis (per 5 years)	0.94 (0.90, 1.00)
Is_baseline = Yes	0.68 (0.58, 0.79)
Prev_TNF = Yes	1.13 (0.92, 1.39)
Type_TNF = IFX	0.87 (0.74, 1.03)
CRP_baseline (per doubling)	1.02 (0.98, 1.07)
FC (per doubling)	1.10 (1.05, 1.16)
L4 = Yes	1.15 (0.89, 1.49)
c-statistic	*0.61*
c-statistic internal-external validation	*0.60*

FC: fecal calprotectin, HR: hazard ratio, TNF: tumor necrosis factor, IFX: infliximab, L4: upper gastrointestinal involvement.

Methods

Study design

This study is a stepped wedge center-randomized non-inferiority trial which is performed at the departments of Gastroenterology and Hepatology in nineteen centers in the Netherlands, including six academic and thirteen non-academic hospitals. Every center will be randomly allocated to one of the three different study arms. As the Erasmus Medical Center and Amsterdam University Medical Centers have the largest CD population, both will be serving as two clusters of patients.

Each study arm is divided in a control group and intervention group. In the control group, patients with a low risk of relapse will continue their anti-TNF treatment. After six, twelve or eighteen months of follow-up, depending on the study arm to which each center was

randomized, each center will implement the anti-TNF stop strategy. Patients with a low risk of relapse who will stop their anti-TNF treatment will be included in the intervention group. The intervention group will also have a follow-up duration of six, twelve or eighteen months depending on the study arm the center has been randomized to (**figure 1**, **table 2**; see also under 'Randomization'). The entire study duration for each center equals 24 months. Patients who were included in the control group, will not participate in the intervention group, to minimize statistical dependence between the control and intervention groups.

The study design is unblinded. For a full impression of eligible patients, we will register patients who are eligible for participation in the intervention group, but who do not want to stop their treatment. These patients (up to a maximum of six) will be asked to sign informed consent to obtain their baseline characteristics and primary outcome after one year. This enables us to investigate the extent to which the study results can be generalized to all patients clinically eligible for stopping their anti-TNF therapy.

Rationale study design

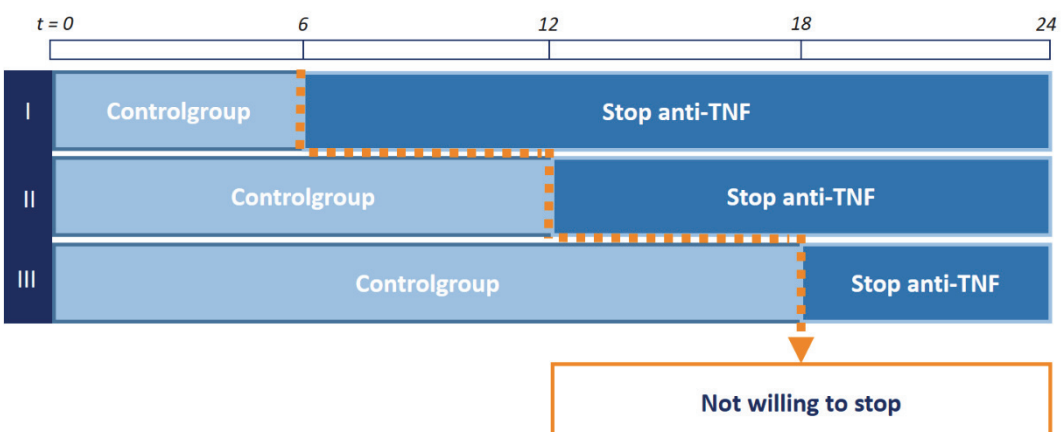
The rationale behind this design is the pragmatic nature of the research goal; to assess the performance and to evaluate the clinical impact of the CEASE tool, and ultimately implementing this in daily practice. Furthermore, using a stepped-wedge design, we will be able to correct for external factors that may arise during the execution of our study, and subsequently affect our primary outcome, e.g., switching from intravenous infliximab to subcutaneous prescription. In addition, the prognostic performance of the CEASE tool will be assessed.

Table 2. Duration of follow-up in each study-arm and group.

	Control group *Continuation of anti-TNF*	**Intervention group** *Cessation of anti-TNF*
Study-arm 1	6 months	18 months
Study-arm 2	12 months	12 months
Study-arm 3	18 months	6 months
Extra group: not willing to stop	NA	12 months

Anti-TNF: anti-tumor necrosis factor.

Figure 1. Flow-chart CEASE phase 2. I: study-arm 1, II: study-arm 2, III: study-arm 3.



Patients and public involvement

This trial was designed in collaboration with the Dutch inflammatory bowel disease (IBD) patient organization (Crohn & Colitis NL).

Inclusion and exclusion criteria

CD patients, aged sixteen years and older, who have received anti-TNF treatment (adalimumab or infliximab) for at least twelve months (stable dose ≥ 3 months) for the primary indication of luminal disease and who are in clinical remission (HBI < 5) are eligible for inclusion. In addition, patients must have a low risk of relapse after anti-TNF cessation, as predicted by the validated CEASE-tool [15]. Low risk of relapse after cessation of anti-TNF therapy is defined as ≤33.3% within the first year. This threshold is motivated by the clinical consideration that stopping treatment in a patient who develops a relapse (undertreatment) is worth treatment of two patients who do not develop a relapse (overtreatment). The 1:2 ratio translates to a decision threshold of 33.3%. Concomitant immunosuppression at a stable dose for at least three months is allowed, and should remain unchanged for the duration of the study. Exclusion criteria include systemic corticosteroid use for luminal CD in the previous six months and comorbidities that are a contraindication to stop anti-TNF treatment (e.g. spondylarthritis, active perianal fistula, etc.).

Cointervention

Concomitant immunosuppression (i.e., mercaptopurin, azathioprin, thioguanin and methotrexate) is permitted if the dose has been stable for at least 3 months, and will be continued throughout the study. Concomitant medication use will be documented on the Case Report Form (CRF) stating type, dosage and duration.

Study schedule, assessments and data collection

Enrolled patients in both groups will visit the outpatient clinic every six months. The number of visits varies from two to four outpatient clinic visits, depending on the centers' randomized starting time of the intervention.

At screening, baseline characteristics will be collected, including gender, age, smoking status, disease characteristics, treatment history and CD-related surgery. Symptoms will be assessed by using the HBI, and to establish the low-risk profile of each patient, blood tests (i.e. hemoglobin, leukocytes, thrombocytes, albumin, C-reactive protein (CRP), mean corpuscular volume (MCV)) and FC are performed. Endoscopic assessment prior to enrollment, as well as at the end of the study period, is highly recommended, but not obligatory. At endoscopy, the simple endoscopic score for CD (SES-CD) will be obtained. Furthermore, four biopsies from the previously active disease location will be collected for histopathology and future analysis. If a colonoscopy was performed in the twelve months before inclusion, data of this procedure can be used. Patients will be asked to complete several questionnaires and a patient diary every three months (‘Secondary outcome measurements’).

At each outpatient clinic visit, symptom assessment (HBI), laboratory tests (i.e. hemoglobin, leukocytes, thrombocytes, albumin, C-reactive protein (CRP), mean corpuscular volume (MCV)) and FC will be performed. Serum samples will be stored at each visit for future analysis. Additionally, patients in both groups will be interviewed by telephone every three months in between outpatient clinic visits to assess symptoms, adverse events and concomitant medication use. All subjects will be asked to complete several questionnaires (IBDQ, EQ-5D-5-L, PRO-2, iPCQ and iMCQ) every three months, for the duration of eighteen months. Subjects who experience a relapse will also be asked to finish the

questionnaires up to eighteen months of follow-up. All questionnaires will be sent automatically via Castor EDC. If subjects have difficulty with completing the questionnaires due to low literacy, questionnaires will be completed by telephone by a member of the research team. An overview of all visits, procedures and assessments are shown in **Table 3**.

Relapse

Patients who present with complaints at the time of a study visit, or in between, will be assessed at the outpatient clinic. Fecal cultures will be taken to rule out infections. Following the primary endpoint, a relapse will be confirmed biochemically on two different time points with two weeks interval. Ulcerations at endoscopy are also confirmative of a relapse. In case of a relapse, treatment can be (re-)started at the discretion of the treating physician.

Secondary outcome measurements

Quality of life

To assess disease-specific quality of life, we will use the validated IBD-Q. This questionnaire measures quality of life in four domains; bowel symptoms, emotional health, systemic systems and social function. In addition, the EuroQol-5D-5L questionnaire (EQ-5D-5L) will be used to assess the generic health status by assessing quality of life in 5 domains; mobility, self-care, usual activities, pain/discomfort and anxiety/depression.

Patient reported outcome

The patient reported outcome 2 (PRO-2) will be used as patient reported outcome measure and consists of two items, abdominal pain and diarrhea

Table 3. Schedule of procedures (Step 1; control group six months of follow-up, intervention group eighteen months of follow-up

Study-arm 1	**Both groups**	**Control group**			**Intervention group**							
Visit	**1**	**2**	**3**	**4**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Flare**
Month	*S*	*E*	*3*	*6*	*E*	*3*	*6*	*9*	*12*	*15*	*18*	*F*
Outpatient clinic visit	X			X			X		X		X	X
Telephone consult		X	X		X	X		X		X		
Informed consent[a]	X											
Assess in-/exclusion criteria	X											
Demographics and relevant medical/surgical history	X											
Blood test: hematology, chemistry and serum[b]	X			X			X		X		X	X
Anti-TNF (trough) level and antibodies	X[c]											
Fecal calprotectin	X			X			X		X		X	X[g]
Stool tests[d]												X

Colonoscopy + biopsies	X[e, f]			X[f]							X[f]	X[f]
Stop anti-TNF treatment					X							
HBI	X	X	X	X	X	X	X	X	X	X	X	X[g]
PRO2		X	X	X	X	X	X	X	X	X	X	X[g]
IBD-Q, EQ-5D-5L, iMCQ, iPCQ[h]		X	X	X	X	X	X	X	X	X	X	X

S = Screening process, E = Enrollment, F = Flare

a When subjects have undergone routine blood tests and fecal calprotectin assessment within 4 weeks before signing the informed consent form in the context of standard care, it is allowed to use these data as screening procedures and subjects will not have to repeat these screening procedures during the screening visit in this study.

B Including Albumin, CRP, hemoglobin, leukocytes, MCV and thrombocytes. Serum will be stored for future purposes.

c If anti-TNF trough level and antibodies have been measured within 6 months prior to screening, these results can be used.

d Including Salmonella, Shigella, Yersinia and Campylobacter stool cultures, C. difficile toxin assessment, Double Feces Test (parasitology; only when indicated).

e Subjects who have undergone a colonoscopy within 12 months before signing the informed consent form, subjects may consent to the use of the data (if a SES-CD score can be obtained from report and/or photo's).

f Colonoscopy + biopsies advised but not obligated

g If a flare is suspected, fecal calprotectin and HBI must be measured twice, with two weeks between the measurements.

h Patients are asked to complete questionnaires up to eighteen months after inclusion.

Costs

Direct intra- and extramural costs will be calculated based on the information from the electronic patient records (e.g. medication, days of hospital admission, side effects etc.). Indirect costs will be measured using the institute of Medical Technology Assessment (iMTA) productivity cost questionnaire (iPCQ) and iMTA medical consumption questionnaire (iMCQ). The iMCQ measures all relevant healthcare related costs like hospital admissions, imaging procedures and outpatient visits at any medical specialist or primary care. The indirect healthcare costs as loss of productivity due to illness in patients younger than 67 years will be estimated based on patient reported absences from paid (or unpaid) labor measured with the iPCQ.

Safety

(Serious) adverse events ((S)AE) will be registered during follow-up. All SAEs related to the intervention (cessation of anti-TNF) will be reported to the Medical Ethical Committee Rotterdam, the Netherlands.

Randomization

Randomization was performed at center level. Prior to randomization, seven triplets of centers were constituted based on comparable numbers of CD patients on anti-TNF therapy. The two largest centers would include double the number of patients in the study, hence these centers were each included in two triplets (triplets 6 and 7). The other centers were randomized in a 1:1:1 ratio (using R version 3.6.3 (2020-02-29) to implement the strategy of discontinuing anti-TNF therapy after respectively six, twelve or eighteen months. (Figure 1). To prevent center by calendar time bias, the timing of the intervention was balanced over the two largest centers in the 6th and 7th triplets. Randomisation was performed by an

independent expert from the department of Epidemiology and Data Science at the Amsterdam UMC.

Sample size

The yearly relapse rate while on anti-TNF therapy was estimated as 13% [16]. Since patients without relapse will presumably remain free from anti-TNF associated side-effects and drug discontinuation will generate considerable cost savings, we considered a 43% relapse risk after treatment withdrawal a reasonable non-inferiority boundary. Therefore, the non-inferiority margin was set to an absolute difference between the group proportions of 30%. This margin aligns with the decision threshold of 1/3 for the relevance of undertreatment versus overtreatment. In Phase 1 of the CEASE project, we found that the proportion of low-risk patients experiencing a relapse after stopping anti-TNF therapy was 28% at 1 year. This was taken as the expected proportion of relapsing patients in the intervention group of this study. The hypothesis of non-inferiority of the relapse proportion at 1 year, estimated with Kaplan-Meier, will be tested using a one-sided Z-test (unpooled) with a 0.05 significance level. Sample sizes of 114 in the continued anti-TNF therapy group and 114 in the stopping anti-TNF therapy group - obtained in both groups by sampling nineteen centers with six subjects each - will achieve at least 80% power to detect an absolute non-inferiority margin difference between the group proportions of 30%, accounting for intracluster correlations in the stepped-wedge study design up to 0.02. The total number of evaluable patients to be included equals 228.

The two largest centers will double their patient numbers (from six to twelve) to account for potential loss to follow-up. The total cohort will consist of 252 subjects (17 clusters * 6 patients * 2 treatment groups plus 2*12*2 respectively).

Planned data analyses

Primary study parameter

The clinical impact of stopping anti-TNF in the low-risk subjects will be assessed by comparing the estimated risk of relapse of CD or CD complications within one year after cessation. If follow-up is incomplete, subjects who do not relapse during the cessation period will be censored at study end. Statistical analysis will adjust for baseline risk according to the CEASE tool. Calendar time will be included as a continuous variable. The clustering nature of the data (i.e., patients within centers) will be accounted for by using a frailty term in a Cox regression model (lme4 in R). Uncertainty will be indicated by 95% confidence intervals from this model.

Secondary study parameters

The prognostic performance of the CEASE tool will be quantified according to discrimination, calibration and clinical usefulness [17]. Discrimination will be quantified with a c-statistic (similar to the area under the receiver operating characteristic (ROC) curve). Calibration will be assessed graphically, and summarized by recalibration statistics: intercept and calibration slope. Uncertainty in each measure will be indicated by 95% confidence intervals based on bootstrap resampling.

Secondary endpoints will be compared between both stages using non-parametric and parametric tests as appropriate, including chi-square statistics for categorical data, t-tests for continuous data, and regression analyses, accounting for the clustering nature of the data. Calendar time will be included as a continuous variable. Quality of life scores and cost data will be analyzed with linear regression models for expected mean quality of life and costs. Cost data will also be modelled by Cox regression to address the expected skewed nature of cost distributions.

Data will be analyzed according to intention-to-treat. For descriptive statistics, mean (standard deviation [SD]) will be used in case of a normal distribution of variables, median (interquartile range [IQR]) will be used for variables with a skewed distribution. Categorical variables will be presented as frequencies with percentages. For biomarker analysis, descriptive statistics of both raw and change from baseline data will be displayed where appropriate (n, arithmetic mean, geometric mean, standard deviation, minimum, median and maximum). Graphical displays will be produced over time if deemed appropriate. Formal statistical analyses may be conducted if deemed appropriate, for within subject comparisons. For the univariate analysis of unpaired continuous variables, either an unpaired t-test or independent 2 group Mann-Whitney U test will be used. A paired t-test or dependent 2-group Wilcoxon signed rank test will be used for paired continuous variables as appropriate. For the univariate analysis of discrete variables, the Fisher's exact test or chi-squared test will be used where appropriate.

Cost-effectiveness analysis

An economic evaluation will be performed from a societal perspective following the Dutch guidelines for

economic evaluations [18]. The time horizon will be 18 months follow-up after cessation to include all relevant costs and effects regarding the stop criteria into account. Both a cost-effective (CEA) and cost-utility (CUA) analysis will be performed. Direct intramural and extramural care costs as well as indirect non-medical costs will be calculated. A change in extramural costs in this trial is not anticipated. Data on medical resource use will be collected from the electronic hospital information systems, based on the iMCQ. For the calculation of medical costs, we will use charges as published in Dutch guidelines as a proxy of real costs. The unit price of applying the stop criteria for anti-TNF treatment in CD patients in remission

will be calculated with the micro-costing method. Productivity loss will be measured with the iPCQ. The economic evaluation of the diagnostic tool compared to usual care will be calculated as the incremental cost-effectiveness ratio (ICER). The primary effect outcome measures will be a relapse for the CEA and quality adjusted life years (QALY) for the CUA. QALYs will be measured for 18 months and extrapolated to a lifelong period, based on the Dutch tariff for the EQ-5D [19]. The lifelong outcomes will be modelled for the CUA, based on relevant literature. The sensitivity analysis will assess the robustness of the results to changes in costs and effect parameters. Bootstrapping with 5000 replications will be used to estimate 95% confidence intervals around cost differences and the uncertainty surrounding the ICERs. This will be graphically presented on cost-effectiveness planes and acceptability curves using the net benefit framework [20]. For the time horizon of 18 months, discounting is not necessary.

Ethics and dissemination

This study is approved by the Medical Ethical Committee Rotterdam, the Netherlands (registration number NL71860.078.19). Protocol adjustments are assessed and approved by the MEC, and will be communicated to all participating investigators. The most recent study protocol version 5 (14-10-2021) is presented in this manuscript. The study has been registered at the Netherlands Trial Register (NL8891). Data of all participating centers will be collected by Castor EDC and will be coded and kept, based on the rules for good clinical practice (GCP). The collection of data and all the study procedures will be monitored following GCP. Results will be presented at (inter-)national conferences and published in peer-reviewed journals. The first patient was enrolled on March 6th 2021.

Discussion

With our previously developed, updated and validated prediction model, we aim to estimate the risk of relapse after cessation of anti-TNF therapy in patients with Crohn's disease to provide a useful tool for physicians to safely cease anti-TNF therapy and to facilitate shared decision-making. In this prospective center-randomized trial, the clinical impact of the model will be assessed, before implementation in daily practice.

Design rationale

In this study, we have adopted a stepped wedge center-randomized design for several reasons. Firstly, in the IBD-research field, the diagnostic landscape and medical options are constantly evolving, ultimately changing the disease course in IBD patients. These involve, but are not limited to, the introduction of subcutaneous infliximab or the FC home test, both of which will be introduced in daily practice in the Netherlands during this trial. Such changes can impact the primary outcome of our study. By adopting a stepped wedge center-randomized design, the implementation of the intervention occurs at different time points, spanning a year's time. In this way, external factors can be controlled for in the statistical analyses. Secondly, this study was designed in cooperation with the Dutch IBD patient organization who expressed their preference for a design where patients will not be randomized to either stopping or continuing their current treatment. Due to ethical, as well as logistic reasons, we instead randomized at center level, to allow for transparency in the treatment course, while still maintaining a level of independent distribution of patient characteristics. Lastly, this design allows for an equal spread of the workload as the intervention is introduced at different time points

Even though the prediction model might be a useful clinical tool, it requires further refinement. Biomarkers closely associated with the pathophysiology of CD might be highly

promising and therefore more data on biochemical, endoscopic and histologic biomarkers, associated with lower or higher risk of relapse after anti-TNF cessation, are warranted. To this end, we will collect serum samples of all patients at predefined time points to improve and update our prediction model. In addition, the predictive value of endoscopic and histological remission will be assessed as a possible predictor of relapse.

Our prediction model includes multiple variables such as patient characteristics, disease specific information and biochemical markers. The willingness of a patient to cease their anti-TNF treatment is crucial in the process of shared decision making, though it is a subjective parameter and therefore difficult to measure. Consequently, this was not taken into account in the development of the model. The (un)willingness of patients to cease their anti-TNF therapy may introduce selection bias in our study, due to the study's unblinded nature. To prevent such bias from affecting our primary analyses, patients who do not want to stop their treatment when asked for participation are asked permission to collect their baseline data and primary outcome after one year.. By collecting this data, we will be able to indicate the level of generalizability of the intention-to-treat analysis.

As CD is a chronic disease, patients can be exposed to years of anti-TNF treatment to maintain remission. Previous studies reported that healthcare costs of CD patients are mainly driven by medication costs, most importantly by anti-TNF therapy [21]. Despite the introduction of biosimilars, the total healthcare costs of anti-TNF therapy remain significant [7, 8]. Therefore, an alternative strategy to safely cease anti-TNF therapy might aid in cost reduction of this significant expenditure, both direct as indirect costs.

Limitations

Despite randomization at center level, we are still aware of the possibility of selection bias while inclusion is ongoing. This might result from a patient's preference to either continue or stop the medication, leading to two different patient groups. To minimize this risk, we screened and enrolled patients based on the order of the next appointment at the outpatient clinic. Furthermore, to stimulate homogeneity between all centers, and again eliminating selection bias as much as possible, screening will be performed by the coordinating researchers in order of the outpatient clinic visits of each patient. After initiation of the study, the willingness to participate in the control group was lower than expected, which was mainly due to the mandatory colonoscopy at screening. Due to the impact of the procedure, we decided to change the screening colonoscopy to an optional procedure. However, this may lead to less information on endoscopic and histologic predictors on relapse.

Conclusion

In conclusion, the CEASE trial is a pragmatic randomized control trial designed to assess our previous developed and updated prediction model to support clinical decision making and optimize patient selection in whom anti-TNF can be ceased. In addition, serological, endoscopic and histologic biomarkers will be collected to further improve and update the CEASE model.

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9

Chapter 9

Discontinuation of Anti-Tumour Necrosis Factor Therapy in Patients with Perianal Fistulizing Crohn's Disease: Individual Participant Data Meta-Analysis of 309 patients from 12 studies

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Abstract

Background The risk of relapse after anti-tumour necrosis factor [TNF] therapy discontinuation in Crohn's disease patients with perianal fistulas [pCD] is unclear. We aimed to assess this risk.

Methods A systematic literature search was conducted to identify cohort studies on the incidence of relapse following anti-TNF discontinuation in pCD patients. Individual Participant Data were requested from the original study cohorts. Inclusion criteria were age ≥16 years, pCD as (co)indication for start of anti-TNF therapy, >3 doses, and remission of luminal and pCD at anti-TNF discontinuation. Primary outcome was the cumulative incidence of CD relapse using Kaplan-Meier estimates. Secondary outcomes included response to retreatment and risk factors associated with relapse as assessed by Cox regression analysis.

Results 309 patients from 12 studies in 10 countries were included. Median duration of anti-TNF treatment was 14 months [IQR 5.8 – 32.5]. Most patients were treated for pCD without active luminal disease [89%], received first line anti-TNF therapy [87%] and continued immunomodulatory following anti-TNF discontinuation [78%]. Overall cumulative incidence of relapse was 36% [95% CI 25-48%] and 42% [95% CI 32-53%] at 1 and 2 years after anti-TNF discontinuation. Risk factors for relapse included smoking [HR 1.5 (1.0, 2.1)] and history of proctitis [HR 1.7 (1.1, 2.5)]. Overall retreatment response rate was 82%.

Conclusions This IPD-MA, on predominantly patients with pCD without active luminal disease and first line anti-TNF therapy, shows that over half of patients remain in remission 2 years after anti-TNF discontinuation. Therefore, anti-TNF discontinuation may be considered in this subgroup.

Introduction

Perianal fistulas are associated with considerable morbidity and affect up to half of Crohn's disease [CD] patients during the disease course [1]. Treatment of perianal fistulizing CD [pCD] has evolved considerably over the last decades. Anti-TNF agents in combination with surgery are the mainstay of treatment [2, 3]. However, despite its efficacy, safety profiles are concerns of long-term exposure to anti-TNF agents, and include infusion reactions, infections, skin diseases and possibly increased risk of melanoma [4]. In addition, treatment with anti-TNF therapy is associated with work productivity loss and chronic fatigue. Direct and indirect health care costs remain considerable despite the advent of biosimilars [5]. Altogether, the decision on discontinuation of anti-TNF therapy remains a dilemma.

In routine practice, anti-TNF therapy is infrequently withdrawn in patients with pCD, due to several reasons including risk of relapse, possible lower response rates after retreatment with anti-TNF therapy and limited remaining treatment options. Available studies reported inconsistent results on the relapse rates following anti-TNF discontinuation in patients with pCD. Some previous studies showed that pCD was associated with an increased risk of relapse as compared to luminal CD. Other studies did not report a difference between both phenotypes [6-9]. Important drawbacks for interpretation of the available literature includes small sample size, varying endpoints and combined analysis of perianal and other [entero-enteric] fistulas.

Therefore, the risk of relapse following anti-TNF discontinuation in patients with pCD is still debated. In this study we performed a meta-analysis of individual participant data [IPD] and aimed to assess the risk of relapse after anti-TNF therapy discontinuation in patients with

pCD in remission. The secondary aim was to evaluate response after retreatment and to identify risk factors for relapse.

Material and methods

An IPD-MA of published studies was conducted using the Meta-analysis Of Observational Studies in Epidemiology [MOOSE] checklist including specifications for the reporting of a meta-analysis of observational studies [10]. Additionally, the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-analysis [PRISMA] were followed [11]. The study protocol was approved by the Medical Ethical Review Committee of Erasmus MC - University Medical Center Rotterdam [number: MEC-2019-0359].

Search strategy

A comprehensive systematic literature search was conducted until July 2022 in Medline, Embase, the Cochrane database, Google scholar and Web of Science in collaboration with the Medical School Library of the Erasmus University Rotterdam, the Netherlands. The literature search was conducted using controlled vocabulary supplemented with key words **[supplementary Figure 1]**. Studies reporting on the effect of anti-TNF therapy discontinuation in CD patients with perianal fistulas were considered eligible. The retrieved studies were screened and selected by three independent reviewers [STBH, MC and LJ].

Study selection and IPD database

Studies reporting on the incidence of relapse after discontinuation of anti-TNF therapy in patients with CD were selected. Studies were included if full text was available in English language. Abstracts published on international congresses were included as well. In case of

incomplete data in abstracts, corresponding authors were contacted and requested for the complete data. Editorials and [systematic or narrative] reviews were excluded. For each selected study, the corresponding authors of the eligible cohort studies were contacted to request IPD.

After obtaining the IPD, inclusion criteria for further analyses comprised patients aged ≥ 16 years, perianal fistulizing disease as the (co)indication for start of anti-TNF therapy, ≥3 infusions of anti-TNF therapy, and remission of both luminal and pCD at time of discontinuation of anti-TNF therapy. Patients with rectovaginal fistulas, non-fistulizing perianal lesions or fistulas unrelated to CD were excluded. In addition, patients who discontinued anti-TNF therapy due to other reasons, i.e. primary or secondary non-response, were excluded as well. In case IPD reported only perianal disease, authors were asked to specify for perianal fistulas.

CD luminal remission was defined as clinical, biochemical or endoscopic/radiologic disease remission at baseline, i.e. Crohn's Disease Activity Index (CDAI) <150 / Physicians' Global Assessment (PGA) 0 / Harvey Bradshaw Index (HBI) <5; and/or fecal calprotectin (FC) <150 µg/g / C-reactive protein (CRP) <10 mg/L; and/or endoscopic remission defined as a simple endoscopic score for Crohn's disease (SES-CD) 0-2 / Rutgeerts' score 0-1 / no ulcerations or mucosal healing Crohn's disease index of severity (CDEIS) <3.

Remission of perianal fistula was defined as complete fistula closure at clinical examination, i.e. absence of anal pain and draining fistula despite gentle compression [Fistula Drainage Assessment score [12]] of the track by the examiner's finger without new fistulizing episode, no further discharge from the fistula on firm finger pressure, nor signs of perianal inflammation. Radiological remission of perianal fistula was defined as complete resolution

of previous high signal tract or a subtle, narrow calibre intermediate signal residual tract or if pelvic magnetic resonance imaging demonstrated that the perianal fistulas tracts showed no signs of activity and were without local complications.

Eventually, patients were subdivided into 2 groups with regard to disease activity at start of anti-TNF therapy: a. patients with parallel luminal disease activity and b. without luminal disease activity at the start of anti-TNF therapy.

Data collection

Patient characteristics and disease specific demographics [including gender, age, disease characteristics according to the Montreal classification, smoking status, treatment history, maintenance of immunosuppressive therapy after anti-TNF discontinuation and history of IBD related surgery] and fistula characteristics [including type of perianal fistula [simple or complex], number of fistulae, prior antibiotics as treatment for perianal fistulas, perianal surgery (incision and drainage of perianal abscess, examination under anesthesia, seton insertion, fistuolotomy, defunctioning surgery and proctectomy/proctocolectomy), prior abscess, seton or proctitis] were collected. Indication to start anti-TNF therapy was collected including both active luminal en perianal fistulizing disease or only perianal fistulizing disease without active luminal disease. Simple fistula was defined as fistula with only single external opening without pain or fluctuation suggestive of perianal abscess, and was low in position (superficial or low inter-sphincteric or low transsphincteric origin) and had no evidence of anorectal stricture. Complex fistulas were defined as fistula(s) with multiple external openings with associated perianal abscess and were high in position [high inter-sphincteric or high trans-sphincteric or extra-sphincteric or supra-sphincteric origin of the fistula tract] according to American Gastroenterological Association (AGA) [13].

Outcomes and definitions

The primary outcome was perianal or luminal relapse. As a secondary outcome, perianal and luminal relapses were assessed separately. Perianal fistulizing relapse was defined as recurrence of draining perianal fistula related to previous or the development of new fistula tracks, or abscess. Luminal relapse was defined as a clinical, biochemical, endoscopic, and/or radiological relapse requiring treatment or dose optimization of IBD medication or surgery. Other secondary outcomes included success of retreatment with anti-TNF therapy and predictors of relapse. Success of retreatment was defined as the absence of clinical symptoms [HBI <5 or CDAI <150 points], biochemical remission [FC <250ug/g and CRP < 5mg/l] or endoscopic/radiologic remission [no sign of active inflammation] or complete fistula closure [no further discharge from the fistulas after manual pressure] during follow-up.

IPD integrity

Data were checked on inconsistency, invalid, missing or out-or-range values and these were queried and solved with the corresponding authors. Data management was performed following published guidelines supported by Amsterdam University Medical Centre directive for data management and incorporation of new European legislation on privacy protection [14].

Quality of evidence assessment and Risk of bias

Quality of evidence assessment and risk of bias were assessed by three investigators [STBH, MC and LJ] using the prediction model risk of bias assessment tool [PROBAST] [15] and the Newcastle-Ottawa Quality Assessment Form for Cohort Studies [NOS] [16].

Statistical analyses

Descriptive statistics were used for baseline characteristics. Continuous variables were summarized with medians and interquartile ranges (IQR) and categorical data were

summarized with frequencies and percentages. Missing values were assumed to be missing at random and were imputed using the mice algorithm [17], exploiting the correlations between variables. As some of the data was interval-censored, the Turnbull estimator was used to estimate the cumulative incidence of relapse within each cohort. Subsequently, the 1- and 2-year relapse rates were pooled across cohorts in a random effects meta-analysis. The heterogeneity between cohorts was quantified using the I^2-statistic [18]. In an exploratory secondary analysis, we estimated the cumulative incidence of each type of relapse in the pooled cohorts with available relapse type data, using a Fine and Gray model to account for competing risks [19]. To identify predictors for relapse after discontinuation of anti-TNF therapy, univariable hazard ratios (HR) with 95% confidence intervals (CI) were estimated. A stratified Cox proportional hazards model was used that accounted for interval censored data [20]. Predictors whose univariable HR had a p-value below 0.2 were included in a multivariable stratified Cox model to estimate multivariable (adjusted) HRs. Small cohorts with fewer than 18 patients were merged in Cox regression analyses. Finally, we investigated the association between individual fistula characteristics and time to relapse in the subgroup of patients with active perianal fistula and in luminal remission at start of anti-TNF. To this end, we again used a stratified Cox model to estimate univariable HRs, in cohorts where fistula characteristics were available. A p-value of <0.05 was considered statistically significant, without correction for multiple comparisons. Data analyses were performed using IBM SPSS Statistics for Windows, version 25.0 and and R version 4.0.3 [21].

Results

Identification of studies

The electronic search retrieved a total of 418 publications, of which 113 articles were excluded due to duplication **[Supplementary Figure 2]**. In total, 305 articles were selected for a more thorough review. Sixteen studies fulfilled the eligible criteria after screening of titles and abstracts. After contacting the corresponding authors, the IPD were obtained from 12 studies **[Supplementary Figure 2]**. Four studies were excluded due to unavailability of IPD, no response or no database received **[Supplementary table 1, Supplementary Figure 2]**. The cohorts were from Europe (10 studies) and Asia (2 studies) **[Supplementary Table 2]**. Two studies were considered prospective and ten retrospective. Finally, IPD were obtained from 366 patients, of whom 309 patients were included **[Supplementary Figure 3]**. With regards to the methodological quality, studies scored between 6 and 7 stars (maximum of nine) according to the NOS **[supplementary table 3]**.

Patient characteristics

A minority of patients [n = 34/307, 11%] had active luminal disease at time of start anti-TNF treatment. The median follow-up time after discontinuation was 29 months [Inter Quartile Range [IQR] 12 – 62] [**Table 1a]**. For further analyses, percentages are mentioned on patients with available data. In total, 129/287 [45%] patients were male. Median age at anti-TNF discontinuation was 34 years [IQR 26 – 44] and the median disease duration was 6 years [IQR 3 – 11] **[table 1a].** Thirty-seven of 281 [13%] patients were previously exposed to anti-TNF therapy [second or third line of anti-TNF therapy]. Median duration of anti-TNF exposure before discontinuation was 14 months [IQR 6.0 – 33]. Regarding anti-TNF agent use prior discontinuation, 259/309 [84%] patients discontinued infliximab and 49/309 [16%] adalimumab. Concomitant therapy with an immunomodulatory was continued in a majority

of patients [234/300, 78%] following anti-TNF discontinuation [**table 1a]**. Based on the available data, complex fistulas were reported in 85/133 [64%] patients. In the past medical history, 56/96 [58%] patients had been diagnosed with proctitis, 69/101 [68%] had a seton and 75/140 [54%] had an abscess in the past **[table 1b]**.

Outcomes after discontinuation of anti-TNF therapy

A total of 168/309 [54%] CD patients relapsed after discontinuation of anti-TNF therapy with a median time to relapse of 11.0 months [IQR 5.0 – 26.9]. The nonstratified cumulative incidence of relapse was estimated at 0.31 [0.28, 0.35] and 0.43 [0.40, 0.47], respectively, at 1 and 2 years after treatment discontinuation **[Figure 1]**. A meta-analysis of the pooled cohorts resulted in overall cumulative incidence estimates of 0.36 [0.25, 0.48] and 0.42 [0.32, 0.53] at 1 and 2 years **[Figure 2a and 2b]**. The heterogeneity in observed relapse rates was high between studies (I^2 = 91% and 90% respectively).

Table 1a. baseline patient characteristics

Parameter		**n = 309**
Age	Median [IQR]	33.6 [26.4 – 43.9]
Sex, female, n = 287	n [%]	129 [45]
Smoking, n = 293	n [%]	97 [33]
Disease duration, years	Median [IQR]	6.0 [2.80 – 11.3]
Follow-up time, months	Median [IQR]	29.0 [12.0 – 621.5]
Age Montreal classification		
<16 years [A1]	n [%]	36 [12]
16 – 39 years [A2]	n [%]	229 [74]
≥ 40 years [A3]	n [%]	44 [14]
Disease location, n = 298		
Ileum [L1]	n [%]	44 [15]
Colon [L2]	n [%]	121 [41]
Ileocolonic [L3]	n [%]	133 [45]
+ upper GI involvement [L4]	n [%]	14 [5]
Disease behaviour, n = 269		
Non stricturing, non-penetrating [B1]	n [%]	150 [56]
Stricturing [B2]	n [%]	37 [14]
Penetrating [B3]	n [%]	82 [31]
Only perianal fistulizing disease	n [%]	11 [4]
Previous intestinal resections*, n = 286	n [%]	139 [49]
Use of anti-TNF agent prior cessation, n = 308		
Adalimumab	n [%]	49 [16]
Infliximab	n [%]	259 [84]
Previous anti-TNF exposure, n = 279	n [%]	37 [13]
Concomitant medication continued after anti-TNF cessation, n = 234		
Thiopurines	n [%]	215 [92]
MTX	n [%]	11 [5]
Unknown	n [%]	8 [3]

* including surgery for perianal diseases [i.e. incision and drainage of perianal abscess, examination under anaesthesia, seton insertion, fistuolotomy, defunctioning surgery and proctectomy/proctocolectomy]

N; numbers of patients, CD Crohn's disease, L; location, B; behaviour, E; extent, TNFα; tumor necrosis factor alpha

Table 1b. Fistula characteristics

Parameters		**n = 309**
Type, n = 133		
Simple	n [%]	48 [36]
Complex	n [%]	85 [64]
Missing	n [%]	178 [58]
Previous use of antibiotics, yes, n = 122	n [%]	82 [67]
Missing	n [%]	189 [61]
Number of fistula, n = 87	Median [IQR]	1 [1 – 2]
Missing	n [%]	224 [72]
Previous surgery for fistulizing disease, yes n = 128	n [%]	79 [62]
Missing	n [%]	183 [59]
Abscess in past, n = 140	n [%]	75 [54]
Missing	n [%]	171 [55]
Seton in past, n = 101	n [%]	69 [68]
Missing	n [%]	210 [68]
Proctitis in past, yes n = 96	n [%]	56 [58]
Missing	n [%]	215 [69]

Figure 1. Cumulative probability of relapse after discontinuation of anti-TNF therapy in perianal fistulizing CD patients (all cohorts pooled)

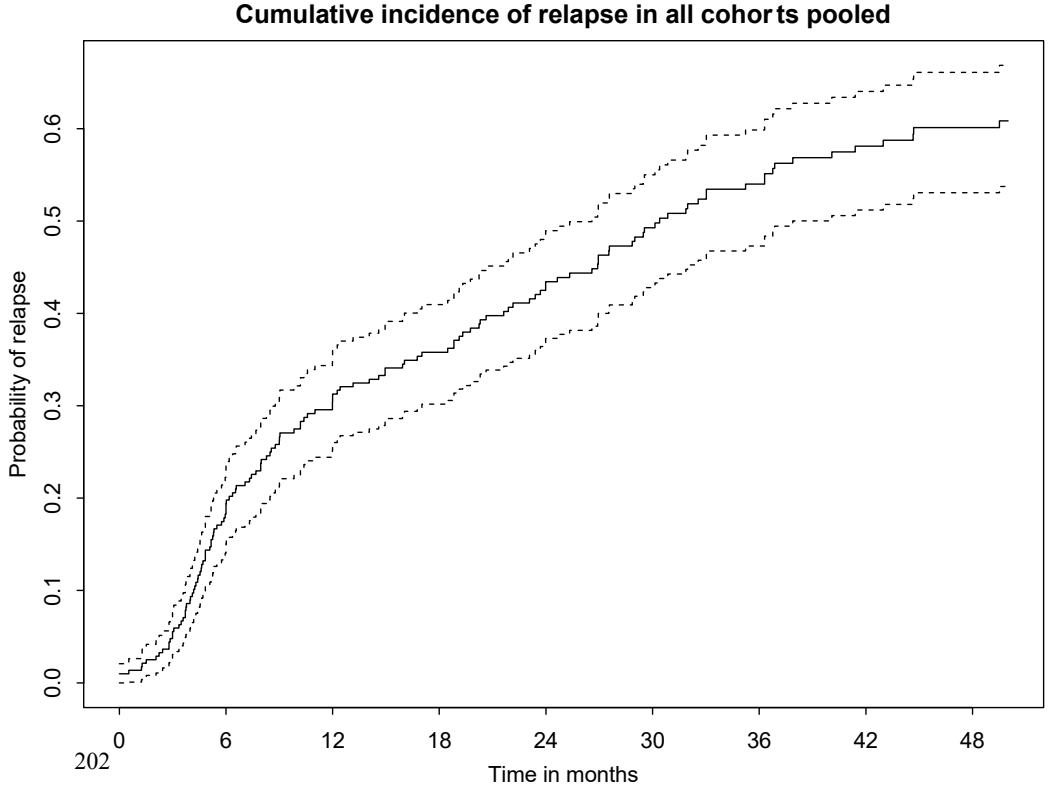
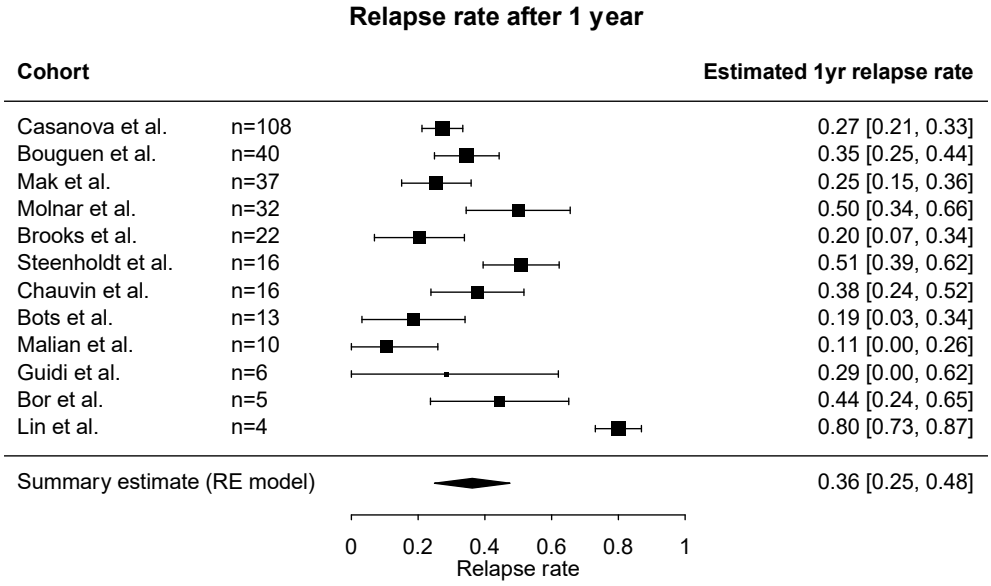
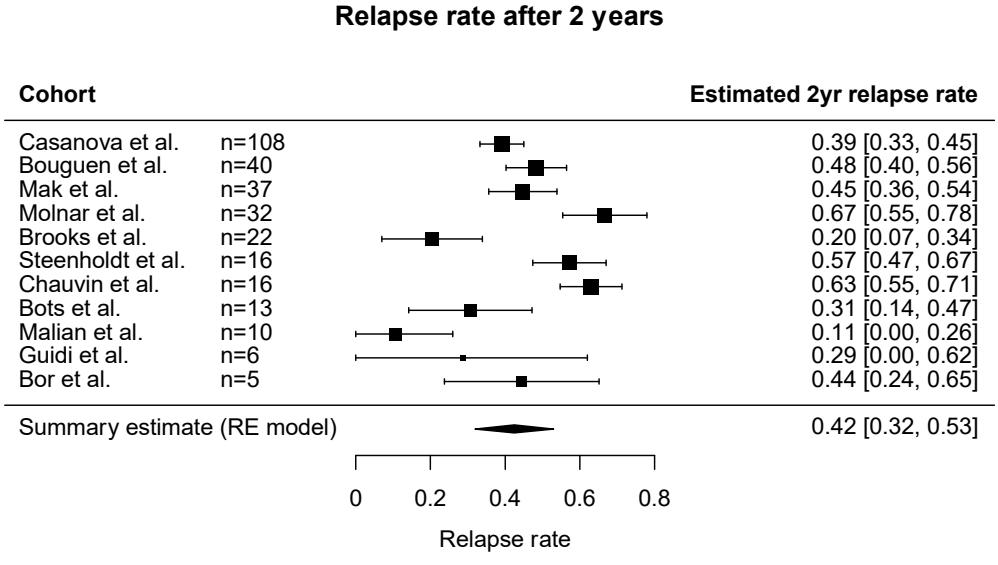


Figure 2. Stratified meta-analysis of relapse rates at 1 year (a) and 2 years (b) after cessation of anti-TNF therapy.

(a)



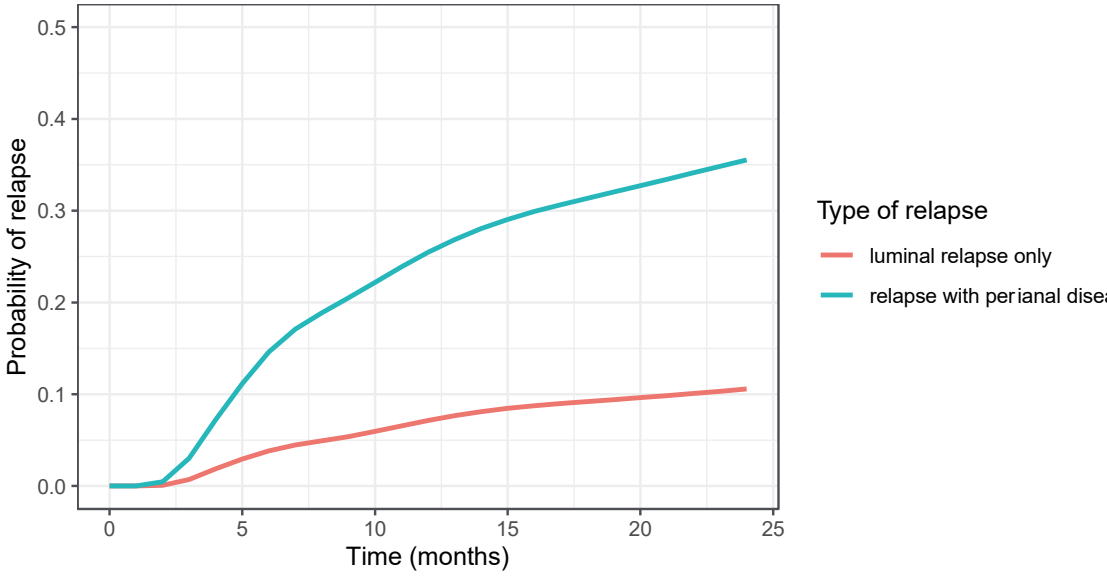
(b)



Relapse of perianal and luminal CD

Regarding type of relapse, 75/168 [45%] patients developed a relapse of pCD after discontinuation of anti-TNF therapy after a median follow-up of 11 months [IQR 2.8 – 24.3]. Among these patients, 58/75 [77%] experienced perianal fistulizing relapse, 16/75 [21%] had both perianal fistulizing relapse and anal abscess whereas 1/75 [1%] had an anal abscess only. In total, 25/168 [15%] patients experienced relapse of pCD in combination with relapse of luminal CD after discontinuation of anti-TNF therapy. In the pooled cohorts where relapse type was recorded, cumulative incidences for relapse of perianal disease were 25% [21% - 34%] and 36% [30% - 46%] at 1 and 2 years **[Figure 3]**.

Figure 3. Cumulative probability of relapse after discontinuation of anti-TNF therapy in perianal fistulizing CD patients split by relapse type (n = 179).



Regarding luminal relapses, 32/168 [19%] patients developed a relapse of only luminal CD after discontinuation of anti-TNF therapy with a median time to relapse of 13.1 months [IQR 5.1 – 41.4]. Estimated cumulative incidences for luminal relapse were 7% [4% - 12%] and 11% [6% - 14%] at 1 and 2 years after discontinuation of anti-TNF therapy**,** in the cohorts where relapse type was known **[Figure 3]**.

Sensitivity analyses were performed including all patients in whom the type of relapse was unknown. During follow-up, 60 patients [19%] experienced a relapse, however without data specifying type of relapse. We examined two scenarios. In the first scenario, we analysed all cohorts, assuming that all patients with unknown type of relapse experienced a relapse of perianal fistulizing CD. This resulted in estimated cumulative incidences of relapse with perianal disease of 22% and 31% at 1 and 2 years, respectively. The cumulative incidence of exclusively luminal relapses was 5% at one year and 7% at two years in this scenario.

Secondly, we investigated the scenario where all patients with unknown type of relapse did not experience perianal disease. These patients are assumed to have experienced only luminal relapses. In this case, the cumulative incidence of relapse with perianal disease was 15% at 1 year and 21% at 2 years. For luminal relapse without perianal disease, the cumulative incidence was estimated to be 15% at one year and 22% at two years.

Factors associated with relapse

The association between baseline characteristics and the rate of relapse was evaluated in a univariable analysis **[Figure 4, supplementary table 4]**. Our multivariable analysis included age at diagnosis, the duration of anti-TNF therapy in months, gender, smoking, disease behaviour, disease location and upper GI involvement (L4). By using multivariable analysis, smoking was significantly associated with relapse [HR 1.48 (1.04, 2.10)]. **[figure 4,**

supplementary table 4]. Maintenance therapy with immunomodulators and prior surgery were not associated with the risk of relapse.

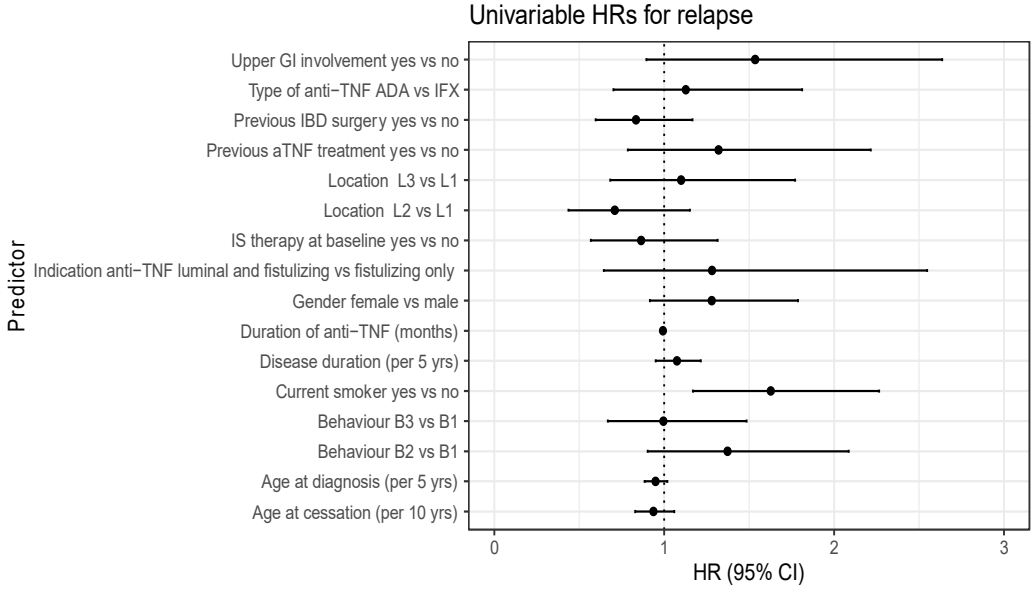
The association between fistula characteristics and relapse risk was assessed in a univariable analysis of the cohorts where at least one fistula characteristic had been recorded (n = 166) **[Figure 5a, supplementary table 5a]**. The number of fistulas [HR 1.11 (0.96, 1.28) per additional fistula], a history of perianal abscess [HR 1.50 (0.99, 2.28)] and a history of proctitis [HR 1.65 (1.09, 2.48)] were associated ($p<0.2$) with relapse. In a subgroup of patients with only perianal fistulizing disease and in luminal remission at the time of start of anti-TNF therapy (n = 144), a history of abscesses [HR 1.39 (0.89, 2.16)] and a history of proctitis [HR 1.62 (1.02, 2.59)] were associated with increased risk of relapse. **[Figure 5b, supplementary table 5b]**. A history of proctitis significantly increased the risk of relapse in both analyses.

Retreatment with anti-TNF therapy

Among the patients with either fistulizing or luminal relapse after discontinuation of anti-TNF therapy, 109 patients were retreated with an anti-TNF agent. Infliximab was used in 77/109 [71%] patients and adalimumab in 32/109 patients [29%]. Median duration of follow-up after retreatment with anti-TNF therapy was 3.5 years [IQR 0.73 – 7.22]. Overall, anti-TNF retreatment was effective in 82% of the patients [79/96] **[Supplementary table 6]**. In total, 90/109 [83%] patients were retreated with the same anti-TNF agent. Retreatment was effective in 67/79 [85%] and 12/17 [71%] for patients treated with the same anti-TNF agent and patients treated with another agent, respectively [$p = 0.174$].

Figure 4. Forest plot of the predictors and their hazard ratios (HR) for relapse resulting from univariable (A) and multivariable (B) stratified Cox-regression analysis of all included cohorts (n = 311).

(A)



(B)

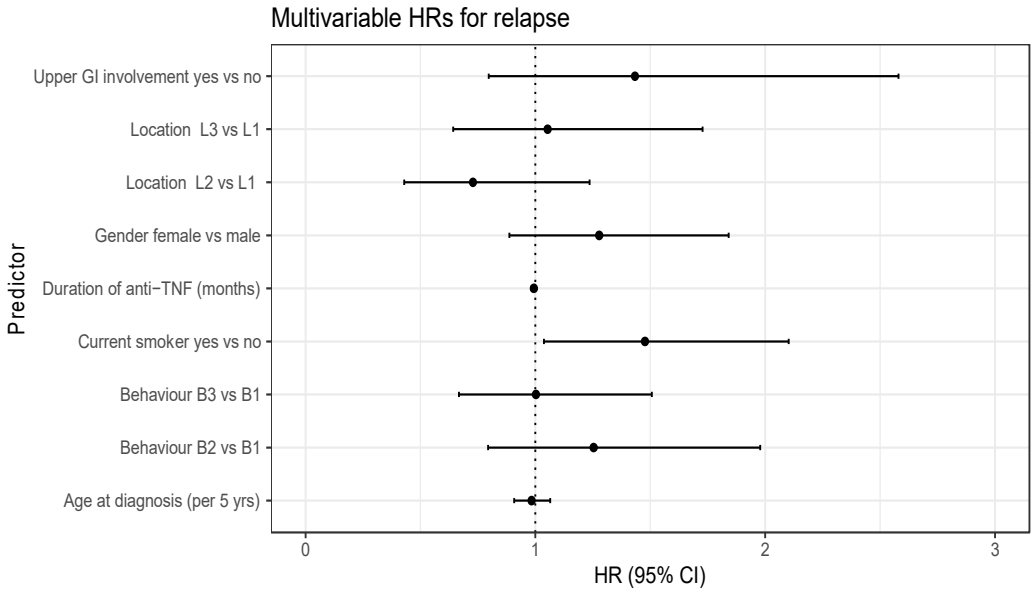


Figure 5a. Forest plot of fistula characteristics and their hazard ratios (HR) for relapse in all cohorts where fistula characteristics were recorded (n = 166).

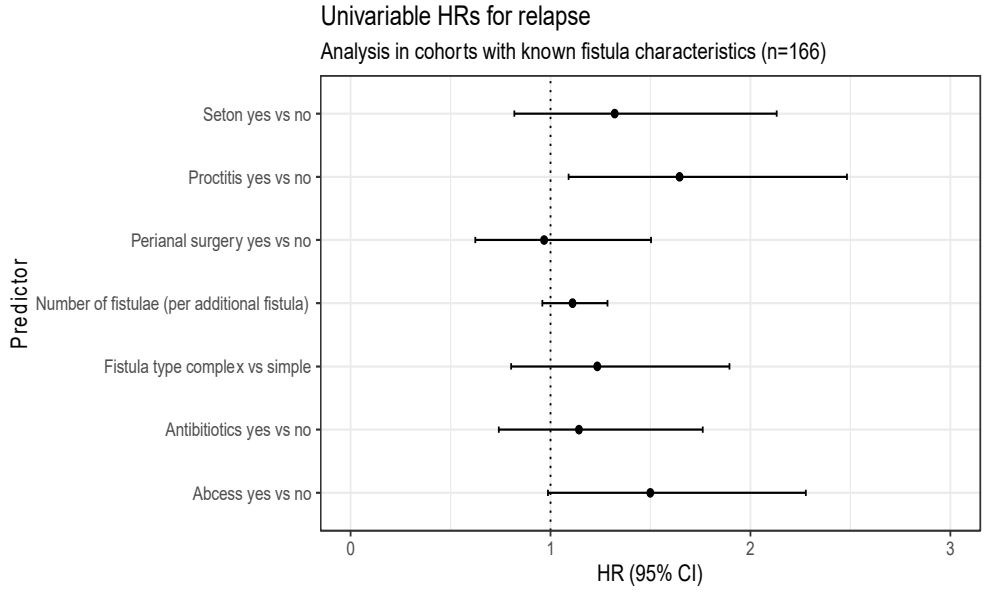
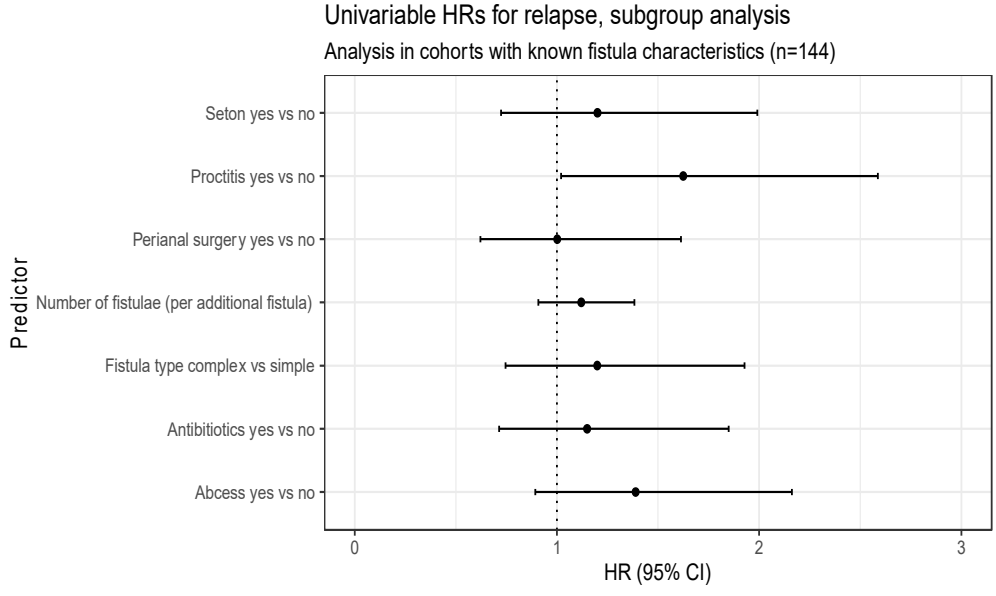


Figure 5b. Forest plot of fistula characteristics and their hazard ratios (HR) for relapse resulting in all cohorts where fistula characteristic were recorded, in the subgroup of patients with perianal fistulizing disease and in luminal remission at time of start anti-TNF therapy (n = 144).



Discussion

Since perianal fistulizing CD is associated with a high disease burden, withdrawal of anti-TNF therapy following disease remission remains a clinical dilemma. According to this IPD-MA, approximately half of the patients with perianal fistulizing disease experience a relapse of either luminal or pCD within two years following anti-TNF discontinuation. Retreatment with anti-TNF agents in patients who experienced a relapse following anti-TNF discontinuation was effective in the vast majority of patients. Risk factors for disease relapse comprised smoking and a history of proctitis. Since the presented data are mostly from patients in remission with perianal fistula without active luminal disease at start of anti-TNF therapy tract and after receiving a first line anti-TNF treatment, a strategy of anti-TNF discontinuation may be considered for this selected sub-group of patients. To further assess this strategy, more data on the comparison of discontinuation of anti-TNF therapy with continuation of therapy are required.

The majority of the included patients in this IPD-MA may have had a favourable prognostic phenotype of pCD at baseline, which is illustrated by for instance the relatively short duration of anti-TNF therapy, the first line of anti-TNF therapy and in a subgroup of patients a median number of fistula tracts of one. A higher rate of sustained remission after anti-TNF discontinuation in this subgroup of patients, as compared to the total population of pCD patients seems likely, since the fistula complexity (both the number of fistula tracts as well as the anatomic location, classified into simple versus complex fistula tracts) determines the effectiveness of anti-TNF therapy on pCD [29]. Therefore, it must be acknowledged that the findings in this study refer to a selected patient population and cannot be generalized to all pCD patients.

Previous studies showed that patients with a higher number of fistula tracts were less likely to achieve clinical remission following anti-TNF treatment and complex fistulas were associated with a decreased change of long-term healing as compared to simple fistulas [22, 23]. Since this IPD-MA included also patients with only one fistula tract at baseline, these results may not be extrapolated to the population with perianal fistulizing CD as a whole since in patients with multiple fistula tracts, anti-TNF therapy is less likely to be discontinued. Further data on patients with more complex perianal fistulizing CD with details on the fistula tracts are required to enhance risk stratification as a prerequisite for clinical decision making on anti-TNF therapy discontinuation.

Healing of the skin but prior complete closure of the internal fistula tract is a relevant clinical issue in treating perianal fistulas. Further discrimination of patients with pCD for anti-TNF discontinuation may, therefore, be guided by the finding of closure of the fistula tract at imaging, with either anal endosonography [AE] or Magnetic Resonance Imaging [MRI]. A previous study showed that radiological healing is slower than clinical healing with a time lag of one year [22]. After one year of follow-up, low disappearance of fistula tracts on adequate AE or MRI despite clinical remission on therapeutic response to anti-TNF agents was reported. In addition, patients with persistent fistula tracts showed higher fistula recurrence rates than patients with disappearance at imaging. In addition, a small prospective study showed that once internal fistula healing was observed on MRI, the fistulas remain healed after anti-TNF discontinuation [24]. Since AE and MRI are both sensitive methods to assess this deep tissue healing, it is suggested to use these imaging techniques rather than clinical remission and/or physical examination alone prior to anti-TNF discontinuation [22, 25]. Unfortunately, this IPD-MA is hampered by the lack of MRI data. It may well be that patients in clinical remission without radiological remission are at an increased risk of relapse. The

predictive value of fistula closure on MRI prior to anti-TNF discontinuation requires further study.

In this IPD-MA, smoking is associated with a higher risk of relapses of perianal or luminal disease activity in patients with CD after anti-TNF discontinuation. Our results once again underline the importance to emphasize the negative consequences of smoking. This is in line with observations on a more complicated disease course of CD in smokers, including a higher rate of relapse, need for biologic therapy, and hospitalization [26]. In addition, active proctitis in the presence of perianal disease might indicate a more severe disease phenotype that decreases the chance of sustained remission after anti-TNF therapy discontinuation, according to this IPD-MA. Finally, most patients continued concomitant immunotherapy when anti-TNF therapy was ceased in this IPD-MA. Relapse rates may be expected to be higher after discontinuation of anti-TNF monotherapy. However, we could not demonstrate a beneficial effect of concomitant therapy with immunomodulator for the total pCD cohort in this IPD-MA. This finding is in line with the ECCO guideline, which states insufficient evidence for fistula healing induced by immunomodulators or adding immunomodulators to anti-TNF therapy on fistula healing [27].

Retreatment with anti-TNF agents in patients who experienced a relapse following anti-TNF discontinuation was effective in over 80% of the patients. This response rate is similar to the response rates of luminal disease only, which was around 80% in earlier reports [28]. These data seem reassuring for a decision on anti-TNF discontinuation. In addition, new therapeutic strategies for pCD have been introduced over the past years, including a possible beneficial effect of ustekinumab on pCD and local stem cell therapy which have shown to be an effective and safe treatment for perianal fistulas [29, 30]. These therapeutic options could be an

alternative for patients in whom no response to retreatment with anti-TNF therapy is achieved.

In the light of a recently published new classification system for perianal fistulizing Crohn's disease which suggests a treatment strategy per class, anti-TNF discontinuation might be considered as an active treatment strategy for patients in class 1 [minimal symptoms and anorectal disease burden, requiring minimal intervention over time] and possibly class 2a over time [symptomatic fistulae suitable for combined medical and surgical closure or repair with fistula closure as main goal] . However, further discrimination of patients in class 2 is required, guided by radiological healing, into those likely to suffer from relapse and those less at risk which would allow rational treatment choices.

To our knowledge, this is the first IPD-MA evaluating the risk of relapse after anti-TNF discontinuation in patients with pCD. In addition, this IPD-MA comprises a large patient cohort from different countries. However, some limitations need to be considered when interpreting our data. Due to the retrospective design of most of the included cohorts and the variety of original study aims, some databases of the original study cohorts did not include fistula characteristics. It is most likely that these patients had complex fistulas at time of anti-TNF introduction since anti-TNF agents are generally indicated for complex perianal fistulas. As mentioned above, data on radiological healing at time of anti-TNF discontinuation were lacking in most studies. This has limited the evaluation of predictors of a relapse. In addition, given the high rate of immunomodulator continuation following anti-TNF discontinuation, data regarding allergic reactions or immunogenicity would be of interest for further decision making on treatment discontinuation. Secondly, the type of relapse was not recorded in most available cohorts. In these cases, it was not possible to distinguish between perianal fistulizing and luminal relapse. To provide insight into the impact of these missing data, we

chose to perform a worst case – best case scenario analysis. Finally, the studies included in this IPD-MA enclosed patients over a period of 11 years, probably leading to differences in treatment strategies over time. This may have influenced the varying relapse rates between the included cohorts.

In conclusion, half of patients with perianal fistulizing CD without active luminal disease at start of anti-TNF therapy and who achieve remission after the first line of anti-TNF therapy, remain in remission 2 years after discontinuation of anti-TNF therapy. In addition, the majority of these patients respond to retreatment with anti-TNF therapy after relapse. Therefore, discontinuation of anti-TNF therapy may be considered in this subgroup of patients with perianal fistulizing CD. Individualized estimation of relapse risk for all other patients with perianal fistulizing CD requires further investigation, with a specific focus on healing of fistula tracts on imaging.

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10

Chapter 10

Mucosal immunological landscape allowing anti-TNFα discontinuation in Crohn's disease

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In progress

Abstract

Background Anti-TNFα therapy may be discontinued without a relapse in a subpopulation of patients with long-standing quiescent Crohn's disease (CD). Currently, identification of these patients with available clinical, biochemical and histological predictors is inaccurate. New biomarkers are required to guide this clinical decision.

Methods The study cohort comprised patients with CD in complete endoscopic and histologic remission who discontinued anti-TNFα therapy. Mucosal biopsies were contrasted between patients who experienced disease relapse and who remained in remission during follow-up ≥ 2 years. The biopsies (FFPE material) were used for deep immunoprofiling by measuring the expression of 772 immunologically-relevant genes using sequence-specific mRNA probes to directly detect gene expression and return counts of each target molecule.

Results Biopsy samples from 22 patients were analyzed (females (55%); median age at anti-TNF discontinuation 40 years (IQR 30 – 56); median disease duration until anti-TNF discontinuation 7.6 years (IQR 4.1 – 14.7)). Among selected patients, 11 successfully discontinued anti-TNFα therapy, while 11 experienced a relapse. Median total follow-up following anti-TNFα therapy discontinuation was 3.3 years (IQR 2.1 – 4.7). RNA isolated from the selected biopsies was of high quality. In the top 10 most upregulated gene expression, THOP1, CGAS, RIPK2, and GAB2 were statistically different between patient groups. Regarding patients who remain in remission after anti-TNFα therapy discontinuation, highest gene expression was observed for THOP1 [average (SD) 1.48 (0.87). In the top 10 most downregulated gene expression, RBPJ, ACKR3, IFITM1 and CCL8 were statistically different between patient groups. IFITM1 reported the highest gene expression [average (SD) 0.96 (0.85)] in the total cohort, as well as in patients who experienced a relapse after anti-TNFα therapy discontinuation.

Conclusion High expression of genes associated with NLRP3 inflammasome signaling predicts successful discontinuation, whereas high expression of genes associated with interferon-γ/λ signaling predicts a relapse after discontinuation of anti-TNFα therapy. This gene index may contribute to better prediction of the risk of relapse after discontinuation of anti-TNFα therapy in individual patients with CD in deep remission.

Introduction

The introduction of anti-tumour necrosis factor (anti-TNFα) therapy revolutionized the treatment for moderate to severe Crohn's disease (CD). Despite the introduction of numerous novel biological agents and pharmacological inhibitors, anti-TNFα remains the primary approach for clinical management of these patients. Current clinical guidelines discourage episodic use of anti-TNFα due to the rapid provocation of severe disease flares upon therapy cessation and the subsequent induction and build-up of anti-anti-TNFα antibody titers. This poses a serious impediment to the reintroduction of anti-TNFα medication when the clinical course necessitates it, ultimately leading to resistance to future anti-TNFα therapy. Consequently, the majority of moderate to severe CD patients in Western countries are maintained on anti-TNFα therapy.

Prolonged anti-TNFα therapy comes with a host of individual and societal disadvantages. This treatment can be associated with significant side effects, such as a heightened susceptibility to serious infections of bacterial, fungal, viral, or atypical nature, an increased risk of malignancies, congestive heart failure, drug-induced lupus, and demyelinating disorders. Additionally, skin reactions to anti-TNFα drugs are frequent, and the use of such drugs complicates pregnancies in women with CD who desire to conceive. Moreover, the high costs of anti-TNFα drugs impose a crippling financial burden on many healthcare systems, and the economic costs associated with drug administration, particularly for intravenous anti-TNFα drugs, are also substantial. Therefore, continuing the practice of constant administration of anti-TNFα medication to all patients with moderate-to-severe Crohn's disease is considered by many to be an unsustainable strategy, especially considering the global rise in disease incidence.

Although it is well recognized that anti-TNFα medication can be safely discontinued in many patients with long-standing quiescent disease, it is currently not possible to adequately select such patients. [1] Improved understanding of the mucosal intestinal immune system is widely considered to be the way forward. Over the past decade, research has led to the formulation of the deep remission concept, wherein clinical, endoscopic, biochemical, and microscopic pathological examinations all demonstrate that the mucosa is comfortably within the normal healthy range. The impact of deep remission in CD patients has been evaluated in discontinuation studies and remains highly controversial. Previous studies have shown an association between deep remission and a reduced risk of relapse after infliximab discontinuation, while another study reported no difference in relapse over time between patients in deep remission and patients in either clinical or endoscopic remission.[2-4] Importantly, previous studies have demonstrated that up to 30% of patients with CD will still relapse while considered to be in deep remission, as indicated by low faecal calprotectin and mucosal healing.[4, 5] Therefore, an improved understanding of the mechanistic factors that drive a patient's ability to successfully discontinue anti-TNFα medication is urgently needed in contemporary medicine.

Currently, identification of patients in whom anti-TNF therapy can be safely ceased without a relapse with available clinical, biochemical and histological predictors is inaccurate. New biomarkers are required to guide this clinical decision. Therefore, we aimed to perform deep immunoprofiling of mucosal biopsies from CD patients under anti-TNFα maintenance therapy who were in deep remission to uncover biological pathways associated with the success of halting anti-TNFα therapy and pathways whose activation predisposes to clinical failure in discontinuing anti-TNFα medication.

Methods

Design

In order to compare the mucosal immune system of patients who successfully halted anti-TNFα therapy to those who failed this intervention, we utilized our access to the patients of the retrospective CEASE cohort.[6] Within the retrospective CEASE cohort, a subgroup of patients who underwent a colonoscopy with biopsies at the time of anti-TNF cessation was identified. Biopsies of patients who experienced a relapse following anti-TNF cessation were analyzed [all biopsies were taken from the same location where the inflammation was observed during a next colonoscopy] and compared with biopsies of patients who remained in remission. Relapse was defined as a relapse of luminal disease activity or the occurrence of (new) CD complications (i.e. extra-intestinal manifestations (EIM), (perianal) fistula and/or abscess) that necessitated introduction of additional treatment including biologicals, corticosteroids, immune-suppressants or surgery. All biopsies were archival biomaterial and were collected from patients from 5 centers, including two academic and three teaching hospitals.

Collection of the ileal or colonic biopsies

All endoscopic material taken at time of anti-TNF cessation of the included patients were collected via the PALGA portal which is a nationwide network and registry of histo- and cytopathology in the Netherlands. In case the intestinal biopsy material were available, the formalin-fixed paraffin embedded (FFPE) tissue blocks from the selected intestinal biopsy material were anonymous transferred to the Erasmus MC and stored at the gastroenterology department until the analyses were completed.

Study procedures

The collected intestinal biopsy material was analyzed by nanostring immunoprofiling (an RNA-based technology that provides cost-effective bulk immunophenotyping of samples with unprecedented detail). Results were validated using digital spatial profiling. This is a technique in which antibodies are labelled with DNA barcodes which allows spatial visualization of immune cell distribution with a theoretically unlimited number of phenotyping antibodies and thus markedly superior to competing technologies like Tissue-cyt of or Vectra analysis. Immune profiling with an immune host RNA panel was performed on the biopsy tissue to determine whether specific differences in immunoprofiling on the level of RNA could predict a CD relapse. Results were correlated to clinical outcome (no relapse vs. relapse).

Deep immunoprofiling

Deep immunoprofiling using RNA of mucosal biopsies requires the quantification of expression for essentially all immunological genes. While various techniques exist for such analysis, most suffer from technological drawbacks, such as the lack of sequencing depth associated with scRNAseq or artifacts induced by enzymatic multiplication of the transcripts. Consequently, we opted to use the Nanostring 360° platform, which directly quantifies the expression levels of 785 immuno-relevant genes through hybridization to specific probes.

Nanostring immunoprofiling

Bioanalyzer

The concentration and integrity of the RNA samples were measured with the Bioanalyzer RNA 6000 Nano assay (#5067-1511 Agilent) according to the manufacturer's protocol. For each sample we determined the percentage of the total RNA that is between 300 and 4000 nucleotides. This percentage was multiplied by the total concentration to give us the corrected

concentration. Only samples with a corrected concentration of higher than 42.8 ng/µL were used for gene expression profiling.

nCounter immune Profiling

Gene expression quantification was performed using the nCounter Host Response Profiling containing 785 genes. For each sample we used 300 ng RNA input, and samples were processed using the nCounter FLEX System (GLMX_ST0002 NanoString). The RNA was hybridized with the Host Response panel capture and reported probes at 65°C for 17 hours in a SimpliAmp Thermal Cycler (Applied Biosciences), before loading the samples into the nCounter system. The gene counts were acquired using the nCounter Digital Analyzer 5s (NanoString) by scanning 490 Fields of View, and the data was extracted from the RCC files using nSolver analysis software v4.0 (NanoString).

Statistics

Categorical variables were provided with frequencies and percentages. Continuous variables were provided with median and interquartile range [IQR]. The time to event was defined as the time between anti-TNF cessation and relapse. Gene expression is provided with relative expression of immunorelevant mRNAs as compared to the results obtained to IO 360 panel standard for each lot.

Gene algorithm for deciding of continuation or discontinuation of anti-TNF therapy

Gene expression is linked to household genes (genes that are always expressed) since gene expression is corrected to the expression of the IO 360 panel standard standard. However, in practical terms, there is technical systematic variation as well, introducing complexity when using gene expression data for clinical purposes. To circumvent this issue, we searched for a gene signature comprising genes that are upregulated and genes that are downregulated in

patients who may discontinue anti-TNF therapy. The reason for this choice was to minimize artefacts and reduce dependence on individual household genes, making the data more robust and less reliant on chance occurrences. Such a gene signature should be as straightforward as possible while providing sufficient discriminative power to effectively distinguish between low and high-risk patients for relapse. The following algorithm was developed to discriminate between patients at low or high risk of relapse; $\sum$ = (Exp. downregulated [gene 1] + [gene 2] + [gene 3] + [gene 4] <5) Λ (exp. upregulated [gene 1] + [gene 2] + [gene 3] + [gene 4] >4).

Ethical approval

The ethical committee of the Erasmus MC approved this study (reference number MEC-2020-0576). Since all biopsies were obtained during routine out clinic visits, no informed consent was needed and all the biopsies were considered as rest-material. Biopsies of patients who specifically signed not to use their rest-material, were excluded.

Results

Baseline characteristics

A total of 192 patients who underwent a colonoscopy at time of anti-TNF cessation were identified. Of these patients, 33/192 (17%) underwent a colonoscopy with intestinal biopsies, of whom 22/192 (11%) were included **[Figure 1]**.

The majority of the included patients were female (55%) with an median age at time of anti-TNF cessation of 40 (IQR 30 – 56). Median disease duration until anti-TNF cessation was 7.6 (IQR 4.1 – 14.7). Median total follow-up following anti-TNF cessation was 3.3 years (IQR 2.1 – 4.7). In total, 13 (59%) and 9 (41%) patients were exposed to infliximab and adalimumab prior anti-TNF cessation. Following anti-TNF cessation, 10 (45%) patients received concomitant immunomodulatory therapy (9 (90%) thiopurines, 1 (10%) methotrexate) after anti-TNF cessation. The two groups were similar in their baseline characteristics **[Table 1]**.

All included patients were reported to be in deep microscopic remission at the time of cessation, and this was confirmed by a specialized gastrointestinal pathologist (MD) upon reappraisal of the histological material. Among the selected patients, 11 successfully discontinued anti-TNFα treatment, while 11 experienced a relapse at some point following cessation. RNA isolated from the selected biopsies was of high quality, allowing for the successful dissection of the immunological parameters driving the response to the discontinuation of anti-TNFα therapy.

Figure 1. Flow chart included patients

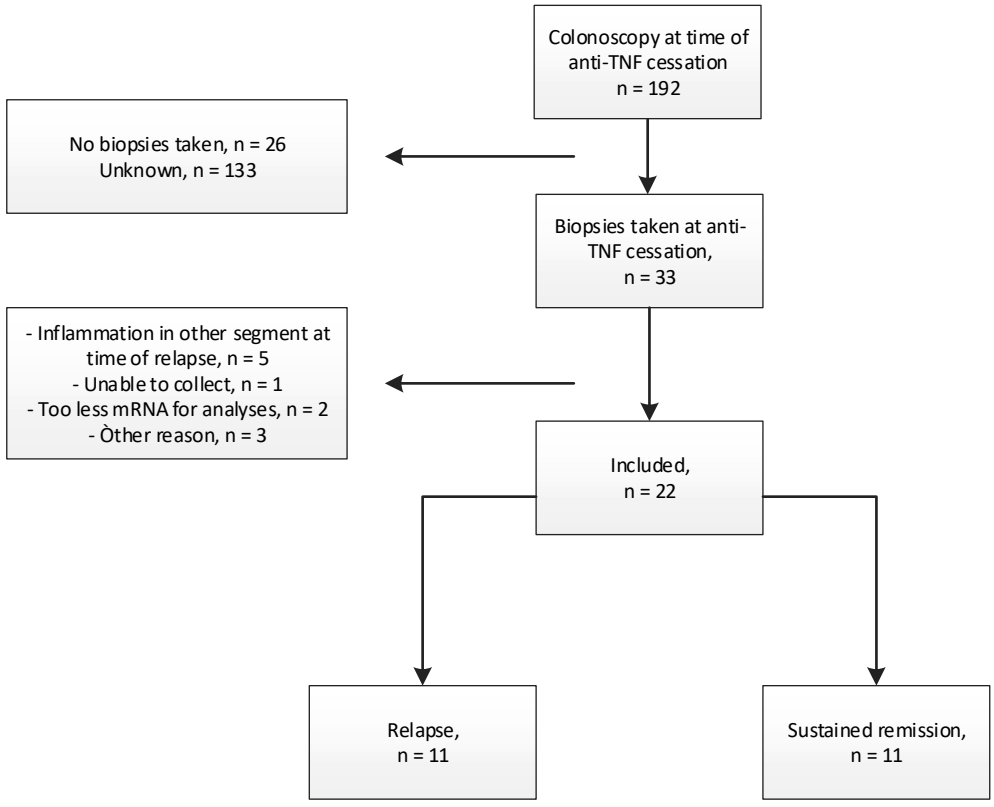

Table 1. Baseline characteristics

Factor		**Total cohort, n = 22**	**Relapse, n = 11**	**Remission, n = 11**	***P-Value***
Follow-up time, years	Median, [IQR]	3.3 [2.1 – 4.7]	3.5 [3.3 – 5.2]	2.5 [1.7 – 4.0]	0.082
Age, years	Median, [IQR]	40 [30 – 56]	39 [28 – 59]	40 [31 – 55]	0.900
Female	n, [%]	12 [55]	5 [46]	7 [64]	0.39
Active smoker	n, [%]	6 [27]	4 [36]	2 [18]	0.60
Disease duration, years	Median, [IQR]	7.6 [4.1- 14.7]	8.2 [6.7 – 11.7]	6.9 [3.2 – 17.7]	0.622
Montreal classification, n = 20					
Age at diagnosis					0.491
A1 ≤ 16 years	n, [%]	4 [18]	2 [18]	2 [18]	
A2 17-40 years	n, [%]	13 [59]	7 [64]	6 [55]	
A3 > 40 years	n, [%]	3 [14]	2 [18]	1 [9]	
Location					0.820
L1 Terminal ileum	n, [%]	6 [30]	3 [27]	3 [27]	
L2 Colon	n, [%]	2 [10]	1 [9]	1 [9]	
L3 Ileocolonic	n, [%]	12 [60]	7 [64]	5 [54]	
L4 Upper GI involvement	n, [%]	-	-	-	-
Behaviour					0.193
B1 Non-stricturing, non-penetrating	n, [%]	12 [60]	8 [73]	4 [36]	
B2 Stricturing	n, [%]	6 [30]	3 [27]	3 [27]	
B3 Penetrating	n, [%]	2 [10]	-	2 [18]	
Perianal disease	n, [%]	8 [35]	3 [25]	5 [46]	0.160
Prior intestinal resection	n, [%]	7 [32]	2 [18]	2 [18]	0.635

Table 1. continued

Treatment history					
Biological (ADA/IFX)	n, [%]	2 [10]	2 [18]	-	0.476
Duration anti-TNF therapy, years	Median, [IQR]	5.2 [3.0 – 7.3]	5.7 [4.2 – 6.7]	3.9 [1.9 – 8.6]	0.670
Concomitant medication continued after anti-TNF cessation					
Thiopurine	n, [%]	9 [41]	4 [36]	5 [46]	1.0
Methotrexate	n, [%]	1 [5]	-	1 [9]	1.0
Haemoglobin, mmol/L	Median, [IQR]	8.7 [8.3 – 9.4]	8.9 [8.5 – 9.7]	8.6 [8.1 – 8.8]	0.157
Leukocytes, * 10^9/L	Median, [IQR]	7.0 [5.7 – 9.4]	7.3 [6.8 – 9.7]	6.1 [5.5 – 9.2]	0.254
Thrombocytes, * 10^3/mm^3	Median, [IQR]	234 [179 – 333]	223 [176 – 377]	244 [191 – 290]	0.870
Albumin, g/L	Median, [IQR]	44 [40.0 – 45.0]	42 [40.0 – 44.5]	44 [44 – 46]	0.306
CRP, mg/L	Median, [IQR]	5 [0.9 – 6.2]	5.0 [3.0 – 6.5]	2.5 [0.8 – 5.5]	0.234
Calprotectin, mg/kg	Median, [IQR]	38 [21 – 93]	63.5 [25 – 196]	30 [15 – 64]	0.078

Relapse

In total, 7/11 [64%] patients who experienced a relapse, were retreated with a biological [ADA 2/7 [18%]; IFX 4/7 [36%]; other 1/7 [9%]. Of these patients, 4/7 [36%] patients restarted anti-TNF in combination with thiopurines. 3/11 [27%] patients started corticosteroids. Retreatment was effective in 7/11 [64%] of the patients.

An NLPR3 gene expression signature associated with successful cessation of anti-TNFα treatment

We used the Nanostring 360° platform, which directly quantifies the expression levels of 785 immuno-relevant genes through hybridization to specific probes. The results obtained for the entire cohort, as well as the stratification based on the response to anti-TNFα cessation, are listed in **Supplementary Table 1.** In the top 10 most upregulated gene expression, 4 genes were statistically different between patients who experienced a relapse and patients who remained in remission including THOP1, CGAS, RIPK2, and GAB2 [**Table 2a**]. In the total cohort, the highest gene expression were observed in both CGAS and GAB2 [average (SD) 1.07 (0.72) and 1.07 (0.8)] whereas the lowest gene expression was observed in THOP1 [average (SD) 1.02 (0.85)] [**Table 3a**]. Regarding patients who remain in remission following anti-TNF cessation, THOP1 reported the highest gene expression [average (SD) 1.48 (0.87) [**Table 3a**].

An interferon-γ/λ expression signature associated with unsuccessful cessation of anti-TNFα treatment

in the top 10 most downregulated gene expression, four genes were statistically different between patients who experienced a relapse and patients who remained in remission including RBPJ, ACKR3, IFITM1 and CCL8 [**Table 2b**]. IFITM1 reported the highest gene

expression [average (SD) 0.96 (0.85)] in the total cohort, as well as in patients who experienced a relapse following anti-TNF cessation [**Table 3b**].

Use of algorithm

Empirically, it was found that selecting four genes that were statistically different between patients who experienced a relapse and patients who remained in remission, and were the most upregulated and downregulated, yielded most robust results. Based on our algorithm, all of patients who experienced a relapse are identified as high risk patients and would not have discontinued anti-TNF therapy [**Table 4a**]. In addition, our algorithm showed justified discontinuation in 8/11 [82%] of the patients who remained in remission following anti-TNF cessation. In 2/11 [18%] patients, anti-TNF therapy would have continued unjustified as these patients remained in remission during follow-up following anti-TNF cessation [**Table 4b**].

Table 2a. top 10 most upregulated gene expression

	Absolute ratio	P-value parametric
THOP1-mRNA	**0.43**	**0.01**
SOCS1-mRNA	0.45	0.06
CGAS-mRNA	**0.46**	**0.01**
RIPK2-mRNA	**048**	**0.01**
CEACAM3-mRNA	0.48	0.08
PSAP-mRNA	0.49	0.10
GAB2-mRNA	**0.49**	**0.03**
HLA-DPA1-mRNA	0.51	0.10
PIK3R5-mRNA	0.51	0.07
SELE-mRNA	0.52	0.06

Table 2b. top 10 most downregulated gene expression

	Absolute ratio	P-value parametric
MLKL-mRNA	2.26	0.02
IL3-mRNA	2.28	0.06
ITGAL-mRNA	2.33	0.09
GZMA-mRNA	2.35	0.06
SOCS3-mRNA	2.43	0.05
STAT2-mRNA	2.47	0.05
RBPJ-mRNA	**2.50**	**0.02**
ACKR3-mRNA	**2.64**	**0.03**
IFITM1-mRNA	**2.73**	**0.01**
CCL8-mRNA	**2.77**	**0.03**

Table 3a. Overview included upregulated genes

Innate mRNA		Total cohort, n = 22	Relapse, n = 11	Remission, n = 11
THOP1-mRNA	Average [SD]	1.02 [0.85]	0.57 [0.56]	1.48 [0.87]
CGAS-MRNA	Average [SD]	1.07 [0.72]	0.69 [0.40]	1.45 [0.78]
RIPK2-mRNA	Average [SD]	1.04 [0.68]	0.65 [0.47]	1.42 [0.65]
GAB2-mRNA	Average [SD]	1.07 [0.80]	0.73 [0.69]	1.40 [0.80]

Table 3b. Overview included downregulated genes

T-cell mRNA		Total cohort, n = 22	Relapse, n = 11	Remission, n = 11
RBPJ-mRNA	Average [SD]	0.95 [0.87]	1.35 [0.96]	0.55 [0.58]
ACKR3-Mrna	Average [SD]	0.90 [0.93]	1.26 [0.98]	0.53 [0.74]
IFITM1-mRNA	Average [SD]	0.96 [0.85]	1.40 [0.89]	0.52 [0.56]
CCL8-mRNA	Average [SD]	0.90 [0.97]	1.29 [1.24]	0.51 [0.30]

Table 4a. Results algorithm regarding patients who experienced a *relapse* following anti-TNF cessation

Patient	RBPJ-mRNA	ACKR3-mRNA	IFITM1-mRNA	CCL8-mRNA	Total sum	THOP1-mRNA	CGAS-mRNA	RIPK2-mRNA	GAB2-mRNA	Total sum	Result algorithm
SEB-01	2.58	2.72	1.89	0.43	7.62	1.07	1.23	0.18	0.02	2.49	Con. anti-TNF
SEB-04	0.41	0.53	0.41	0.60	1.95	0.32	0.59	1.46	1.03	3.41	Con. anti-TNF
SEB-07	2.05	2.02	2.71	2.17	8.95	0.31	0.37	0.12	0.41	1.22	Con. anti-TNF
SEB-10	0.49	0.37	0.28	0.86	2.00	0.21	0.75	0.15	1.77	2.88	Con. anti-TNF
SEB-12	2.53	2.51	2.53	0.30	7.86	1.51	0.42	0.79	0.27	2.98	Con. anti-TNF
SEB-02	2.40	0.59	0.36	3.46	6.80	0.31	1.38	0.70	0.29	2.69	Con. anti-TNF
SEB-03	1.69	1.76	0.72	2.88	7.05	0.06	0.27	0.09	0.38	0.80	Con. anti-TNF
SEB-05	0.40	0.44	1.07	0.05	1.96	1.51	0.43	1.01	0.15	3.10	Con. anti-TNF
SEB-06	0.32	0.34	2.23	0.39	3.29	0.36	0.32	0.88	1.94	3.50	Con. anti-TNF
SEB-08	1.82	2.38	1.52	2.93	8.66	0.11	0.54	0.61	0.36	1.62	Con. anti-TNF
SEB-11A	0.34	0.36	1.89	0.41	3.00	0.29	1.00	0.95	1.22	3.45	Con. anti-TNF

Con.; continuation

Table 4b. Results algorithm regarding patients who remained in *remission* following anti-TNF cessation

Patient	RBPJ-mRNA	ACKR3-mRNA	IFITM1-mRNA	CCL8-mRNA	Total sum	THOP1-mRNA	CGAS-mRNA	RIPK2-mRNA	GAB2-mRNA	Total sum	Result algorithm
SEB-13	0.42	0.52	0.95	1.01	2.89	2.23	1.89	1.19	1.47	6.78	Dis. anti-TNF
SEB-16	0.41	2.71	0.03	0.87	4.02	1.63	0.41	1.86	0.15	4.04	Dis. anti-TNF
SEB-17	0.43	0.05	2.05	0.12	2.65	1.97	2.22	1.29	1.76	7.25	Dis. anti-TNF
SEB-19	0.17	0.04	0.10	0.22	0.53	2.04	1.98	0.93	1.97	6.92	Dis. anti-TNF
SEB-20	0.47	0.58	0.43	0.94	2.42	0.06	0.55	2.00	0.13	2.74	Con. anti-TNF
SEB-24	0.36	0.42	0.51	0.51	1.80	0.54	0.57	2.16	1.64	4.90	Dis. anti-TNF
SEB-14	0.27	0.55	0.28	0.53	1.63	0.27	1.27	1.98	1.82	5.34	Dis. anti-TNF
SEB-15	0.71	0.05	0.54	0.14	1.44	2.05	1.98	1.72	1.87	7.61	Dis. anti-TNF
SEB-18	0.28	0.35	0.40	0.47	1.49	2.30	2.08	0.91	1.47	6.76	Dis. anti-TNF
SEB-22	2.30	0.33	0.17	0.51	3.31	0.78	1.93	0.15	2.35	5.22	Dis. anti-TNF
SEB-23A	0.37	0.33	0.32	0.40	1.42	1.78	0.47	0.90	0.39	3.54	Con. anti-TNF

Dis; discontinuation; Con: continuation

Discussion

Following the launch of anti-TNF therapy, twenty years ago, biologicals have become a pivotal treatment for moderate to severe CD. The impact of deep remission in CD patients remains controversial. Previous studies reported no difference in relapse over time between patients in deep remission and patients in both clinical or endoscopic remission [2-4]. Importantly, previous studies have shown that up to 30% patients with CD will still relapse while considered to be in deep remission with low faecal calprotectin and mucosal healing [4, 5]. We identified a signature of genes related to the activation of NLRP3 inflammasome, with high expression being associated with patients who became anti-TNFα independent in terms of disease control. The identification of this NLRP3 signature provides important evidence for the notion that the NLRP3 inflammasome plays a major role in protecting against chronic inflammation in the human intestine. Additionally, we identified high expression of a group of genes related to interferon-γ/λ signaling as predisposing to clinical failure in discontinuing anti-TNFα medication. Thus, this pathway emerges as a critical pathway aggravating CD, potentially amenable to pharmacological intervention (e.g., using JAK inhibitors). Overall, our results not only allow for the selection of patients under anti-TNFα therapy in whom discontinuation is safe but also prompt a critical reappraisal of the immunological pathways involved in maintaining remission in patients with Crohn's disease.

The underlying pathophysiology of CD relapse is poorly understood and highlights the difficulty of predicting the risk of relapse in CD patients who are in remission. Therefore, more accurate biomarkers, including histologic markers are essential to identify patients who are less likely to relapse. In this study, we aimed to determine whether prediction of a relapse based on the mucosal immunological landscape may contribute to the individual patient decision whether stop or not to stop anti-TNF therapy.

To better understand relapse and how to interact with medical treatment options depends on the manifestation of disease activity in general. The determination of whether innate immunity or adaptive immunity plays a pivotal role in CD pathogenesis holds significant importance. Two prevailing hypotheses exist: first, those who think that the disease is caused by genetic defects that trigger exaggerated innate responses to the gut flora, resulting in excessive inflammation as a consequence; second, those who suggests that CD may manifest as a form of immunodeficiency caused by impaired innate immunity.[7] This impaired innate immunity would enable the accumulation of potential immune inducers including commensal bacteria. Consequently, this leaves the secondary lines of defense of the body (i.e. adaptive immunity) the task of bacterial resolution. However, the adaptive immunity is by nature much less precisely controlled in comparison to innate responses. This less-regulated reaction subsequently gives rise to the characteristic intestinal inflammation observed in CD.

Central to this hypothesis are observations revealing reduced neutrophil accumulation and interleukin 8 production in CD patients, leading to compromised pathogen clearance from tissues. It has been suggested that in the absence of sufficient neutrophil numbers for effective bacterial clearance, the adaptive immune system, including macrophages, phagocytizes these bacteria. This process results in the formation of granulomas and focal areas of chronic inflammation, distinctive features of CD. This scenario may imply a primary immunodeficiency within macrophages, leading to insufficient secretion of pro-inflammatory cytokines upon bacterial challenge. The precise molecular mechanisms involved are challenging. Some environmental and genetic factors are acknowledged to influence susceptibility to CD. Moreover, the clinical significance of the innate immunodeficiency is determined by the ensuing detrimental adaptive immune response.

In this current study, a group of genes including RBPJ-, ACKR3-, IFITM1- and CCL8-mRNA were identified which are associated with T-cell expression and may predict relapse. RBPJ-dependent Notch signaling initiates the T-cell program in a subset of thymus-seeding progenitors whereas ACKR3 (atypical chemokine receptor) is related to the internalization and degradation of chemokines as well as to the inflammation control. [8, 9] Previous literature reported that IFITM1 can be found in CD45-positive inflammatory cells, CD68-positive macrophages/activated microglia, and astrocytes.[10, 11] lastly, receptor-expressing cell type of CCL8 may be associated with inflammatory response based on monocyte and T-lymphocyte attractant properties.[12] consequently, high expression of genes related to T-cell influx and activation are associated with failure of anti-TNF cessation.

In addition, we also identified a group of genes including THOP1, CGAS, RIPK2 and GAB2 which are related to the innate system and which may be associated with success (i.e. no relapse). This group are consistent with quiescent mucosa, exhibiting relatively low expression of genes associated with the granulocyte compartment (indicative of acute inflammation) or the plasma cell compartment (a characteristic signal of distorted villial tissue architecture in chronic inflammatory bowel disease) of which expression was related to successful cessation.[13-16] The observation of reduced gene expression of genes related to the innate immune system in patients who experienced a relapse compared with patients who remain in remission suggests an impaired innate immune system. This, together with the finding of an overactive adaptive immune system in the same patients experiencing a relapse (i.e. elevated gene expression of genes associated with increased T-cell response) suggests the plausibility of the proposed theory that a weak innate immunity (in contrast to acquired immunity) is in general associated with the development of CD symptoms.

Regarding future perspectives, a gene index has been identified including a group of genes that predicts relapse, and a group of genes associated with treatment success (i.e., no relapse). A combination these two groups of genes is a new predictor that discriminate more precisely between patients at low or high risk of relapse.

In current practice, predicting relapse for individual patients remains challenging given the high relapse rate following anti-TNF cessation. Our preliminary data showed unjustified discontinuation of anti-TNF therapy in 18% of patients as these patients remained in remission during follow-up. Contrary, according to our gene index, all patients who experienced a relapse would not have ceased anti-TNF treatment. Relapse leads to a reduced quality of life, may eventually result in surgery with all associated costs. Moreover, in patients who restart anti-TNF therapy may develop insensitivity for anti-TNF therapy.

We suggested that this gene index may contribute to better predicting the risk of relapse in the individual patients with CD in remission since a vast majority of patients who were identified as low risk remained in remission. in addition, all patients who developed a relapse were identified as high risk and therefore, relapse would have been avoided as anti-TNF therapy would not have been ceased.

Based on the preliminary results of this study, future research is warranted and can be categorized into two primary directions. The first involves further examination of the basis of innate immune defects in relation to CD. This entails a more comprehensive delineation of innate immune deficiencies and the broader implications of the innate immune system in CD. The second involves assessment whether expression gene or histologic coloring is useful in guiding clinical decision making in the individual patient with CD in whom anti-TNF was

discontinued due to remission. Confirmation of these promising results is necessary through a large-scale Randomized Controlled Trial.

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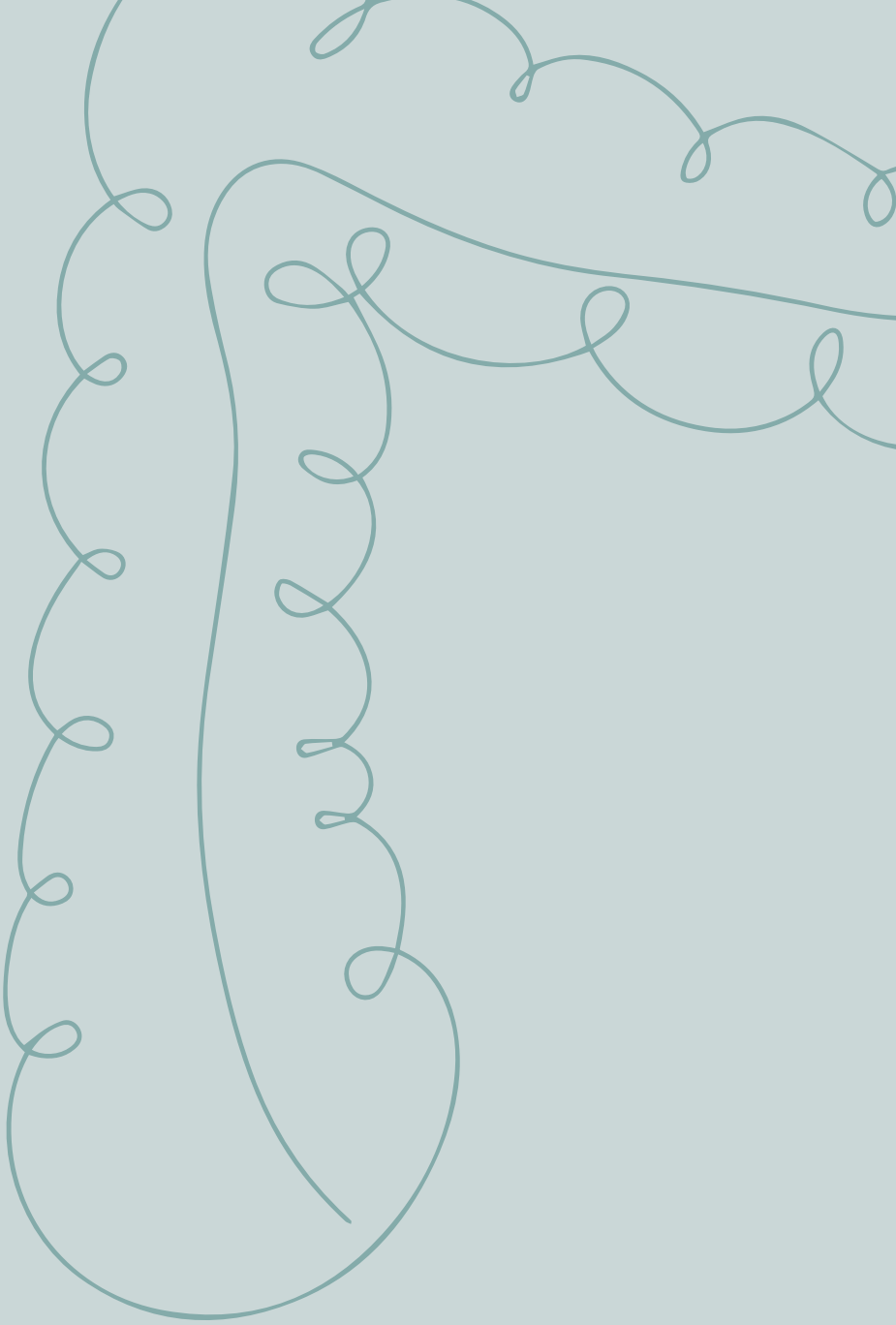

Part IV

Discussion

Chapter 11

General discussion and future perspectives


11

Chapter 11

General discussion and future perspectives

This thesis aimed to provide more insight into different disease optimization strategies in patients with CD at different stadia during the disease course. The findings of this thesis may aid in the guidance of current clinical decisions on optimization or de-escalation of biological strategies. In **Part I** of this thesis, optimization strategies were assessed in patients treated with ustekinumab. **Part II** described optimization strategies in patients who underwent an ileocolonic resection [ICR]. **Part III** of this thesis focused on de-escalation strategies in patients in stable remission during anti-TNF therapy.

Part I Optimization strategies during Ustekinumab therapy

Although anti-TNF has proven its efficacy, the risk of disease progression into complicated disease has not changed in the past decades.[1] In addition, anti-TNF agents may be withdrawn due to primary or secondary non-response or adverse events.[2, 3] Although treatment with a second anti-TNF could be an alternative treatment strategy, it has been shown that response to a second or third line of anti-TNF agent is less effective.[4, 5] Ustekinumab [UST] has a different mechanism of action [i.e. targeting IL-12 and IL-23].

UST demonstrated rapid symptom improvement, however, a delay in endoscopic response has been reported following the induction phase.[6-8] Although endoscopic response evaluation plays an essential role in the management and treatment of CD and is recommended by international guidelines following the start of new medical therapies to identify mucosal improvement, a non-invasive response evaluation including fecal calprotectin [FC] is preferred due to the disadvantages of endoscopic response evaluation including invasiveness and costs.[9, 10] In **Chapter 2**, we assessed whether FC levels following UST induction are related to endoscopic response. In total, 59 patients, all refractory to anti-TNF therapy of whom 51% previously failed both anti-TNF therapy and vedolizumab, were prospectively followed after initiation of UST. A significant association was found between an absolute decrease of ≥500 µg/g in FC levels from baseline to week 8 and endoscopic response at week 16. In addition, the absence of a decrease in FC levels at week 8 was associated with the absence of endoscopic response. For this subgroup of patients, FC measurement at week 8 may guide therapeutic decisions making regarding the continuation of UST treatment in patients with CD. In all other patients, endoscopic evaluation remains currently the golden standard for therapeutic decisions.

Contrary to the above findings, a post-hoc analysis of the registration trials reported that early FC measurements [FC < 250µg/g at week 6 following UST initiation] predict endoscopic response at week 52.[11] This discrepancy could be partly explained by the fact that our cohort included highly refractory patients who failed multiple classes of biologics which results in lower effectiveness of UST.[12] In addition, since an increase in FC levels was observed after week 8, it is hypothesized that low serum levels of UST might contribute to low rates of endoscopic response rates at week 16. It is therefore suggested that peak levels rather than through levels are needed to achieve optimal effect of UST. This is supported by a prospective study showing that peak concentrations [≤1 hour following infusion] are associated with both biochemical and endoscopic response and may be an promising predictor for response to UST treatment.[13] More studies on pharmacokinetics mechanisms of UST are highly needed to elucidate this issue, especially in refractory patients. Furthermore, previous study showed that baseline risk for poor outcomes with UST to be multifactorial, involving multiple additive risk factors, with no single factor sufficiently explaining poor response by itself.[14] It was shown that, in particularly, prior exposure to anti-TNF, or prior exposure to anti-integrins is correlated with poor outcomes, suggesting that earlier initiation of UST, might induce better outcomes. This hypothesis, however, need to be validated in a randomized control trial.

As demonstrated, FC levels might be used to guide endoscopic response evaluation. However, in a subgroup of patients, endoscopic response evaluation is required for therapeutic decisions. In these patients, early differentiation between delayed endoscopic response and primary non-response remains challenging. Therefore, further research is needed with prolonged treatment followed by systematic information regarding endoscopic response. Furthermore, optimal dosing regimen needs to be established to further optimize non-invasive response evaluation strategies for UST including peak concentration.

Although reported effectiveness of UST in short- and long-term studies, loss of response is not uncommon. Registration trials reported a high percentage [up to 40%] of the patients developing secondary loss of response after primary response to treatment, particularly among patients with anti-TNF refractory CD.[15] Moreover, observational real-world cohorts [up to 100% anti-TNF refractory] reported a secondary loss of response rate up to 34% after approximately 52 weeks of treatment.[16, 17] Therefore, optimizing strategies are needed

following secondary loss of response to UST, especially in patients who have failed multiple treatment options. However, up to date, few studies have reported on the effectiveness and safety of a second dose of intravenous UST. **Chapter 3** contributes data on the effectiveness and safety of re-induction with intravenous UST in clinical practice. The effectiveness was retrospectively assessed in 31 patients with loss of response following initial response to UST and who received a second intravenous dose of UST. In this 100% anti-TNF refractory CD population, re-induction with UST may be considered an important rescue treatment strategy since UST therapy was continued in approximately three-quarters of patients and resulted in clinical remission in half of the patients with refractory CD.

Current literature showed a clinical benefit of a second dose of intravenous UST in up to 50% of patients. However, these studies are limited by short-term follow-up. A multicenter study evaluated the short-term efficacy of intravenous re-induction and reported a clinical remission rate of 49% and 43% at weeks 8 and 16, respectively.[18] Additionally, two other real-world cohort studies reported comparable overall response rates of 43% and 50%.[19, 20] The variability of the response rates could be explained by the differences in endpoints used for evaluation, short follow-up periods and small study populations. Our results confirmed these clinical remission rates following a second dose of intravenous UST on the short-term. In addition, our cohort follow-up was extended reporting the maintenance of UST therapy in 74% and 71% of the patients at weeks 20 and 52, respectively.

It is hypothesized that secondary loss of response during UST treatment may be related to low levels of UST suggesting again the need for peak levels rather than through levels. Therapeutic drug monitoring could help to identify a subset of patients who benefit from high peak levels. However, shortening of the interval and dose intensification, to obtain peak levels, in patients with a primary response did not correlate with statistically significant better effectiveness outcomes.[21] A second dose of intravenous UST following secondary loss of response might be needed for this subset of patients in order to obtain a peak concentration not reached with more frequent subcutaneous maintenance dosing for a maximal response of UST and may be considered an important rescue treatment option.

These results suggest that re-induction with a second dose of UST may be effective treatment strategy for therapy-refractory patients with CD. However, less treatment strategies remains in case of treatment failure. UST is associated with superiority effectiveness outcomes

compared to vedolizumab in CD patients with prior failure to anti-TNF therapy making vedolizumab not preferred to start with following treatment failure with UST.[22] In addition, concomitant use of immunomodulators in combination with UST was no more effective than monotherapy in induction or maintenance of remission.[21, 23, 24] In future perspectives, it needs to be determined whether not to start a new medical treatment since the effectiveness of treatment decreases each following step as it is well known that there is less response to treatment when multiple biologicals have failed. Hypothetically, if this trend continues and patients experience persistent severe disease symptoms following initiation of every new treatment, surgery, even in an earlier stage, might be a better treatment strategy.

Given the absence of sufficient data regarding the optimal therapeutic window of UST, additional real-world pharmacokinetics trials are necessary to evaluate the significance of therapeutic peak levels in both induction and maintenance therapy. Implementing therapeutic drug monitoring based on UST peak concentration could potentially identify a subgroup of CD patients who specifically benefit from higher peak levels, necessitating pulsed intravenous ustekinumab maintenance therapy.

Part II Postoperative optimization strategies

Since a majority of patients with CD who underwent an ileocecal resection [ICR] will subsequently experience postoperative recurrence, different management strategies have been suggested in order to prevent postoperative recurrence. An important clinical question regarding postoperative CD management is whether treatment, rather than prevention, of postoperative clinical recurrence can be effectively managed with a second exposure of anti-TNF therapy in patients who failed anti-TNF therapy preoperatively. Therefore, In **Chapter 4**, we assessed the effectiveness of retreatment of anti-TNF therapy in patients with postoperative clinical recurrence following ICR. In this retrospective cohort of 66 patients, we demonstrated that in patients retreated with anti-TNF therapy, postoperative treatment failure rates at 1 and 2 years were 28% and 47%, respectively. In addition, anti-TNF therapy in combination with an immunomodulator results in continuation of therapy in approximately two-third of the patients. Therefore, retreatment with anti-TNF therapy, especially in combination with an immunomodulator, may be an effective strategy for postoperative clinical recurrence of CD in patients treated with an anti-TNF agent preoperatively.

Previous literature on treatment with anti-TNF therapy to prevent postoperative recurrence have shown beneficial effect.[25-27] Importantly, these cohorts included mostly anti-TNF naïve patients.[25-27] One other study, including paediatric patients who failed anti-TNF therapy preoperatively, showed no difference in clinical remission rate between children who were refractory for anti-TNF therapy prior surgery as compared with children who did not receive an anti-TNF agent preoperatively.[28] This might suggest that paediatric patients who failed anti-TNF therapy preoperative can be retreated with the same agent for postoperative recurrence with a success rate similar to that of anti-TNF naïve patients. This is in line with our results confirming beneficial effect of retreatment with anti-TNF therapy for postoperative recurrence in adults.

In addition, significantly lower rate of treatment failure rate at 2 years were reported in patients receiving combination therapy with an immunomodulator as compared to anti-TNF monotherapy (30% vs 49%, $p = 0.016$). This is in line with a previous literature reporting that patients on combination treatment were more likely to stay on infliximab at every 8 weeks compared to those on infliximab mono-therapy following ICR.[29] Our study suggest that postoperative recurrence following primary ICR can be effectively managed with a second line of anti-TNF therapy in combination with an immunomodulator in patients with anti-TNF refractory disease course preoperatively.

In daily practice, prophylactic therapy is recommended in patients at high risk of postoperative recurrence whereas in patients at low risk current guidelines recommend endoscopic evaluation at 12 months following ICR and therapy optimization based on endoscopic findings.[30-32] With regard to therapy optimization, based on these results, retreatment with combination therapy could be an effective treatment strategy in those patients who are in need for treatment, however failed anti-TNF therapy preoperatively.

To conclude on a critical note, a subgroup of patients still develop recurrence following initiation of treatment. For these patients, possible contributing factors to improve this postoperative disease course, including strict postoperative monitoring of disease activity and new treatment options tailored to high risk patients of CD recurrence, needs further investigation since these factors may drive improvement of the postoperative CD course. With regard to new treatment options, prospective studies investigating the efficacy of UST and vedolizumab as treatment option of postoperative recurrence would be of added value to

current literature. In addition, several factors could contribute to anti-TNF treatment failure including pharmacokinetics, immunogenic and pharmacodynamics failure. Further research, including pharmacokinetics trials routinely assessing antidrug antibodies levels and trough levels, is warranted to differentiate between these factors since they could be tackled by different optimization strategies.

Another postoperative management strategy include prophylactic treatment. Prophylactic treatment, including immunomodulators or anti-TNF therapy, is recommended in patients with CD at high risk and reduce endoscopic recurrence as compared to placebo.[33, 34] However, since most studies focused on short-term outcomes, the long-term prognosis of patients receiving postoperative prophylactic treatment are scarce.[35-37] In a large real-world cohort study [n = 811], described in **Chapter 5**, we evaluated the effectiveness of postoperative prophylactic treatment [immunomodulators and/or biologicals within 12 weeks following ICR] on long-term outcomes, including surgical and severe endoscopic recurrence. Both outcomes were significantly reduced up to 10 years following primary ICR in patients who received prophylactic treatment as compared to patients without prophylaxis. The benefit of prophylaxis for patients with CD following primary ICR, as observed in this study, is in line with current literature on early treatment of CD. It is suggested that early treatment with anti-TNF agents during the disease course increases the probability of achieving deep remission which is associated with an improved prognosis with regard to complications or risk of surgery.[38] Similarly, other CD medication trials showed better outcomes in patients with short CD duration as compared with a less robust response in those patients with longer disease duration.[39-42] An explanation that CD patients with longer disease duration have a reduced medication response has yet to be found, but may reflect, structural bowel damage, irreversible vascular changes or possibly an altered microbiome and cytokine profile due to longstanding chronic inflammation.

Although prophylactic treatment following ICR is recommended in patients at high risk, identification of these patients remain challenging due to the lack of consistent and strong predictors of recurrence.[30, 31, 43] In this study, prophylaxis was identified as protective factor for both surgical and severe endoscopic recurrence. Contradictory, active smoking at surgery was identified as risk factor for surgical and severe endoscopic recurrence, which is in line with available literature, underlining the importance of smoking cessation.[44-46] In addition,

preoperative exposure to biologicals and ileocolic disease were associated with surgical recurrence. Contrary, penetrating behavior at surgery was found to be protective factor for the development severe endoscopic recurrence.

Despite abundant data of treatment trials reporting its efficacy of anti-TNF therapy for the prevention of postoperative recurrence of CD, recurrence remains common underlining the complicated disease course to achieve remission following ICR. Therefore data on other biologicals in order to prevent postoperative recurrence are required. The efficacy and safety of UST and vedolizumab has been limited to retrospective studies.[47, 48] New data are eagerly awaited.[49]

Risk stratification may guide clinicians to identify patients who may benefit from prophylactic treatment following ICR. To this end, reliable markers of prognosis with regard to postoperative recurrence are highly needed. In addition to long-term evaluation on severe endoscopic and surgical recurrence, prediction of these outcomes could provide value insights for personalized decision making. The prognostic value of the modified Rutgeerts'score [mRS] in relation to these long-term outcomes remains uncertain. The most important matter of debate concerns anastomotic lesions. A recently published individual participant data meta-analysis [IPD-MA] found no difference between anastomotic lesions [i2a] and lesions in the neoterminal ileum [i2b] on the outcomes of clinical or surgical recurrence. However, these analyses did not account for known risk factors associated with recurrence. Furthermore, this study did not assess the progression to severe endoscopic recurrence. Consequently, in **Chapter 6**, we aimed to assess the prognostic value of the mRS with correction for known clinical risk factors to predict the risk of progression to severe endoscopic recurrence and a re-resection following primary ICR. In this cohort including 654 patients, the ascending index of the mRS closely corresponds with the risk of re-resection in patients with CD following a primary IC, however not with the risk of progression to severe endoscopic recurrence.

Our findings are consistent with previous retrospective multicenter studies demonstrating a less aggressive disease course in patients with anastomotic lesions [i2a] as compared to those with ileal inflammation [i2b] in terms of progression to severe endoscopic lesions.[50, 51] In our cohort, after adjusting for known clinical risk factors, anastomotic lesions [i2a] were not associated with re-resection and progression to severe endoscopic recurrence, whereas mild

lesions in the neoterminal ileum [i2b] lesions were associated with both outcomes. A more conservative management seems indicated in postoperative CD patients with anastomotic lesions [i2a]. Close surveillance and postoperative prophylaxis are warranted for patients with lesions in the neoterminal ileum [i2b]. Current international guidelines recommend escalation of medication in patients with a RS ≥i2.[31, 32] Based on our findings as well as previous observations on the long-term outcomes of anastomotic lesions, refinement of these recommendations into mRS ≥ i2b seems indicated. [50-52] Further research is warranted to identify risk factors and biomarkers for postoperative recurrence in order to effectively manage patients with anastomotic lesions [i2a]. The need for more accurate biomarkers is underscored by the absence of an association between clinical risk factors and long-term outcomes in multivariable analysis in this study.

Part III de-escalation strategies

Since mucosal remission prevents long-term disease complications, avoids surgery and hospitalization and is associated with Quality of Life in patients with CD, treatment goals have changed from symptom improvement to sustained endoscopic remission.[53, 54] However, due to the uncertainty of the risk of relapse in the individual patient, cessation of anti-TNF therapy in patients in remission is still debated.[55, 56] Since guidelines on when to cease anti-TNF therapy are lacking, a valid tool for patient identification to safely cease anti-TNF therapy is highly needed. Therefore, a prediction model has been developed to identify patients with CD with a low risk of relapse following anti-TN therapy cessation.[57] In **Chapter 7**, this previously developed prediction model has been externally validated and updated to estimate the risk of relapse in individual patients following anti-TNF cessation. In this external cohort, which comprised 486 patients with CD who ceased anti-TNF therapy due to remission, relapse rates were 35% and 54% after 1 and 2 years which was in line with previous literature.[58-63] The discrepancy in relapse rate, varying between 26% to 44%, could partly be explained by differences in definitions of remission and relapse. Although the prediction model still showed a moderate discriminative ability [c-statistic of 0.6] performance after the update, several indications can be considered for using the prediction model. First, in other fields, including fertility and oncology research, similar c-statistics [0.58 – 0.64] are reported whilst these prediction models are frequently used in daily practice as a guide for individual decision making.[64-76] Second, our updated model may support a better cessation strategy as compared to current guidelines stating that cessation of anti-TNF

therapy is recommend only in patients with long-standing and stable deep remission [clinical, biochemical and endoscopic].[77] Third, using the prediction model could avoid unjustified cessation of anti-TNF in a subgroup of patients who are identified as high-risk of relapse. Last indication for using this prediction model could be the high rate of successful retreatment [81%] with anti-TNF agents following relapse, reported in this study, which is consistent with available literature.[58, 59, 62] Consequently, the use of this prediction model may support clinical decision-making to optimize patient selection in whom anti-TNF can be safely ceased. However, further improvement of the prognostic performance of the model is necessary before using it in daily practice. Identification of new biomarkers for a better discrimination between patients at high- and low risk is highly necessary. Further research is warranted to refine and update the prediction model.

Therefore a stepped wedge center randomized non-inferiority trail was designed in **Chapter 8** which will provide prospective data for further updating the prediction model with biochemical, endoscopic and histologic and new serological data as well as insight in the cost-effectiveness of the new strategy of anti-TNF cessation based on the prediction model. This randomized control trial [RCT] aims to study the clinical impact of this tool and to assess its prognostic performance. In total, 19 Dutch hospital will be randomly allocated for implementation of the prediction model. Based on this design, prognostic performance of the tool, adverse outcomes, disease-related quality of life and cost-effectiveness will be assessed to support clinical decision making to optimize patient selection in whom anti-TNF therapy can be safely ceased. In addition, biomarkers including serological, endoscopic and histological, will be collected to further improve and update this prediction model to safely cease anti-TNF therapy in patients with CD. Identification of new biomarkers closely related to the pathophysiology of CD are warranted for further discrimination between patients prone for relapse and those who remain in remission following anti-TNF cessation. To this end, more biochemical, histological, serological and genetic markers are needed for the prediction of the individual risk of relapse in clinical practice. In future perspective, cessation of anti-TNF therapy following individual risk estimation might improve the quality of care to patients with CD.

Our results highlights the difficulty of predicting the individual risk of relapse in patients with CD in remission in whom anti-TNF therapy was ceased. Currently, real-world testing

are required to implement the model in clinical practice and in (inter)national guidelines. Following further improvement of the prognostic performance of the model, a cost-effectiveness analysis will be performed as well. Finally, the prediction model will be implemented as an online diagnostic tool for shared decision making in daily practice. Eventually, the implementation of uniform treatment protocols for cessation of anti-TNF therapy following optimal individual risk estimation will improve quality of care in patients with CD.

Another clinical issue regarding anti-TNF cessation is cessation in patients with perianal fistulizing disease [pCD]. This phenotype of CD is associated with a high disease burden and, therefore, cessation of anti-TNF therapy following disease remission is even more controversial. Available literature reported inconsistent results regarding relapse rates following anti-TNF discontinuation.[59, 78-80] Therefore, the risk of relapse following anti-TNF discontinuation in patients with pCD is still debated. In **Chapter 9**, a large IPD-MA including 309 participants from 12 studies in 10 countries was performed to assess the risk of relapse following anti-TNF cessation in patients with pCD in remission. After 1 year, 36% of the patients experienced a relapse [either luminal or pCD] which increased to approximately half of the patients within two years following anti-TNF cessation.

Based on this IPD-MA, the negative consequences of smoking once again underline the importance of quitting as smoking was associated with a higher risk of relapse which is in line with previous literature reporting need for biologic therapy, hospitalization and higher rate of relapse in smokers.[81] In addition, a new risk factor was identified including history of proctitis which was associated with higher risk of relapse. Eventually, most patients continued concomitant immunotherapy when anti-TNF therapy was ceased in this IPD-MA. Relapse rates may be expected to be higher after discontinuation of anti-TNF monotherapy. However, no beneficial effect of concomitant therapy with an immunomodulator was demonstrated. This is in line with the European guideline, which states insufficient evidence for fistula healing induced by immunomodulators or adding immunomodulators to anti-TNF therapy on fistula healing [82].

It may well be that patients in clinical remission without radiological remission are at an increased risk of relapse since healing of the skin, however, before complete closure of the

internal fistula tract is a clinical issue in the treatment of perianal fistula. This is supported by the fact that radiological healing is slower than clinical healing with a time lag of one year.[83] Therefore, the predictive value of fistula closure on MRI prior to anti-TNF discontinuation requires further investigation.

As mentioned earlier, the therapeutic goal, in patients with luminal disease, has been changed with its main focus to achieve deep remission. However, deep remission in order to reduce the risk of relapse following anti-TNF discontinuation have not yet been validated and previous studies reported a relapse rate up to 30% in patients considered to be in deep remission with low FC and mucosal healing.[59, 60] This highlights the importance of additional factors, rather than deep remission, to identify CD patients likely to relapse.

It is generally recognized that a significant fraction of patients can discontinue anti-TNFα therapy without experiencing a relapse. Currently, the clinical factors and biomarkers that allow for discontinuation of anti-TNFα therapy remain obscure at best. **In Chapter 10**, mucosal biopsies obtained just before the discontinuation of anti-TNFα therapy were contrasted between patients who did not show relapse during a two-year follow-up and who experienced disease relapse. To this end, the biopsies were used for deep immunoprofiling by measuring the expression of 772 immunologically-relevant genes using sequence-specific mRNA probes to directly detect gene expression and return counts of each target molecule. We identified a signature of genes related to the activation of NLRP3 inflammasome, with high expression being associated with patients who became anti-TNFα independent in terms of disease control. The identification of this NLRP3 signature provides important evidence for the notion that the NLRP3 inflammasome plays a major role in protecting against chronic inflammation in the human intestine. Additionally, we identified high expression of a group of genes related to interferon-γ/λ signaling as predisposing to clinical failure in discontinuing anti-TNFα medication. Thus, this pathway emerges as a critical pathway aggravating CD, potentially amenable to pharmacological intervention (e.g., using JAK inhibitors). Overall, our results not only allow for the selection of patients under anti-TNFα therapy in whom discontinuation is safe but also prompt a critical reappraisal of the immunological pathways involved in maintaining remission in patients with Crohn's disease.We suggest that this gene index may contribute to better predicting the risk of relapse in the individual patients with CD in remission Future research is warranted to assess whether gene expression is useful in

guiding clinical decision making in the individual patient with CD in whom anti-TNF was discontinued due to remission. Confirmation of these promising results is necessary through an external validation cohort followed by large-scale Randomized Controlled Trial.

Future perspectives

Based on **Part I**, there is a need for continued research to refine and optimize the use of UST in the treatment of CD. Various challenges and opportunities in the use of UST for CD treatment are highlighted, including optimizing response evaluation strategies, managing secondary loss of response and refining treatment strategies for refractory patients. As the effectiveness of UST may vary among patients, especially those who have failed multiple classes of biologics, future research might explore personalized or stratified treatment approaches based on individual patient characteristics, such as prior exposure to specific therapies. In addition, research should focus on understanding optimal peak concentrations and dosing regimens for UST to maximize its effectiveness, especially in refractory patients. Comparative studies and a focus on the optimal therapeutic window will contribute to enhancing the overall management of CD with UST.

In addition, as discussed in **Part II**, the future of postoperative CD management involves an ongoing exploration of diverse aspects, including personalized treatment decision making to optimize outcomes for individual patients, identification of reliable prognostic markers, refinement of current strategies, and the refinement of guidelines to improve patient outcomes.

In future perspective, cessation of anti-TNF therapy following individual risk estimation might improve the quality of care to patients with CD. However, our results in **Part III** underline the challenges in predicting the individual risk of relapse in patients with CD in remission following anti-TNF cessation. Ongoing research, real-world testing, and improvement of the prediction model are essential for realizing its potential in personalized CD management. Identification of new biomarkers closely related to the pathophysiology of CD are needed for better discrimination between patients prone for relapse and those who remain in remission following anti-TNF cessation. To this end, more biochemical, histological, serological and genetic markers are warranted for the prediction of the individual risk of relapse in clinical practice. Our ongoing multicenter and randomized

CEASE-trial [NL8891] aims at achieving these objectives. These results are eagerly awaited as real-world testing are required to implement the model in clinical practice and in (inter)national guidelines. The ultimate aim is to improve patient outcomes and the overall quality of care.

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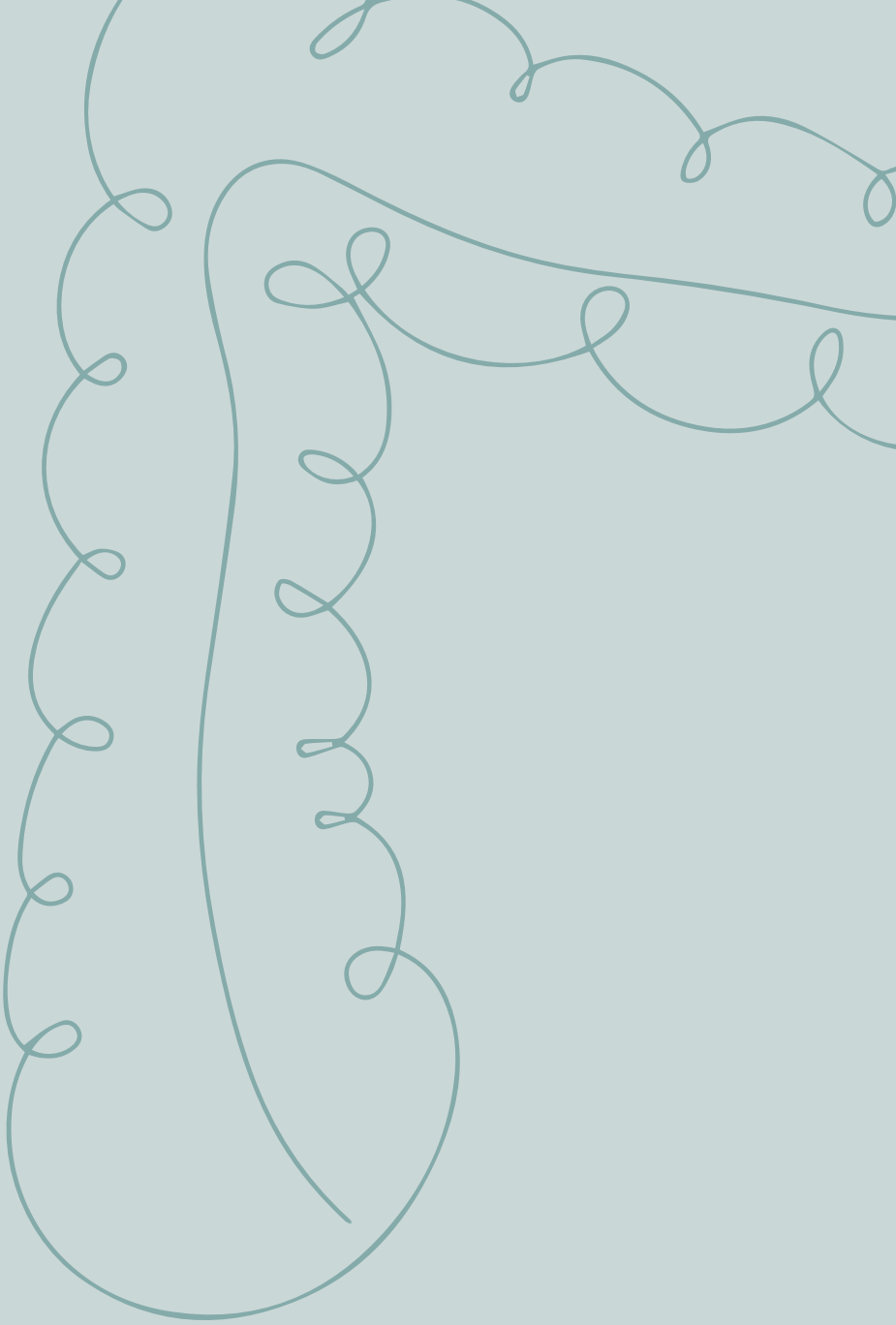

Part V

Appendices

Nederlandse samenvatting

List of contributing authors

Curriculum Vitae

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PhD portfolio

Dankwoord

Nederlandse samenvatting

Dit proefschrift heeft als doel meer inzicht te bieden in verschillende optimalisatiestrategieën voor de behandeling van de ziekte van Crohn [CD] in verschillende stadia van de ziekte. In **deel I** van dit proefschrift worden optimalisatiestrategieën beschreven bij CD patiënten die behandeld werden met ustekinumab. **Deel II** beschrijft optimalisatiestrategieën bij patiënten die een ileocecaal resectie [ICR] hadden ondergaan. **Deel III** richt zich op de-escalatiestrategieën bij patiënten die in remissie zijn met anti-tumor necrosis factor [anti-TNF] therapie.

Deel I: Optimalisatiestrategieën tijdens Ustekinumab-therapie

Hoewel anti-TNF therapie haar effectiviteit heeft bewezen, kan het gestaakt worden vanwege primair of secundair non-respons of bijwerkingen. Omdat Ustekinumab [UST] een ander werkingsmechanisme betreft is de switch naar UST een voor de hand liggende vervolgstap. Endoscopische responsbeoordeling speelt een essentiële rol in de management en behandeling van de ziekte van CD en wordt aanbevolen door internationale richtlijnen om mucosale verbetering te identificeren. Echter heeft niet invasieve respons beoordeling, zoals fecale calprotectine [FC], de voorkeur vanwege de nadelen van endoscopische responsbeoordeling. In **Hoofdstuk 2** is onderzocht of FC levels gerelateerd zijn aan endoscopische respons na het starten van UST. Er werd een significante associatie gevonden tussen een absolute afname van ≥500 µg/g in FC-levels vanaf de start van UST tot week 8 en een endoscopische respons op week 16. Bovendien werd geen afname in FC-levels op week 8 geassocieerd met de afwezigheid van endoscopische respons. Voor deze subgroep van patiënten kunnen FC-levels op week 8 therapeutische besluitvorming begeleiden met betrekking tot het voortzetten van UST behandeling. Bij alle andere patiënten blijft

endoscopische evaluatie momenteel de gouden standaard voor therapeutische besluitvorming.

Hoewel de effectiviteit van UST is aangetoond in zowel korte- als langtermijnstudies, is verlies van respons een veelvoorkomend probleem. Daarom zijn optimalisatiestrategieën nodig, met name bij patiënten bij wie meerdere behandelingsopties hebben gefaald. Uit **Hoofdstuk 3**, dat de effectiviteit en veiligheid van een 2e gift met intraveneus UST in de klinische praktijk onderzoekt, blijkt dat een 2e gift met UST kan worden beschouwd als een belangrijke optimalisatiesstrategie gezien de behandeling werd voortgezet bij ongeveer driekwart van de patiënten en resulteerde in klinische remissie bij de helft van de patiënten met refractaire CD.

Deel II Postoperatieve optimalisatiestrategieën

Een belangrijke klinische vraag, met betrekking tot de management van postoperatieve CD, is of de behandeling van een postoperatief klinische recidief effectief kan worden behandeld met een tweede blootstelling aan anti-TNF therapie bij patiënten die preoperatief niet reageerden op anti-TNF-therapie. In **Hoofdstuk 4** is aangetoond dat relatief weinig patiënten, die postoperatief opnieuw werden behandeld met anti-TNF therapie, faalden op deze behandelstrategie. Bovendien resulteert anti-TNF therapie in combinatie met een immunomodulator in voortzetting van de therapie bij ongeveer twee derde van de patiënten. Daarom kan opnieuw behandelen met anti-TNF therapie, in combinatie met een immunomodulator, een effectieve strategie zijn in de behandeling van een postoperatief recidief van CD bij patiënten die preoperatief zijn behandeld met een anti-TNF therapie.

In **Hoofdstuk 5** is de effectiviteit van postoperatieve profylactische behandeling onderzocht in het voorkomen van een chirurgische en ernstige endoscopische recidief. Beide uitkomsten

waren significant verminderd tot 10 jaar na primaire ICR bij patiënten die profylactische behandeling kregen in vergelijking met patiënten zonder profylaxe.

Naast langer termijn evaluatie van ernstige endoscopische en chirurgische recidieven kan voorspelling van deze uitkomsten waardevolle inzichten bieden voor gepersonaliseerde besluitvorming. In **hoofdstuk 6** is de prognostische waarde van de mRS onderzocht met correctie voor bekende klinische risicofactoren om het risico op progressie naar ernstig endoscopisch recidief en een nieuwe operatie na primaire ICR te voorspellen. In deze cohortstudie correleerde de oplopende index van de mRS nauw met het risico op een nieuwe operatie, maar niet met het risico op progressie naar een ernstig endoscopisch recidief.

Deel III - Strategieën voor de-escalatie

Gezien het feit dat er geen richtlijnen zijn over wanneer patiënten kunnen stoppen met anti-TNF-therapie, is een tool voor het identificeren van patiënten om veilig te stoppen met anti-TNF-therapie noodzakelijk. Daarom is een predictiemodel ontwikkeld om patiënten met de ziekte van Crohn te identificeren met een laag risico op terugval na stopzetting van anti-TN-therapie. In **Hoofdstuk 7** is dit eerder ontwikkelde voorspellende model extern gevalideerd en bijgewerkt om het risico op terugval bij individuele patiënten na het staken van anti-TNF te schatten. In **Hoofdstuk 8** is een Randomized Control Trial opgezet, dat prospectieve gegevens zal verschaffen om het predictiemodel te verbeteren, evenals inzicht te bieden in de kosteneffectiviteit van de nieuwe strategie voor het staken van anti-TNF op basis van het voorspellende model. Bovendien worden serologische, endoscopische en histologische biomarkers verzameld om dit voorspellende model verder te verbeteren en bij te upgraden.

Een andere klinische kwestie met betrekking tot het stoppen van anti-TNF is het staken bij patiënten met perianale fistelvormende ziekte [pCD]. In **hoofdstuk 9** werd een grote IPD-MA uitgevoerd, waaraan 309 deelnemers uit 12 studies in 10 landen deelnamen, om het risico

op terugval na stopzetting van anti-TNF bij patiënten met pCD in remissie in kaart te brengen. Na 1 jaar ervoer 36% van de patiënten een terugval [zowel luminaal als pCD], wat toenam tot ongeveer de helft van de patiënten binnen twee jaar na stopzetting van anti-TNF.

Het is algemeen erkend dat een aanzienlijk aantal patiënten anti-TNFα-therapie kan stoppen zonder een terugval te ervaren. Momenteel blijven de klinische factoren en biomarkers die het staken van anti-TNFα-therapie mogelijk maken onduidelijk. In **hoofdstuk 10** onderzocht we of genexpressie, uitgevoerd op darmbiopten, van toegevoegde waarde kan zijn in het voorspellen van een relapse. De resultaten tonen aan dat een hoge expressie van een groep genen geassocieerd met NLRP3-inflammasoomsignalering [innate immuunsysteem] succesvolle staken van anti-TNFα-therapie voorspelt, terwijl een hoge expressie van een groep genen geassocieerd met interferon-γ/λ-signalering [adaptief immuunsysteem] juist relapse na het staken van anti-TNFα-therapie voorspelt. Toekomstig onderzoek is nodig om te beoordelen of genexpressie nuttig is bij het begeleiden van klinische besluitvorming bij de individuele patiënt met CD bij wie anti-TNF is gestaakt vanwege remissie. Bevestiging van deze veelbelovende resultaten is nodig via een externe validatiecohort gevolgd door een grootschalig gerandomiseerd gecontroleerd onderzoek.

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Curriculum Vitae

Sebastiaan ten Bokkel Huinink werd geboren op 21 september 1990, te Woerden, en groeide op Zeist, te Utrecht. Na het afronden van de middelbare school, en tweemaal te zijn uitgeloot, startte hij in 2011 met de studie Geneeskunde aan de Vrije Universiteit, te Amsterdam. Aan het einde van zijn studie geneeskunde verrichte hij zijn masteronderzoek bij de afdeling Maag-, Darm- en leverziekten bij prof. R. Gearry in Christchurch in Nieuw Zeeland. Daaropvolgend was hij werkzaam als promovendus onder supervisie van prof. C.J. van der Woude en dr. A. C. de Vries waarbij de basis werd gelegd voor dit proefschrift. In afwachting van het afronden van zijn promotieonderzoek startte hij in maart 2022 met werken in de kliniek als arts niet in opleiding tot specialist (ANIOS) met als doel het verkrijgen van een goede basis aan klinisch ervaring. Initieel bij de Interne Geneeskunde in het Haaglanden MC, en later bij de Maag-, Darm- en Leverziekten in het Leids Universitair Medisch Centrum. Heden werkt hij met veel passie en enthousiasme bij Lilly Nederland B.V. waar hem een mooie toekomst te wachten staat.

List of publications

1. Sadaf Asl Baakhtari, Andrew McCombie, **Sebastiaan Ten Bokkel Huinink**, Peter Irving, Corey A Siegel, Roger Mulder, Chris J Mulder, Richard Gearry, *Observational study: Perspectives of IBD patients concerning the use of corticosteroids.*
   Dig Dis. 2018;36(1):33-39.

2. Tim L Kortlever, **Sebastiaan Ten Bokkel Huinink**, Marleen Offereins, Clarice Hebblethwaite, Leigh O'Brien, Julie Leeper, Chris Mulder, Jacqueline S Barrett, Richard B Gearry, *The effect of the low FODMAP diet on long-term health outcomes in IBS patients.* Nutr Clin Pract. 2019 Aug;34(4):623-630.

3. Morag Wright-McNaughton, **Sebastiaan Ten Bokkel Huinink**, Christopher M A Frampton, Andrew M McCombie, Nicholas J Talley, Paula M L Skidmore, Richard B Gearry, *Measuring Diet Intake and Gastrointestinal Symptoms in Irritable Bowel Syndrome: Validation of the Food and Symptom Times Diary.*
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4. **Sebastiaan Ten Bokkel Huinink**, Vince Biemans, Marjolijn Duijvestein, Marieke Pierik, Frank Hoentjen, Rachel L West, Christien J van der Woude, Annemarie C de Vries, *Re-induction with Intravenous Ustekinumab after Secondary Loss of Response is a Valid Optimization Strategy in Crohn's Disease.*
   Eur J Gastroenterol Hepatol. 2021 Jul 30 1;33 (1S Suppl 1):e783 – e788.

5. **Sebastiaan Ten Bokkel Huinink**[*], Djuna de Jong*, Daan Nieboer, Doranne Thomassen, Ewout Steyerberg, Marcel Dijkgraaf, Alexander Bodelier, Rachel West, Tessa Römkens, Frank Hoentjen, Rosalie Mallant, Bas van Tuyl, Wout Mares, Frank Wolfhagen, Gerard Dijkstra, Jurriën Reijnders, Nanne de Boer, Adriaan Tan, Petra van Boeckel, Greetje Tack, Dirk van Asseldonk, Geert D'Haens, Janneke van der Woude, Marjolijn Duijvestein, Annemarie de Vries, *Validation and Update of a Prediction Model for Risk of Relapse After Cessation of Anti-TNF Treatment in Crohn's Disease.*
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6. **Sebastiaan Ten Bokkel Huinink**, Evelien M J Beelen, Thomas Ten Bokkel Huinink, Frank Hoentjen, Alexander G L Bodelier, Gerard Dijkstra, Marielle Romberg-Camps, Nanne K de Boer, Laurents P S Stassen, Andrea E van der Meulen, Rachel West, Oddeke van Ruler, C Janneke van der Woude, Annemarie C de Vries, *Anti-TNFα Refractory Patients Respond to Retreatment With Anti-TNFα Therapy for Postoperative Recurrence in Crohn's Disease.*
   Eur J Gastroenterol Hepatol. 2023 Jan 1;35(1):45 – 51.

7. **Sebastiaan Ten Bokkel Huinink**[*], Renske W M Pauwels[*], Christien J van der Woude, M Doukas, L Oudijk, Annemarie C de Vries, *Early fecal calprotectin levels at week 8 may guide therapeutic decisions on Ustekinumab therapy in patients with Crohn's disease.*
Scan J of Gastroenterology. 2023 Mar 27:1-8.

8. **Sebastiaan Ten Bokkel Huinink**, Doranne Thomassen, Ewout Steyerberg, Renske W M Pauwels, Maria J Casanova, Guillaume Bouguen, Joyce W Y Mak, Tamas Molnár, Alan J Lobo, Jacob B Seidelin, Aurelien Amiot, Geert D'Haens, Pauline Rivière, Luisa Guidi, Renata Bor, Wei-Chen Lin, Laurent Peyrin-Biroulet, Javier P Gisbert , C Janneke van der Woude, Annemarie C de Vries, *Discontinuation of Anti-Tumour Necrosis Factor Therapy in Patients with Perianal Fistulizing Crohn's Disease: Individual Patient Data Meta-Analysis of 309 patients from 12 studies.*
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9. Michiel T J Bak, **Sebastiaan Ten Bokkel Huinink**, Nicole S Erler, Alexander G L Bodelier, Gerard Dijkstra, Mariëlle Romberg-Camps, Nanne K H de Boer, Frank Hoentjen, Laurents P S Stassen, Andrea E van der Meulen-de Jong, Rachel L West, Oddeke van Ruler, C Janneke van der Woude, Annemarie C de Vries, *Prognostic value of the modified Rutgeerts' score for long-term outcomes after primary ileocecal resection in Crohn's disease.*
Am J of Gastroenterol. (): 10.14309 2023 Nov 1

10. **S. ten Bokkel Huinink***, D.C. de Jong*, D. Thomassen, M.J.C. Devillers, M.J. van der Hoff, E.W. Steyerberg, S.C.M. Heemskerk, S. Polinder, M.G.W. Dijkgraaf, G.R.A.M. D'Haens, C.J. van der Woude, M. Duijvestein, A.C. de Vries, *Diagnostic tool to Safely CEASE Anti-TNF Therapy in Crohn's Disease: a Stepped Wedge Cluster Randomized Trial.*
Submitted to BMJ Open

11. **S. ten Bokkel Huinink**, M.T.J. Bak, E.M.J. Beelen, MD N.S. Erler, F. Hoentjen, MD A.G.L. Bodelier, G. Dijkstra, M. Romberg-Camps, N.K. de Boer, L.P.S. Stassen, A.E. van der Meulen – de Jong, R. West, C.J. van der Woude, O. van Ruler, A.C. de Vries, *Postoperative prophylaxis prevents surgical and severe endoscopic recurrence after primary ileocecal resection in Crohn's disease patients*
Submitted to Am J of Gastroenterol

[*] Shared first authorship

PhD portfolio

Name PhD student:	Sebastiaan ten Bokkel Huinink
PhD period:	February 2019 – February 2021
Promotor:	Prof. dr. C.J. van der Woude
Co-promotor:	Dr. A.C. de Vries
Department Erasmus MC:	Gastroenterology and Hepatology

Course and workshops	**Year**
Good Clinical Practice (BROK). Erasmus MC, Rotterdam	2019
Basic Introduction Course on SPSS	2019
English course B2.2	2019
Workshop on Microsoft Excel 2010: Basic	2019
Biomedical English Writing	2020
Scientific Integrity	2021
Academic Integrity & Responsible Research	2021
Oral presentations	
Prediction model to safely CEASE anti-TNF therapy in Crohn's disease: Validation of a predictive diagnostic tool for cessation of anti-TNF therapy in CD in a Dutch cohort Regionaal IBD-symposium, Ridderkerk, the Netherlands	2019
Prediction model to safely CEASE anti-TNF therapy in Crohn's disease: Validation of a predictive diagnostic tool for cessation of anti-TNF therapy in CD in a Dutch cohort Digestive Disease Days, Velthoven, the Netherlands	2020
Prediction model to safely CEASE anti-TNF therapy in Crohn's disease: Validation of a predictive diagnostic tool for cessation of anti-TNF therapy in CD in a Dutch cohort Webinar – IBD nascholing, online	2020
To stop or not to stop: predicting relapse after anti-TNF cessation om patients with CD Initiative on Crohn and Colitis, Amsterdam, the Netherlands	2021
Cessation of anti-Tumour Necrosis factor Therapy in Patients with Perianal Fistulizing Crohn's Disease Digestive Disease Days, Velthoven, the Netherlands	2021

Poster presentations	
Prediction model to safely CEASE anti-TNF therapy in Crohn's disease: Validation of a predictive diagnostic tool for cessation of anti-TNF therapy in CD in a Dutch cohort 20th European Crohn's and Colitis Organisation (ECCO), Wenen, Oostenrijk	2020
Cessation of anti-Tumour Necrosis factor Therapy in Patients with Perianal Fistulizing Crohn's Disease: Individual Patient Data Meta-Analysis of 323 patients from 12 studies 21th European Crohn's and Colitis Organisation (ECCO), virtual congress	2021
Attended (inter)national conferences	
19th - 21th European Crohn's and Colitis Organisation (ECCO)	2019 - 2021
Digestive Disease Days, Velthoven, the Netherlands	2019 - 2021
Attended seminars	
Journal Clubs, dept. of Gastroenterology and Hepatology, Erasmus MC	2019 - 2021
Regionale IBD-symposium, Ridderkerk, the Netherlands	2019 - 2021
Initiative on Crohn and Colitis, Amsterdam, the Netherlands	2019 - 2022
NVGE Casuistische Conferentie Jaarbeurs, Utrecht	2019
Young Initiative on Crohn and Colitis (Y-ICC) post ECCO symposia	2019 – 2021
T- Pensant Rotterdam Gastrology, Rotterdam	2019 – 2021
Teaching	
Supervision bachelor students (tutor), Medicine Erasmus University	2019 - 2022
Supervision keuzeonderwijs (KOW) inflammunity, Medicine Erasmus University	2019 – 2022
Supervision scientific research project bachelor students, Medicine Erasmus University	2020
Supervision scientific research project master student, Medicine Erasmus University	2021 - 2022

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