UiT Norges arktiske universitet

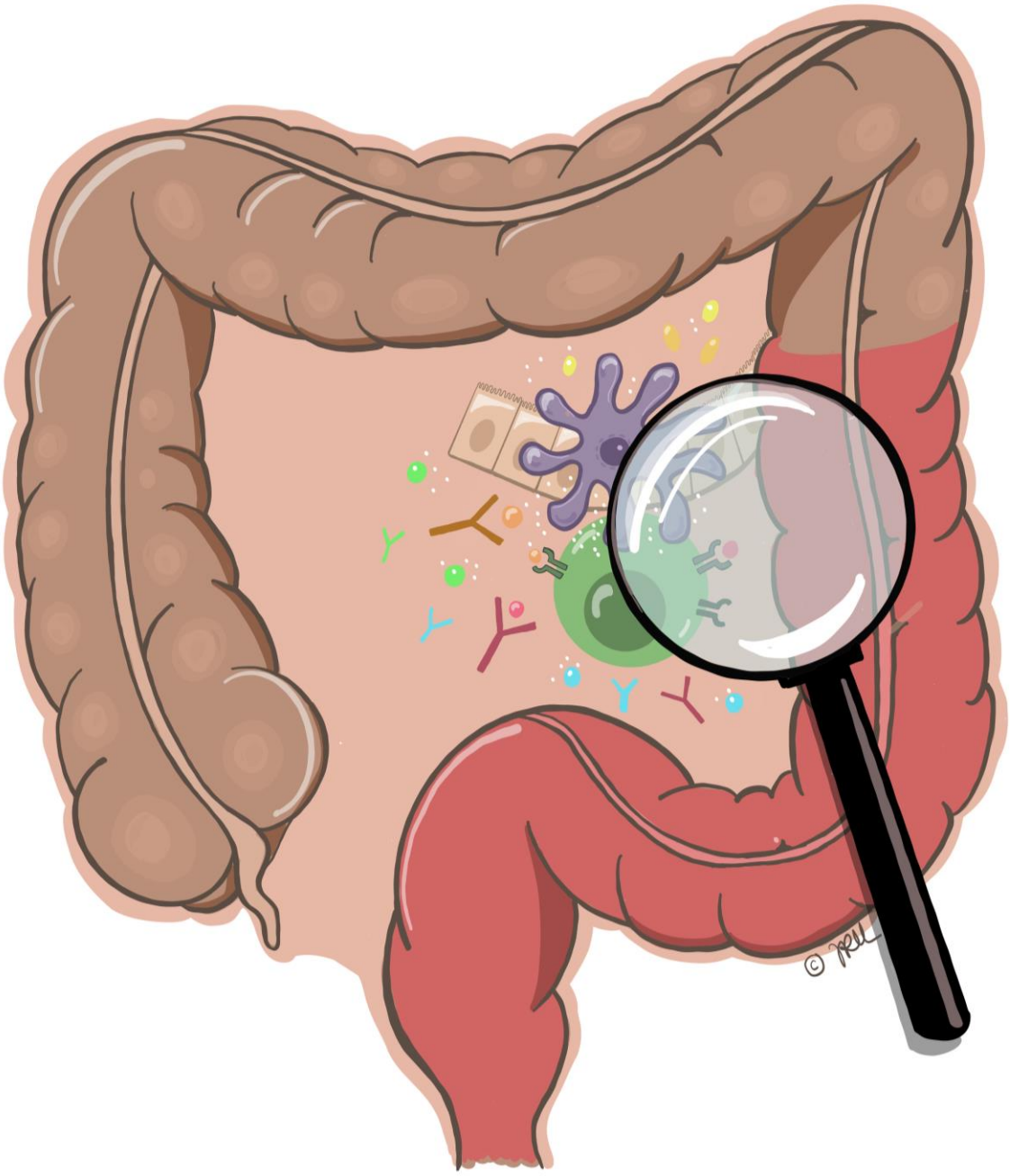
FACULTY OF HEALTH SCIENCE

Towards personalized medicine in ulcerative colitis.

Long-term follow-up after biological treatment and prediction of clinical outcomes.

Kay-Martin Johnsen

A dissertation for the degree of Philosophiae Doctor – February 2024



Contents

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGMENTS

This research was carried out at the Research Group of Gastroenterology and Nutrition, Department of Clinical Medicine, UiT The Arctic University of Norway, and Department of Gastroenterology, University Hospital of North Norway.

I extend my sincere gratitude to my primary supervisor, Rasmus Goll, whose Danish charm and sense of humour made our collaboration not only productive but also enjoyable. Your guidance and expertise have been invaluable throughout this doctoral journey.

I am equally indebted to Jon Florholmen, my co-supervisor. Your visionary leadership has played a pivotal role in establishing our research group. The boundless capacity you possess is matched only by your patience and support for those of us who may not share your superhuman abilities. Your scientific mind and unwavering positivity have been a constant source of inspiration.

A heartfelt thank you to my colleagues in the research group, the camaraderie and the collective effort, have contributed significantly to this thesis. Your dedication has created a stimulating and supportive environment.

Special appreciation goes to, my office mates during different stages of this journey. Our unacademic conversations and shared sense of humour provided a welcome respite during intense periods.

I extend my heartfelt gratitude to my colleagues at the gastrointestinal unit, whose collaboration has not only shaped me into a more proficient clinician but has also significantly contributed to the clinical aspects of this thesis. Your and active participation in the clinical components of the thesis have been invaluable.

I owe a debt of thanks to my forever fiancée, Ina Rye-Holmboe, for your unwavering financial support during my Ph.D. years with modest income. Additionally, your artistic talents are showcased in the illustrations within the thesis, adding a personal touch to the scientific endeavour. I am immensely thankful to for your unwavering support in managing all aspects of our home life, allowing me the focus and time needed to prepare for the defence of this thesis

My family, the foundation of my journey, deserves heartfelt acknowledgment. To my parents, Jan-Eirik Johnsen and Hanne Mari Johnsen, thank you for the upbringing that instilled the essential qualities for a “successful” researcher. To my brothers, Peter Holger Johnsen and Haagen Andreas Johnsen, your toughness has undoubtedly made me stronger.

Lastly, to my children, Mari and Konrad, thank you for simply being yourselves. Your presence has been a reminder of a purpose beyond the pursuit of knowledge.

ABBREVIATIONS

Throughout this text, the following abbreviations have been used

ACTB	Actin Beta
ADAs	Anti-drug antibodies
AGA	American Gastroenterological Association
ASIB	Advanced Study of Inflammatory Bowel disease
ASUC	Acute sever colitis
CD	Crohn's disease
cDNA	complementary Deoxyribonucleic Acid
CRP	C-reactive protein
CT	Cycle Threshold
CR	Clinical remission
CS	Corticosteroids
DAI	Disease Activity Index
ECCO	European Crohn's and Colitis Organization
ER	Endoscopic remission
GI	Gastrointestinal
GS	Geboes Score
HI	Histological remission
HC	Healthy controls
IBD	Inflammatory bowel disease
IFX	Infliximab
IL	Interleukin
IL1RL1	Interleukin-1-like-receptor 1
IMiDs	Immunomodulating drugs
IQR	Interquartile Range
JAK	Janus kinase
LTR	Long-term remission
MES	Mayo Endoscopic score
mRNA	messenger Ribonucleic Acid
PMS	Partial Mayo Score
PNR	Primary non-responder
RCT	Randomized controlled trial
RHI	Robarts histopathology Index
RIN	RNA Integrity Number
RNA	Ribonucleic Acid
RPLP0	Ribosomal Protein Lateral Stalk Subunit P0
RT-qPCR	real-time quantitative polymerase chain reaction
SNR	secondary non-responder
STAT	Signal transducers and activators of transcription
STRIDE	Selecting Therapeutic Targets in Inflammatory Bowel Disease
TDM	Therapeutic drug monitoring
TLR	Toll-like receptor
TH	T-helper
TNF	Tumor necrosis factor
TYK	Tyrosine kinase
TWAS	Transcriptome wide association studies
UC	Ulcerative colitis
UCEIS	Ulcerative Colitis Endoscopic Index of Severity
UCDAI	Ulcerative Colitis Disease Activity Index
6-MP	Mercaptopurine

LIST OF PAPERS

The thesis is bases on the following papers:

I. Repeated intensified infliximab induction - results from an 11-year prospective study of ulcerative colitis using a novel treatment algorithm.
Johnsen KM, Goll R, Hansen V, Olsen T, Rismo R, Heitmann R, Gundersen MD, Kvamme JM, Paulssen EJ, Kileng H, Johnsen K, Florholmen J.
Eur J Gastroenterol Hepatol. 2017 Jan;29(1):98-104.

II. Discovery and validation of mucosal TNF expression combined with histological score - a biomarker for personalized treatment in ulcerative colitis
Jon R. Florholmen, Kay-Martin Johnsen, Renate Meyer, Trine Olsen, Øystein K. Moe, Petter Tandberg, Mona D. Gundersen, Jan-Magnus Kvamme, Knut Johnsen, Terje Løitegård, Gabriele Raschpichler, Cecilia Vold, Sveinung W. Sørbye, Rasmus Goll
BMC Gastroenterol 20, 321 (2020).

III. Prediction of long-term remission in patients following discontinuation of anti-TNF therapy in ulcerative colitis: a 10 year follow up study.
Johnsen Kay-Martin, Florholmen Jon, Gundersen Mona, Sørbye Sveinung and Goll Rasmus
BMC Gastroenterol **22**, 459 (2022).

NORSK SAMMENDRAG

Ulcerøs kolitt (UC) er en kronisk tarmsykdom kjennetegnet med betennelse i tykktarmen som forårsaker symptomer som magesmerter, blodig avføring og diaré. Å forstå årsakene til UC er utfordrende og involverer en kompleks blanding av faktorer som immunsystemproblemer, eksponering for mikrober og genetiske påvirkninger.

Et gjennombrudd i UC-behandling kom med anti-tumor nekrose faktor (anti-TNF) medisiner. Denne behandlingen har ført til betydelig bedring i behandlingen med tanke på å oppnå kontroll på tarmbetennelsen.

Personlig medisin, tilpasning av behandling basert på markører for å forutsi sykdomforløp vil være til stor nytte for å vurdere hvordan behandlingen skal tilpasses den enkelte pasient.

I de tidlige stadiene av sykdommen følges vanligvis en gradvis opptrapping av behandling for å oppnå kontroll på betennelse. På grunn av risiko for at mange vil overbehandles startes man ikke i motsatt ende med den mest effektive behandlingen for så å trappe ned. Dette kan igjen føre til at noen pasienter opplever lengre perioder med høy betennelse og forverring av sykdommen før man får trappet opp.

Nåværende retningslinjer favoriserer ofte vedlikeholds behandling på ubestemt tid grunnet manglende kriterier for å stoppe anti-TNF-behandling. Fraværet av biomarkører og begrenset kunnskap om hvordan terapi endrer sykdomsforløpet bidrar til denne utfordringen.

Å innføre behandlingsalgoritmer med biomarkører som kan bidra til å velge det riktige tidspunktet for å avslutte dyr målrettet behandling, kan gjøre denne type behandlinger både mere tilgjengelig samt og redusere bivirkninger. På samme måte vil biomarkører være til stor nytte om de kan forutsi hvilke pasienter som vil være i behov for anti-TNF behandling, og dermed kunne starte med den mest effektive behandlingen for så å trappe ned. Tidlig kontroll på betennelse vil sannsynligvis også bedre prognosene.

Avhandlingen konkluderer med at tidlig og intensiv behandling, spesielt med anti-TNF behandling kan bidra til å raskt oppnå sykdomskontroll. Langsiktige resultater antyder en endring mot en mildere sykdomsgrad, muligens er normalisering av TNF-uttrykk i tarmslimhinnen en viktig markør for dette. Avslutning av anti-TNF-behandling er gjennomførbart for de fleste, og for de som for oppbluss vil re oppstart med anti-TNF behandling ha god effekt for de fleste. Biomarkører, spesielt TNF-uttrykk i tarmslimhinnen kombinert med mikroskopiundersøkelse av prøver fra tarmslimhinnen kan bidra til å identifisere pasienter som er egnet for tidlig oppstart med målrettet behandling.

ENGLISH SUMMARY

Ulcerative colitis (UC) is a chronic inflammation of the colon that causes symptoms such as abdominal pain, bloody stools, and diarrhea. Understanding the causes of UC, an inflammatory bowel disease (IBD), is challenging and involves a complex interplay of factors such as immune system issues, exposure to microbes, and genetic influences.

A breakthrough in UC treatment came with targeted therapies like anti-tumor necrosis factor (anti-TNF) medications. This treatment has significantly improved the management of intestinal inflammation. Personalized medicine, tailoring treatment based on biomarkers to predict disease progression, would be beneficial in assessing how treatment should be customized for each patient.

In the early stages of the disease, a gradual escalation of treatment is typically followed to gain control over inflammation. Due to the risk of overtreating, the most effective treatment is not initiated immediately and then tapered down. This, in turn, may lead to some patients experiencing prolonged periods of high inflammation and worsening of the disease before treatment escalation. The lack of tools to select suitable patients is a challenge.

Current guidelines often favor indefinite maintenance treatment due to the absence of criteria to discontinue anti-TNF treatment. The lack of biomarkers and limited knowledge of how therapy alters the course of the disease contribute to this challenge.

The high cost of IBD treatment is a significant challenge for healthcare and a burden for patients in terms of frequent hospital visits and side effects. Introducing treatment algorithms with biomarkers that can help choose the right time to discontinue expensive targeted therapy can make such treatments more accessible and reduce side effects. Similarly, biomarkers would be beneficial if they could predict which patients will need anti-TNF treatment, enabling the initiation of the most effective treatment followed by tapering. Early control of inflammation will likely improve prognoses.

The thesis concludes that early and intensive treatment, especially with infliximab, can contribute to achieving rapid disease control. Long-term results suggest a shift toward a milder disease course, possibly with the normalization of TNF expression in the intestinal lining as a key marker. Discontinuation of anti-TNF treatment is feasible for most, and for those experiencing relapses, re-treatment with the same medication is effective. Biomarkers, especially mucosal TNF expression combined with histological assessment, can help identify patients suitable for early initiation of targeted treatment.

1 INTRODUCTION

In this introduction follows a presentation of ulcerative colitis clinical features, natural course of disease, pathophysiology, diagnosis, definition of disease severity and general principles for medical management. Subsequently, the focus will shift towards the overarching aim of this thesis, exploring precision medicine in UC, examining current biomarkers, and identifying knowledge gaps within this field.

1.1 Ulcerative colitis and inflammatory bowel disease

Ulcerative colitis (UC) represents one of the primary disease entities within the broader category of inflammatory bowel disease (IBD), which also includes Crohn's disease (CD), inflammatory bowel disease unclassified (IBDU), and microscopic colitis (MC). The term IBD-U is designated for cases where it is challenging to distinguish between UC and CD[1]. Microscopic colitis displays significant differences in both clinical features and endoscopic findings, limited to histological changes. As this thesis focuses on UC in adults, microscopic colitis will not be further discussed. Although the primary emphasis is on UC, some extrapolated knowledge may apply between UC and CD.

1.2 Epidemiology of ulcerative colitis

The prevalence of UC varies considerably between and within in geographic regions. A recent systematic review reports world-wide prevalence estimates ranging from below 50/100 000 in Africa, South America, and parts of Asia to over 250/100 000 in Northern Europe.[2] Collectively data indicates increasing incidence rates following the industrialisation and westernisation of society and that the incidence rate of IBD is stalling and reaching a plateau in westernized countries.[2,3] In Europe there is a north-west/south-east gradient of IBD incidence with a total maximum-estimated 178 000 new cases of UC each year. In total, there might be as many as 1.5 million persons suffering from UC and 2.6 million including CD in Europe.[4] In Norway data from early 1990-2003 showed an incidence of UC in 12,8 per 100 000 in south eastern Norway.[5] The incidence of UC in North Norway was found to 13/100 000 in the 1983-1986 and the most northern county, Finnmark 26/100 000 from 2000-2002.[6,7] The latest estimates from patient registry data in a nationwide registry showed an prevalence of 0.5 percent for UC.[8] IBD is diagnosed in all ages, but mean ranges are reported from 33-45 years for CD and about 5-10 years later for UC.[3]

1.3 Natural disease course of ulcerative colitis

UC is regarded as a lifelong chronic inflammatory disease, although the disease course at an individual level varies greatly. One of the most referred studies for clinical outcomes and disease course of UC are from the IBSEN group. The patient-self reported symptom burden during a 10-year period after diagnosis was described by four pre-defined curves reflecting the clinical course. The majority (55%) reported a decline in intestinal symptoms, while 1%, 6% and 37% reported increase, chronic continuous, and chronic relapsing respectively. From initial diagnosis 7,5 and 9,8 percent underwent surgery during the first five and 10 years after diagnosis.[9] The extension of disease also changed during the course from proctitis and left sided to further proximal in 42 and 21 percent respectively.[10]

Introduction of targeted therapy (biological-, anti-adhesion-, or small molecule-drugs) with the highly effective anti-tumor necrosis factor drugs for UC represented a paradigm shift in treatment of UC in form of response rates, achieving remission, and inflammation control.[11,12] In Norway, nine percent of UC patients received targeted therapy within one year of diagnosis.[13] A five year's follow-up of UC patients in Europe reports that 11 percent of the newly diagnosed patients needed biological treatment during this period. The study indicated no differences in colectomy rates in the decade of new treatment options with immunomodulators (IMiDs) and biological therapy compared to previous two decades. However, there was a reduction in the risk of hospitalization for patients treated with IMiDs.[14] Reports on colectomy rates affected by targeted therapy have been conflicting, there are Scandinavian studies reporting decreased colectomy rates for UC, maybe due to new treatment options, while an US cohort reports higher rates. [15-17] A recent large retrospective cohort study from US reported a steady decrease of colectomy rates from 2007-2014 with a clear time interruption with a more precipitous decrease in colectomy rates following the 2014 introduction of 3 new biologics.[18] Ulcerative colitis is associated with an increased risk of developing colorectal cancer (CRC), but a decrease in GI malignancy during the twenty-first century have been reported, maybe due to better treatment options. [19,20] Thus the therapy itself with use of IMiDs and anti-TNF therapy is considered as risk factor for cancer, but increased risk for CRC have not been shown. see ECCO guidelines malignancy concencsus.[21]

So far there is no general agreement on if and when to discontinue anti-TNF therapy, thus lacking knowledge on the long-term outcomes after discontinuation, and further need of anti-TNF therapy. Relapse rates in a systematic review indicated a 36 percent risk during a period of 12-24 months after discontinuation of anti-TNF treatment.[22] In case of relapse, there is a lack of knowledge whether the previous treatment should be reinstated or the natural disease course may change to a milder phenotype allowing initiation of re-treatment on a lower treatment level.

To find new predictors for disease course, such as demographic, clinical, biochemical,

histological or mucosal gene transcripts, it is important to gain further knowledge of the natural- or therapy-modified -disease course.

1.4 Pathophysiology of ulcerative colitis

The pathophysiology of IBD and UC is complex and far from understood. It involves dysregulation of the immunotolerance in the gut, exposure to microbes and luminal antigens, in addition to environmental-, and genetic factors. The interaction between these factors result in an overwhelming complexity. The first use of the term ulcerative colitis was in a case report in 1859, later CD was recognized as an entity separate from UC in 1932.[23] Moreover, the individual phenotypes within those two diseases exhibit significant heterogenicity. To fully understand the pathophysiology of UC, we probably have to integrate all the pathophysiological components and correlate to the clinical phenotypes.[24,25] The following chapter will provide a brief introduction to the main identified pathophysiological components of UC.

1.4.1 The role of tumor necrosis factor in inflammation

TNF is a cytokine produced and expressed by most cells in the immune system, but macrophages are considered the main contributor in the concept of IBD. The production of TNF can be induced by a wide variety of stimuli including bacteria, viruses, cytokines, complement factors, basically all cell stress. As a mediator TNF leads to a cascade of cellular responses, by inducing the expression of other pro-inflammatory cytokines, adhesion molecules, and enzymes that contribute to tissue damage.[26] Conversely, TNF may also act as an effector molecule, directly contributing to the tissue injury seen in IBD. TNF induces apoptosis of epithelial cells, disrupts the intestinal barrier function, and promotes the infiltration of inflammatory cells into the intestinal mucosa.[27]

TNF is present in two forms, soluble (sTNF) and transmembrane (tmTNF), which have different functions, Figure 1. Transmembrane TNF is considered important in the innate immune response to infections and tolerance to auto-antigens. There are two types of TNF receptors, TNFR1 and TNFR2, respectively. Both forms of TNF bind to both receptor types, but pairing of sTNF to TNFR1 and tmTNF to TNFR2 are favoured. It is believed that sTNF bound to TNFR2 is quickly released and passes on to TNFR1. Soluble TNF bound to TNFR1 is regarded as the major mediator of inflammation and apoptosis, while TNFR2 is shown to induce antiviral response.[28-30] In UC it is shown that elevated transcription of TNF at the site of inflammation correlates with the grade of inflammation. [31]

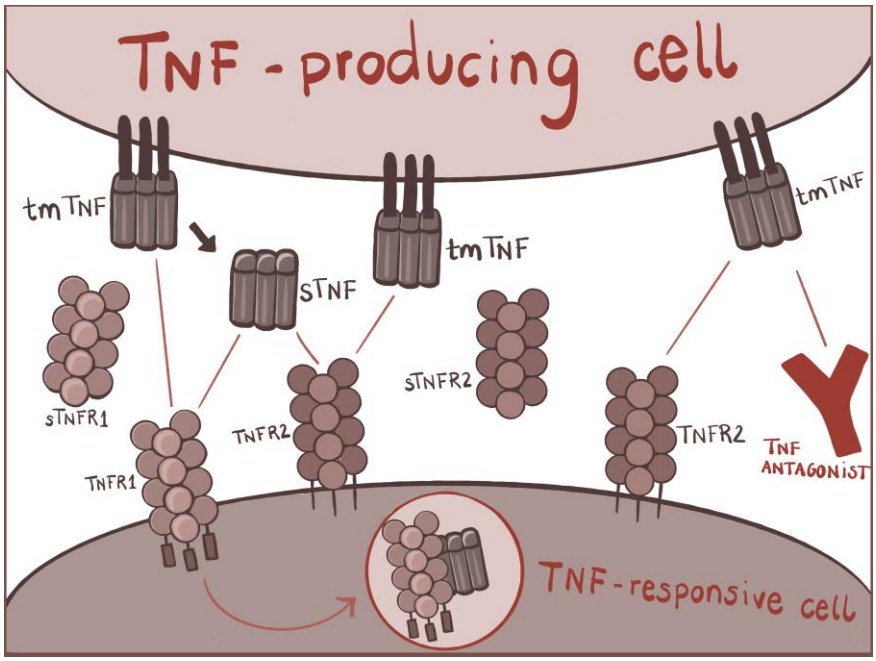


Figure 1: TNF forms and receptors (Ina Rye-Holmboe)
TNF = Tumor necrosis factor tmTNF = transmembrane TNF sTNF = soluble TNF
TNFR2= TNF receptor 2

1.4.2 Ulcerative colitis inflammasome

IBD is a heterogenous polygenetic disease where various molecular mechanisms are involved reflecting the inflammasome first described in 2002 and later characterized as the molecular sequence of f the genome, exposome/epigenome, transcriptome, proteome and metabolome.[32] Familiar predisposition to UC was first demonstrated in 1938 by Lewisohn's.[23] Since the introduction of genome wide association studies (GWAS), a method of screening the entire genome in connection to a disease, GWAS has delivered new insight into the genetic mechanism of UC. For CD, variants of NOD2 are associated with small bowel disease.[33] The largest GWAS study conducted including 30 000 patients with UC or CD found mostly genetic associations between disease location, suggesting three distinct groups of IBD, ileal CD, colonic CD and UC. For UC, the strongest association with extensive disease was on the ancestral 8.1 HLA haplotype, which is also associated with primary sclerosing cholangitis.[34,35] So far more than 200 genetic loci have been identified in IBD, explaining 20-25% of all IBD cases, indicating that genetics alone cannot explain the full occurrence of IBD. Regarding disease course, genotype data have not shown utility as a predictor.[35]

The genetic susceptibility in a population is relatively stable, therefore other factors must have contributed to the rising incidence following the westernization of countries. There

is general agreement that the increasing incidence of IBD is partly explained by changes in environmental factors. The sum of all environmental factors a human is exposed to, from conception and during a lifetime is termed the exposome.[36] The exposomes can cause changes in gene functions, by alterations such as DNA methylation, this is known as epigenetics- a concept most used in molecular biology defined as the study of the complete set of epigenetic modifications on the genetic material of a cell.[37,38] Epigenome-wide association studies (EWAS) of UC patients have shown altered expression in genes involving homeostasis, defence and immune response.[37,39] In IBD, environmental factors as smoking, breastfeeding, diet, pollution, microbes and antibiotics may induce epigenetic changes leading to phenotypic expression of IBD, for more details see nature review by Ananthakrisnan and Figure 2.[40,41] .

The transcriptome is the transcript of genetic codes into RNA. As with GWAS, RNA sequencing methods have opened the way for characterization whole transcriptome wide association studies (TWAS). Integrating TWAS with GWAS has allowed a deeper understanding of the functional elements of the genome and genetic susceptibility. [42-44] Earlier paradigms that categorized UC with a TH2 cytokine profile and CD with TH1/TH17 profiles have been challenged by new findings that suggest a more complex interplay of immune responses in both diseases. Single-cell RNA sequencing has revealed unique immune cell and cytokine expression signatures for UC and CD, indicating that the two conditions may have distinct pathophysiological mechanisms.[45,46] Studies have identified various T helper cell subsets, as well as cytokines like TNF-α, mediate inflammation differently in CD and UC.[47-49] The clinical impact of these studies is currently limited.

In addition to the transcriptome, we have the proteome and metabolome. The proteome encompasses all proteins expressed by a cell or organism and is not directly proportional to mRNA levels from the transcriptome, due to complex regulatory mechanisms governing mRNA translation.[50,51] The metabolome, consisting of the end products of cellular processes, reflects the cell's biochemical activity. The metabolome could potentially indicate the state of inflammation and the efficacy of various metabolic pathways. Hence metabolic profiling might reflect disease activity and may offer a tool for diagnosis and monitoring of IBD. [52] The metabolome is a significant effector of inflammation, which could play a key role in the pathogenesis and progression of IBD, it warrants further investigation to fully understand its impact.

The genome, transcriptome, proteome, and metabolome are intricately linked and form the core of systems biology, also known as multi-omics, which seeks to decipher the complex interactions within biological systems. Overcoming the challenges of data integration and the

development of sophisticated bioinformatics tools are essential for advancing our understanding and improving the prediction of the clinical course of diseases like ulcerative colitis.[53] This thesis does not delve further into systems biology as a whole

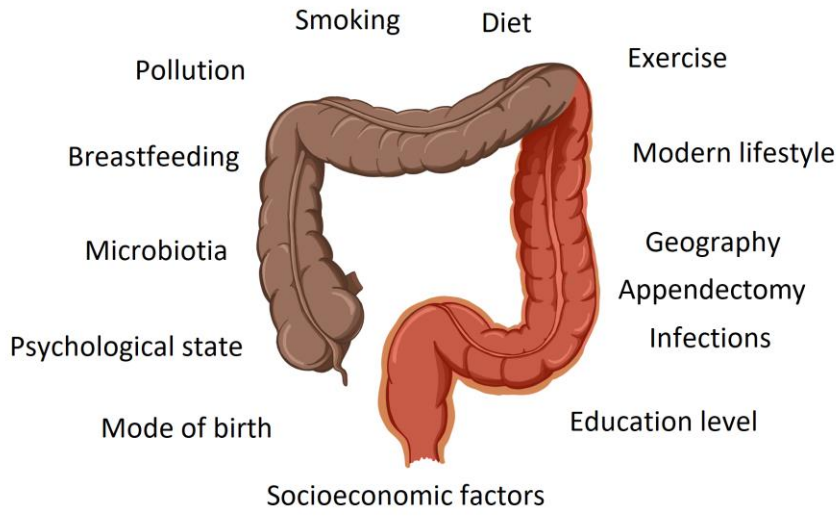


Figure 2: The IBD exposome (Ina Rye-Holmboe)

1.4.3 Role of gut microbiota in IBD

The link between modern lifestyle such as diet, use of antibiotics, cesarean delivery, less contact with soil microorganisms and alterations in the microbial colonization of the gut is well established.[54-57] There is a general agreement that environmental factors are influencing the increasing incidence of IBD. The gut microbiota and their interaction with the host is identified as a contributor to the development of IBD.[56,58,59] Studies have shown that commensal bacteria in the mucosal tissue is essential to the maintenance of the homeostasis in the immune system, and a dysregulated immune response leads to a susceptibility to immune-mediated diseases such as IBD, Figure 3.

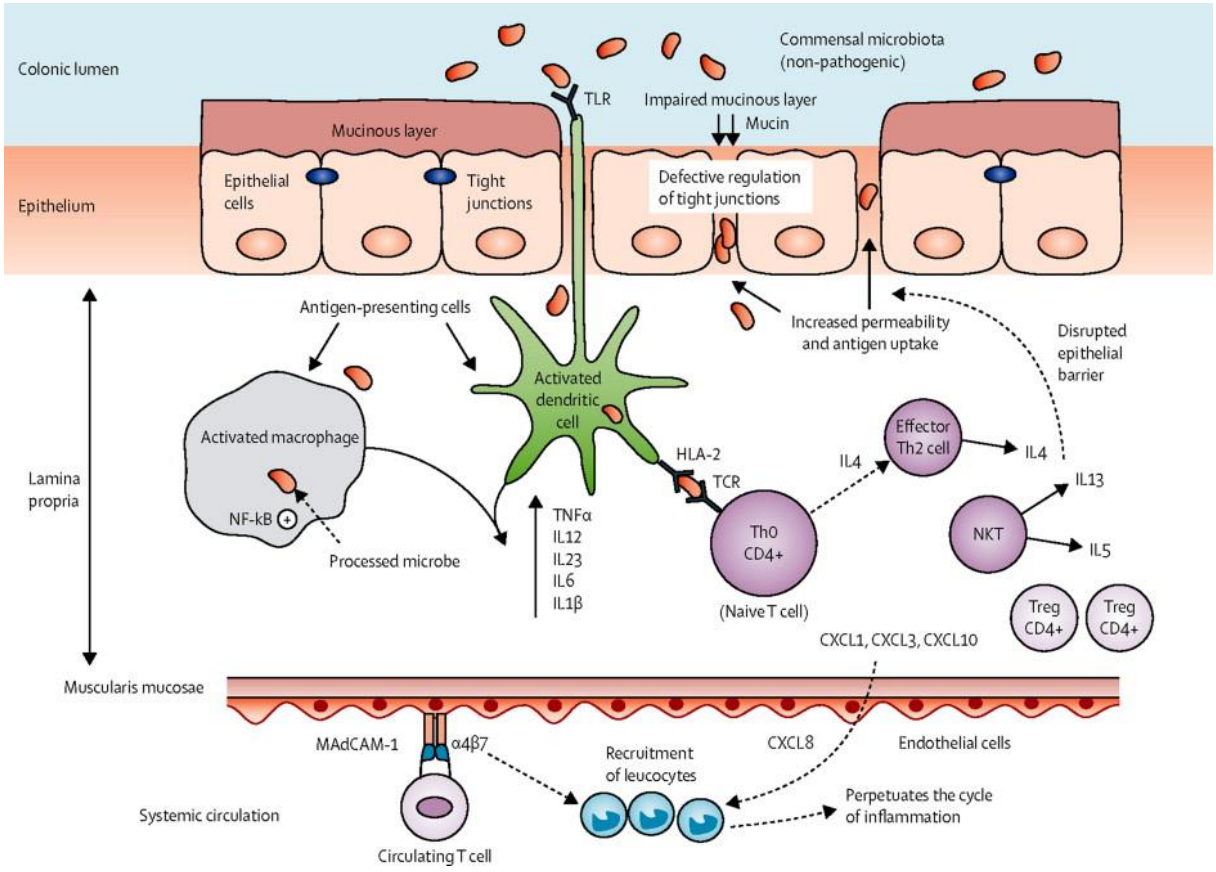


Figure 3. Gut microbiota in the pathophysiology of UC: Disruption of tight junctions and the mucus film covering the epithelial layer causes increased permeability of the intestinal epithelium. Activation of dendritic cells by recognition of commensal bacteria through molecular pattern-recognition receptors and macrophages by NF-kB resulting in proinflammatory cytokines (TNF-α, interleukins 12, 23, 6, and 1Lβ). Further antigen presentation to naïve CD4-T cells promoting differentiation into Th2 effector cells and production of IL4. IL13 and IL5 is produced by natural killer T-cells which disrupts the epithelial barrier. mucosal vascular addressin-cell adhesion molecule 1 receptors in the mucosal vessels endothelium binds circulating T-cells which passes over in the intestinal endothelium.[60] (Reprinted and text adopted with permission from Elsevier publishing company)

1.4.4 Dysregulated immune response

As previous described, it is a complex interaction between multiple factors that results in the development of IBD. The sum of these interactions leads to an inappropriate response in both the adaptive and innate immune system. The following events numbered below are identified as primary or secondary events resulting in IBD.[61]

I. The intestinal epithelium plays a crucial role as a barrier, separating the microbes within the lumen from the inflammatory cells in the lamina propria. Studies indicate a reduction in epithelial resistance and an increase in permeability in both inflamed and non-inflamed mucosa in individuals with IBD.[62,63]

II. The innate immune mechanism of the epithelial layer is disrupted, characterized by altered TLR expression and heightened NOD2 expression. This leads to the inappropriate recognition of commensal bacteria by dendritic cells, inducing a pro-inflammatory Th1 and Th17 response, typically reserved for pathogenic microbes.[64,65]

III. Antigen recognition and processing by professional antigen-presenting cells are disturbed. Dendritic cells incorrectly identify commensal bacteria, resulting in the loss of regulatory capacity. This failure to control overactive T-cell populations may lead to sustained inflammation.[66,67]

IV. Atypical antigen-presenting cells, such as intestinal epithelial cells, activate T cells instead of inducing anergy.[68,69]

V. The clearance of overactive or auto-reactive T-cell populations is disturbed, with activated T-cells persisting and failing to undergo apoptosis. [70]

VI. There is an imbalance between regulatory and effector T-cells.[61]

VII. Overactivation of the sympathetic nervous system has been shown to increase colonic paracellular permeability, overproduction of interferon-gamma, and altered expression of tight junction proteins. [71,72]

1.5 Diagnosis and definition of outcomes

1.5.1 Diagnosis and classification

The clinical hallmark of UC is the chronic inflammation of the colon mucosa, accompanied by characteristic symptoms including abdominal pain, frequent bloody stool, and diarrhoea. Similar symptoms are observed in numerous other inflammatory diseases, with overlapping biochemical, stool test, endoscopic appearance, and histological findings. These may include immune, vascular, infectious, and non-infectious diseases. According to European guidelines, there is no single reference standard for the diagnosis of UC.[73] Therefore, the diagnosis relies on a comprehensive evaluation of clinical, biochemical, stool, endoscopic, histologic, and radiologic findings. Key features of UC are summarized in Table 1.

Table 1: Characteristics of ulcerative colitis

Characteristic	
Disease location	• Limited to colon (except backwash ileitis) • Usually rectal involvement.
Endoscopic findings	• Loss of vascular pattern • Erythema • Friability • Ulcerations • Spontaneous bleeding • Pseudo polyps
Histological findings	• Distortion of crypt architecture • Mucosal or submucosal inflammation • Lamina propria cellular infiltrate (plasma cells, eosinophils, lymphocytes) • Mucin depletion • Lymphoid aggregates • Crypt abscesses
Clinical features and complications	• Bloody diarrhoea/rectal bleeding • Tenesmus • Abdominal pain • Fever (severe cases) • Toxic megacolon • Extraintestinal manifestations • Increased risk of colorectal cancer.

Following the Montreal classification of disease extension, UC is classified as either proctitis, left-side colitis of extensive colitis, Figure 4.[1]

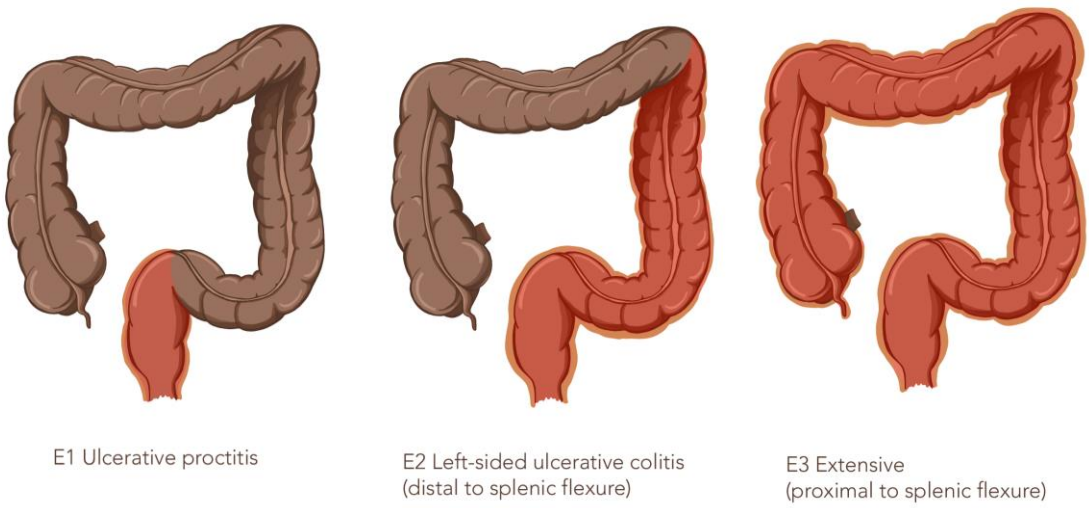


Figure 4: Montreal classification of ulcerative colitis (Ina Rye-Holmboe)

1.5.2 Clinical scoring and grading of disease activity

Several clinical scoring systems have been developed to assess disease severity and treatment response in UC. These systems incorporate symptoms related to stool frequency, rectal bleeding, urgency, and are combined with either patients' self-reported well-being or the physician's judgment of disease severity. Commonly used symptom scores include the Ulcerative Colitis Clinical Score (UCCS), Simple Colitis Clinical Activity Index (SCCAI), and Partial Mayo Score (PMS).[74-76] Although these symptom scores provide cut-off values for disease activity as a tool to escalate or de-escalate treatment, they are insufficient to reflect the underlying mucosal inflammation.[73] To address this limitation, the symptom scores are often combined with endoscopic appearance indices and biochemical biomarkers such as calprotectin.

There are several developed endoscopic indices, but the two most commonly applied are the Mayo endoscopic score (MES) and Ulcerative Colitis Endoscopic Index of Severity (UCEIS). Both indices encompass similar aspects, including the vascular pattern, erythema, bleeding in the mucosa, and erosions or ulcers. Among these, only the UCEIS score is validated. However, the MES, when combined with the PMS, referred to as Mayo Score, Disease Activity Index (DAI), or UCDAI score, is likely the most frequently employed as an endpoint in various trials.[74,77]

Table 2: Mayo score and clinical grading

Parameter	*Clinical evaluation*	*Score*
1. Stool frequency (per day)	Normal number of stools	0
	1-2 more than normal	1
	3-4 more than normal	2
	≥ 5 more than normal	3
2. Rectal bleeding	None	0
	Streaks of blood with stool in in less than half of the cases	1
	Obvious blood with stools in most cases	2
	Blood alone passes	3
Endoscopic findings	Normal mucosa or inactive disease	0
	Mild activity (erythema, decreased vascular pattern, mild friability)	1
	Moderate activity (marked erythema, lack of vascular pattern, friability, erosions)	2
	Severe activity (spontaneous bleeding, large ulcerations)	3
Physician's global assessment	Normal	0
	Mild disease	1
	Moderate disease	2
	Severe disease	3

Decoding: 0-2 Remission (Provided no sub-score greater than 1. 3-5 Mild activity 6-10 Moderate activity > 10 Severe activity

1.5.3 Histological scoring

Currently, the treatment goals for UC emphasize achieving both endoscopic and clinical remission.[78] It is known that histologic activity persist in an apparent endoscopic normal mucosa. The clinical utility of histologic indices (HI) is gaining prominence with growing evidence that histological remission is associated with a lower risk of relapse, colectomy, hospitalization, and a reduced risk of IBD-related neoplasia.[79,80] There is a plethora HI, and the most used; Geboes Score (GS), Nancy Score (NS) and Robarts histopathology Index (RHI) does not correlate well endoscopic score.[80,81] Of these NS and RHI have undergone some validation, but none are fully validated.[82] Due to the lack of validation, histologic remission remains to be recommended as a treatment target according to the Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE) program.[83]

1.5.4 Definition of remission

The concept of remission can be intricate, given the dynamic nature of therapeutic goals influenced by advancements in targeted therapy for treating UC and enhanced insights into optimizing drug efficacy. In diverse drug trials, distinct endpoints have been outlined, including clinical remission, endoscopic remission, histological remission, and mucosal healing. Throughout the years, there have been varied suggestions on precisely defining these terms (Table 3). Notably, existing guidelines lack a definitive clarification of the term "remission" as a therapeutic target.[84,85] Additional terms such as "deep remission" and "immunologic remission" have been introduced; however, a precise definition for these terms is yet to be established.[86,87] Studies have consistently demonstrated enhanced long-term outcomes when pursuing elevated treatment targets, underscoring the importance of refining and adopting higher standards in remission criteria.

Table 3: Evolution of remission definitions

Remission 2005	*Mayo Score ≤ 2 points and no sub-score >1*[11]
Endoscopic remission 2005	MES ≤ 1[11]
Mucosal healing 2011	MES ≤ 1[88]
Endoscopic remission 2019	MES =0[89]
Histological remission 2019	Geboes score < 2[89]
Mucosal healing 2019	Histological and endoscopic remission with MES=0 and Geboes score < 2[89]
Remission 2021	MS with SFS ≤ 1 RBS = 0 and ESS ≤1[90]
Disease clereance 2022	Simultaneous clinical, endoscopic and histological remission (partial-Mayo≤2 and subscore =0, Nancy index =0)[91]

Mayo score = MS, Stool frequency score = SFS, Rectal bleeding score: RBS, Mayo endoscopic subscore = MES

1.5.5 Definition of treatment refractory ulcerative colitis

Even though treatment with biological therapy have improved the clinical outcomes in UC, there are studies indicate that 20-50 percent of patients show non-responsiveness to initial anti-TNF treatment, termed primary non-responders (PNR). Similarly, an equivalent number of initially responsive patients experience a later loss of effect, known as secondary non-responders (SNR), though exact estimates are not available.[92,93] There is no general agreement on how to define both PNR and SNR, and this might explain lack of epidemiologic data on therapeutic failure Proposed criteria for defining PNR and SNR encompass factors such as the appropriate duration of treatment (12 weeks), the absence of anti-drug antibodies (ADAs) along with adequate drug levels, the absence of pathogenic bacteria in fecal samples, and the confirmation of disease activity through either endoscopic or clinical evaluation. This lack of standardized definitions highlights the complexity of assessing treatment responses and emphasizes the importance of establishing clear and consistent criteria for characterizing non-responsive cases.[94]

1.6 Current treatment strategies and medical management

Ulcerative colitis is a heterogenous disease manifesting with several phenotypes, and various response to the different treatment alternatives. Consequently, a personalized approach is essential to achieve the treatment target. In cases of acute severe UC, an initial, aggressive intervention is imperative. This may involve high-dose corticosteroids, antibiotic therapy, early initiation of anti-TNF treatment, and in certain instances, immediate consideration of colectomy.[95] Management of hospitalized patients with acute severe colitis is not further elaborated in this thesis.

1.6.1 Treatment goals

As previously described, trials employ a multitude of definitions for remission and endpoints. For patients, relief from symptoms is a crucial factor in their daily lives. However, achieving remission based on subjective measurements, such as patients' reported outcomes, has resulted in relatively high remission rates, even in placebo groups. Notably, persisting endoscopic inflammation can be observed even when patients report normalization of rectal bleeding and stool frequency.[96,97] With emerging evidence indicating that stricter endpoints may positively alter the disease course, leading to more favourable outcomes such as sustained remission and lower rates of colectomy and hospitalization, it becomes evident that treatment beyond symptom relief is necessary. Currently there is general agreement to aim for an endoscopic healed mucosa, with a MES of ≤ 1.[83] Recent studies have even suggested that achieving an MES of 0 might further improve clinical outcomes.[98,99] Recently a new definition of a composite outcome have been introduced, termed “Disease clearance,” which includes

complete histological and endoscopic remission along with clinical remission. This approach has demonstrated a lower risk of hospitalization and surgery in UC patients.[91]

1.6.2 Step up and top down

The two most applied guidelines in treatment of UC, The European Crohn's and Colitis Organization (ECCO) guidelines and American Gastroenterological Association (AGA) clinical care pathway. Both advocate treatment based on location and severity of disease.[85,95,100] In general, a medical step-up strategy is followed, starting with local 5-ASA for mild proctitis or left-sided colitis, and escalating to per oral 5-ASA, systemic or prednisolone, or locally acting budesonides for more extensive disease, as illustrated in Figure 5. Disease activity and treatment effects should be closely monitored, and treatment escalation with IMiDs or targeted treatments (biological, anti-adhesion, or small molecule drugs) should be considered to achieve the treatment goal, following a treat-to-target strategy.[83]

In contrast to step-up, a top-down strategy has been proposed and shown to yield favourable outcomes in CD. [101,102] In UC there are few studies on early initiation of targeted treatment. However, one study has demonstrated a reduced risk of surgery and hospitalization associated with the early attainment of combined CR, ER and HI.[91] As previously described there is emerging evidence indicating lower colectomy rates during over the recent decades, coinciding the introduction of advanced treatment alternatives. This points toward a therapeutic window to prevent severe outcome of disease.
The main challenge in a top down approach in UC is overtreatment due to the lack of clinical, biochemical or molecular -biomarkers to predict disease course and accurately pick the correct patients with a high risk of severe outcome.[103,104]

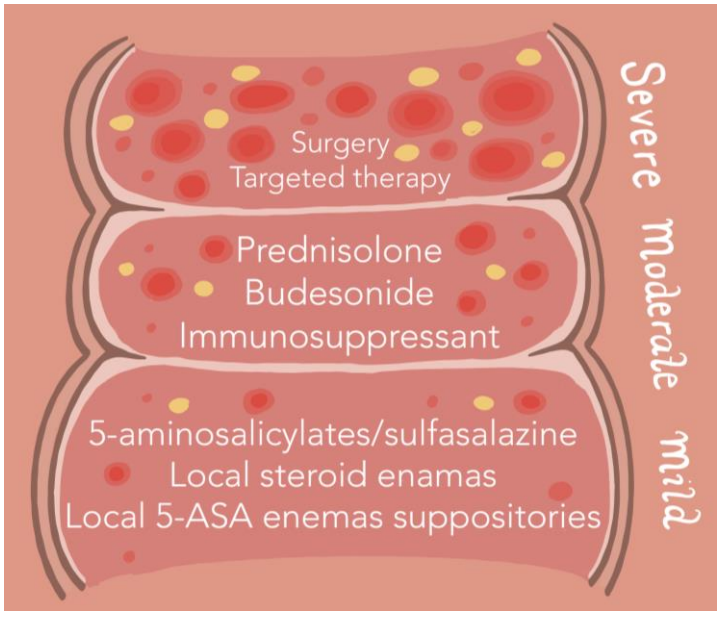


Figure 5: Therapeutic treatment escalation according to disease severity. (Ina Rye-Holmboe)

1.6.3 Medical management

As previous described the most widely accepted treatment strategy is aiming for endoscopic remission MES≤1 and treat to target approach. Over the past two decades, there has been significant evolution in the medical management of UC, particularly with the advent of targeted therapies. However, traditional untargeted drug classes such as 5-ASA and corticosteroids, which have been available since the 1940s to 1990s, continue to play a pivotal role in both the induction and maintenance of remission (see Figure 6). The following outlines of medical management is based on the AGA and ECCO guidelines, with additional insights from other sources referenced throughout the text. [84,105,106]

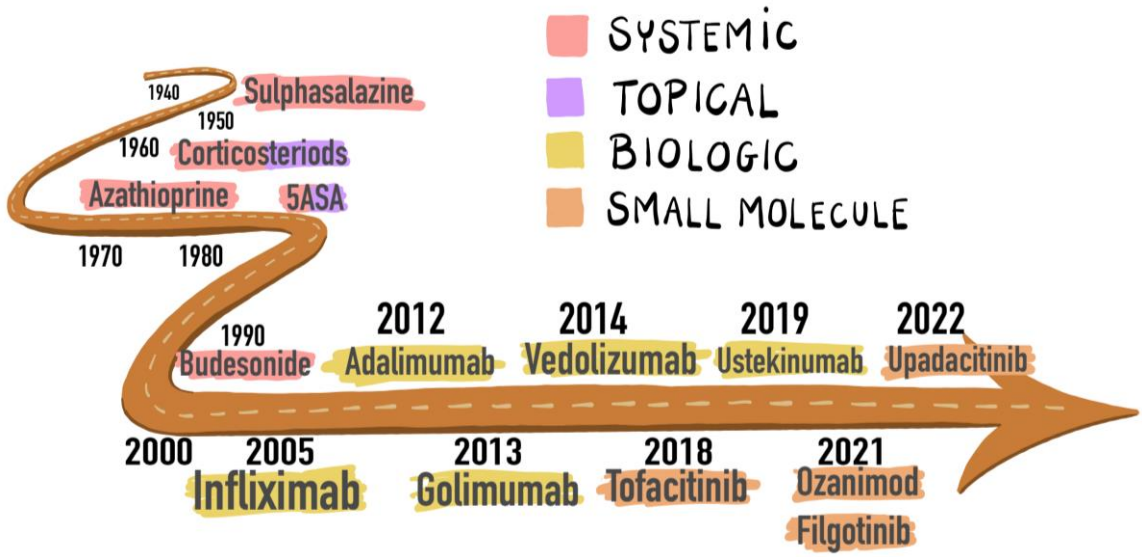


Figure 6: Timeline of introduction of therapy in ulcerative colitis (Ina Rye-Holmboe)

1.6.3.1 Untargeted treatment (5-ASA, corticosteroids, IMiDs, surgery)

Both 5-ASA and corticosteroids are accessible in both systemic and topical formulations. Typically, topical formulations are employed for patients with distal disease distribution and mild to moderate disease activity. If there is suboptimal effectiveness or a lack of adherence to local therapy, the initial course of action involves escalating to systemic 5-ASA and/or corticosteroid compounds, with or without concurrent use of topical treatments. This stepwise approach allows for a tailored and responsive adjustment in treatment strategies based on individual patient responses and therapeutic needs.

5-aminosaliculic acid (5-ASA): Sulfasalazine (5-ASA linked to sulfapyridine) was the first drug used and became standard therapy in the treatment of UC between 1940-1960s.[107] The mechanism of action is not fully understood but it is suggested that 5-ASA acts on nuclear receptors in the epithelial cells involved in the control of inflammation, cell proliferation and apoptosis.[108] Sulfasalazine has later

been replaced with other formulas without sulfapyridine due to adverse reaction, but can still be beneficial in patients with IBD associated joint pain. As a first-line therapy for all UC patients, 5-ASA is strongly recommended. For patients with distal disease distribution, topical 5-ASA treatment is considered superior to systemic 5-ASA and locally acting corticosteroids. In cases of rectal disease, the use of 5-ASA suppositories has demonstrated high efficacy, achieving an 84% efficiency in inducing endoscopic remission at 4 weeks.[109] With more extensive disease per oral 5-ASA alone or in combination with topical treatment is recommended. Systemic 5-ASA have shown to be effective in inducing and maintain clinical remission in patients with mild to moderate UC.[110,111]. Common adverse events associated with 5-ASA occur in up to 15% of patients, with flatulence, abdominal pain, nausea, diarrhea, headache, rash, and thrombocytopenia being the most prevalent. A rare, more serious, adverse effect is renal failure due to nephrotoxicity.[112] Moreover, 5-ASA possibly reduce the risk of CRC in UC patients with disease distribution beyond rectum, and is recommended to be continued indefinitely if well tolerated as concomitant therapy.[113]

Corticosteroids (CS): CS in the treatment of UC were established in the 1950s. [114] A pivotal moment in the management of acute severe colitis occurred during the 1970s with studies involving intravenous methylprednisolone. Conducted by Truelove and colleagues, these studies marked a paradigm shift in the approach to treating acute severe colitis.[115,116]

Corticosteroids bind to glucocorticoid receptors present in all human cells, translocating to the nucleus, where they downregulate transcription factors responsible for inflammatory cytokines. This mechanism results in a potent immunosuppressive effect. However, due to the abundance of glucocorticoid-responsive targets in the genome, corticosteroids also induce a broad spectrum of severe adverse effects Common side effects encompass insomnia, acne, weight gain with cushingoid features, hypertension, hyperglycemia, glaucoma, dyspepsia and ulcer, psychiatric complications, osteoporosis, and an overall increased risk of mortality.[117-119]

Similar to 5-ASA, CS is available in different modes of delivery, intravenous, per oral prednisolone and budesonide multimatrix-structure (MMX) and topical treatment with enemas that act locally. Budesonide MMX, characterized by low systemic bioavailability, is associated with fewer adverse effects compared to other corticosteroids. While budesonide MMX may be considered less potent than prednisolone, it is recommended as an alternative in specific patient populations where a reduced systemic impact is desirable.[105,120] In the introduction studies budesonide MMX showed a combined clinical and endoscopic remission rate of 17.7 percent in UC at 8 weeks, proving superior to placebo. However, a systematic review indicates that its effectiveness is not significant in cases of extensive disease.[120,121] Corticosteroids are recommended as first line therapy in addition to 5-ASA in patients with moderate to severe UC and second line therapy for mild-moderate UC who have failed to achieve remission on 5-ASA. The use of oral corticosteroids for maintaining remission is discouraged due to their adverse effects, making CS-free remission a crucial outcome in studies. Despite the increasing availability of corticosteroid-sparing therapeutic alternatives in the last two decades,

numerous studies reveal that the use of corticosteroids, prolonged courses, and repeated treatments remains widespread and has not distinctly decreased over time.[14,122-125] Much of the exposure to corticosteroids seems avoidable, emphasizing the need for strategies to mitigate excess use.[119]

Immunomodulating drugs (IMiDs): The first IMiD to show efficacy in UC was mercaptopurine (6-MP) during the 1960s.[126,127] Subsequently, azathioprine, a prodrug of 6-MP, was introduced in the 1970s.[128] The mechanism of action for thiopurines is intricate, with the primary mode likely being the inhibition of DNA synthesis, particularly impacting rapidly dividing cells such as inflammatory cells and cancerous cells. The discovery of 6-MP was acknowledged with the Nobel Prize in 1988.[129]

The IMiDs share similar side effects, and the frequency of discontinuation due to adverse events can vary, with reports of up to 40 percent. Adverse events associated with IMiDs encompass bone marrow suppression, hepatotoxicity, infectious complications, pancreatitis, general malaise, arthralgia, and gastrointestinal symptoms. Thiopurines, in particular, are linked to an increased risk of malignancy, including non-melanoma skin cancer and lymphoma.[130-133] In line with ECCO guidelines, the use of thiopurines is discouraged in patients aged above 65 years due to safety concerns.

It is not recommended to use IMiDs in monotherapy to induce remission due to no clear benefit compared to 5-ASA, safety, tolerability and delayed onset of effect.[105,134] The primary indication for IMiD use is to maintain remission induced by CS, 5-ASA or targeted treatment. Dosing of thiopurines are adjusted by measuring of 6-MP metabolites. In combination therapy with anti-TNF, dose reduction of thiopurines might me equally effective.[135]

Surgery: During the disease course some patients may exhibit non-responsiveness to treatment, develop high-grade dysplasia or CRC, necessitating colectomy. The 5- and 10 year cumulative risk of colectomy in UC is reported to range between 7.8-9.8 percent.[9] In UC patients with moderate to severe disease with need of biological therapy, the 36-month risk of surgery is reported to be approximately 40 percent.[136] For patients receiving surgery, there are mainly two options, proctocolectomy with ileal pouch-anal anastomosis or end-ileostomy. For further details see ECCO guideline.[137]

1.6.3.2 Targeted treatment (anti-TNF, Anti-integrin, small molecule)

The introduction of anti-TNF therapy represented a paradigm shift in efficacy of UC treatment but also represented a transition from nonspecific (untargeted) drugs to targeting selected cytokines and inflammatory signalling pathways, as illustrated in Figure 7. According to guidelines, targeted therapy is recommended in patients who fail du achieve remission on CS or are CS dependent to maintain remission. In Norway which order to select targeted therapy is decided by the joint procurement of pharmaceuticals between health regions.[138] There are limited head-to-head comparisons regarding the efficacy and safety of various targeted therapies. Several ongoing or planned head-to-head trials aim to address this gap in comparative data.[139]

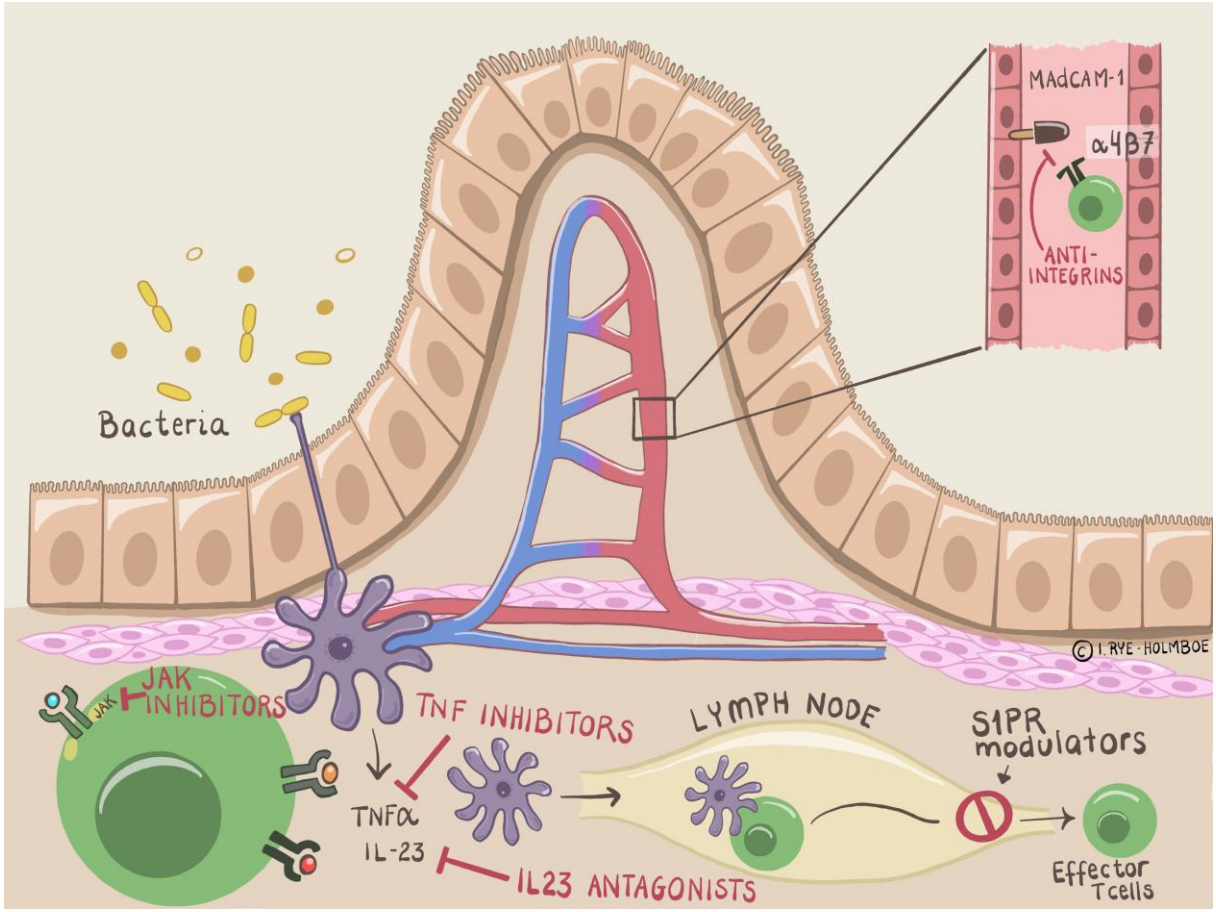


Figure 7: Targeted therapy mechanism of action (Ina Rye-Holmboe)

Ant-TNF: Current approved anti-TNF drugs for UC, infliximab, adalimumab and golimumab, inhibits both sTNF and tmTNF.[140] Infliximab distinguishes itself from adalimumab and golimumab through its chimeric antibody nature, characterized by the incorporation of both human and murine components. In contrast, adalimumab and golimumab are exclusively comprised of fully human monoclonal antibodies. This variance in composition holds notable implications for factors such as immunogenicity and potential side effects during clinical use. [141] The formation of ADAs due to

immunogenicity can be reduced by concomitant treatment with IMiDs.[142] While there are no direct head-to-head studies comparing anti-TNF drugs, a meta-analysis demonstrated a preference for infliximab over adalimumab after 8 weeks of treatment. However, it's noteworthy that by week 52, no statistically significant difference between the two was observed.[143]

Anti-integrin: Vedolizumab, approved for UC in 2014, marked a milestone as the first drug to target a cytokine other than TNF.[144] It operates by inhibiting leukocyte trafficking by blocking gut-selective $\alpha_4\beta_7$ integrin on T cells, thereby preventing their binding to cell adhesion molecules on endothelial cells in the gut blood vessels. In a head-to-head study, vedolizumab demonstrated superiority over adalimumab in its primary endpoint, revealing a statistically significant difference in remission rates at week 52. Vedolizumab also achieved a higher clinical response at week 6 during the induction period and demonstrated a lower rate of exposure-adjusted infections, with few disparities regarding serious adverse events.[145] However, a notable limitation of the study was the absence of dose adjustment for either drug, a practice recommended by guidelines that has demonstrated efficacy in the treatment of IBD.[146-148]

IL12/IL23 inhibitor: Ustekinumab, a monoclonal antibody, binds to the p40 subunit of IL-12 and IL-23, thereby preventing downstream signalling, gene activation and the production of inflammatory cytokines.[149] The introduction studies showed higher efficacy than placebo and similar adverse events.[150] Alongside vedolizumab, ustekinumab is the only targeted therapy that has undergone a double-blinded head-to-head comparison. In the SEAVU trial of ustekinumab versus adalimumab in CD there was no difference in the primary endpoints. Recently a selective IL23 inhibitor, riskankizumab have showed better results in head to head trial versus ustekinumab in CD, and phase III studies in UC is announced to have met the primary and secondary outcomes, neither studies are currently published.[151]

Janus kinase inhibitor: Several Janus kinase (JAK) inhibitors have received approval for the treatment of UC. In contrast to monoclonal antibody drugs, JAK inhibitors differs in being small molecules with a short half-life, rapid onset of action, no immunogenicity and being administrated orally. The mechanism of action involves blocking one or more intracellular tyrosine kinase (Tyk) and consequently downregulating Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway involved in inflammatory cytokine production. Currently, three JAK inhibitors have gained approved, tofacitinib, upadacitinib, and filgotinib. These inhibitors vary in their selectivity towards different tyrosine kinases, namely JAK1-3 and Tyk3.[152] Across introduction studies conducted between 2017 and 2021, all three JAK inhibitors demonstrated superior efficacy compared to placebo.[90,153,154] A network meta-analysis revealed no significant differences in adverse events.[155] However, there is a lack of direct head-to-head comparisons, necessitates further studies to assess and compare the efficacy and safety between JAK inhibitors, and in relation to monoclonal antibodies.

Sphingosine-1-phosphate receptor (S1P) modulator: Ozanimod being the sole approved agent currently demonstrating efficacy. Its mechanism of action involves mitigating the migration of lymphocytes from lymph nodes to inflammatory sites.[156] However, it's noteworthy that no head-to-head clinical studies have yet compared Ozanimod to other targeted therapies in the context of UC.

1.6.4 Personalized medicine

"Personalized medicine refers to… the tailoring of medical treatment to the individual characteristics of each patient . . . to classify individuals into subpopulations that differ in their susceptibility to a particular disease or their response to a specific treatment"

President's Council on Advisors on Science and Technology 2008 p. 7[157]

The terms "precision medicine" and "personalized medicine" are often used interchangeably. Various opinions exist about their definitions, with some suggesting that "personalized medicine" might be misconstrued to imply uniquely tailored treatments for each person, while "precision" is this context could suggests a meticulous and highly targeted approach.[158] In this thesis, both terms, "precision" and "personalized medicine," are considered synonymous, representing the same conceptual framework..

1.6.4.1 Biomarkers and predicting clinical outcome

There is an increasing knowledge of the pathogenesis and inflammatory cytokines involved in the mucosal inflammation in IBD. [159] Following the guidelines mentioned earlier, it is evident that not all patients require early intensive treatment. It is apparent that improved tools are necessary to accurately identify and select the right patients for more aggressive treatment.[104] Certain risk factors for a more severe disease course in patients with UC are recognized, including male gender, young age, and elevated levels of pANCA in the serum. However, none of these factors are fully validated or integrated into the treatment algorithm.[159] In IBD, measurement of the enzyme Thiopurine S-methyltransferase have shown to identify patients with higher risk of adverse effects of thiopurine treatment.[160] In CD HLA-DQA1*05 allele have shown increased risk of ADAs, while individuals with a homozygous risk allele TL1A -358 C/C have shown reduced risk of surgery.[161,162]

1.6.5 Summary of introduction

Ulcerative colitis involves mucosal inflammation with varied distribution in the colon, leading to diverse inflammatory and symptom burdens. The natural disease course, or perhaps more accurately described as the "therapy-modified," exhibits heterogeneity. In patients with severe disease requiring targeted therapy, long-term outcomes after discontinuation remain poorly described. Despite advancements in new drugs, many patients still experience insufficient treatment effects.

The current medical approach adopts a step-up strategy, aiming to achieve mucosal healing. However, a notable gap exists in the absence of biomarkers that can accurately predict the disease course and identify patients who would benefit from initial aggressive treatment. An improved management approach involves tailoring therapy, often referred to as personalized medicine, utilizing biomarkers to predict the risk of severe outcomes.

While the goal is to attain and sustain remission, there is a conspicuous lack of clear guidelines on how to de-escalate treatment. This uncertainty stems, in part, from inadequate studies demonstrating the long-term outcomes after discontinuation of targeted therapy and the absence of biomarkers that could indicate the opportune time for treatment de-escalation.

2 AIMS OF THE THESIS/RESEARCH QUESTIONS

2.1 Hypotheses

1. Early achievement of endoscopic remission could potentially alter the individual phenotype, steering it away from a more aggressive disease course.

2. Indefinite maintenance of targeted treatment is not necessary, reinitiating targeted treatment during periods of heightened disease activity could be a viable approach.

3: Assessment of inflammation severity based on biomarkers has the potential to aid in identifying patients suitable for top-down treatment at the onset of the disease.

2.2 Aims

Aim 1: Present a treatment algorithm with an intensified induction treatment with infliximab and discontinuation on remission, and to identify possible biomarkers for a more personalized medical management. (Paper 1)

Aim 2: Present the effect after reintroduction of anti-TNF therapy in patients that relapse after anti-TNF discontinuation. (Paper 1)

Aim 3: Discover and validate possible biomarkers at the onset of the disease to accurately identify patients suitable for an initial top-down treatment strategy. (Paper 2)

Aim 4: Characterize the therapy-modified disease course of patients treated with infliximab, and discover potential prognostic biomarkers for long-term outcomes. (Paper I and III)

3 MATERIAL AND METHODS

3.1 Study population

Inclusion: Patients enrolled in Paper I and the calibration cohort in Paper II were selected from the Advanced Study of Inflammatory Bowel Disease (ASIB) at the University Hospital of Northern Norway during the period 2004-2014. The patient cohort in Paper III comprises a subset drawn from those included in Paper I. The validation cohort in Paper II, as well as individuals for cytokine measurements of early relapse and healthy controls (HC) in Paper II, were recruited from ASIB and collaborating gastrointestinal units at Kirkenes, Hammerfest, Bodø, Drammen and Hønefoss - hospitals. Recruitment took place between 2014 and 2018.

Healthy controls included individuals referred for colonoscopy to rule out cancer, with normal findings on colonoscopy. Exclusion criteria encompassed serious medical conditions, immunological disorders, irritable bowel symptoms, polyps, cancer, or abnormal histology in colonic biopsies.

Table 4: Patients included and study design

	Included	*Disease severity*	*Study type*
Paper I	Intention to treat N= 116 Per protocol N= 101	Moderate - severe UC	Prospective cohort study
Paper II	Calibration cohort N= 66 validation cohort N= 89	Mild to severe UC Mild to severe UC	Prospective cohort study
Paper III	Long-term outcome N=75 Early relapse N= 9 Healthy controls N= 24	Moderate - severe UC Moderate – severe UC	Prospective cohort study

Ethics, permissions and funding: All participants were informed and signed a written consent. The study was performed according to the Helsinki declaration. The protocol including establishment of the project biobank, was recommended by the Regional Committee of Medical Ethics of Northern Norway Ref No: 1349/2012 and 14/2004. The studies were founded by Northern Norway Regional Health Authority, ID SFP-50-04, SFP-888-09 and SFP-1136-13.

Definition of disease severity: The degree of illness was evaluated using the Ulcerative Colitis Disease Activity Index (UCDAI/Mayo) scoring system. Colonic mucosal inflammation was graded 0–3 using the UCDAI endoscopic subscore (MES). Moderate to severe UC was defined as an UCDAI of 6–12. [163]

Definition of remission: Remission was defined as a reduction in the UCDAI score to less than 3 or together with an endoscopic subscore of 0 or 1.[164] In the clinical evaluation and

follow up, remission was defined by the partial mayo < 2.

Definition of relapse: In Paper I, relapse was defined as an increase of UCDAI greater than 3 and endoscopic score greater than 1.[164] In Paper III, relapse was defined as clinical, biochemical, endoscopic signs of disease activity leading to a therapeutic intervention as escalation of medical therapy or surgery.

Definition of clinical outcomes: In Paper II, clinical outcomes were defined on by the required treatment level one year after diagnosis of UC to obtain disease remission, using the step-up algorithm guidelines ECCO and the three levels proposed by Danese et al.[84,165]

Criteria for discontinuation of anti-TNF treatment: In Paper I the criteria were clinical or endoscopically remission, as previous defined. In Paper III some patients who discontinued anti-TNF after 2014 had an additional criteria of normalized mucosal TNF gene expression.[166]

3.2 Tissue handling

Biopsies for histological assessment and mucosal gene expression measurement were sampled with a standard biopsy forceps 2.8 mm. In active UC, biopsies were sampled form the region with most inflammation and in remission from the same region. In the HC, biopsies were sampled from the sigmoid colon. For histology, biopsies were immediately contained in 10% formalin and for gene expression measurements in RNA later (Ambion, Austin, TX/ Qiagen). The RNA later was stored in room temperature, maximum overnight, then at -20 °C until RNA isolation.

3.3 Quantitative Polymerase Chain Reaction

In this thesis quantitative polymerase chain reaction (qPCR) is used to measure the transcription activity of a gene, by quantifying messenger ribonucleic acid (mRNA). mRNA is a copy of a gene that contains the instruction (recipe) for a protein for example a cytokine. The amount of copies of a gene reflects the activity of gene expression. The qPCR method can be described in the following steps.[167]

1. Extraction of total RNA from the cells in the specimen.
2. Reverse transcription in which the mRNA is copied in equal number copies of complementary DNA (cDNA)

3. Amplification of cDNA with a dual-labelled TaqMan hydrolysis probe (fluorescence dye and quencher) where the number of cycles needed to reach a detectable threshold for the fluorescent signal corresponds to the number of copies in the original sample (low cycle threshold number corresponds to high initial copy number and vice versa). Use of an absolute standard curve enables quantification to copy number containing dyes is performed real time.

The RT-qPCR analysis in Paper I-III were performed at our laboratory by a specialized bioengineer. For further details regarding the RT-qPCR, see methods part Paper I-III.

3.4 Histology

In Paper II, we present a biomarker for predicting severe outcomes in UC, which includes a histological score. Initially all histopathological samples were evaluated by a experienced gastrointestinal pathologist. The assessment involved scoring based on the Geboes Score, utilizing white light microscopy. Given that the Geboes Score lacks a scaled scoring system, we converted the scores to the Robarts Histopathology Index (RHI), which is scaled. This conversion was undertaken to streamline the statistical analysis process. Notably, the RHI encompasses the same parameters as the Geboes Score (refer to Table 5 for details)

Table 5: Robarts Histopathology Index conversion from Geboes Score [97]

	1x	*Chronic inflammatory infiltrate level (4 levels)*
Sum of	2x	Lamina propria neutrophils (4 levels)
	3x	Neutrophils in epithelium (4 levels)
	5x	x Erosion or ulceration (4 levels after combining Geboes 5.1 and 5.2)
		Total score range: 0-33 (no disease activity to severe disease activity)

3.5 Statistical methods

All statistical analyses and graphs in Paper I-III were performed and created in IBM SPSS statistics version 22, and 24 respectively (IBM Corporation, Armonk, New York, USA).

Paper I: Survival (time to relapse) were first analysed using Kaplan-Meier with different potential predictors. Possible predictors (log rank $p<0.10$) were then retained in a multiple regression by Cox proportional hazard analysis were factors were reduced until all were significant. For prediction of outcomes multiple logistic regression were performed.

Paper II: Difference in baseline characteristics were performed by non-parametric testing, first performing a global Kruskal Wallis test, and then Mann-Whitney U with Bonferroni correction. For categorical variables, Chi-square with Bonferroni correction were performed. To evaluate predictors

of outcomes, receiver operating characteristics curves (ROC) were constructed, thereafter picking the optimal cut-off by maximal Youden's J. For further description se article.

Paper III: To find difference in expression of mucosal cytokine transcript between groups, two-way ANCOVA were performed adjusted sex, age, disease distribution. The ΔΔCT fold change difference were calculated and converted to a positive scale and indicated whether down or up regulated compared to reference group. To find predictors of outcome Cox regression was performed and ROC curves constructed from significant predictors from the Cox regression. By selecting the optimal cut-off by maximal Youden's J sensitivity and specificity were calculated.

4 SUMMARY OF RESULTS

4.1 Paper 1

Repeated intensified infliximab induction - results from an 11-year prospective study of ulcerative colitis using a novel treatment algorithm. ***European Journal of Gastroenterology and Hepatology. 2017***

In this study of moderate to severe UC, we performed a novel treatment algorithm with an intensified induction treatment with IFX until remission, followed by anti-TNF discontinuation. In short, patients underwent the initial standard treatment with IFX at weeks 0, 2, and 6, followed by subsequent administrations every 4 weeks until achieving endoscopic remission (Mayo 0-1). In the event of a relapse, patients received retreatment with anti-TNF based on clinical judgment, and the same algorithm was repeated. Additionally, patients received concomitant treatment with IMiDs, 5-ASA and CS tapering.

The aim of this study was to evaluate the efficacy of the intensified infliximab induction treatment, evaluate the outcomes after IFX discontinuation, and compare this algorithm to outcomes reported for maintenance treatment in terms of colectomy rates and loss of effectiveness with IFX. The impact of retreatment was also explored. Furthermore, the study aimed to identify biomarkers with high clinical utility for selecting the optimal time for IFX discontinuation.

A total of 116 patients were enrolled in the intention-to-treat analysis. The intensified induction demonstrated high remission rates with 83% of the patients in the intention-to-treat group and 95% in the per-protocol group achieving remission.
Reintroducing IFX also show high efficacy to induce remission in the second and third

induction treatment, with 93- and 91 percent achieving remission per protocol and 56 and 59% of the patients intended. Other important findings were that 29, 42 and 21 percent of those who achieved remission in the first, second and third induction treatment did not relapse during the observation time (median 52 months). This highlights the importance of finding biomarkers that can select the correct patients to down escalate treatment as many patients will not relapse during the long-term course.

Of the initial 116 patients 24 underwent colectomy, 12 during the first induction therapy. This is according to previous described outcomes. The relatively high numbers of patients that needed colectomy during the first induction therapy reflect the high disease severity in this cohort. We also found that the algorithm did not clearly increase the risk of colectomy during the long-term course. Male sex and high age were det only identified risk factors for surgery. Regarding other aspects of safety, the results showed that 9 percent experienced severe allergic reactions with the highest percentage in the second induction therapy. Infectious complications were observed, but only temporary withhold of treatment were required.

In the analysis of possible predictors, mucosal TNF mRNA expression was a significant predictor of time in remission and remission without relapse. Patients with normalized TNF expression were median time in remission 33 months versus 11 months is those with elevated levels at IFX discontinuation Non-smoking and lower pre-treatment UCDAI score were also predictive of prolonged time in remission and remission without relapse.

In summary, this study demonstrated the high efficacy of our algorithm in inducing remission during initial and repeated inductions, with colectomy rates comparable to those reported for maintenance treatment. The normalization of mucosal TNF mRNA expression emerged as a potentially biomarker for predicting prolonged time in remission and remission without relapse after IFX discontinuation.

4.2 Paper 2

Discovery and validation of mucosal TNF expression combined with histological score - a biomarker for personalized treatment in ulcerative colitis. ***BMC Gastroenterology 2020.***

The aim of this study of newly diagnosed patients with UC, was to find biomarkers at disease debut

that could accurately predict the clinical outcome within the first twelve months. Discovery and validation of biomarkers to predict severe outcome, defined as need of targeted therapy or colectomy, would be of highest interest. It would be of high clinical utility and a step toward personalized therapy if a test could select the patients with the most severe disease phenotype for more aggressive treatment from debut of disease, in contrast to the general step-up management.

The study encompassed two patient cohorts: one for the discovery and calibration of potential biomarkers and a second cohort for validating these biomarkers. Sixty-six patients were included in the discovery cohort, and 89 patients were included in the validation cohort. Twelve months after the onset of the disease, clinical outcomes were categorized as mild, moderate, or severe based on the highest treatment levels required to achieve clinical remission during this initial 12-month period (Figure 5).

In the calibration cohort, mucosal TNF mRNA expression demonstrated high test performance as a single factor in predicting severe outcomes, with sensitivity and specificity values of 0.81 and 0.91, respectively. To enhance specificity at the expense of sensitivity, a combined test was developed by first selecting those positive for the cut-off value for mucosal TNF expression. When combined with the RHI, the specificity improved. Conversely, common clinical factors such as the UCDAI score, endoscopic score, and calprotectin values exhibited low performance in predicting the disease course. In the validation cohort this two-step test by first selecting mucosal TNF expression positives and then RHI test positives still showed high specificity 0.99 and a sensitivity of 0.44. The positive predictive, negative predictive value and the diagnostic odds ratio of the test were 0.89, 0.87 and 54. This can be explained as, the test would classify 54 patients correctly as severe outcome for every wrong classification.

In summary, this study aimed to identify biomarkers at the onset of UC for predicting clinical outcomes within the first year. Mucosal TNF mRNA expression, especially when combined with RHI, demonstrated promising potential as a predictor of severe outcomes, offering a step towards personalized therapy by identifying patients who may benefit from more aggressive treatment from the early stages of the disease.

4.3 Paper 3

Prediction of long-term remission in patients following discontinuation of anti-TNF therapy in ulcerative colitis: a 10 year follow up study. *BMC Gastroenterology 2022*

The aim of the study was to describe the therapy-modified disease course of patients with moderate to severe UC and to discover potential biomarkers for the long-term disease course.

Patients treated to remission following the treatment algorithm in Paper I were included to describe the long-term disease course. Patients withdrawn in Paper I due to switch to other biological

therapy was also included. The patient's outcomes were grouped after the highest treatment level needed the last three years of the observation time. In addition, HC and a cohort of patients treated to remission on anti-TNF who relapsed within the first year after anti-TNF discontinuation. These two groups were included for mucosal cytokine mRNA expression measurements to describe the difference in the molecular signalling between HC (N=24), patients from Paper I who are in long-term remission without medication or 5 ASA only, and patients in remission who relapse within twelve months after anti-TNF discontinuation (N=9). From the initial 116 patients in Paper I, 75 patients were included in this study with a median (IQR) observation time of 10(8-11) years. Of the 41 patients from the original cohort who were excluded from the present study, 11 were due to loss off follow up, 14 patients received treatment for other diseases, and 16 patients were primary non-responders.

The long-term disease course analysis revealed that 61% of patients achieving remission on anti-TNF therapy remained in remission without requiring targeted therapy for a median (IQR) time of 95 (71-128) months. Notably, a unique subset of patients in long-term remission without signs of relapse, managed with 5-ASA or even no medication, was identified. Mucosal TNF expression measured during remission after anti-TNF treatment emerged as a predictor of sustained remission and remission without the need for targeted therapy. Nineteen percent of patients underwent colectomy during the observation period, with young age and low mucosal TNF expression in remission associated with a significantly lower risk of colectomy. However, high values of mucosal TNF expression demonstrated a specificity of 0.80 for predicting colectomy risk, albeit with low sensitivity (0.10)

The previous mentioned phenotype of patients in long-term remission (LTR) without any signs of relapse had a histological healed mucosa. Despite this and no difference in mucosal TNF expression compared to HC, there was a clear upregulation of both pro- and anti-inflammatory mucosal gene expression. This indication that the immune activity in patients with UC will never fully normalize. When compared to other patients in remission who relapsed within the first twelve months, the LTR group exhibited normalized IL-17 and IL-23 pathways, but elevated levels compared to HC. The only signalling pathway that was normalized in the LTR group compared to HC and significant lower compared to the relapsing group were where IL1RL1, a ligand receptor of IL-33. This indicates L1RL1 as a potential fingerprint of a patient with a phenotype close to "cured" from UC.

In summary, the study provided insights into the prolonged disease course of UC patients following discontinuation of anti-TNF therapy and identified potential biomarkers, such as mucosal TNF expression and the IL1RL1 pathway, associated with long-term remission and risk of colectomy.

5 DISCUSSION

5.1 Methodological considerations

5.1.1 Study design

This thesis includes patients with UC that were included in the prospective ASIB study at the University Hospital of Northern Norway.

The patients in Paper I were treated following a determined algorithm and then followed over a relatively long period of time in a prospective cohort design. The individual clinical judgments were done by the gastroenterologist at the hospital and alterations in treatment were done without conferring the study group, in other words this thesis includes patients with real life outcomes in contrast to clinical trials outcomes.

There was also no control group to compare the treatment algorithm in Paper 1. The sample size of patients was limited by the number of IBD patients in the clinic, patients that agreed inclusion in the study, number of samples in the IBD biobank and some patients were excluded due to loss off follow-up. A large sample size would reduce the interference of individual gastroenterologist judgment on the outcomes and reduce the risk of statistical errors. Measurements of ADAs and though levels were not available at the time of this study, and could potentially contribute to explain primary and secondary loss off effect to IFX.

Paper II have a retrospective case control design of two patient cohorts. One for the discovery and calibration of predictors of clinical outcomes, and a second cohort to validate the candidate

biomarkers. This improves the reliability of the findings. In this study of patients at debut of disease were also included from participating centres in Northern Norway, and the clinical judgment of the diagnosis was done by the local gastroenterologist.

Paper III includes patients from Paper I in a similar study design with further follow-up. In addition, HC and a group of patients with relapsing UC were included from the IBD biobank for comparison in the gene expression analysis.

5.1.2 Internal validity

The internal validity in a study can be explained as to which extent the finding in the study can be explained by what is measured and cannot be explained by other interfering factors. Accurate measurements are of importance to avoid informational bias, this is further discussed in the sections below.

Selection bias: Non-IBD diagnosed with IBD is not uncommon.[168] In Paper I recruiting moderate to severe UC, each patient's diagnosis of IBD was revised by an experienced gastroenterologist, and the risk of wrong diagnosis is probably small. More common is confusing UC vs CD. The observation time of the study and confirmation of the diagnosis probably reduces the risk of false classification of UC in Paper I. In Paper II of newly diagnosed UC including mild – severe UC, wrongly diagnosed UC or classification of IBD is possible, but the resulting test for predicting UC with severe outcome would still apply in a similar real-life clinical setting. The sample size is also considered large enough to adjust for this bias.

The indication for anti-TNF treatment were according national and European guidelines. The treatment algorithm performed were according to local established guidelines for anti-TNF treatment and scheduled follow-up. Therefore, participation or reluctance to participate in the study would not cause difference in the treatment options. In general, the only difference was the number of biopsies sampled during normal scheduled colonoscopies. Participating patients could differ from other patients, very few patients refused to participate and effect of this is probably minimal on the measured results.

In Paper II HC were included. These were of older age than the UC group. The HC would ideally be participants without any symptoms or disease. Most commonly they were referred to colonoscopy due to suspicion of cancer. Symptoms of irritable bowel syndrome, polyps larger than 5 mm and signs of inflammation and abnormal histological score were exclusion criteria to ensure that they represented as healthy as possible colon. The sample size of the comparing groups in Paper II is small, this limits the strength of this data, and increases the risk of statistical errors. Hence, the findings from the mucosal gene expressions analysis require further investigations.

Confounding variables: Evaluating the clinical indication for anti-TNF treatment required two

experienced gastroenterologists. All gastroenterologist at the department are involved in the treatment of UC, therefore the clinical outcomes cannot be explained by interference of individual clinicians. Patients were included over a long period of time 2004-2014 in Paper I and III. In the initial inclusion period, the experience with anti-TNF treatment for UC was scarce among the gastroenterologist, and this could have influenced some of the clinical judgements in the time period. It is well known that that increasing knowledge on how to optimize the effect of anti-TNF, in example; concomitant IMiDs and therapeutic drug measurements have improved the care. Some patients intended to treat did not even receive the first 3 doses of anti-TNF implicating that they were probably very close to colectomy but were decided to receive anti-TNF as "a last resort" or that the clinicians did not show enough patience to await the effect of the treatment before deciding upon colectomy. Simplified, the time of inclusion and the physicians' performances might be an unmeasured variable that could affect the clinical outcomes.

Limitations of transcript analyses: RT-qPCR includes many steps that can influence the results. Therefore, guidelines are developed to improve the accuracy of the measurements.[167,169] The biopsies were immediately put on RNA later that reduces degradation of RNA. The samples were put in-20° freezer within 24 hours until RNA isolation. In Paper II and III the RNA isolation was performed by using automated column purification that reduces the risk of contamination. Other steps to prevent DNA contamination were different location for sample preparation and pre and post amplification. All mucosal gene expression analysis was performed by experienced bioengineers, performing these analyses on regular basis. Our method includes the use of reference genes previous validated and interplate calibration to adjust for technical variability.[31] In Paper II and III two reference genes was used ACTB and RPLP0 in contrast to only ACTB in Paper I. The quality of the RNA was controlled in Paper II and III by measuring RNA integrity number (RIN) value. After RNA isolation the samples were stored in -70°C freezer.

Limitation of histology assessment:

In Paper II, RHI was used in development of the two-step biomarker tool to predict severe outcome. As previous described RHI is one of the UC scores that have undergone most validation. In our study one experienced GI pathologist rated all histological samples, this may affect the cut of value in Paper II. Studies has shown an inter-rater correlation coefficient of 0.73-0,86, indicating moderate-good reliability, the intraclass correlation coefficient of RHI is shown to be excellent.[96,170,171]

Clinical and endoscopic factors:

The clinical evaluation of remission and relapse were as previous described according to acknowledged definitions, but the interpretation of the selected endoscopic and clinical scores relies

on subjective interpretation by the physicians and the patients. It is theoretically possible that patients in clinical remission were for example misclassified as MES 1 instead of MES 2 therefore discontinued anti-TNF treatment, but for this to happen systematically to a degree affecting the result is less likely. In some cases, a clear MES score was not defined in the medical record, therefore a MES score were composed by an experienced gastroenterologist by information in journal entry and photos from the endoscopy.

The diagnosis of UC was according to ECCO guidelines, hence there are no clear criteria. The studies do not include any patients classified as IBDU. The explanation for this is probably the long observation time in Paper I and III. It is likely that a clearer phenotype manifested itself during this observation time. In Paper II, newly diagnosed IBDU were not included. The incidence of IBDU have recently been reported to be only 4 % of patients with IBD, and would probably not interfere with the final results.[138]

5.1.3 External validity

External validity refers to the extent to which the results of a study can be generalized to other populations, settings, and time periods.[172] The indication for anti-TNF treatment were according to generally accepted criteria, and in Norway due to the national healthcare system, it is probably correct to state that the availability of this treatment is not influenced by neither patient nor hospital economy. Northern-Norway consist mainly of Caucasians and Sami ethnicity, but whether genetic factors influence the treatment response in unknown. The thesis in focused on UC and anti-TNF treatment and cannot be generalized to all IBD and targeted therapy in general. During the observation time in long-term studies the medical treatment has evolved, and therefore, the therapy-modified natural outcomes might be different in the following time period.

5.1.4 Statistical considerations

In the studies of predictors of clinical outcomes in Paper I and Paper II, the main limitations are probably sample size. In general, a larger sample size would reduce the risk of statistical errors. For example, in Paper I most patients were started with IMiDs during the induction treatment, we did not find any effect of IMiDs (unpublished) on the clinical outcomes; this might be due to few patients not receiving this medication. In Paper II, the discovery cohort is of small size, and the two-step test performed better in the discovery cohort. This can probably to some extent be explained by sample size. The sample size also limits the number of covariates in the statistical models. In Paper III we performed statistical analyses exploring potential important mucosal cytokine expression to define a healed mucosa and possible predictors of relapse. The small sample size is an obvious weakness and reduces the precision of estimates. Because of the exploratory approach, we did not perform correction

for family-wise error rate to reduce the risk of type I errors (mistakenly reject the null hypothesis). As a statistical consequence of this carries a greater chance of type II errors. Hence, these findings are not conclusive pending further validation studies.

5.2 Discussion of main results

In the introduction part if this thesis, the current management of UC treatment following the generally accepted guidelines with a step-up approach are described. The overall aim of this thesis is to get one step closer to a more personalized treatment approach due to the current challenges with a lack of tools, like clinical, biochemical, genetic or histological biomarkers to stratify patients for increased risk of different clinical outcomes.

In the papers composing this thesis we present a possible treatment algorithm in three important clinical scenarios of UC care:

1. Selecting the correct patients at debut of disease for a selective top-down treatment strategy with early introduction of anti-TNF therapy by using a combined biomarker of mucosal mRNA gene expression and histology score.
2. Achieving early and high remission rates following an intensified treatment algorithm with anti-TNF therapy.
3. Selecting the correct patients and time for discontinuation of anti-TNF therapy.

In addition to this, the therapy-modified disease course of UC is described which underlines the utility of the points above. This and other important findings will be further discussed in the following part.

5.2.1 The therapy-modified disease course

Current guidelines lack clear criteria for the discontinuation of anti-TNF therapy, and favour maintenance treatment. So far, few studies describe how therapy by anti-TNF modifies the long-term disease course. Generally, most patients maintain clinical remission on maintenance treatment within the first year. In the introductory studies for IFX, ACT I-II, a 77% response rate was reported after 1 year of maintenance treatment.[11] The annual loss of response can vary widely, ranging from 10-50%.[173] Following the intensified treatment algorithm in Paper I, the annual loss of effect was 8% the first five years. The emphasis on achieving mucosal healing (at that time defined as MES≤1) might be one explanation for the low loss of response to anti-TNF. There have also been concerns about ADAs formation with cyclic anti-TNF treatment. ADAs measurement and trough levels were not available at the time the study was performed, but the intensified treatment algorithm would obviously provide high trough levels which has shown an inverse relationship to ADAs formation. Most patients did also

receive IMiDs which also reduces the risk of ADAs. A retreatment strategy in case of relapse after discontinuation of anti-TNF treatment have also been studied in an RCT in CD. It showed that retreatment was highly effective and for the patients discontinuing anti-TNF treatment, the overall time in remission were only 2 weeks shorter compared to the group receiving standard therapy with maintenance and dose escalation in case of flares. There was also no difference in the ADAs levels between the two groups. Of note, all patients in that study received IMiDs. [173]

In Paper I, we observed that 51% of patients did not relapse by the end of the study, with a median observation time of over 4 years in remission. Approximately 70% of those who remained relapse-free received no more than two induction treatments, while the remaining 30% received a third retreatment.

In Paper III, we further described the long-term outcomes after anti-TNF discontinuation with a median observation time of 10 years after discontinuation. In contrast to the general agreement that maintenance treatment is required to maintain remission, this study showed that around 60 % of the patients who achieved remission in the first induction therapy, maintained remission without need of targeted therapy. The median(range) observation time was 91(71-128) months. Moreover 50% of these patients had no history of clinical relapse during this time, and the remaining 50% only needed escalation of untargeted treatment. Notably, in 48 percent of the patients who relapsed without need of targeted therapy, 5-ASA dose escalation or 5-ASA suppositories were sufficient to obtain remission. (unpublished). Of special note, in the group of patients with no history of relapse after the last induction therapy, there was a special subgroup of patients in long-term remission receiving 5-ASA only or no medical treatment. This indicates that the disease activity in moderate-severe UC might shift towards a milder phenotype in those treated with anti-TNF. The most comparable reports are the IBSEN studies, a larger cohort including UC from mild-severe disease activity. The 10 years follow up in this study reported decline in intestinal symptoms in 55% of patients which is similar to our results.[9] This further underlines that even patients with moderate to severe UC may in time show improvement towards a less aggressive phenotype, hence reduced need for indefinite biologic maintenance therapy.

Regarding colectomy, most patients in our studies received colectomy during the first induction therapy (14%). In the group of patients achieving remission on anti-TNF treatment, additional 12% of the initial patients intended to treat received colectomy during the 10 years follow up. The median time to colectomy was 38 months, emphasizing the highest risk of the most severe outcome, colectomy, during the first induction therapy. As previous described there is a clear trend towards declining colectomy rates following the introduction of targeted therapy. There is general agreement about a step-up approach with gradual escalating medical therapy to obtain the treatment target. The cumulative risk of targeted therapy in UC is reported to be ~10% in a Danish study. A study from Norway showed that 13% started with targeted therapy within 12 months after diagnosis. [13,174] During this phase of gradual escalation some patients experience poor effect and higher

inflammatory burden. A hypothesis for further investigation is whether a top-down strategy with early initiation of targeted therapy might reduce the risk of colectomy in these patients. Favourable outcomes have been reported with a top-down approach in Crohn's disease with early therapy using IMiDs, leading to a reduced risk of hospitalization.[175] Also, improved outcomes is reported when achieving early mucosal healing in UC.[88]

Given the relatively low cumulative risk for initiating targeted therapy within the first year after a UC diagnosis, a top-down strategy with targeted therapy could lead to massive overtreatment. Therefore, it is crucial to identify patients at the highest risk of severe outcomes for this strategy, necessitating a personalized therapy approach, as further discussed in the next section.

5.2.2 The need for a personalized treatment

Ulcerative colitis is heterogeneous in terms of disease severity and in which treatment is needed to obtain disease remission. The previous described step-up strategy, and care according to AGA and ECCO guidelines are probably most widely accepted. Due to the unpredictable response to treatment and various disease courses, some patients experience a prolonged time with high inflammatory burden and even worsening of disease until they either achieve response to treatment or receive surgical treatment. In patients obtaining remission on anti-TNF therapy, the general accepted strategy is to prescribe maintenance treatment. The decision on treatment intensity must carefully consider the balance between the risk of adverse effects and the potential for disease worsening. Emerging evidence suggests that not all patients require this ongoing maintenance treatment.[176] From a clinical point of view, it would be of high utility to find precision predictors for different disease courses, for example remission without need of biological therapy and patient in need of biological at debut of disease or in case of relapse.

The high cost of IBD care represents a major challenge to health-care systems in distribution of limited resources on limited budgets. De-escalation of unnecessary treatments that repeatedly require follow-up in first line healthcare would probably have a high impact on both patient's health and healthcare-satisfaction. As previously described, the use of CS is still widespread, and the global use have not decreased even tough targeted treatment is clearly a more favourable option in contrast to repeated treatment with CS. A possible explanation to this could be increasing prevalence of IBD in low and middle-income countries. Treatment algorithms with an exit strategy from high cost treatment would probably increase the availability of targeted treatment that is both more effective and includes less severe side-effects. A personalized treatment would therefore have a considerable contribution to alleviating the global burden of IBD.

5.2.3 Selecting patients for top-down treatment

The previously described current guidelines represent a step-up treatment approach in UC. Data from Norway has shown that 13 percent of patients will require targeted therapy within the first year after diagnosis.[13] The impact of early biological treatment in UC has not been fully investigated in RCTs. Meta-analysis has demonstrated favourable outcomes in CD with lower rates of surgery, but higher in UC. The results in UC might be explained by confounding disease severity.[177] In a retrospective study, early disease clearance in UC indicated significantly lower risk of hospitalization and surgery. An observation study of time from diagnosis of UC to initiation treatment with vedolizumab, revealed a higher response rate in the group of patients that received treatment within 30 days of diagnosis.[178]

The main benefit of a step-up approach is the avoidance of over-treatment and the risk of unnecessary side-effects, high treatment cost, regular follow-ups, and the possible development of antibodies to biological treatment. A drawback of this approach is the gradual worsening during the prolonged time to find the optimal treatment level, and perhaps an increased risk of more severe outcomes due to possibly missing the optimal therapeutic window to achieve disease remission.

To overcome these obstacles, biomarkers to select the correct patients that will require biological treatment to obtain remission would be of high clinical utility. So far, no biomarkers have been able to predict the clinical course with accuracy. In Paper II, possible clinical, histological, and biochemical signatures were investigated in a discovery and validation cohort. The established clinical parameters, including endoscopic score, symptom score, faecal calprotectin, and histological score, individually exhibited poor performance in selecting the correct patients for a top-down strategy. Mucosal TNF expression was identified as the most accurate single predictor with high sensitivity of 81% and a specificity of 91%. When combining histological score (RHI) and mucosal TNF expression, specificity improved at the cost of sensitivity. This was a deliberate choice to avoid starting top-down treatment in patients who would not need biologic therapy. In the validation cohort, the proposed combined biomarker with mucosal TNF and RHI showed a specificity of 99 percent and a sensitivity of 44%. This yields a diagnostic odds ratio of 54. This implies that mucosal TNF expression combined with RHI could serve as a possible biomarker to select the correct patients for a top-down strategy.

5.2.4 Intensified treatment algorithm

In the previous section a strategy to select the correct patients for targeted therapy were described. The next step would be to achieve a high rate of remission and early disease control. Prolonged time to find the optimal treatment is associated with more severe disease outcomes and prolonged time with disease burden for the patients. During the last two decades following the introduction of anti-TNF therapy with infliximab, knowledge has been improved on how to optimize the drug effect, including therapeutic drug monitoring (TDM) and ADAs measurements. Low drug levels and ADAs are

associated with loss of effect to infliximab.[179-182].

Some patients will not achieve an optimal drug level on infliximab with the standard dose regimen of infliximab 5 mg/kg at week 0, 2 and 6, thereafter every 8 weeks. Especially the group of patients with more severe disease. It is shown that patients with acute severe colitis (ASUC) have lower drug levels.

In Paper I, we evaluated the effect of a treatment algorithm with a similar strategy, in contrast to dose escalation we performed an intensified induction treatment with the standard dose 5 mg/kg but the patients received treatment every 4 week instead of every 8 weeks after the initial 3 infusions. The treatment algorithm performed in this study also included discontinuation of infliximab with endoscopic remission as criteria. Of notice, at the time of the study, measurements of TDM and ADAs were not available. The intensified treatment algorithm showed high efficacy in inducing remission with 83% of the patients intended to treat and 95% per protocol achieving remission during the first infliximab treatment. In patients that relapsed after discontinuation, retreatment with the same algorithm were still effective in inducing remission.

The intensified treatment strategy in our study represents a potential alternative to the standard dose regime. It aligns with a top-down approach, sharing the rationale of expediting optimal treatment levels to minimize the duration of high disease burden. Given that the highest risk of colectomy occurs during periods of elevated inflammatory burden, as previously discussed, optimizing treatment has the potential to reduce the rate of colectomies. Therefore, adopting a more aggressive initial approach, not only with ASCUC, but for all patients with moderate to severe UC requiring targeted therapy, could mitigate the elevated risk of severe outcomes. However, it's important to acknowledge potential drawbacks, such as an increased likelihood of severe adverse events associated with intensified anti-TNF treatment. Despite these concerns, our study did not observe high rates of severe adverse events. Notably, all patients with infections were able to continue anti-TNF treatment, and the incidence of loss of effect due to allergic reactions was generally low.

A similar intensified strategy has been tested for adalimumab in an RCT, SERENE-UC trial that showed a clinically meaningful benefit of receiving treatment with standard dose every week, compared to every other week. The safety profile was similar in the two groups.[157] Currently there is an ongoing RCT comparing standard induction dose infliximab 5 mg/kg versus 10mg/kg in patients with ASUC (Predict-UC study).

5.2.5 Treatment de-escalation

Novel strategies to enhance and intensify medical treatments emerge; these involve measuring drug levels for dose escalation, monitoring ADAs, and transitioning to alternative targeted therapies in the event of therapeutic failure. Additionally, recent findings suggest that adopting more stringent criteria for remission as a treatment target yields improved clinical outcomes. However, in notable contrast,

our understanding of safe de-escalation of therapy remains limited.

There is no definitive cure for UC, and its inherently relapsing nature results in significantly higher relapse rates upon discontinuation of targeted therapy. This constitutes a compelling argument against discontinuation of targeted therapy. Studies indicate that maintenance treatments fall short of ensuring immunity against disease flares, with a cumulative relapse rate of 61% observed over a five-year follow-up period.[183] In the SPARE trial involving anti-TNF discontinuation in CD, the duration of remission was found to be less than two weeks longer in the maintenance group compared to the anti-TNF discontinuation group over the two-year observation period.[173] Theoretical and conflicting concerns have arisen regarding immunogenicity following anti-TNF discontinuation. There is also a theoretical argument suggesting that withdrawing anti-TNF may diminish the development of true drug resistance by alleviating pressure on other inflammatory pathways, such as IL-23, considered a potential mechanism for resistance in CD.

The primary arguments in favour of targeted therapy discontinuation include the reduction of overtreatment, mitigating side effects, cost savings for both patients and the healthcare system, and integrating discontinuation as an alternative in shared decision-making. According to a survey conducted by the BIOCYCLE consortium, patients with CD expressed willingness to accept a 20 percent risk of relapse and allocate 5% of their time to active disease to facilitate the de-escalation of therapy, whether it involves discontinuing IMIDs or anti-TNF therapy.[184]

In Paper 1, anti-TNF discontinuation was performed according to the previously described algorithm. These patients were treated to endoscopic remission, Mayo Score ≤ 2 points and no sub-score >1. The median time to relapse after the first induction therapy were 16 months. Notably, while relapse is a common outcome, 23% of patients in this study did not experience relapse, with a median observation time exceeding 4 years. In addition, 42% and 21% did not relapse after a second and third induction therapy. In total, 50% of the patients who initially achieved remission did not require additional targeted therapy, with a median overall observation time of 4 years. Analysis of possible predictors for remission without relapse and time in remission were performed. Normalization of mucosal TNF mRNA expression were a significant predictor with a OR 3.8 (1.0–14.0) for remission without relapse. Patients with normalized mucosal TNF mRNA expression had a median time in remission of 33 months versus 11 months in the group with only endoscopic remission.

As shown in table 3, the criteria and definition of remission has evolved over time, mostly toward a stricter definition due to studies showing better outcomes. It is well known that clinical remission is not a sufficient treatment target, and studies have shown lower risk of relapse with a endoscopic score of 0. It is shown that histological inflammation persist in a normalized mucosa and that histological scoring does not significant discriminate between MES 0 and 1. Further the inter-rater agreement varies from poor to excellent.[170] In our study histological remission/resolution were not assessed, Paper III reported that patients with normalized mucosal TNF expression were also in histological remission. Interestingly, most other pro and anti-inflammatory gene expressions measured

in this study remained elevated. This suggests that normalization of mucosal TNF mRNA expression may represent a more advanced stage beyond histological remission. Consequently, the normalization of mucosal TNF mRNA expression emerges as a potential treatment target and criterion for discontinuation of anti-TNF therapy.

5.2.6 Prediction of clinical outcomes (others than above)

In Paper I, active smoking was identified as a factor increasing the risk of relapse after anti-TNF discontinuation, with a HR of 2.9 (95% CI: 1.2–7.1).

Additionally, patients with the lowest clinical score (UCDAI) before anti-TNF treatment were more likely to achieve long-term remission, with an OR of 1.4 (95% CI: 1.0–2.0). Concerning surgery, male sex and older age emerged as significant predictors, with an OR of 3.4 (95% CI: 1.1–10.7) for male sex and 1.05 (95% CI: 1.01–1.08) per year. In the follow-up study (Paper III), normalized mucosal TNF expression after the first induction therapy was identified as a predictor for long-term remission, remission without the need for further biological therapy, and a lower risk of colectomy throughout the observation period. Notably, patients with particularly high mucosal TNF expression at inclusion were at a heightened risk of undergoing colectomy.

5.2.7 Mucosal immune activity in long-term remission

In Paper III, known mucosal gene expression of known IBD associated cytokines were measured in a selected group of patients previously treated with anti-TNF in long-term remission without any signs of relapse and receiving no treatment or only 5-ASA. This group was compared to both HC and patients in remission who experienced a relapse within 12 months.

The findings revealed a persistent activation of most gene transcripts, suggesting that the mucosal immune system does not fully normalize in this patient subgroup. The degree of activation, however, was generally lower than in patients in remission who later experienced a relapse. Notably, only the gene expression of IL1RL1 showed normalization in the long-term remission group compared to HC, and it was significantly lower compared to the group that experienced a relapse. While acknowledging the study's small sample size and the exploratory nature of the analysis, these results suggest that the normalization of IL1RL1 should be explored further as a potential predictor of a more favourable long-term outcome when combined with the normalization of mucosal TNF mRNA. This normalization of IL1RL1 could potentially serve as a treatment target.

5.3 Conclusions

In conclusion, this doctoral thesis, comprised of three studies, may represent a significant advancement in the field of precision medicine for UC. The overarching aim of this research was to address the limitations of recommended treatment strategies, find new biomarkers and, ultimately pave the way for more personalized and effective treatment strategies.

The findings from these studies collectively support the notion that personalized medicine can significantly improve outcomes for individuals with UC. The identification of patients at the highest risk of severe outcomes through the assessment of mucosal TNF mRNA expression and histological scores at the onset of the disease is a possible breakthrough. This knowledge allows for the early implementation of targeted therapy in selected patients. A more intensified initial treatment particularly with infliximab has been demonstrated to result in improved rates of remission.

Furthermore, the research provides an insight into the long-term outcomes of UC patients in remission. By targeting normalized mucosal TNF expression as a treatment goal, the majority of patients can successfully discontinue anti-TNF therapy. The extensive follow-up studies reveal that these patients largely remain in remission, or no longer require targeted therapies. This suggests a phenotypic shift, potentially induced by the normalization of mucosal TNF expression. In case of disease relapse, re-introduction of targeted therapy remains effective.

In summary, the research presented in this thesis represents a step toward the realization of personalized medicine in UC. By implementing an intensified treatment algorithm including novel biomarkers, the field is moving closer to tailoring therapies to individual patient needs. This potentially enhances the quality of life for UC patients, improves patient outcomes, hence may represent a significant advancement in the management of this chronic condition.

5.4 Summary of hypotheses and conclusions

1. **Early achievement of endoscopic remission and prediction of long-term outcomes:**
    - **Hypothesis:** Achieving endoscopic remission early could alter the disease phenotype, steering it away from a more aggressive course.
    - **Study Conclusion:** Early intensive treatment, particularly with infliximab, result in improved rates of remission. Long-term outcomes suggest a phenotypic shift towards a milder phenotype, maybe induced by the normalization of mucosal TNF expression.
2. **Indefinite maintenance vs. discontinuation of targeted treatment:**
    - **Hypothesis:** Indefinite maintenance of targeted treatment is not necessary; reinitiating treatment during periods of heightened disease activity is a viable approach.

- **Study Conclusion:** Discontinuation of anti-TNF therapy is feasible for the majority, with the option to reintroduce if needed.

3. **Biomarkers for top-down treatment:**
    - **Hypothesis:** Assessing inflammation severity using biomarkers can identify patients suitable for top-down treatment at the onset of the disease.
    - **Study Conclusion:** Biomarkers, specifically mucosal TNF expression combined with histological scores, are valuable for identifying high-risk patients.

5.5 Clinical implications and knowledge beyond state of the art

- Mucosal TNF mRNA expression combined with RHI is a possible biomarker to select patients for at top-down treatment approach.
- An intensified anti-TNF induction treatment might improve patients' outcomes.
- Normalization om mucosal TNF mRNA expression is possible biomarker to select patients for treatment de-escalation.

5.6 Research implications

The findings in these studies should be tested in further RCT studies. Currently a multicentre RCT with normalization of mucosal TNF mRNA expression compared to maintenance is planned. A multicentre RCT comparing the standard step-up approach vs selected top-down treatment based on mucosal TNF mRNA expression combined with RHI is also a planned. IL1RL should be investigated in further studies as a biomarker predicting long-term remission.

6 REFERENCES

1 Satsangi, J., Silverberg, M. S., Vermeire, S. & Colombel, J.-F. The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. *Gut* **55**, 749-753, doi:10.1136/gut.2005.082909 (2006).

2 Ng, S. C. *et al.* Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *Lancet (London, England)* **390**, 2769-2778, doi:10.1016/s0140-6736(17)32448-0 (2018).

3 Loftus, E. V., Jr. Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. *Gastroenterology* **126**, 1504-1517 (2004).

4 Burisch, J., Jess, T., Martinato, M. & Lakatos, P. L. The burden of inflammatory bowel disease in Europe. *Journal of Crohn's & colitis* **7**, 322-337, doi:10.1016/j.crohns.2013.01.010 (2013).

5 Moum, B. *et al.* Inflammatory bowel disease: re-evaluation of the diagnosis in a prospective population based study in south eastern Norway. *Gut* **40**, 328-332, doi:10.1136/gut.40.3.328 (1997).

6 Kildebo, S. *et al.* The Incidence of Ulcerative Colitis in Northern Norway from 1983 to 1986. *Scandinavian Journal of Gastroenterology* **25**, 890-896, doi:10.3109/00365529008997609 (1990).

7 Høybjør, G. *Insidens av sykdommene ulcerøs kolitt & Crohn sykdom i Finnmark*, <(https://munin.uit.no/bitstream/handle/10037/728/student.pdf?sequence=1&isAllowed=y> (2004).

8 Lirhus, S. S., Høivik, M. L., Moum, B., Anisdahl, K. & Melberg, H. O. Incidence and Prevalence of Inflammatory Bowel Disease in Norway and the Impact of Different Case Definitions: A Nationwide Registry Study. *Clin Epidemiol* **13**, 287-294, doi:10.2147/clep.S303797 (2021).

9 Solberg, I. C. *et al.* Clinical course during the first 10 years of ulcerative colitis: results from a population-based inception cohort (IBSEN Study). *Scandinavian Journal of Gastroenterology* **44**, 431-440, doi:10.1080/00365520802600961 (2009).

10 Henriksen, M. *et al.* Ulcerative colitis and clinical course: results of a 5-year population-based follow-up study (the IBSEN study). *Inflammatory bowel diseases* **12**, 543-550, doi:10.1097/01.MIB.0000225339.91484.fc (2006).

11 Rutgeerts, P. *et al.* Infliximab for induction and maintenance therapy for ulcerative colitis. *The New England journal of medicine* **353**, 2462-2476, doi:10.1056/NEJMoa050516 (2005).

12 Rutgeerts, P., Vermeire, S. & Van Assche, G. Mucosal healing in inflammatory bowel disease: impossible ideal or therapeutic target? *Gut* **56**, 453-455, doi:10.1136/gut.2005.088732 (2007).

13 Anisdahl, K. *et al.* First-line biologic treatment of inflammatory bowel disease during the first 12 months after diagnosis from 2010 to 2016: a Norwegian nationwide registry study. *Scandinavian Journal of Gastroenterology* **56**, 1163-1168, doi:10.1080/00365521.2021.1955147 (2021).

14 Burisch, J. *et al.* Natural Disease Course of Ulcerative Colitis During the First Five Years of Follow-up in a European Population-based Inception Cohort-An Epi-IBD Study. *Journal of Crohn's & colitis*, doi:10.1093/ecco-jcc/jjy154 (2018).

15 Eriksson, C. *et al.* Changes in medical management and colectomy rates: a population-based cohort study on the epidemiology and natural history of ulcerative colitis in Orebro, Sweden, 1963-2010. *Alimentary pharmacology & therapeutics* **46**, 748-757, doi:10.1111/apt.14268 (2017).

16 Vester-Andersen, M. K. *et al.* Disease course and surgery rates in inflammatory bowel disease: a population-based, 7-year follow-up study in the era of immunomodulating therapy. *The American journal of gastroenterology* **109**, 705-714, doi:10.1038/ajg.2014.45 (2014).

17 Samuel, S. *et al.* Cumulative incidence and risk factors for hospitalization and surgery in a population-based cohort of ulcerative colitis. *Inflammatory bowel diseases* **19**, 1858-1866, doi:10.1097/MIB.0b013e31828c84c5 (2013).

18 Barnes, E. L. *et al.* Decreasing Colectomy Rate for Ulcerative Colitis in the United States Between 2007 and 2016: A Time Trend Analysis. *Inflammatory bowel diseases* **26**, 1225-1231, doi:10.1093/ibd/izz247 (2019).
19 Jess, T., Rungoe, C. & Peyrin-Biroulet, L. Risk of colorectal cancer in patients with ulcerative colitis: a meta-analysis of population-based cohort studies. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association* **10**, 639-645, doi:10.1016/j.cgh.2012.01.010 (2012).
20 Kappelman, M. D. *et al.* Risk of cancer in patients with inflammatory bowel diseases: a nationwide population-based cohort study with 30 years of follow-up evaluation. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association* **12**, 265-273.e261, doi:10.1016/j.cgh.2013.03.034 (2014).
21 Annese, V. *et al.* European Evidence-based Consensus: Inflammatory Bowel Disease and Malignancies. *Journal of Crohn's and Colitis* **9**, 945-965, doi:10.1093/ecco-jcc/jjv141 (2015).
22 Gisbert, J. P., Marin, A. C. & Chaparro, M. The Risk of Relapse after Anti-TNF Discontinuation in Inflammatory Bowel Disease: Systematic Review and Meta-Analysis. *The American journal of gastroenterology* **111**, 632-647, doi:10.1038/ajg.2016.54 (2016).
23 Mulder, D. J., Noble, A. J., Justinich, C. J. & Duffin, J. M. A tale of two diseases: The history of inflammatory bowel disease. *Journal of Crohn's and Colitis* **8**, 341-348, doi:10.1016/j.crohns.2013.09.009 (2014).
24 de Souza, H. S. P., Fiocchi, C. & Iliopoulos, D. The IBD interactome: an integrated view of aetiology, pathogenesis and therapy. *Nature Reviews Gastroenterology &Amp; Hepatology* **14**, 739, doi:10.1038/nrgastro.2017.110 (2017).
25 Ray, K. Genotypes and phenotypes of IBD. *Nature Reviews Gastroenterology &Amp; Hepatology* **12**, 672, doi:10.1038/nrgastro.2015.188 (2015).
26 Neurath, M. F. Cytokines in inflammatory bowel disease. *Nature reviews. Immunology* **14**, 329-342, doi:10.1038/nri3661 (2014).
27 Billmeier, U., Dieterich, W., Neurath, M. F. & Atreya, R. Molecular mechanism of action of anti-tumor necrosis factor antibodies in inflammatory bowel diseases. *World journal of gastroenterology* **22**, 9300-9313, doi:10.3748/wjg.v22.i42.9300 (2016).
28 Levin, A. D., Wildenberg, M. E. & van den Brink, G. R. Mechanism of Action of Anti-TNF Therapy in Inflammatory Bowel Disease. *Journal of Crohn's and Colitis* **10**, 989-997, doi:10.1093/ecco-jcc/jjw053 (2016).
29 Sands, B. E. & Kaplan, G. G. The Role of TNFα in Ulcerative Colitis. *The Journal of Clinical Pharmacology* **47**, 930-941, doi:https://doi.org/10.1177/0091270007301623 (2007).
30 Kalliolias, G. D. & Ivashkiv, L. B. TNF biology, pathogenic mechanisms and emerging therapeutic strategies. *Nature Reviews Rheumatology* **12**, 49-62, doi:10.1038/nrrheum.2015.169 (2016).
31 Olsen, T. *et al.* Tissue levels of tumor necrosis factor-alpha correlates with grade of inflammation in untreated ulcerative colitis. *Scand J Gastroenterol* **42**, 1312-1320, doi:10.1080/00365520701409035 (2007).
32 Martinon, F., Burns, K. & Tschopp, J. The Inflammasome: A Molecular Platform Triggering Activation of Inflammatory Caspases and Processing of proIL-&#x3b2. *Molecular Cell* **10**, 417-426, doi:10.1016/S1097-2765(02)00599-3 (2002).
33 Cuthbert, A. P. *et al.* The contribution of NOD2 gene mutations to the risk and site of disease in inflammatory bowel disease. *Gastroenterology* **122**, 867-874 (2002).
34 Spurkland, A. *et al.* HLA class II haplotypes in primary sclerosing cholangitis patients from five European populations. *Tissue antigens* **53**, 459-469 (1999).
35 Cleynen, I. *et al.* Inherited determinants of Crohn's disease and ulcerative colitis phenotypes: a genetic association study. *Lancet (London, England)* **387**, 156-167, doi:10.1016/s0140-6736(15)00465-1 (2016).
36 Wild, C. P. Complementing the genome with an "exposome": the outstanding challenge of environmental exposure measurement in molecular epidemiology. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* **14**, 1847-1850, doi:10.1158/1055-9965.epi-05-0456 (2005).

37 Murphy, T. M. & Mill, J. Epigenetics in health and disease: heralding the EWAS era. *Lancet (London, England)* **383**, 1952-1954, doi:10.1016/s0140-6736(14)60269-5 (2014).
38 Xu, J. *et al.* New Insights Into the Epigenetic Regulation of Inflammatory Bowel Disease. *Frontiers in pharmacology* **13**, 813659, doi:10.3389/fphar.2022.813659 (2022).
39 Taman, H. *et al.* Genome-wide DNA Methylation in Treatment-naive Ulcerative Colitis. *Journal of Crohn's & colitis* **12**, 1338-1347, doi:10.1093/ecco-jcc/jjy117 (2018).
40 Ananthakrishnan, A. N. *et al.* Environmental triggers in IBD: a review of progress and evidence. *Nature Reviews Gastroenterology &Amp; Hepatology* **15**, 39, doi:10.1038/nrgastro.2017.136 (2017).
41 Andersson, R. E., Olaison, G., Tysk, C. & Ekbom, A. Appendectomy and protection against ulcerative colitis. *The New England journal of medicine* **344**, 808-814, doi:10.1056/nejm200103153441104 (2001).
42 Wang, Z., Gerstein, M. & Snyder, M. RNA-Seq: a revolutionary tool for transcriptomics. *Nat Rev Genet* **10**, 57-63, doi:10.1038/nrg2484 (2009).
43 Gusev, A. *et al.* Integrative approaches for large-scale transcriptome-wide association studies. *Nat Genet* **48**, 245-252, doi:10.1038/ng.3506 (2016).
44 Díez-Obrero, V. *et al.* Transcriptome-Wide Association Study for Inflammatory Bowel Disease Reveals Novel Candidate Susceptibility Genes in Specific Colon Subsites and Tissue Categories. *Journal of Crohn's & colitis* **16**, 275-285, doi:10.1093/ecco-jcc/jjab131 (2022).
45 Mitsialis, V. *et al.* Single-Cell Analyses of Colon and Blood Reveal Distinct Immune Cell Signatures of Ulcerative Colitis and Crohn's Disease. *Gastroenterology* **159**, 591-608.e510, doi:10.1053/j.gastro.2020.04.074 (2020).
46 Zheng, H. B. Application of single-cell omics in inflammatory bowel disease. *World journal of gastroenterology* **29**, 4397-4404, doi:10.3748/wjg.v29.i28.4397 (2023).
47 Gonzalez Acera, M. *et al.* Comparative Transcriptomics of IBD Patients Indicates Induction of Type 2 Immunity Irrespective of the Disease Ideotype. *Frontiers in medicine* **8**, 664045, doi:10.3389/fmed.2021.664045 (2021).
48 Hu, C., Liao, S., Lv, L., Li, C. & Mei, Z. Intestinal Immune Imbalance is an Alarm in the Development of IBD. *Mediators of inflammation* **2023**, 1073984, doi:10.1155/2023/1073984 (2023).
49 Agrawal, M., Allin, K. H., Petralia, F., Colombel, J. F. & Jess, T. Multiomics to elucidate inflammatory bowel disease risk factors and pathways. *Nature reviews. Gastroenterology & hepatology* **19**, 399-409, doi:10.1038/s41575-022-00593-y (2022).
50 Assadsangabi, A., Evans, C. A., Corfe, B. M. & Lobo, A. Application of Proteomics to Inflammatory Bowel Disease Research: Current Status and Future Perspectives. *Gastroenterol Res Pract* **2019**, 1426954, doi:10.1155/2019/1426954 (2019).
51 Vogel, C. & Marcotte, E. M. Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. *Nat Rev Genet* **13**, 227-232, doi:10.1038/nrg3185 (2012).
52 Bjerrum, J. T., Wang, Y. L., Seidelin, J. B. & Nielsen, O. H. IBD metabonomics predicts phenotype, disease course, and treatment response. *eBioMedicine* **71**, 103551, doi:https://doi.org/10.1016/j.ebiom.2021.103551 (2021).
53 Sudhakar, P. *et al.* Tailoring Multi-omics to Inflammatory Bowel Diseases: All for One and One for All. *Journal of Crohn's and Colitis* **16**, 1306-1320, doi:10.1093/ecco-jcc/jjac027 (2022).
54 Bager, P., Simonsen, J., Nielsen, N. M. & Frisch, M. Cesarean section and offspring's risk of inflammatory bowel disease: a national cohort study. *Inflammatory bowel diseases* **18**, 857-862, doi:10.1002/ibd.21805 (2012).
55 De Filippo, C. *et al.* Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 14691-14696, doi:10.1073/pnas.1005963107 (2010).
56 Hviid, A., Svanstrom, H. & Frisch, M. Antibiotic use and inflammatory bowel diseases in childhood. *Gut* **60**, 49-54, doi:10.1136/gut.2010.219683 (2011).
57 Jernberg, C., Lofmark, S., Edlund, C. & Jansson, J. K. Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. *The ISME journal* **1**, 56-66, doi:10.1038/ismej.2007.3 (2007).

58 Bernstein, C. N. & Shanahan, F. Disorders of a modern lifestyle: reconciling the epidemiology of inflammatory bowel diseases. *Gut* **57**, 1185-1191, doi:10.1136/gut.2007.122143 (2008).
59 Paun, A. & Danska, J. S. Immuno-ecology: how the microbiome regulates tolerance and autoimmunity. *Current Opinion in Immunology* **37**, 34-39, doi:https://doi.org/10.1016/j.coi.2015.09.004 (2015).
60 Ordás, I., Eckmann, L., Talamini, M., Baumgart, D. C. & Sandborn, W. J. Ulcerative colitis. *The Lancet* **380**, 1606-1619, doi:10.1016/S0140-6736(12)60150-0 (2012).
61 Baumgart, D. C. & Carding, S. R. Inflammatory bowel disease: cause and immunobiology. *The Lancet* **369**, 1627-1640, doi:https://doi.org/10.1016/S0140-6736(07)60750-8 (2007).
62 Söderholm, J. D. *et al.* Augmented increase in tight junction permeability by luminal stimuli in the non-inflamed ileum of Crohn's disease. *Gut* **50**, 307, doi:10.1136/gut.50.3.307 (2002).
63 Irvine, E. J. & Marshall, J. K. Increased intestinal permeability precedes the onset of Crohn's disease in a subject with familial risk. *Gastroenterology* **119**, 1740-1744, doi:https://doi.org/10.1053/gast.2000.20231 (2000).
64 Franchimont, D. *et al.* Deficient host-bacteria interactions in inflammatory bowel disease? The toll-like receptor (TLR)-4 Asp299gly polymorphism is associated with Crohn's disease and ulcerative colitis. *Gut* **53**, 987, doi:10.1136/gut.2003.030205 (2004).
65 Cario, E. & Podolsky, D. K. Differential Alteration in Intestinal Epithelial Cell Expression of Toll-Like Receptor 3 (TLR3) and TLR4 in Inflammatory Bowel Disease. *Infection and Immunity* **68**, 7010, doi:10.1128/IAI.68.12.7010-7017.2000 (2000).
66 Steinman, R. M. & Nussenzweig, M. C. Avoiding horror autotoxicus: The importance of dendritic cells in peripheral T cell tolerance. *Proceedings of the National Academy of Sciences* **99**, 351, doi:10.1073/pnas.231606698 (2002).
67 Papadakis, K. A. *et al.* Dominant Role for TL1A/DR3 Pathway in IL-12 plus IL-18-Induced IFN-γ Production by Peripheral Blood and Mucosal CCR9⁺ T Lymphocytes. *The Journal of Immunology* **174**, 4985-4990, doi:10.4049/jimmunol.174.8.4985 (2005).
68 Cruickshank, S. M., McVay, L. D., Baumgart, D. C., Felsburg, P. J. & Carding, S. R. Colonic epithelial cell mediated suppression of CD4 T cell activation. *Gut* **53**, 678, doi:10.1136/gut.2003.029967 (2004).
69 Nakazawa, A. *et al.* The expression and function of costimulatory molecules B7H and B7-H1 on colonic epithelial cells. *Gastroenterology* **126**, 1347-1357, doi:https://doi.org/10.1053/j.gastro.2004.02.004 (2004).
70 Ina, K. *et al.* Resistance of Crohn's Disease T Cells to Multiple Apoptotic Signals Is Associated with a Bcl-2/Bax Mucosal Imbalance. *The Journal of Immunology* **163**, 1081-1090 (1999).
71 Furlan, R. *et al.* Sympathetic overactivity in active ulcerative colitis: effects of clonidine. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **290**, R224-R232, doi:10.1152/ajpregu.00442.2005 (2006).
72 Demaude, J., Salvador-Cartier, C., Fioramonti, J., Ferrier, L. & Bueno, L. Phenotypic changes in colonocytes following acute stress or activation of mast cells in mice: implications for delayed epithelial barrier dysfunction. *Gut* **55**, 655-661, doi:10.1136/gut.2005.078675 (2006).
73 Maaser, C. *et al.* ECCO-ESGAR Guideline for Diagnostic Assessment in IBD Part 1: Initial diagnosis, monitoring of known IBD, detection of complications. *Journal of Crohn's and Colitis* **13**, 144-164K, doi:10.1093/ecco-jcc/jjy113 (2018).
74 Schroeder, K. W., Tremaine, W. J. & Ilstrup, D. M. Coated Oral 5-Aminosalicylic Acid Therapy for Mildly to Moderately Active Ulcerative Colitis. *New England Journal of Medicine* **317**, 1625-1629, doi:10.1056/nejm198712243172603 (1987).
75 Walmsley, R. S., Ayres, R. C. S., Pounder, R. E. & Allan, R. N. A simple clinical colitis activity index. *Gut* **43**, 29-32, doi:10.1136/gut.43.1.29 (1998).
76 Lewis, J. D. *et al.* Use of the noninvasive components of the mayo score to assess clinical response in Ulcerative Colitis. *Inflammatory bowel diseases* **14**, 1660 1666 (2008).
77 Lobatón, T. *et al.* The Modified Mayo Endoscopic Score (MMES): A New Index for the Assessment of Extension and Severity of Endoscopic Activity in Ulcerative Colitis Patients. *Journal of Crohn's and Colitis* **9**, 846-852, doi:10.1093/ecco-jcc/jjv111 (2015).

78 Peyrin-Biroulet, L. *et al.* Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE): Determining Therapeutic Goals for Treat-to-Target. *Official journal of the American College of Gastroenterology | ACG* **110**, 1324-1338, doi:10.1038/ajg.2015.233 (2015).
79 Christensen, B. *et al.* Histologic Normalization Occurs in Ulcerative Colitis and Is Associated With Improved Clinical Outcomes. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association* **15**, 1557-1564 e1551, doi:10.1016/j.cgh.2017.02.016 (2017).
80 Yvellez, O. V. *et al.* Cumulative Histologic Inflammation Predicts Colorectal Neoplasia in Ulcerative Colitis: A Validation Study. *Inflammatory bowel diseases* **27**, 203-206, doi:10.1093/ibd/izaa047 (2021).
81 Shah, J. *et al.* Relationship between Mayo endoscopic score and histological scores in ulcerative colitis: A prospective study. *JGH Open* **4**, 382-386, doi:https://doi.org/10.1002/jgh3.12260 (2020).
82 Mosli, M. H. *et al.* Histologic scoring indices for evaluation of disease activity in ulcerative colitis. *Cochrane Database of Systematic Reviews*, doi:10.1002/14651858.CD011256.pub2 (2017).
83 Ungaro, R., Colombel, J.-F., Lissoos, T. & Peyrin-Biroulet, L. A Treat-to-Target Update in Ulcerative Colitis: A Systematic Review. *Official journal of the American College of Gastroenterology | ACG* **114**, 874-883, doi:10.14309/ajg.0000000000000183 (2019).
84 Dignass, A. *et al.* Second European evidence-based consensus on the diagnosis and management of ulcerative colitis part 2: current management. *Journal of Crohn's & colitis* **6**, 991-1030, doi:10.1016/j.crohns.2012.09.002 (2012).
85 Feuerstein, J. D. *et al.* AGA Clinical Practice Guidelines on the Management of Moderate to Severe Ulcerative Colitis. *Gastroenterology* **158**, 1450-1461, doi:10.1053/j.gastro.2020.01.006 (2020).
86 Zallot, C. & Peyrin-Biroulet, L. Deep remission in inflammatory bowel disease: looking beyond symptoms. *Curr Gastroenterol Rep* **15**, 315, doi:10.1007/s11894-013-0315-7 (2013).
87 Florholmen, J. Mucosal healing in the era of biologic agents in treatment of inflammatory bowel disease. *Scandinavian Journal of Gastroenterology* **50**, 43-52, doi:10.3109/00365521.2014.977943 (2015).
88 Colombel, J. F. *et al.* Early mucosal healing with infliximab is associated with improved long-term clinical outcomes in ulcerative colitis. *Gastroenterology* **141**, 1194-1201, doi:10.1053/j.gastro.2011.06.054 (2011).
89 Sandborn, W. J. *et al.* OP14 Improved endoscopic outcomes and mucosal healing of upadacitinib as an induction therapy in adults with moderately to severely active ulcerative colitis: data from the U-ACHIEVE study. *Journal of Crohn's and Colitis* **13**, S009-S009, doi:10.1093/ecco-jcc/jjy222.013 (2019).
90 Sandborn, W. J. *et al.* Efficacy of Upadacitinib in a Randomized Trial of Patients With Active Ulcerative Colitis. *Gastroenterology* **158**, 2139-2149.e2114, doi:10.1053/j.gastro.2020.02.030 (2020).
91 D'Amico, F. *et al.* Ulcerative colitis: Impact of early disease clearance on long-term outcomes - A multicenter cohort study. *United European gastroenterology journal* **10**, 775-782, doi:10.1002/ueg2.12288 (2022).
92 Papamichael, K. *et al.* Role for Therapeutic Drug Monitoring During Induction Therapy with TNF Antagonists in IBD: Evolution in the Definition and Management of Primary Nonresponse. *Inflammatory bowel diseases* **21**, 182-197, doi:10.1097/mib.0000000000000202 (2014).
93 Roda, G., Jharap, B., Neeraj, N. & Colombel, J.-F. Loss of Response to Anti-TNFs: Definition, Epidemiology, and Management. *Clinical and Translational Gastroenterology* **7**, e135, doi:10.1038/ctg.2015.63 (2016).
94 Goll, R. *et al.* Pharmacodynamic mechanisms behind a refractory state in inflammatory bowel disease. *BMC Gastroenterol* **22**, 464, doi:10.1186/s12876-022-02559-5 (2022).

95 Harbord, M. *et al.* Third European Evidence-based Consensus on Diagnosis and Management of Ulcerative Colitis. Part 2: Current Management. *Journal of Crohn's and Colitis* **11**, 769-784, doi:10.1093/ecco-jcc/jjx009 (2017).
96 Jairath, V. *et al.* Development of interim patient-reported outcome measures for the assessment of ulcerative colitis disease activity in clinical trials. *Alimentary pharmacology & therapeutics* **42**, 1200-1210, doi:10.1111/apt.13408 (2015).
97 Colombel, J. F. *et al.* Discrepancies between patient-reported outcomes, and endoscopic and histological appearance in UC. *Gut* **66**, 2063-2068, doi:10.1136/gutjnl-2016-312307 (2017).
98 Karstensen, J. G. *et al.* Confocal laser endomicroscopy in ulcerative colitis: a longitudinal study of endomicroscopic changes and response to medical therapy (with videos). *Gastrointestinal Endoscopy* **84**, 279-286.e271, doi:https://doi.org/10.1016/j.gie.2016.01.069 (2016).
99 Chang, J. *et al.* Impaired Intestinal Permeability Contributes to Ongoing Bowel Symptoms in Patients With Inflammatory Bowel Disease and Mucosal Healing. *Gastroenterology* **153**, 723-731.e721, doi:10.1053/j.gastro.2017.05.056 (2017).
100 Ko, C. W. *et al.* AGA Clinical Practice Guidelines on the Management of Mild-to-Moderate Ulcerative Colitis. *Gastroenterology* **156**, 748-764, doi:10.1053/j.gastro.2018.12.009 (2019).
101 D'Haens, G. R. Top-down therapy for IBD: rationale and requisite evidence. *Nature reviews. Gastroenterology & hepatology* **7**, 86-92, doi:10.1038/nrgastro.2009.222 (2010).
102 Khanna, R. *et al.* Early combined immunosuppression for the management of Crohn's disease (REACT): a cluster randomised controlled trial. *The Lancet* **386**, 1825-1834, doi:https://doi.org/10.1016/S0140-6736(15)00068-9 (2015).
103 Flamant, M. & Roblin, X. Inflammatory bowel disease: towards a personalized medicine. *Therapeutic advances in gastroenterology* **11**, 1756283x17745029, doi:10.1177/1756283x17745029 (2018).
104 Siegel, C. A. Refocusing IBD Patient Management: Personalized, Proactive, and Patient-Centered Care. *The American journal of gastroenterology* **113**, 1440-1443, doi:10.1038/s41395-018-0246-x (2018).
105 Bressler, B. *et al.* Clinical Practice Guidelines for the Medical Management of Nonhospitalized Ulcerative Colitis: The Toronto Consensus. *Gastroenterology* **148**, 1035-1058.e1033, doi:https://doi.org/10.1053/j.gastro.2015.03.001 (2015).
106 Raine, T. *et al.* ECCO Guidelines on Therapeutics in Ulcerative Colitis: Medical Treatment. *Journal of Crohn's and Colitis* **16**, 2-17, doi:10.1093/ecco-jcc/jjab178 (2021).
107 Svartz, N. Ett nytt sulfonamidpreparat? Forelopande meddelande [Swedish]. *Nordisk Medicin*, 554-555 (1941).
108 DESREUMAUX, P. & GHOSH, S. Review article: mode of action and delivery of 5-aminosalicylic acid – new evidence. *Alimentary pharmacology & therapeutics* **24**, 2-9, doi:https://doi.org/10.1111/j.1365-2036.2006.03069.x (2006).
109 Watanabe, M. *et al.* Randomised clinical trial: evaluation of the efficacy of mesalazine (mesalamine) suppositories in patients with ulcerative colitis and active rectal inflammation – a placebo-controlled study. *Alimentary pharmacology & therapeutics* **38**, 264-273, doi:https://doi.org/10.1111/apt.12362 (2013).
110 Murray, A., Nguyen, T. M., Parker, C. E., Feagan, B. G. & MacDonald, J. K. Oral 5‐aminosalicylic acid for induction of remission in ulcerative colitis. *Cochrane Database of Systematic Reviews*, doi:10.1002/14651858.CD000543.pub5 (2020).
111 Murray, A., Nguyen, T. M., Parker, C. E., Feagan, B. G. & MacDonald, J. K. Oral 5‐aminosalicylic acid for maintenance of remission in ulcerative colitis. *Cochrane Database of Systematic Reviews*, doi:10.1002/14651858.CD000544.pub5 (2020).
112 Gisbert, J. P., González-Lama, Y. & Maté, J. 5-Aminosalicylates and renal function in inflammatory bowel disease: a systematic review. *Inflammatory bowel diseases* **13**, 629-638, doi:10.1002/ibd.20099 (2007).
113 Qiu, X., Ma, J., Wang, K. & Zhang, H. Chemopreventive effects of 5-aminosalicylic acid on inflammatory bowel disease-associated colorectal cancer and dysplasia: a systematic review with meta-analysis. *Oncotarget* **8** (2016).

114 Dearing, W. H. & Brown, P. W. Experiences with cortisone and ACTH in chronic ulcerative colitis. *Proc Staff Meet Mayo Clin* **25**, 486-488 (1950).
115 Truelove, S. & Jewell, D. Intensive intravenous regimen for severe attacks of ulcerative colitis. *The Lancet* **303**, 1067-1070 (1974).
116 Truelove, S., Lee, E., Willoughby, C. & Kettlewell, M. Further experience in the treatment of severe attacks of ulcerative colitis. *The Lancet* **312**, 1086-1088 (1978).
117 Brattsand, R. & Linden, M. Cytokine modulation by glucocorticoids: mechanisms and actions in cellular studies. *Alimentary pharmacology & therapeutics* **10**, 81-90 (1996).
118 Ramamoorthy, S. & Cidlowski, J. A. Corticosteroids: mechanisms of action in health and disease. *Rheumatic Disease Clinics* **42**, 15-31 (2016).
119 Dorrington, A. M. *et al.* The Historical Role and Contemporary Use of Corticosteroids in Inflammatory Bowel Disease. *Journal of Crohn's and Colitis* **14**, 1316-1329, doi:10.1093/ecco-jcc/jjaa053 (2020).
120 Rosiou, K. *et al.* Comparative Outcomes of Budesonide MMX versus Prednisolone for Ulcerative Colitis: Results from a British Retrospective Multi-Centre Real-World Study. *Journal of Clinical Medicine* **10**, 4329 (2021).
121 Travis, S. P. *et al.* Once-daily budesonide MMX in active, mild-to-moderate ulcerative colitis: results from the randomised CORE II study. *Gut* **63**, 433-441 (2014).
122 Cha, J. M. *et al.* Long-term prognosis of ulcerative colitis and its temporal changes between 1986 and 2015 in a population-based cohort in the Songpa-Kangdong district of Seoul, Korea. *Gut* **69**, 1432-1440 (2020).
123 Burisch, J. *et al.* Natural disease course of Crohn's disease during the first 5 years after diagnosis in a European population-based inception cohort: an Epi-IBD study. *Gut*, doi:10.1136/gutjnl-2017-315568 (2018).
124 Waljee, A. K. *et al.* Corticosteroid use and complications in a US inflammatory bowel disease cohort. *PloS one* **11**, e0158017 (2016).
125 Siegel, C., Yang, F., Eslava, S. & Cai, J. DOP060 real-world treatment pathway visualizations show low use of biologic therapies in Crohn's disease and ulcerative colitis in the United States. *Journal of Crohn's & colitis* **11**, S61-S62 (2017).
126 Bean, R. The treatment of chronic ulcerative colitis with 6-mercaptopurine. *The Medical Journal of Australia* **49**, 592-593 (1962).
127 Bean, R. Treatment of ulcerative colitis with antimetabolites. *British medical journal* **1**, 1081 (1966).
128 Jewell, D. & Truelove, S. Azathioprine in ulcerative colitis: final report on controlled therapeutic trial. *Br Med J* **4**, 627-630 (1974).
129 Chabner, B. A. In Celebration of a Nobel Prize. *JNCI: Journal of the National Cancer Institute* **80**, 1512-1513, doi:10.1093/jnci/80.19.1512 (1988).
130 Jharap, B. *et al.* Thiopurine therapy in inflammatory bowel disease patients: Analyses of two 8-year intercept cohorts. *Inflammatory bowel diseases* **16**, 1541-1549, doi:10.1002/ibd.21221 (2010).
131 Smith, M. A., Irving, P. M., Marinaki, A. M. & Sanderson, J. D. Review article: malignancy on thiopurine treatment with special reference to inflammatory bowel disease. *Alimentary pharmacology & therapeutics* **32**, 119-130, doi:10.1111/j.1365-2036.2010.04330.x (2010).
132 Kotlyar, D. S. *et al.* A systematic review of factors that contribute to hepatosplenic T-cell lymphoma in patients with inflammatory bowel disease. *Clinical Gastroenterology and Hepatology* **9**, 36-41. e31 (2011).
133 Osterman, M. T. *et al.* Increased risk of malignancy with adalimumab combination therapy, compared with monotherapy, for Crohn's disease. *Gastroenterology* **146**, 941-949, doi:10.1053/j.gastro.2013.12.025 (2014).
134 Gisbert, J. P., Linares, P. M., McNicholl, A. G., Maté, J. & Gomollón, F. Meta-analysis: the efficacy of azathioprine and mercaptopurine in ulcerative colitis. *Alimentary pharmacology & therapeutics* **30**, 126-137, doi:10.1111/j.1365-2036.2009.04023.x (2009).
135 Roblin, X. *et al.* Azathioprine dose reduction in inflammatory bowel disease patients on combination therapy: an open-label, prospective and randomised clinical trial. *Alimentary pharmacology & therapeutics* **46**, 142-149, doi:https://doi.org/10.1111/apt.14106 (2017).

136 Thorne, K. *et al.* Colectomy rates in patients with ulcerative colitis following treatment with infliximab or ciclosporin: a systematic literature review. *European Journal of Gastroenterology & Hepatology* **28**, 369-382, doi:10.1097/meg.0000000000000568 (2016).
137 Øresland, T. *et al.* European evidence based consensus on surgery for ulcerative colitis. *Journal of Crohn's and Colitis* **9**, 4-25, doi:10.1016/j.crohns.2014.08.012 (2014).
138 Kristensen, V. A. *et al.* Inflammatory bowel disease in South-Eastern Norway III (IBSEN III): a new population-based inception cohort study from South-Eastern Norway. *Scandinavian Journal of Gastroenterology* **56**, 899-905, doi:10.1080/00365521.2021.1922746 (2021).
139 Macaluso, F. S. *et al.* Head-to-head comparison of biological drugs for inflammatory bowel disease: from randomized controlled trials to real-world experience. *Therapeutic advances in gastroenterology* **14**, 17562848211010668, doi:10.1177/17562848211010668 (2021).
140 Tracey, D., Klareskog, L., Sasso, E. H., Salfeld, J. G. & Tak, P. P. Tumor necrosis factor antagonist mechanisms of action: A comprehensive review. *Pharmacology & Therapeutics* **117**, 244-279, doi:https://doi.org/10.1016/j.pharmthera.2007.10.001 (2008).
141 Puxeddu, I. *et al.* Hypersensitivity reactions during treatment with infliximab, etanercept, and adalimumab. *Annals of Allergy, Asthma & Immunology* **108**, 123-124, doi:https://doi.org/10.1016/j.anai.2011.11.004 (2012).
142 Vermeire, S., Gils, A., Accossato, P., Lula, S. & Marren, A. Immunogenicity of biologics in inflammatory bowel disease. *Therapeutic advances in gastroenterology* **11**, 1756283X17750355 (2018).
143 Thorlund, K., Druyts, E., Mills, E. J., Fedorak, R. N. & Marshall, J. K. Adalimumab versus infliximab for the treatment of moderate to severe ulcerative colitis in adult patients naïve to anti-TNF therapy: An indirect treatment comparison meta-analysis. *Journal of Crohn's and Colitis* **8**, 571-581, doi:10.1016/j.crohns.2014.01.010 (2014).
144 Poole, R. M. Vedolizumab: first global approval. *Drugs* **74**, 1293-1303, doi:10.1007/s40265-014-0253-1 (2014).
145 Sands, B. E. *et al.* Vedolizumab versus Adalimumab for Moderate-to-Severe Ulcerative Colitis. *New England Journal of Medicine* **381**, 1215-1226, doi:10.1056/NEJMoa1905725 (2019).
146 Paul, S. *et al.* Therapeutic drug monitoring of infliximab and mucosal healing in inflammatory bowel disease: a prospective study. *Inflammatory bowel diseases* **19**, 2568-2576, doi:10.1097/MIB.0b013e3182a77b41 (2013).
147 Papamichael, K. *et al.* Proactive Therapeutic Drug Monitoring of Adalimumab Is Associated With Better Long-term Outcomes Compared With Standard of Care in Patients With Inflammatory Bowel Disease. *Journal of Crohn's & colitis* **13**, 976-981, doi:10.1093/ecco-jcc/jjz018 (2019).
148 Restellini, S. & Afif, W. Update on TDM (Therapeutic Drug Monitoring) with Ustekinumab, Vedolizumab and Tofacitinib in Inflammatory Bowel Disease. *Journal of Clinical Medicine* **10**, 1242 (2021).
149 Benson, J. M. *et al.* Discovery and mechanism of ustekinumab: a human monoclonal antibody targeting interleukin-12 and interleukin-23 for treatment of immune-mediated disorders. *MAbs* **3**, 535-545, doi:10.4161/mabs.3.6.17815 (2011).
150 Sands, B. E. *et al.* Ustekinumab as Induction and Maintenance Therapy for Ulcerative Colitis. *The New England journal of medicine* **381**, 1201-1214, doi:10.1056/NEJMoa1900750 (2019).
151 Thomas, H. UEG Week 2023. *The Lancet Gastroenterology & Hepatology* **8**, 1076, doi:10.1016/S2468-1253(23)00371-0 (2023).
152 Sedano, R., Ma, C., Jairath, V. & Feagan, B. G. Janus Kinase Inhibitors for the Management of Patients With Inflammatory Bowel Disease. *Gastroenterol Hepatol (N Y)* **18**, 14-27 (2022).
153 Sandborn, W. J. *et al.* Tofacitinib as Induction and Maintenance Therapy for Ulcerative Colitis. *The New England journal of medicine* **376**, 1723-1736, doi:10.1056/NEJMoa1606910 (2017).
154 Feagan, B. G. *et al.* Filgotinib as induction and maintenance therapy for ulcerative colitis (SELECTION): a phase 2b/3 double-blind, randomised, placebo-controlled trial. *The Lancet* **397**, 2372-2384, doi:10.1016/S0140-6736(21)00666-8 (2021).

155 Li, Y. *et al.* Network meta-analysis on efficacy and safety of different Janus kinase inhibitors for ulcerative colitis. *Journal of Clinical Pharmacy and Therapeutics* **47**, 851-859, doi:https://doi.org/10.1111/jcpt.13622 (2022).
156 Sandborn, W. J. *et al.* Ozanimod as Induction and Maintenance Therapy for Ulcerative Colitis. *New England Journal of Medicine* **385**, 1280-1291, doi:10.1056/NEJMoa2033617 (2021).
157 Panés, J. *et al.* Higher vs Standard Adalimumab Induction and Maintenance Dosing Regimens for Treatment of Ulcerative Colitis: SERENE UC Trial Results. *Gastroenterology* **162**, 1891-1910, doi:https://doi.org/10.1053/j.gastro.2022.02.033 (2022).
158 National Research Council Committee on, A. F. f. D. a. N. T. o. D. in *Toward Precision Medicine: Building a Knowledge Network for Biomedical Research and a New Taxonomy of Disease* (2011).
159 Ungaro, R., Mehandru, S., Allen, P. B., Peyrin-Biroulet, L. & Colombel, J.-F. Ulcerative colitis. *The Lancet* **389**, 1756-1770, doi:https://doi.org/10.1016/S0140-6736(16)32126-2 (2017).
160 Dong, X.-W., Zheng, Q., Zhu, M.-M., Tong, J.-L. & Ran, Z.-H. Thiopurine S-methyltransferase polymorphisms and thiopurine toxicity in treatment of inflammatory bowel disease. *World journal of gastroenterology: WJG* **16**, 3187 (2010).
161 Sazonovs, A. *et al.* HLA-DQA1* 05 carriage associated with development of anti-drug antibodies to infliximab and adalimumab in patients with Crohn's disease. *Gastroenterology* **158**, 189-199 (2020).
162 Endo, K. *et al.* TL1A (TNFSF15) genotype affects the long‐term therapeutic outcomes of anti‐TNFα antibodies for Crohn's disease patients. *JGH Open* **4**, 1108-1113 (2020).
163 D'Haens, G. *et al.* A review of activity indices and efficacy end points for clinical trials of medical therapy in adults with ulcerative colitis. *Gastroenterology* **132**, 763-786, doi:10.1053/j.gastro.2006.12.038 (2007).
164 Nilsson, A. *et al.* Olsalazine versus sulphasalazine for relapse prevention in ulcerative colitis: a multicenter study. *The American journal of gastroenterology* **90**, 381-387 (1995).
165 Danese, S., Siegel, C. A. & Peyrin-Biroulet, L. Review article: integrating budesonide-MMX into treatment algorithms for mild-to-moderate ulcerative colitis. *Alimentary pharmacology & therapeutics* **39**, 1095-1103, doi:10.1111/apt.12712 (2014).
166 Olsen, T. *et al.* Normalization of mucosal tumor necrosis factor-alpha: A new criterion for discontinuing infliximab therapy in ulcerative colitis. *Cytokine* **79**, 90-95, doi:10.1016/j.cyto.2015.12.021 (2016).
167 Nolan, T., Hands, R. E. & Bustin, S. A. Quantification of mRNA using real-time RT-PCR. *Nature Protocols* **1**, 1559-1582, doi:10.1038/nprot.2006.236 (2006).
168 Henriksen, M. *et al.* Change of diagnosis during the first five years after onset of inflammatory bowel disease: Results of a prospective follow-up study (the IBSEN Study). *Scandinavian Journal of Gastroenterology* **41**, 1037-1043, doi:10.1080/00365520600554527 (2006).
169 Bustin, S. A. *et al.* The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin Chem* **55**, 611-622, doi:10.1373/clinchem.2008.112797 (2009).
170 Arkteg, C. B. *et al.* Real-life evaluation of histologic scores for Ulcerative Colitis in remission. *PLOS ONE* **16**, e0248224, doi:10.1371/journal.pone.0248224 (2021).
171 Landis, J. R. & Koch, G. G. The Measurement of Observer Agreement for Categorical Data. *Biometrics* **33**, 159-174, doi:10.2307/2529310 (1977).
172 Allen, M. The SAGE Encyclopedia of Communication Research Methods. doi:10.4135/9781483381411 (2017).
173 Allez, M. *et al.* Report of the ECCO pathogenesis workshop on anti-TNF therapy failures in inflammatory bowel diseases: Definitions, frequency and pharmacological aspects. *Journal of Crohn's and Colitis* **4**, 355-366, doi:10.1016/j.crohns.2010.04.004 (2010).
174 Zhao, M. *et al.* Trends in the use of biologicals and their treatment outcomes among patients with inflammatory bowel diseases – a Danish nationwide cohort study. *Alimentary pharmacology & therapeutics* **55**, 541-557, doi:https://doi.org/10.1111/apt.16723 (2022).

175 D'Haens, G. *et al.* Early combined immunosuppression or conventional management in patients with newly diagnosed Crohn's disease: an open randomised trial. *Lancet (London, England)* **371**, 660-667, doi:10.1016/s0140-6736(08)60304-9 (2008).
176 Kobayashi, T. *et al.* Discontinuation of infliximab in patients with ulcerative colitis in remission (HAYABUSA): a multicentre, open-label, randomised controlled trial. *Lancet Gastroenterol Hepatol* **6**, 429-437, doi:10.1016/s2468-1253(21)00062-5 (2021).
177 Law, C. C. Y. *et al.* Early Biologic Treatment Decreases Risk of Surgery in Crohn's Disease but not in Ulcerative Colitis: Systematic Review and Meta-Analysis. *Inflammatory bowel diseases*, doi:10.1093/ibd/izad149 (2023).
178 Cleveland, N. *et al.* EARLY VERSUS DELAYED INITIATION OF VEDOLIZUMAB IN ULCERATIVE COLITIS: TREATMENT RESPONSE IN THE REAL WORLD (RALEE). *Gastroenterology* **162**, S105-S106, doi:10.1053/j.gastro.2021.12.218 (2022).
179 Ordás, I., Mould, D. R., Feagan, B. G. & Sandborn, W. J. Anti‐TNF monoclonal antibodies in inflammatory bowel disease: pharmacokinetics‐based dosing paradigms. *Clinical Pharmacology & Therapeutics* **91**, 635-646 (2012).
180 Casteele, N. V. *et al.* The relationship between infliximab concentrations, antibodies to infliximab and disease activity in Crohn's disease. *Gut* **64**, 1539-1545 (2015).
181 Moore, C., Corbett, G. & Moss, A. C. Systematic review and meta-analysis: serum infliximab levels during maintenance therapy and outcomes in inflammatory bowel disease. *Journal of Crohn's and Colitis* **10**, 619-625 (2016).
182 Casteele, N. V. *et al.* Trough concentrations of infliximab guide dosing for patients with inflammatory bowel disease. *Gastroenterology* **148**, 1320-1329. e1323 (2015).
183 Arias, M. T. *et al.* A panel to predict long-term outcome of infliximab therapy for patients with ulcerative colitis. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association* **13**, 531-538, doi:10.1016/j.cgh.2014.07.055 (2015).
184 Siegel, C. A. *et al.* Perspectives From Patients and Gastroenterologists on De-escalating Therapy for Crohn's Disease. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association* **19**, 403-405, doi:10.1016/j.cgh.2019.11.062 (2021).

7 APPENDIX OF PAPER I-III

7.1 Paper 1

7.2 Paper 2

BMC Gastroenterology

RESEARCH ARTICLE Open Access

Discovery and validation of mucosal TNF expression combined with histological score - a biomarker for personalized treatment in ulcerative colitis

Jon R. Florholmen[1,2,3], Kay-Martin Johnsen[1,2*], Renate Meyer[1], Trine Olsen[1,2], Øystein K. Moe[1,4], Petter Tandberg[3], Mona D. Gundersen[1,2], Jan-Magnus Kvamme[1,2], Knut Johnsen[1,4], Terje Løitegård[5], Gabriele Raschpichler[6], Cecilia Vold[7], Sveinung W. Sørbye[8] and Rasmus Goll[1,2]

Abstract

Background: There are no accurate markers that can predict clinical outcome in ulcerative colitis at time of diagnosis. The aim of this study was to explore a comprehensive data set to identify and validate predictors of clinical outcome in the first year following diagnosis.

Methods: Treatment naive-patients with ulcerative colitis were included at time of initial diagnosis from 2004 to 2014, followed by a validation study from 2014 to 2018. Patients were treated according to clinical guidelines following a standard step-up regime. Patients were categorized according to the treatment level necessary to achieve clinical remission: mild, moderate and severe. The biopsies were assessed by Robarts histopathology index (RHI) and TNF gene transcripts.

Results: We included 66 patients in the calibration cohort and 89 patients in the validation. Mucosal TNF transcripts showed high test reliability for predicting severe outcome in UC. When combined with histological activity (RHI) scores the test improved its diagnostic reliability. Based on the cut-off values of mucosal TNF and RHI scores from the calibration cohort, the combined test had still high reliability in the validation cohort (specificity 0.99, sensitivity 0.44, PPV 0.89, NPV 0.87) and a diagnostic odds-ratio (DOR) of 54.

Conclusions: The combined test using TNF transcript and histological score at debut of UC can predict severe outcome and the need for anti-TNF therapy with a high level of precision. These validated data may be of great clinical utility and contribute to a personalized medical approach with the possibility of top-down treatment for selected patients.

Keywords: antiTNF, Calprotectin, Cytokines, Diagnostic odds ratio, Robarts histopathology index

* Correspondence: kay-martin.johnsen@unn.no
[1]Research Group of Gastroenterology and Nutrition, Department of Clinical Medicine, University of Tromsø, Tromsø, Norway
[2]Department of Gastroenterology, Division of Internal Medicine, University Hospital of North Norway, Tromsø, Norway
Full list of author information is available at the end of the article

Background

Ulcerative colitis (UC) is one of the two main disease entities of inflammatory bowel disease (IBD). UC is a chronic inflammatory disease believed to result from a dysregulated immune response caused by a combination of environmental and genetic factors causing loss of immunotolerance in the gut [1]. Many patients experience severe outcomes of disease with significant reduction in quality of life. The need for surgery is reported in 8 and 9.7% after 5 and 11 years, respectively [2, 3].

Definitions of clinical outcomes and prognosis in IBD are poorly defined, with little agreement on primary and secondary endpoints [4]. The IBSEN study is one of the most well-known prospective studies on clinical outcomes in UC, where the patients were divided into 4 predefined patterns of disease [2]. In a recently published review, the extent of disease and high disease activity were predictors of a more severe progression of disease [5].

The Montréal guidelines classify UC disease activity into four categories; clinical remission, mild, moderate and severe disease [6, 7]. Different guidelines for medical and surgical treatment are available for both UC and CD in Europe and America, European Crohn's and Colitis Organization (ECCO) guidelines and American Gastroenterological Association (AGA) clinical care pathway repectively [8, 9]. Danese et al. have created a modified algorithm with a medical step-up approach for the treatment of UC with the goal of achieving clinical remission [10]. In short, 5-ASA and local steroids are used in mild disease, with additional oral steroids, immunosuppressive and biological therapy in moderate to severe disease, consecutively. In contrast, a so-called top-down therapy has previously been documented to induce long term clinical remission of Crohn's disease [11].

From a clinical point of view, there is a need to find good predictive markers at onset of disease that enables clinicians to individually tailor therapy. There is an increasing interest for a biomarker approach. In various diseases, such as breast cancer, four gene subtypes of human epidermal growth factor receptor 2 (*HER2)* forms the basis of a molecular reclassification of disease according to risk factors [12]. Although there are an increasing number of reports and reviews for clinical and biochemical biomarkers at onset of disease, none have been able to predict future clinical outcome with great certainty [13–18]. In our research group we have published reports on mucosal transcript levels of tumor necrosis factor (TNF) as a biomarker for response to and when to stop anti-TNF thereapy [19–21], However, most of the studies are of retrospective design and there is a lack of validated studies of prognostic biomarkers to predict the clinical outcome in IBD with high reliability. Moreover, a personalized therapy approach initiated at the time of disease diagnosis, may have an impact on the natural course of IBD. This is so far unsettled due to the lack of long- term studies [22–24].

There is increasing knowledge of the pathophysiological events mediating the mucosal inflammation in IBD including cytokine and chemokine responses [25, 26]. So far there are few reports on how these crucial mediators can be used as biomarkers [19–22]. Therefore, the aims of this study were, first, based on a calibration cohort of newly diagnosed patients with ulcerative colitis from 2004 to 2014, to discover potential clinical, biochemical, histological and mucosal gene transcripts to predict 1 year level of treatment to obtain remission. Second, to validate these parameters in a cohort study from 2014 to 2018.

Methods

The main goal of the study was to detect and validate potential predictors of treatment level 1 year after disease onset of UC. In principle, to do a proper validation of a predictor(s) it is general accepted that this should be a two-step procedure. First, we have to study a calibration (discover) cohort, followed by a study of a validation cohort to validate the candidate predictors from the discovery study. Inclusion criteria for both the discovery and validation cohort were patients with newly diagnosed, treatment- naive UC aged ≥18 years. Patients were excluded if they were lost to follow in the first year after diagnosis, patients with severe medical disease other than UC, pregnancy and lactation; and patients who first were diagnosed UC but later developed an indeterminate form of IBD.

In addition to the UC patients with newly diagnosed, treatment-naïve disease, a group of healthy subjects performing a cancer screening examination with no clinical, endoscopic or histological signs of intestinal disease were included as controls.

Cohorts examined

Calibration cohort

Patients attending the Gastrointestinal Unit at the University Hospital of North Norway, Tromsø, Norway, were recruited from the project *Immunopathogenesis in inflammatory bowel disease* in the time period January 2004 –March 2014. *Validation cohort:* Patients were recruited in the time period March 2014 –March 2018 attending 6 clinical centers in Norway (Gastrointestinal units at the hospitals of Kirkenes, Hammerfest, University Hospital North Norway, Tromsø, Bodø, Vestre Viken (Ringerike and Drammen)) as a part of an ongoing prospective study - *Advanced Study of Inflammatory Bowel disease* (ASIB- study).

Diagnosis, clinical grading and clinical outcome after 1 year

The clinical grading of UC was based on evaluation of clinical activity at 1 year. The biopsies were histologically assessed by an experienced pathologist (SWS) using Robarts histopathology index (RHI) score [27].

The clinical outcomes of UC are based on the required treatment level to obtain disease remission, using the step-up algorithm guidelines ECCO and the three levels proposed by Danese et al. [10, 28] In this study we used three disease outcome levels after 1 year; mild, moderate and severe. These outcomes were defined by the treatment level needed for clinical remission; 5-ASA per oral or local (mild), need of oral steroids and/or thiopurines (moderate) and need of anti-TNF and/or surgery (severe) (see Fig. 1). Clinical remission was defined by ulcerative colitis clinical score (UCCS) < 2 [29] and/or calprotectin level < 100 mg/kg according to Feagan et al. Faecal calprotectin was measured by an ELISA kit from Calpro Norway (Oslo, Norway).

Tissue samples

Colonic mucosal biopsies were sampled from the region with the most severe inflammation. In healthy controls, biopsies were sampled from the sigmoid. Biopsy specimens for RNA extraction were immediately immersed in RNA *later* (Qiagen) and stored at room temperature overnight, then at − 20 °C until RNA isolation.

Cytokine transcript measurements

Total RNA was isolated from patient biopsies using Trizol until July 1, 2008; later the Allprep DNA/RNA Mini Kit (Qiagen, Hilden, Germany, Cat No: 80204) and the automated QIAcube instrument (Qiagen, Hilden, Germany) according to the manufacturer's recommendations. Quantity and purity of the extracted RNA were determined using the Qubit 3 Fluorometer (Cat No: Q33216; Invitrogen by Thermo Fisher Scientific, Waltham, MA, USA). Reverse transcription of the total RNA was performed using the QuantiTect Reverse Transcription Kit (Cat. No: 205314; Qiagen, Hilden, Germany). Mucosal TNF gene transcript was measured by real-time PCR procedures previously described in detail [30–33].

Statistics

The following factors were evaluated as predictors: extent of disease, UCDAI score and endoscopic sub-score, histological activity score, fecal calprotectin and mucosal cytokine transcripts. All baseline predictors were standardized and centered for exploring combinations of two variables. To evaluate predictors of outcome, ROC curves were constructed. Optimal cut-off values were picked by maximal Youden's J [34]. Test characteristics were derived by confusion matrices and diagnostic odds ratios [35]. A sequential test for mucosal TNF transcript and RHI score was constructed: Observations with a positive TNF test were run in a new ROC analysis for RHI score, which resulted in a two-step combined model with one cut-off value for mucosal TNF transcript and another cut-off value for RHI score following a positive TNF test.

As a global test, Kruskal Wallis one-way ANOVA was performed, then Mann-Whitney U test with Bonferroni correction. For categorical values Chi-square test with Bonferroni correction was utilized.

All statistical analyses were carried out in IBM SPSS Statistics 24 (IBM Corporation, Armonk, New York, USA).

Results

Healthy controls

Thirty-eight healthy controls were included, 13 females and 25 men aged 43–69 years. The median TNF value was 4450 copies/μg mRNA.

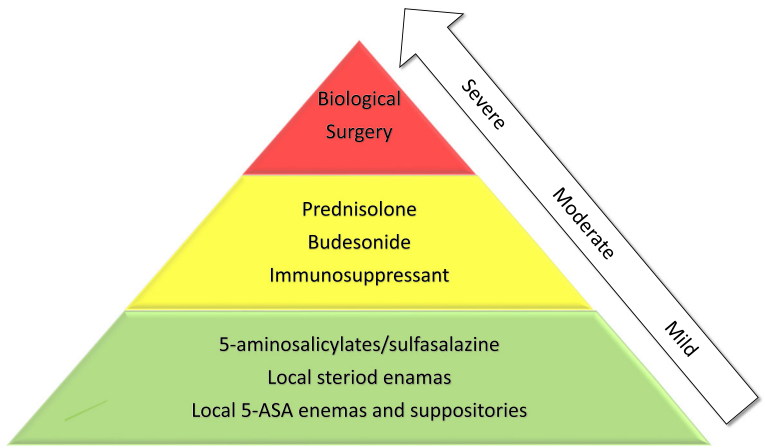


Fig. 1 In this and the following figures data from patients with ulcerative colitis at debut of disease are grouped after 1-year treatment level outcome, Step-up algorithm according to clinical treatment outcomes (mild, moderate, severe). Modified after Danese et al., see ref. [8]

Calibration cohort

Baseline characteristics and outcome groups

Sixty-six patients were included as a follow up mainly from an earlier report [19]. At 1 year follow-up patients were categorized into mild ($n = 23$), moderate ($n = 18$) and severe ($n = 25$) disease outcomes based on a step-up treatment level algorithm. In the moderate outcome group, no patients needed continuous steroid treatment and two patients were treated with azathioprine. In the severe outcome group, all patients were on anti-TNF treatment including one patient that later was in the need of colectomy. Sixteen patients were on concomitant treatment with azathioprine and one patient on methotrexate. An overview of baseline characteristics for each outcome group is shown in Table 1. There were significant differences between the three treatment groups for mucosal TNF and UCDAI scores ($p < 0.017$).

Discovery of potential biomarkers

With three defined treatment outcomes we made two sets of ROC curves, one set to discriminate between mild and moderate/severe and one set to discriminate between mild/moderate and severe. There were no baseline predictors that showed good test performance for discriminations between mild and moderate/severe (data not shown). However, there was a tendency towards increasing concentrations of the mucosal TNF transcripts with increasing treatment level (Fig. 2a).

Severe outcome

Baseline predictors of severe outcome are shown in Table 1 and Fig. 3 presenting clinical parameters (Calprotectin, UCDAI, Mayo endoscopic score), RHI score and mucosal TNF transcripts. Selected predictors including cut off values are shown in Table 3. Of individual factors, mucosal TNF transcript had the best test performance with a sensitivity, specificity and diagnostic odds ratio (DOR) of 0.81, 0.91 and 43 respectively. Clinical data including fecal calprotectin, UCDAI and RHI -score, yielded a high sensitivity but poor specificity (Table 2, Fig. 3), and therefore a poorer test performance than mucosal TNF transcript. To increase the test performance, we then combined mucosal TNF transcript and RHI score in a sequential setup: subjects with mucosal TNF transcript above cut-off were subjected to a second ROC curve using RHI score as predictor. The combined sequential test of mucosal TNF transcript and RHI score showed a superior test performance for specificity and DOR, however lower sensitivity (Table 2). No other clinical, biochemical, histological or immunological combinations could improve the test performance of prediction of severe outcome (supplement material Fig. 4).

Validation cohort

Baseline characteristics and outcome groups

At one year follow up patients were categorized into mild ($n = 36$), moderate ($n = 31$) and severe ($n = 22$) disease outcomes based on a step-up treatment level

Table 1 Baseline characteristics of patients in the calibration cohort with ulcerative colitis according to one-year treatment outcome level

Patients groups	Mild $N = 23$	Moderate $N = 18$	Severe $N = 25$
Age med (IQR)	41 (35–54)	35 (24–55)	41 (27–54)
Sex			
Female	15 (65%)	7 (39%)	9 (36%)
Male	8 (35%)	11 (61%)	16 (64%)
Colonic area involved			
Proctitis	9 (39%)	3 (17%)	3 (12%)
Left side	9 (39%)	7 (39%)	10 (40%)
Extensive	5 (22%)	8 (44%)	12 (48%)
Smoking	14	21	12
Current smoker	4 (29%)	2 (17%)	2 (10%)
Non-smoker	10 (71%)	10 (83%)	18 (90%)
Mucosal TNF*	10,500 (4600–11,900)	12,000 (8000–17,200)	26,900 (18700–40,400)
UCDAI med (IQR)* at debut	7 (5–8)	9 (8–12)	12 (9–12)
Calprotectin med (IQR)	590 (400–1100)	790 (470–1540)	2300 (670–2500)
RHI med (IQR)	9 (5–10)	7 (6–10)	9 (7–12)
UCCS score 1-year med (IQR)	0 (0–0)	0 (0–2)	0 (0–2)
Calprotectin 1-year med (IQR)	60 (25–85)	50 (25–100)	25 (0–160)

*$p < 0{,}017$ between groups, Mann-Whitney U test with Bonferroni correction
Med (IQR) Median (Interquartile range), *RHI* Robarts histopathology index. Mucosal TNF in copies/μg RNA: Fecal calprotectin in mg/kg

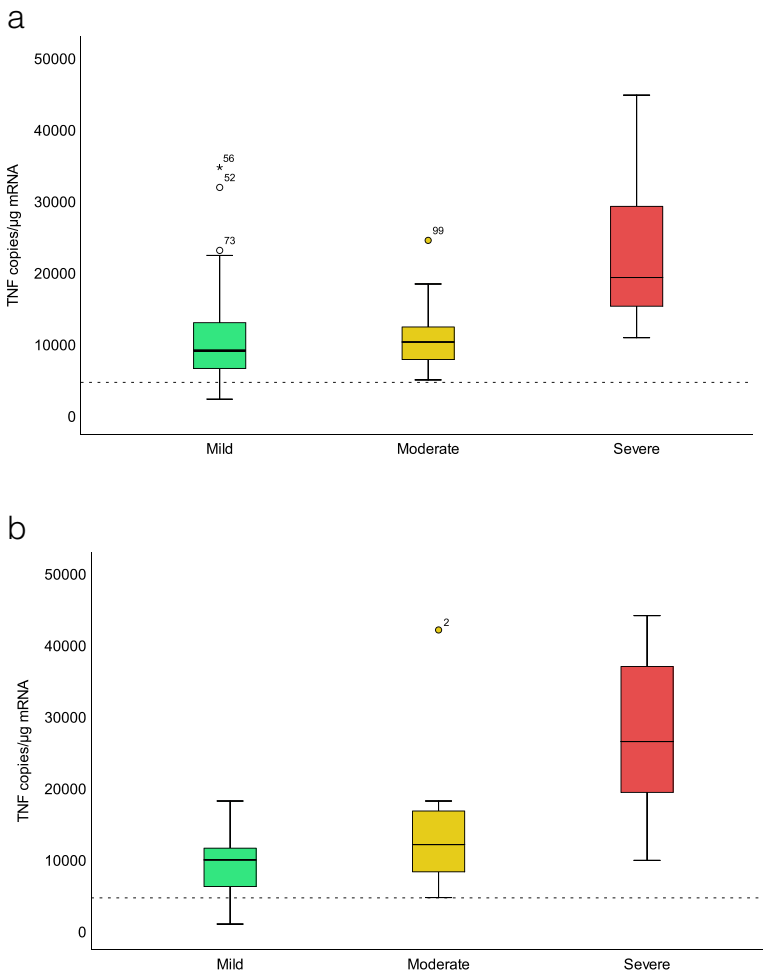


Fig. 2 Mucosal TNF transcript in treatment outcome groups and in healthy normal controls in the calibration cohort (**a**) and the validation group (**b**)

algorithm. In the moderate outcome group, no patients needed continuous steroid treatment and five subjects were treated with azathioprine. In the severe outcome group, 22 patients were on anti-TNF treatment whereas two of these patients were later in the need of colectomy. Thirty-eight healthy controls were included. An overview of baseline characteristics for each outcome group is shown in Table 3. There were significant differences between the three treatment groups for mucosal TNF, UCDAI, RHI scores and fecal calprotectin (Table 3, Fig. 2b).

Validation of predictors of severe outcome

The cut off values from the discovery study (TNF ≥ 18,000, RHI ≥ 9) were used for test performance. The baseline predictors of severe outcome presenting mucosal TNF transcripts and RHI score are shown in Table 4. Mucosal TNF transcript had a test performance with sensitivity, specificity and DOR of 0.5, 0.9 and 9 respectively. RHI transcript had a test performance with sensitivity, specificity and DOR of 0.72, 0.69 and 6, respectively. When combined TNF and RHI the specificity increased to high 0.99, whereas the DOR was still high as 54. Moreover, the low sensitivity of 0.44 represents most likely the overlapping TNF and RHI score values to the mild/moderate outcome groups (Table 3).

Discussion

We present a combined discovery study (from 2004) and a validation study (from 2014) in a prospective design (the transomic Advanced Study of Inflammatory Bowel Disease) where clinical, biochemical, histological and transcript data where retrospectively tested to identify biomarkers of clinical outcome 1 year after disease diagnosis of UC. Mucosal TNF transcripts showed high test reliability for predicting severe outcome after 1 year in UC in both studies but was not ideal to discriminate between mild, moderate and severe disease. Moreover, when the TNF transcripts were combined with histological activity (RHI) scores, the test improved its

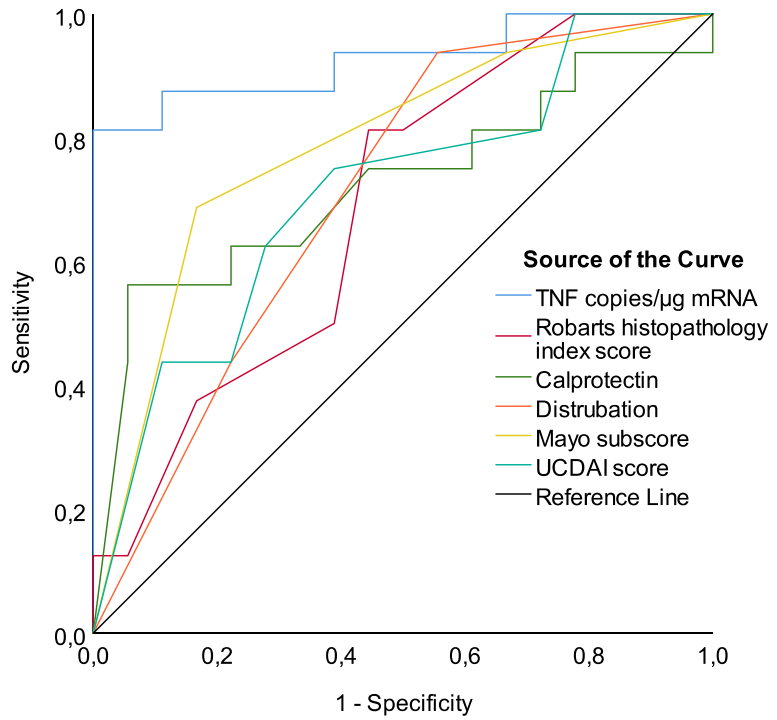


Fig. 3 ROC curves of predictors of severe outcome in calibration cohorte

diagnostic reliability. Mucosal cut-off values for TNF and RHI scores determined in the calibration cohort displayed a high test performance with specificity of 0.99 and a diagnostic odds-ratio (DOR) of 54 in the prospective validation study. Thus, mucosal TNF transcript combined with a histological score at debut of disease can likely identify patients who experience severe outcomes during the first year. This is an important step towards personalizing treatment in IBD and may be used as a criterion for selecting candidates for top-down treatment of anti-TNF. However, this awaits further studies.

We have tested a broad spectrum of potential factors that could, alone, or in combinations, predict clinical outcome in the first year of diagnosis. The clinical outcomes were defined as the highest treatment level required for achieving disease remission during the first year of disease, in a step-up treatment approach. The broad/wide selection of variables including various combinations did not have the necessary precision to discriminate between mild, moderate and severe outcomes. However mucosal TNF transcript in combination with the histological RHI score was able to predict, with high precision, the most severe colitis outcomes needing biological or surgical treatment, within the first year of disease. The validated cut-off values (TNF ≥18,000, RHI ≥ 9) showed a high specificity to predict severe outcome and a DOR as high as 54. From a clinical point of view, these cut–off values indicate a need of anti-TNF therapy during the first year after diagnosis with high reliability, and therefore of high clinical value and utility in the management of IBD/UC. In order to use a biomarker for selection for top-down treatment, a high PPV is

Table 2 Factors at debut of ulcerative colitis in the calibration cohort to predict severe treatment outcomes at one year of disease

Factors	Youden's J	Cut-off value	Sensitivity	Specificity	PPV	NPV	DOR
TNF[a]	0,72	≥18,000	0,81	0,91	0,85	0,89	43
RHI[a]	0,23	≥9	0,71	0,52	0,48	0,74	3
Combined TNF RHI	0,57	≥18,000 and ≥ 9	0,57	1	1	0,79	∞
UCDAI	0,4	≥9	0,79	0,61	0,54	0,83	6
Mayo subscore	0,45	3	0,72	0,73	0,62	0,81	7
Calprotectin	0,51	≥2000	0,6	0,91	0,86	0,72	15

Diagnostic odds ratio PPV: Positive predictive value *NPV* Negative predictive value
[a] copies/µg mRNA [b]Robarts histopathology index score

Table 3 Baseline characteristics of patients with ulcerative colitis in the validation cohort according to one-year treatment outcome level

Patient groups	Mild *N* = 36	Moderate *N* = 31	Severe *N* = 22
Age med (IQR)	36 (24–49)	30 (24–41)	26 (22–47)
Sex			
Female	17 (47%)	8 (26%)	10 (46%)
Male	19 (53%)	23 (74%)	12 (54%)
Colonic area involved			
Proctitis	5 (14%)	1 (3%)	1 (4%)
Left side	25 (69%)	18 (58%)	10 (46%)
Extensive	6 (17%)	12 (39%)	11 (50%)
Smoking	28	21	12
Current smoker	1 (4%)	2 (10%)	1 (8%)
Non-smoker	27 (96%)	19 (90%)	11 (92%)
Mucosal TNF*	8800 (6100–12,800)	10,500 (7400–13,200)	17,400 (15100–26,800)
UCDAI med (IQR)* at debut	7 (5–9)	9 (8–11)	10 (7–11)
Calprotectin med (IQR)*	570 (200–970)	1000 (340–2000)	1100 (830–1400)
RHI med (IQR)*	6 (2–10)	6 (4–11)	14 (9–27)
UCCS score 1-year med (IQR)	0 (0–0)	0 (0–0)	0 (0–8)
Calprotectin 1-year med (IQR)	40 (25–94)	50 (0–140)	25 (20–60)

*$p < 0{,}017$ between groups, Mann-Whitney U test with Bonferroni correction
Med (IQR): median (Interquartile range); RHI: Robarts histopathology index. Mucosal TNF in copies/μg RNA; Fecal calprotectin in mg/kg

necessary to avoid excessive use of biologics. Our proposed biomarker shows a PPV of 0.89 meaning that 9 out of 10 positives will be correctly identified as severe outcome.

A step-up treatment approach represents well-established international guidelines [8–10]. One drawback of this approach is that patients in the severe outcome group often experience a period of poor response during the gradual escalation of treatment intensity until an adequate response is obtained. In some cases, one may lose an important window of opportunity for optimal effect of biologics leading to permanent structural damage and/or need of surgery. The impact of early treatment before development of severe disease is not completely investigated. However, the top down approach published by D'Haens et al. indicated that immunosuppressive therapy was superior to a step-up approach in patients with Crohn's disease [36]. Moreover, it is well documented that induction of treatment to remission reduces later hospitalization, whereas conflicting results exist for colectomy in two studies [37, 38].

The use of molecular data from the mucosa represents a novel approach and is an easily available tool, with high utility for clinicians to individually tailor therapy in UC. Endoscopic biopsies are routinely taken at diagnosis and surveillance of IBD. Thus, the logistics of measuring mucosal TNF transcript are simple, as biopsies are readily available and samples do not require freezing prior to analysis [31].

Our study contributes with new knowledge in the scientific field of personalized therapy in UC [15, 16]. We know that treatment to remission improves long-term clinical outcome [39, 40]. The main question is: Can a top-down therapy of the most severe forms of disease have an effect on the natural course of disease? This awaits future studies.

The strength of this prospective designed, combined discovery and validation study is that we have

Table 4 Factors at debut of ulcerative colitis in the validation cohort to predict severe treatment outcomes at one year of disease based on cut off values from the discovery cohort

Factors	Youden's J	Cutt off value	Sensitivity	Specificity	PPV	NPV	DOR
TNF[a]	0,40	≥18,000	0,50	0,90	0,56	0,87	9
RHI[b]	0,41	≥9	0,72	0,69	0,38	0,90	6
Combined TNF RHI	0,43	18,000 ≥ 9	0,44	0,99	0,89	0,87	54

DOR Diagnostic odds ratio, *PPV* Positive predictive value *NPV* Negative predictive value
[a] copies/μg mRNA [b]Robarts histopathology index score

retrospectively searched for and validated biomarkers for treatment at debut of UC, using a broad search of clinical, histological and analytical factors including mucosal immune transcripts. Moreover, this is part of the transomic Advanced Study of Inflammatory Bowel disease (ASIB) study where parallel studies of the epigenome, transcriptome, proteome and metabolome are ongoing [33, 41–45]. .This transomic approach at debut of UC will be performed and correlated to long-term clinical outcome. Therefore, the upcoming transomic data from the ASIB study and from several ongoing studies such as the PREDICTS study will not only search for therapeutic but also prognostic and natural course biomarkers [17]. The weakness of the study includes the lack of endoscopic diagnosis at 1 year, which would have given insight into endoscopic status and endoscopic remission rates according to treatment levels. Additionally, the decision to use or not use steroids at time of diagnosis is dependent on the subjective decision of the clinicians. This may be one explanation for the small differences detected between the mild and moderate treatment group.

Conclusion

The combined information of mucosal TNF transcription and histological score at debut of UC can predict severe outcome and the need for anti-TNF therapy. This is of great clinical utility and may contribute to a personalized medicine approach in UC.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12876-020-01447-0.

Additional file 1: Figure 4. Supplement figure with ROC curves of predictors of mild outcome from calibration cohort

Abbreviations

RHI: Robarts histopathology index; DOR: Diagnostic odds-ratio; UC: Ulcerative colitis; IBD: Inflammatory bowel disease; TNF: Tumor necrosis factor; ASIB-study: Advanced Study of Inflammatory Bowel disease; UCCS: Ulcerative colitis clinical score

Acknowledgments

The publication charges for this article have been funded by a grant from the publication fund of UiT The Arctic University of Norway. We thank Ingrid Christiansen, Marian Remijn and Line Wilsgaard for expert technical assistance.

Authors' contributions

Planning and conducting: JRF, KMJ, RG, KJ, RM, TO, SWS, ØKM, PT, MDG, JMK, TL, GR, CV Collecting or interpreting data: JRF, KMJ, RG, KJ, RM, TO, SWS, ØKM, PT, MDG, JMK, TL, GR, CV. Drafting of manuscript: JRF, KMJ, RG, KJ, RM, TO, SWS, ØKM, PT, MDG, JMK, TL, GR, CV. The authors read and approved the final manuscript.

Funding

This work was supported by Northern Norway Regional Health Authority, ID SFP-50-04, SFP-888-09 and SFP-1136-13.

Availability of data and materials

Data are available from the authors upon reasonable request due to privacy/ethical restrictions.

Ethics approval and consent to participate

All participants were informed and signed a written consent to participate and publication.
Approval including the use of biobank was granted by the Regional Committee of Medical Ethics of Northern Norway Ref no: 14/2004 and 1349/2012.

Consent for publication

All authors have approved the final manuscript for publication.

Competing interests

None declared from all authors.

Author details

[1]Research Group of Gastroenterology and Nutrition, Department of Clinical Medicine, University of Tromsø, Tromsø, Norway. [2]Department of Gastroenterology, Division of Internal Medicine, University Hospital of North Norway, Tromsø, Norway. [3]Department of Internal Medicine, Vestre Viken Hospital, Hønefoss, Norway. [4]Department of Internal Medicine, Hammerfest Hospital, Hammerfest, Norway. [5]Department of Gastroenterology, Vestre Viken Hospital, Drammen, Norway. [6]Department of Internal Medicine, Kirkenes, Norway. [7]Department of Gastroenterology, Nordland Hospital, Bodø, Norway. [8]Department of Pathology, University Hospital of North Norway, Tromsø, Norway.

Received: 22 April 2020 Accepted: 9 September 2020
Published online: 02 October 2020

References

1. Zhang YZ, Li YY. Inflammatory bowel disease: pathogenesis. World J Gastroenterol. 2014;20:91–9.
2. Henriksen M, Jahnsen J, Lygren I, et al. Ulcerative colitis and clinical course: results of a 5-year population-based follow-up study (the IBSEN study). Inflamm Bowel Dis. 2006;12:543–50.
3. Solberg IC, Hoivik ML, Cvancarova M, et al. Risk matrix model for prediction of colectomy in a population-based study of ulcerative colitis patients (the IBSEN study). Scand J Gastroenterol. 2015;50:1456–62.
4. Williet N, Sandborn WJ, Peyrin Biroulet L. Patient-reported outcomes as primary end points in clinical trials of inflammatory bowel disease. Clin Gastroenterol Hepatol. 2014;12:1246–1256.e6.
5. da Silva BC, Lyra AC, Rocha R, et al. Epidemiology, demographic characteristics and prognostic predictors of ulcerative colitis. World J Gastroenterol. 2014;20:9458–67.
6. Silverberg MS, Satsangi J, Ahmad T, et al. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a working party of the 2005 Montreal world congress of gastroenterology. Can J Gastroenterol. 2005;19 Suppl A:5A–36A.
7. Satsangi J, Silverberg MS, Vermeire S, et al. The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. Gut. 2006;55:749–53.
8. Dassopoulos T, Cohen RD, Scherl EJ, et al. Ulcerative colitis care pathway. Gastroenterology. 2015;149:238–45.
9. Magro F, Gionchetti P, Eliakim R, et al. Third European evidence-based consensus on diagnosis and Management of Ulcerative Colitis. Part 1: definitions, diagnosis, extra-intestinal manifestations, pregnancy, cancer surveillance, surgery, and Ileo-anal pouch disorders. J Crohns Colitis. 2017;11:649–70.
10. Danese S, Siegel CA, Peyrin-Biroulet L. Review article: integrating budesonide-MMX into treatment algorithms for mild-to-moderate ulcerative colitis. Aliment Pharmacol Ther. 2014;39:1095–103.
11. D'Haens GR. Top-down therapy for IBD: rationale and requisite evidence. Nat Rev Gastroenterol Hepatol. 2010;7:86–92.
12. Wolff AC, Hammond ME, Hicks DG, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. J Clin Oncol. 2013;31:3997–4013.

13. Choung RS, Princen F, Stockfisch TP, et al. Serologic microbial associated markers can predict Crohn's disease behaviour years before disease diagnosis. Aliment Pharmacol Ther. 2016;43:1300–10.
14. Hamilton AL, Kamm MA, De Cruz P, et al. Serologic antibodies in relation to outcome in postoperative Crohn's disease. J Gastroenterol Hepatol. 2017;32:1195–203.
15. Spekhorst LM, Imhann F, Festen EAM, et al. Cohort profile: design and first results of the Dutch IBD biobank: a prospective, nationwide biobank of patients with inflammatory bowel disease. BMJ Open. 2017;7:e016695.
16. Stevens TW, Matheeuwsen M, Lonnkvist MH, et al. Systematic review: predictive biomarkers of therapeutic response in inflammatory bowel disease-personalised medicine in its infancy. Aliment Pharmacol Ther. 2018;48:1213–31.
17. Porter CK, Riddle MS, Gutierrez RL, et al. Cohort profile of the PRoteomic evaluation and discovery in an IBD cohort of tri-service subjects (PREDICTS) study: rationale, organization, design, and baseline characteristics. Contemp Clin Trials Commun. 2019;14:100345.
18. Dulai PS, Peyrin-Biroulet L, Danese S, et al. Approaches to integrating biomarkers into clinical trials and care pathways as targets for the treatment of inflammatory bowel diseases. Gastroenterology. 2019;157:1032–1043.e1.
19. Olsen T, Goll R, Cui G, et al. TNF-alpha gene expression in colorectal mucosa as a predictor of remission after induction therapy with infliximab in ulcerative colitis. Cytokine. 2009;46:222–7.
20. Rismo R, Olsen T, Cui G, et al. Normalization of mucosal cytokine gene expression levels predicts long-term remission after discontinuation of anti-TNF therapy in Crohn's disease. Scand J Gastroenterol. 2013;48:311–9.
21. Johnsen KM, Goll R, Hansen V, et al. Repeated intensified infliximab induction - results from an 11-year prospective study of ulcerative colitis using a novel treatment algorithm. Eur J Gastroenterol Hepatol. 2017;29:98–104.
22. Flamant M, Roblin X. Inflammatory bowel disease: towards a personalized medicine. Therap Adv Gastroenterol. 2018;11:1756283x17745029.
23. Siegel CA, Refocusing IBD. Patient management: personalized, proactive, and patient-centered care. Am J Gastroenterol. 2018;113:1440–3.
24. Weimers P, Munkholm P. The natural history of IBD: lessons learned. Curr Treat Options Gastroenterol. 2018;16:101–11.
25. de Souza HSP. Etiopathogenesis of inflammatory bowel disease: today and tomorrow. Curr Opin Gastroenterol. 2017;33:222–9.
26. Kim DH, Cheon JH. Pathogenesis of inflammatory bowel disease and recent advances in biologic therapies. Immune Netw. 2017;17:25–40.
27. Mosli MH, Feagan BG, Zou G, et al. Development and validation of a histological index for UC. Gut. 2017;66:50–8.
28. Dignass A, Lindsay JO, Sturm A, et al. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis part 2: current management. J Crohns Colitis. 2012;6:991–1030.
29. Feagan BG, Greenberg GR, Wild G, et al. Treatment of ulcerative colitis with a humanized antibody to the α4β7 integrin. N Engl J Med. 2005;352:2499–507.
30. Olsen T, Goll R, Cui G, et al. Tissue levels of tumor necrosis factor-alpha correlates with grade of inflammation in untreated ulcerative colitis. Scand J Gastroenterol. 2007;42:1312–20.
31. Cui G, Olsen T, Christiansen I, et al. Improvement of real-time polymerase chain reaction for quantifying TNF-alpha mRNA expression in inflamed colorectal mucosa: an approach to optimize procedures for clinical use. Scand J Clin Lab Invest. 2006;66:249–59.
32. Olsen T, Rismo R, Gundersen MD, et al. Normalization of mucosal tumor necrosis factor-alpha: a new criterion for discontinuing infliximab therapy in ulcerative colitis. Cytokine. 2016;79:90–5.
33. Diab J, Al-Mahdi R, Gouveia-Figueira S, et al. A quantitative analysis of colonic mucosal Oxylipins and Endocannabinoids in treatment-naive and deep remission ulcerative colitis patients and the potential link with cytokine gene expression. Inflamm Bowel Dis. 2019;25:490–7.
34. Youden WJ. Index for rating diagnostic tests. Cancer. 1950;3:32–5.
35. Glas AS, Lijmer JG, Prins MH, et al. The diagnostic odds ratio: a single indicator of test performance. J Clin Epidemiol. 2003;56:1129–35.
36. D'Haens G, Baert F, van Assche G, et al. Early combined immunosuppression or conventional management in patients with newly diagnosed Crohn's disease: an open randomised trial. Lancet. 2008;371:660–7.
37. Colombel JF, Rutgeerts P, Reinisch W, et al. Early mucosal healing with infliximab is associated with improved long-term clinical outcomes in ulcerative colitis. Gastroenterology. 2011;141:1194–201.
38. Burisch J, Kiudelis G, Kupcinskas L; Epi-IBD group, et al. Natural disease course of Crohn's disease during the first 5 years after diagnosis in a European population-based inception cohort: an Epi-IBD study. Gut. 2019;68:423–33.
39. Rutgeerts P, Vermeire S, Van Assche G. Mucosal healing in inflammatory bowel disease: impossible ideal or therapeutic target? Gut. 2007;56:453–5.
40. Arias MT, Vande Casteele N, Vermeire S, et al. A panel to predict long-term outcome of infliximab therapy for patients with ulcerative colitis. Clin Gastroenterol Hepatol. 2015;13:531–8.
41. Schniers A, Anderssen E, Fenton CG, et al. The proteome of ulcerative colitis in colon biopsies from adults - optimized sample preparation and comparison with healthy controls. Proteomics Clin Appl. 2017;11. https://doi.org/10.1002/prca.201700053. https://onlinelibrary.wiley.com/action/showCitFormats?doi=10.1002%2Fprca.201700053.
42. Taman H, Fenton CG, Hensel IV, et al. Genome-wide DNA methylation in treatment-naive ulcerative colitis. J Crohns Colitis. 2018;12:1338–47.
43. Taman H, Fenton CG, Hensel IV, et al. Transcriptomic landscape of treatment-naive ulcerative colitis. J Crohns Colitis. 2018;12:327–36.
44. Schniers A, Goll R, Pasing Y, et al. Ulcerative colitis: functional analysis of the in-depth proteome. Clin Proteomics. 2019;16:4.
45. Diab J, Hansen T, Goll R, et al. Lipidomics in ulcerative colitis reveal alteration in mucosal lipid composition associated with the disease state. Inflamm Bowel Dis. 2019;25:1780–7.

Publisher's Note

7.3 Paper 3

BMC Gastroenterology

RESEARCH

Open Access

Prediction of long-term remission in patients following discontinuation of anti-TNF therapy in ulcerative colitis: a 10 year follow up study

Kay-Martin Johnsen[1,2*], Jon Florholmen[1,2], Øystein K. Moe[1,2], Mona Gundersen[1,2], Julia Beilfuss[1,2], Hege Kileng[1,2], Sveinung W. Sørbye[3] and Rasmus Goll[1,2]

Abstract

Background: The long-term outcomes of Ulcerative colitis (UC) after discontinuation of biological therapy are largely unknown. There is also a lack of accurate and validated markers that can predict outcome after withdrawal accurately. The aims of this study were to describe the long-term outcomes in UC patients following cessation of anti-TNF therapy and explore potential biomarkers as an approach towards precision medicine.

Methods: Seventy-five patients with moderate to severe UC treated to remission with anti-tumor necrosis factor (TNF) were included in the study. This is a follow-up of previously reported UC outcomes. The patients were categorized as either "Remission" or "Relapse". The "Relapse" group was divided into subgroups determined by the highest treatment level needed to obtain remission the last 3 years of observation: non-biological therapy, biological therapy or colectomy. Remission were divided in long term remission (LTR), those using immunomodulating drugs (LTR + imids) and those using only 5-amino-salicylate (5-ASA) treatment (LTR) for the past 3 years. Analyses of mucosal gene expression by real-time PCR were performed.

Results: The median (IQR) observation time of all patients included was 121 (111–137) months. Of the 75 patients, 46 (61%) did not receive biological therapy, including 23 (31%) in LTR ± imids. Of these 23 patients, 16 (21%) were defined as LTR with a median observation time of (IQR) 95 (77–113) months. In total 14 patients (19%) underwent colectomy during the 10 years after first remission. Mucosal TNF copies/µg mRNA < 10 000 at anti-TNF discontinuation predicted long-term remission, biological free remission and lower risk of colectomy with a HR 0.36 (0.14–0.92) for long-term remission, HR 0.17 (0.04–0.78) for biological free remission and HR 0.12 (0.01–0.91) for colectomy. IL1RL1 was normalized in LTR phenotype and higher in relapsing UC.

Conclusion: In this 10-year follow-up of UC of patients with moderate to severe disease, 61% of patients experience an altered phenotype to a milder disease course without need of biological therapy. Twenty-one percent of the patients were LTR without any medication except of 5-ASA. Mucosal TNF gene expression and IL1RL1- transcripts may be of clinical utility for long term prognosis in development of precision medicine in UC.

Keywords: Anti-TNF therapy, Biological therapy, Inflammatory bowel disease, Tumor necrosis factor, Ulcerative colitis

Introduction

Ulcerative colitis (UC) is regarded as a lifelong chronic inflammatory disease most likely caused by genetic and environmental factors where the disease course on an individual level varies greatly [1–4]. One of the

*Correspondence: Kay-Martin.Johnsen@unn.no

[1] Research Group of Gastroenterology and Nutrition, Department of Clinical Medicine, UiT The Arctic University of Norway, Tromsö, Norway
Full list of author information is available at the end of the article

most referred studies for clinical outcomes and disease course of UC is from the IBSEN group [5]. During a 5-year period after diagnosis, 59% reported a decline in self-reported symptom burden, whereas 1%, 9% and 31% reported increasing activity, chronic continuous and, chronic relapsing disease, respectively. In a 10 year follow-up study of UC patients, 55% reported a disease course in remission or only mild symptoms [6].

In a recent review of population-based cohorts mainly including mild and moderate adult UC, 10–15% experienced an aggressive disease course with a 10 years cumulative risk of relapse as high as 70–80%, and a colectomy rate of 15% [7].

Biological therapies for UC, including anti-tumor necrosis factor (anti-TNF) have shown high efficacy in achieving disease remission- after 12 months [8–10]. However, loss of response in UC on biological maintenance therapy have been reported to be in the range of 15 to 20% annually [11]. Moreover, the one-year risk of relapse after ani-TNF withdrawal in UC can be as high as 50% and as low as 6% [12]. There is a lack of reports regarding long-term outcomes after discontinuation.

So far there are no risk factors, neither clinical, immunological, genetic or laboratory markers that can predict outcome after withdrawal accurately [13, 14]. Therefore, neither published AGA or ECCO guidelines include clear recommendations regarding withdrawal of biological therapy [13–15].

We have previously published results showing that normalization (< 10 000 copies/µg mRNA) of mucosal TNF mRNA when discontinuing anti-TNF treatment in both UC and Crohn´s disease prolonged the time in remission. In UC the median time to relapse was 36 months, compared to 11 months in the group with increased mucosal TNF mRNA level [16, 17]. These reports are of great value in the effort of obtaining predictive biomarkers suitable for a precision medicine algorithm with individualized therapy in UC [18–23].

This is a follow-up of the previously reported prospective UC outcome study [16] with 4 years extended outcome registrations until 2019 with the following aims:

1. To define the immunological phenotype (fingerprint) of long-term remission.
2. Define a subgroup of patients who are in long-term remission with only 5-ASA per oral maintenance treatment or no medical treatment (LTR).
3. Describe potential biomarkers to predict long-term remission following discontinuation of anti-TNF therapy; i.e. an approach to development of precision medicine.

Materials and methods

This study is part of the ongoing prospective transregional study Advanced Study of Inflammatory Bowel disease (ASIB- study). All patients included in this follow-up were previously treated to remission with anti-TNF, subsequent discontinuation, and retreatment in case of relapse. In the first report of clinical outcomes in UC patients following this algorithm, 116 patients were included. Of these 116 patients, 96 patients obtained remission and observed until August 2015 [16]. The patients included and follow-up are described in Fig. 1. In addition, 24 healthy controls (HC) and 9 patients with UC that relapses within 12 months after discontinuation of IFX were also recruited (early relapse group). The HC and the early relapse, were included to measure mucosal gene transcripts to compare with patients from the follow up with > 3 years in remission to find potential predictors of remission.

Categorization of long-term outcome after stop of anti-TNF

Patients were divided into predefined groups termed "Relapse" or "Remission". The "Relapse" group was further divided into subgroups determined by the highest treatment level needed to obtain remission in the last 3 years; Biological therapy, colectomy or non-biological therapy including; corticosteroids, immunomodulators, 5-ASA. Corticosteroid use was short term courses when needed. No patients received integrin blockers or JAK inhibitors. Patients in the Remission group, had no relapse during the study period following discontinuation of the last anti TNF treatment. The remission group was divided into two subgroups, long-term remission without any treatment other than 5-ASA (LTR) and with immunomodulating drugs (LTR + imids). The definition of LTR was minimum 3 years in clinical remission after stopping/discontinuing anti-TNF. Clinical remission was defined by a combination of global assessment using ulcerative colitis Clinical Score (UCCS) less than 3 and faecal calprotectin < 100 mg/kg. Patients in LTR were invited to a follow-up endoscopy and clinical evaluation with UCDAI score.

Criteria for discontinuation of anti-TNF treatment

The criteria of discontinuation of anti-TNF treatment were endoscopic remission with a Mayo endoscopic sub-score of 0–1, until these 4 years extended observation time whereas an additional criterium of clinical remission > 6 months and normalized mucosal TNF gene expression were included in 2014 [24, 25].

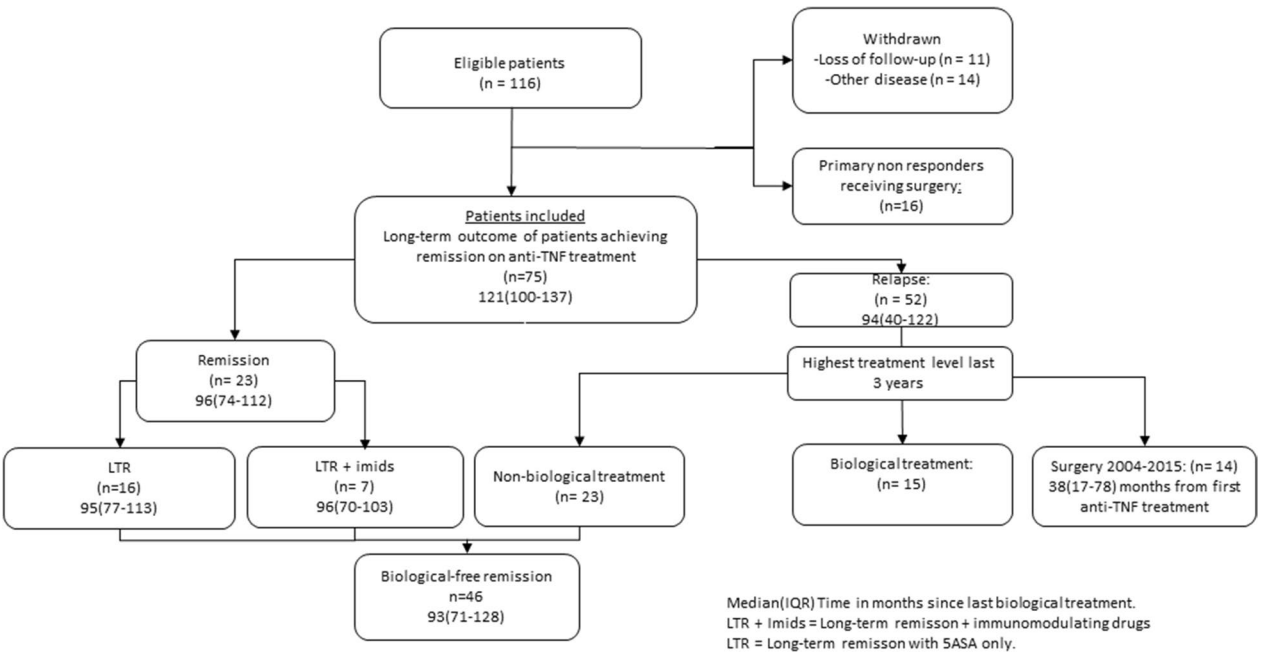


Fig. 1 Flow chart of long-term outcomes of patients treated with anti-TNF treatment

Relapse

Relapse was defined as a clinical, biochemical, endoscopic signs of disease activity leading to a therapeutic intervention as escalation of medical therapy or surgery.

Healthy control group

We included 24 healthy controls, 8 and 16 females and males respectively, with median (25–75 percentile) of 57 (42–67) years. The patients were recruited in the time period March 2014 to March 2018. The healthy control included patients referred for cancer screening where colonoscopy was normal. Exclusion criteria were serious medical conditions including immunological disorders, irritable bowel symptoms, polyps or cancer and abnormal histology in colonic biopsies.

Tissue samples

We performed tissue sampling by using standard forceps and retrieving two mucosal biopsies in witch we performed all cytokine measurements. Colonic mucosal biopsies were sampled from the region with most severe inflammation in patients with active inflammation. In patients in remission, biopsies were sampled from the previously most inflamed region. Biopsies were sampled from the sigmoid in healthy controls. The biopsies were histologically assessed by an experienced pathologist (SWS) using Robarts histopathology index (RHI) score [26]. The samples for RNA extraction were immediately immersed in RNA *later* (Qiagen), and stored at room temperature overnight, then at − 20 °C until RNA isolation.

Cytokine measurements

Real-time PCR procedures have previously been described in detail [25, 27, 28]. Total RNA was isolated from mucosal biopsies using the Allprep DNA/RNA Mini Kit (Qiagen, Hilden, Germany, Cat No: 80204) and the automated QIAcube instrument (Qiagen, Hilden, Germany) according to the manufacturer's recommendations. Quantity and purity of the extracted RNA were determined using the Qubit 3 Fluorometer (Cat No: Q33216; Invitrogen by Thermo Fisher Scientific, Waltham, MA, USA). Reverse transcription of the total RNA was performed using the QuantiTect Reverse Transcription Kit (Cat. No: 205314; Qiagen, Hilden, Germany) and QuantiNova probe RT-PR kit (Quiagen Cat no:208352). Primer sequences are previous published [29, 30]. The following gene transcripts were measured*: IL17a, IL23, IL4, IL5, IL6, IL10, IL21, IL33, IL1B, TGFβ, GATA, IL18, TLR4, IL1RL1, RORC, FOXp3, TBX21* and *TNF*.

Statistics

Two-way ANCOVA models adjusted for sex, disease distribution and age were performed to compare cytokine levels between groups and estimate effect size. For cytokines with a *P*-value of < 0.05, the difference in ΔΔCT fold change were calculated and converted to fold

difference indicating up or down regulated in LTR compared to HC and early relapse group. To evaluate predictors of surgery, biological free remission and remission without relapse, Cox regression analyses were performed and receiver operating characteristics (ROC) curves were constructed. All statistical analyses were carried out in IBM SPSS Statistics 24 (IBM Corporation, Armonk, New York, USA).

Results

Clinical groups and demographic characteristic

All patients included, including subgroup division are presented in Fig. 1. Demographic characteristics of patients included and main selected subgroups are shown in Table 1. The median observation time of all patients included was 121 (111–137 IQR) months.

Long-term remission

Of the 75 patients included in the study 23 (31%) were still in remission without any clinical, or endoscopic signs of relapse after stop of biological therapy (Fig. 1). The median observation time was 96 (74–112 IQR) months. In the previously described subgroup LTR, 16 patients did not receive any medication except per oral 5ASA (Table 1).

Relapse

Of the 75 patients, 52 patients relapsed after the last anti-TNF treatment. Fourteen patients received surgery. Fifteen patients were still in need of biological treatment. In the relapse patient group, twenty-three patients obtained remission on non-biological treatment, and of these, 14 patients received immunosuppressive treatment.

Biological free remission

Forty-six (61%) of the 75 patients included were in biological free remission. This group consists of 23 patients from LTR and 23 patients the "Relapse group" (Fig. 1). The median (IQR) time since discontinuation of biological therapy was 93 (71–128) months.

Surgery free survival

Of the total 116 patients included in the original study, 30 patients underwent colectomy with a median (IQR) time to colectomy of 18 (8–44) months. From the selected group of 75 patients included in the study that achieved remission on anti-TNF treatment, 14 underwent colectomy during the observation period with a median (IQR) time to colectomy of 38 (17–78) months.

Mucosal transcripts in ulcerative colitis phenotypes and healthy controls

In Table 2 we show the mucosal gene expression in colonic biopsies sampled in remission before discontinuation of anti-TNF treatment in the two subgroups LTR, and "Early relapse" (ER). Patients in the ER group relapsed within 12 months after anti-TNF discontinuation. Arrows in Table 2 indicates whether the mucosal gene expression in the LTR group is up or downregulated compared to ER and HC respectively. IL1RL boxplot as Additional file 1: Fig. S1.

Long-term remission versus healthy control

Patients in LTR were invited to a follow up endoscopy and colonic biopsies were sampled. There were no significant differences between HC and LTR for TNF, IL1RL1, IL5 and IL21 indicating normalization in LTR. However, the other proinflammatory cytokines and T-regs were increased in this group compared to healthy controls, despite being in endoscopic remission.

Long-term remission versus early relapse

Colonic biopsies were sampled from the early relapse group at time of discontinuation of anti-TNF treatment

Table 1 Demographic data of patients after stop of anti-TNF

Patient groups	Included N = 75	Remission N = 23	LTR N = 16	Relapse N = 52	Early Relapse N = 9
Age med (IQR)	46 (38–55)	51 (39–56)	41 (26–55)	46 (37–54)	46 (29–56)
Sex Female/Male	29/46	9/14	8/8	20/32	4/5
Colonic area R/L/E	10/30/35	4/9/10	1/9/6	6/21/25	0/5/4
UCDAI score med (Range)	0 (0–4)	0 (0–1)	0 (0–1)	0 (0–4)	0 (0–1)
Observation time (months) med (IQR)	121 (100–137)	96 (74–112)	95 (77–113)	94 (40–122)	4 (4–6)
Medication					
IFX	15	0	0	15	0
5-ASA	55	19	13	23	9
AZA/MTX	30	7	0	23	4
Steroids	4	0	0	4	0

med (IQR) median (interquartile range 25–75), *R/L/E* rectum/Left/Extensive, *AZA* azathioprine, *MTX* methotrexate, *LTR* Long-term remission

Table 2 Results of cytokine measurements comparing LTR to HC and patients in remission with relapse during the first year after anti-TNF discontinuation

Cytokine	LTR vs. HC		LTR vs. Early relapse	
	P value	ΔΔCT FC	*P* value	ΔΔCT FC
IL17	0.026	3.31↑	0.044	5.14↓
IL23	0.000	10.20↑	0.024	2.17↓
IL4	0.024	2.77↑	0.890	ns
IL5	0.329	ns	0.997	ns
IL6	0.001	7.46↑	0.192	ns
IL10	0.000	6.82↑	0.038	2.45↓
IL21	0.062	ns	0.264	ns
IL33	0.001	3.77↑	0.011	2.42↓
TGF	0.007	2.39↑	0.009	1.70↓
GATA	0.000	12.47↑	0.212	ns
TLR4	0.001	3.89↑	0.023	1.69↓
IL1RL1	0.111	ns	0.049	1.93↓
RORC	0.000	6.41↑	0.089	ns
FOX	0.000	8.88↑	0.470	ns
TBX	0.000	5.28↑	0.181	ns
TNF	0.103	ns	0.542	ns

↑ ↓Up or down regulated mucosal gene expression in LTR group

CT FC Cycle threshold fold change, *ns* not significant, *LTR* Long-term remission, *HC* Healthy control

and histologically assessed. With the exception of one patient in the LTR group, all included colonic biopsies were histologically assessed as RHI 0. The patient in the LTR group were assessed as RHI 4. Significantly increased values in the ER group compared to the LTR group were seen for cytokines such as IL10, 1IL17, IL23, IL33, TLR4 and TGF. Of interest, the IL1RL1 transcript was normalized in the LTR, but increased in the ER group compared to the LTR and HC.

Prediction of long-term remission and biological free remission

Analysis by Cox regression was carried out including normalized mucosal TNF gene expression (< 10 000 copies/µgRNA), age, disease location, and smoking habits to predict long-term remission and biological free remission. Mucosal TNF gene expression at remission before discontinuation of anti-TNF treatment, after the first and last induction treatment showed significant prediction for both long-term remission and biological free remission (Figs. 2, 3). Of note, 31 and 61 percent of the patients that achieved remission on initial anti-TNF treatment have been in long-term and biological free remission respectively for approximately the last 8 years.

Prediction of colectomy

Analysis by Cox regression was carried out including normalized mucosal TNF gene expression (< 10,000 copies/µgRNA), age, disease location, and smoking habits to predict colectomy. The resulting model showed a significant effect of normalized mucosal TNF gene expression and young age before the first anti-TNF to predict lower risk of colectomy (Fig. 2). Moreover, ROC analyses were performed by selecting a mucosal TNF gene expression cut off of high 40,000 (copies/µgRNA) at inclusion before start of biological therapy. The test obtained a relative high specificity of 80%, but the sensitivity was as low as 10%. From a clinical point of view this indicates that patients with very elevated mucosal TNF gene expression at debut of disease are in higher risk of colectomy during the disease course.

Discussion

This 10-year observation study of long-term outcomes after anti-TNF discontinuation upon endoscopic remission, shows that 61% of those who achieve remission do not require biological treatment in the long-term. Fifty percent of the patients without anti-TNF treatment were in clinical remission without any signs of relapse with a median observation time of 8 years, whilst the other half only required escalation of non-biological treatment in case of relapse. This may indicate that anti-TNF treatment may alter the natural disease course for patients with moderate to severe ulcerative colitis.

We have previously described mucosal TNF gene expression as a possible biomarker that predicts prolonged time in remission after anti-TNF discontinuation in ulcerative colitis, and mucosal TNF gene expression combined with histological score predicts need of biological treatment wihtin the first year after diagnosis [18]. In this study we have found that normalized mucosal TNF gene expression at discontinuation of anti-TNF treatment also predicted a milder disease course in the a long-term perspective, and moreover a lower need of biological therapy and lower risk of surgery. A hypothesis could be that patiens with low mucosal TNF gene expression reflects milder disease phenotype. In this context, measuring TNF gene expression could be an analogue to measuring the "temperature" of the local immune activation.

In our former study of 116 patients with moderate to severe UC intention treated with biological therapy (26%) underwent colectomy after a median time of 18 months. Moreover, the selected group of 75 patients included in this study, 19% underwent colectomy after a median time of 40 months during the total observation time of approximately 10 years. In a metanalyses of UC patients,

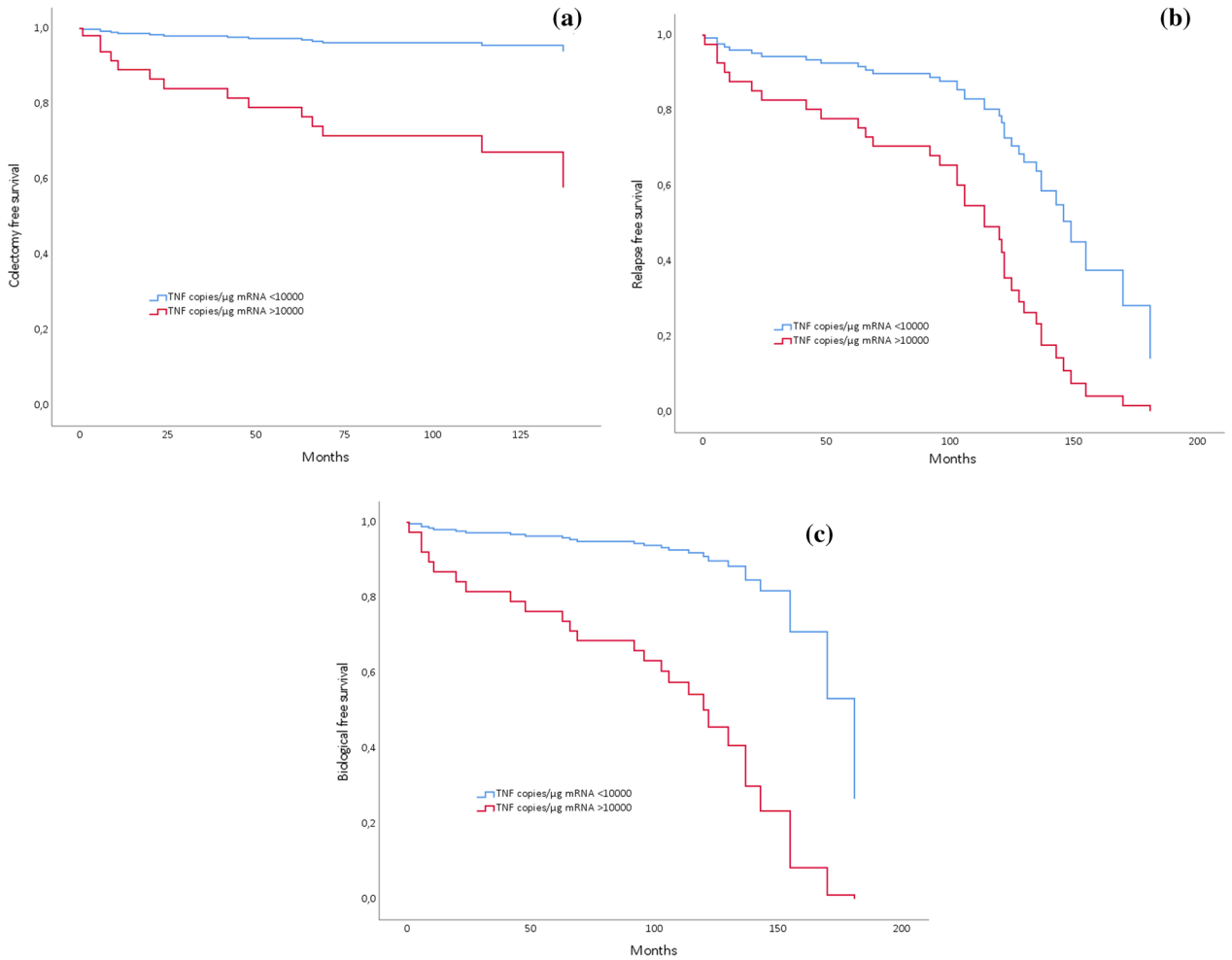


Fig. 2 **a**–**c** Cox regression model entering mucosal TNF gene expression normalization after first anti-TNF treatment and age at inclusion. **a** Model $P=0.003$; low TNF at IFX discontinuation: HR 0.12 (0.01–0.91) for surgery ($P=0.04$); Age baseline HR 1.06 (1.01–1.12) for surgery ($P=0.015$), **b** Model $P=0.005$; low TNF: HR 0.31 (0.13–0.76) for long-term remission ($P=0.01$); **c** Model $P=0.001$; low TNF: HR 0.13 (0.013–0.61) for biological free remission ($P=0.009$). *HR* hazard ratio, *IFX* infliximab, *TNF* tumour necrosis factor

including the majority of the patients with mild to moderate disease, the 10 years cumulative risks of colectomy were 15% [7]. As far as we know there are no clear comparing reports on the risk of colectomy in patients with moderate to severe disease activity, and non-existing with similar observation time after discontinuation biological therapy. Moreover, the need for surgery in patients with moderate to severe UC on maintenance therapy has been reported to be 31% (mean observation time 50 months), 50% (36 months), 27.3% (84 months), 20% (60 months), 53% (60 months) and 17% (33 months), and in a meta-analysis, the mean risk of colectomy after 36 months treatment of IFX in moderate to severe UC was around 40% [31–37]. Normalized mucosal TNF gene expression and young age significantly predicted low risk of surgery. Moreover, at inclusion before start of biological therapy a mucosal TNF gene expression cut off of 40 000 (copies/µgRNA) predicted high risk of surgery with high specificity but low sensitivity. This supports hypothesis of mucosal TNF gene expression reflecting disease severity of UC phenotype. High TNF gene expression levels before start of biological therapy represent patients with high risk of surgery which may be of some clinical utility.

The small subgroups of patients defined as LTR is of special interest according to causal pathophysiological mechanisms of UC. This group was in endoscopic remission with histological healed mucosa. but still not in immunologic remission, i.e. not all mucosal pro-inflammatory cytokines were normalized. In this LTR group there were no differnce in the mucosal TNF gene

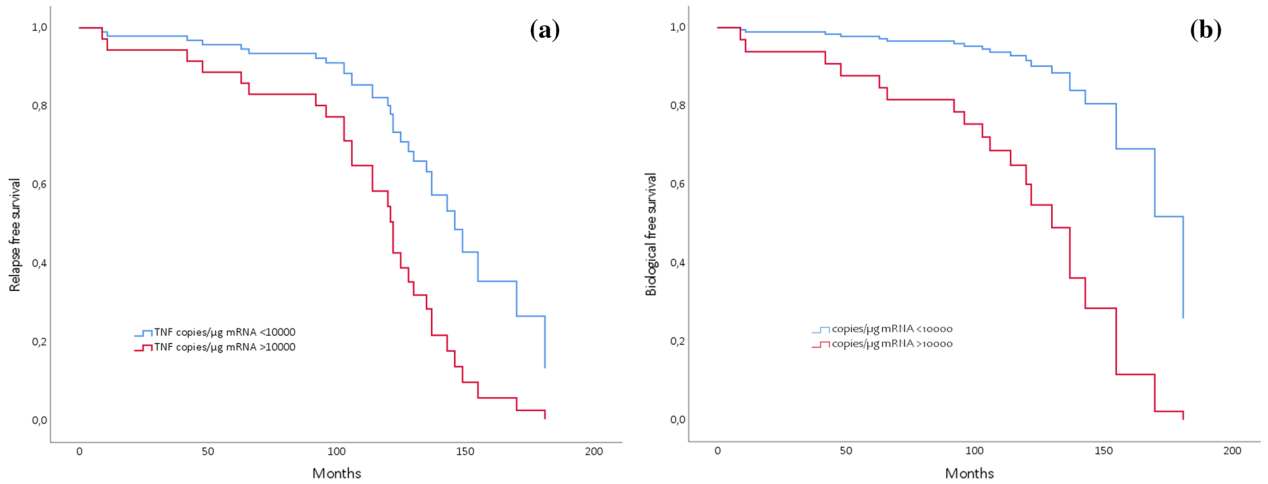


Fig. 3 **a, b** Cox regression model entering mucosal TNF gene expression normalization after last anti-TNF treatment. **a** Model $P=0.021$; low TNF at IFX discontinuation: HR 0.36 (0.14–0.92) for long-term remission ($P=0.03$). **b** Model $P=0.005$; low TNF at IFX discontinuation: HR 0.17 (0.04–0.78) for biological free remission ($P=0.03$). *HR* hazard ratio, *IFX* infliximab, *TNF* tumour necrosis factor

expression compared to normal healty controls, but there were still a clear up-regulation of other pro- and anti-inflammatoric mediator genes. This indicates that even in case of long-term endoscopic remission with apparent histological healed mucoa the immune activity was not completely resolved. Moreover, when the LTR patients were compared to other ulcerative colitis patients in remission with normalized mucosal TNF gene expression that experience a relapse within the first year after anti-TNF discontiuation, there is a significant difference in the pro inflammatory pathways including IL17 and IL 23. Apart from a generally lower inflammatory immune acitivety in LTR, the IL1RL1/IL33 pathway is normalized in LTR compared to healthy controls, contradictory to the relapsing phenotype. IL1RL1 is the ligand receptor to the alarmin IL33, and present on a wide range of cells including immune cells in the gut mucosa. Several splice variants of IL1RL1 exist including a membrane receptor (IL1RL1L) as well as a soluble decoy receptor (sIL1RL1). The relationship of mucosal IL-33 and IL1RL1—gene expression is not completly understood with both pro and anti-inflammatory properties described [38]. Final normalisation of IL1RL1 is the most clear fingerprint that differentiate the LTR phenotype from relapsing type, and might be a possible marker of long-term remission without need of treatment escalation. IL1RL1/IL33 activity have in previous studies shown to be a possible marker of inflammatory activiety and corresponds to fecal calprotecin [38]. Our findings support further investigation into the IL1RL1/IL-33 pathway as interest as biomarkers as a step toward precision medicine in UC. This should be adressed in further studies.

The strength of this study is the long-term follow up with systematic registration of oucomes after discontinuation and re-treatment with biological therapy of moderate to severe UC, and the aspect of using features of molecular immunology for development of precision medicine in UC. The weakness of this study is the sample size of the cohort and lack of endoscopical verfication of disease activity. The sample size of the mucosal gene expression analysis is also small. Moreover, an open RCT study design comparing the specific treatment algorithm to manitenance treatment with biological therapy would definately have increased the scientific value. We have only included patients treathed with anti-TNF therapy, and the results cannot necessarily be translated to other biological therapies or smal molecule drugs. This awaits further studies.

In conlusion the study indicates that anti-TNF treatment may alter the disease severity to a milder phenotype for those who do not need colectomy. Low mucosal TNF gene expression in remisson both after the first and folowing induction treatments predict a lower risk of colectomy in the long-term and long-term remission without biological treatment. The mucosal immunologic gene expression profile of LTR is not normalized but shows in generally lower immune acitivety. IL1RL1 is normalized in LTR phenotype and higher in relapsing UC, and normalization of IL1RL1 might be a possible marker of long-term remission.

Abbreviations

ASIB: Advanced study of inflammatory bowel disease; CD: Crohn's disease; ER: Early relapse; FOXp3: Forkhead box protein3; GATA: Globin transcription

factor; HC: Healthy controls; IBD: Inflammatory bowel disease; IFNG: Interferon Gamma; IFX: Infliximab; IL: Interleukin; IL1RL1: Interleukin-1-like-receptor 1; Imids: Immunomodulating drugs; LTR: Long term remission; mRNA: Messenger ribonucleic acid; NFKB: Nuclear factor Kappa B; RORC: Nuclear receptor related orphan receptor C; TBX21: T-box transcription factor 21; TGFβ: Transforming growth factor Beta; TNF: Tumor necrosis factor; UC: Ulcerative colitis; UCDAI: Ulcerative colitis disease activity index.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12876-022-02522-4.

Additional file 1: Fig. S1. Boxplot of IL1RL cytokine measurements comparing HC, LTR and patients in remission with relapse. CT FC= Cycle threshold fold change, HC= Healthy controls, LTR=Long-term remission.

Acknowledgements
We thank Ingrid Christiansen, Marian Remijn, Line Wilsgaard for expert technical assistance.

Author contributions
Planning and conducting: KMJ, RG, JRF, Collecting or interpreting data: KMJ, RG, JRF, ØKM, MG, JB, HK, SWS, Drafting of manuscript: KMJ, RG, JRF, ØKM, MG, JB, HK, SWS. All authors read and approved the final manuscript.

Funding
Open access funding provided by UiT The Arctic University of Norway (incl University Hospital of North Norway). This work was supported by Northern Norway Regional Health Authority, ID SFP1286-16.

Availability of data and materials
The raw data are available on shared folder: https://data.mendeley.com/datasets/f4mb2c4nnb/1.

Declarations

Ethics approval and consent to participate
All participants were informed and signed a written consent. The study were performed according to the Helsinki declaration. Safety protocols were approved by the institutional review board. Approval including the use of biobank was granted by the Regional Committee of Medical Ethics of Northern Norway Ref No: 1349/2012.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Research Group of Gastroenterology and Nutrition, Department of Clinical Medicine, UiT The Arctic University of Norway, Tromsö, Norway. [2]Division of Internal Medicine, Department of Gastroenterology, University Hospital of North Norway, Tromsö, Norway. [3]Department of Clinical Pathology, University Hospital of North Norway, Tromsö, Norway.

Received: 2 May 2022 Accepted: 23 September 2022
Published online: 16 November 2022

References

1. Baumgart DC, Carding SR. Inflammatory bowel disease: cause and immunobiology. The Lancet. 2007;369:1627–40. https://doi.org/10.1016/S0140-6736(07)60750-8.
2. Ananthakrishnan AN, et al. Environmental triggers in IBD: a review of progress and evidence. Nat Rev Gastroenterol Hepatol. 2017;15:39. https://doi.org/10.1038/nrgastro.2017.136.
3. Paun A, Danska JS. Immuno-ecology: how the microbiome regulates tolerance and autoimmunity. Curr Opin Immunol. 2015;37:34–9. https://doi.org/10.1016/j.coi.2015.09.004.
4. Cleynen I, et al. Inherited determinants of Crohn's disease and ulcerative colitis phenotypes: a genetic association study. Lancet (London, England). 2016;387:156–67. https://doi.org/10.1016/s0140-6736(15)00465-1.
5. Henriksen M, et al. Ulcerative colitis and clinical course: results of a 5-year population-based follow-up study (the IBSEN study). Inflamm Bowel Dis. 2006;12:543–50. https://doi.org/10.1097/01.MIB.0000225339.91484.fc.
6. Solberg IC, et al. Clinical course during the first 10 years of ulcerative colitis: results from a population-based inception cohort (IBSEN Study). Scand J Gastroenterol. 2009;44:431–40. https://doi.org/10.1080/00365520802600961.
7. Fumery M, et al. Natural history of adult ulcerative colitis in population-based cohorts: a systematic review. Clin Gastroenterol Hepatol. 2018;16:343–56. https://doi.org/10.1016/j.cgh.2017.06.016.
8. Rutgeerts P, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. N Engl J Med. 2005;353:2462–76. https://doi.org/10.1056/NEJMoa050516.
9. Reinisch W, et al. Adalimumab for induction of clinical remission in moderately to severely active ulcerative colitis: results of a randomised controlled trial. Gut. 2011;60:780–7. https://doi.org/10.1136/gut.2010.221127.
10. Sandborn WJ, et al. Subcutaneous golimumab maintains clinical response in patients with moderate-to-severe ulcerative colitis. Gastroenterology. 2014;146:96-109.e101. https://doi.org/10.1053/j.gastro.2013.06.010.
11. Casanova MJ, et al. Evolution after anti-TNF discontinuation in patients with inflammatory bowel disease: a multicenter long-term follow-up study. Am J Gastroenterol. 2017;112:120–31. https://doi.org/10.1038/ajg.2016.569.
12. Gisbert JP, Marín AC, Chaparro M. The risk of relapse after anti-TNF discontinuation in inflammatory bowel disease: systematic review and meta-analysis. Am J Gastroenterol. 2016;111:632–47. https://doi.org/10.1038/ajg.2016.54.
13. Feuerstein JD, et al. AGA clinical practice guidelines on the management of moderate to severe ulcerative colitis. Gastroenterology. 2020;158:1450–61. https://doi.org/10.1053/j.gastro.2020.01.006.
14. Doherty G, et al. European Crohn's and Colitis Organisation Topical Review on Treatment Withdrawal ['Exit Strategies'] in Inflammatory Bowel Disease. J Crohn's Colitis. 2017;12:17–31. https://doi.org/10.1093/ecco-jcc/jjx101.
15. [TA329], N. t. a. g. *Infliximab, adalimumab and golimumab for treating moderately to severely active ulcerative colitis after the failure of conventional therapy*, http://www.nice.org.uk/guidance/ta329/chapter/1-Guidance (2015).
16. Johnsen KM, et al. Repeated intensified infliximab induction - results from an 11-year prospective study of ulcerative colitis using a novel treatment algorithm. Eur J Gastroenterol Hepatol. 2017;29:98–104. https://doi.org/10.1097/meg.0000000000000753.
17. Rismo R, et al. Normalization of mucosal cytokine gene expression levels predicts long-term remission after discontinuation of anti-TNF therapy in Crohn's disease. Scand J Gastroenterol. 2013;48:311–9. https://doi.org/10.3109/00365521.2012.758773.
18. Florholmen JR, et al. Discovery and validation of mucosal TNF expression combined with histological score: a biomarker for personalized treatment in ulcerative colitis. BMC Gastroenterol. 2020;20:321–321. https://doi.org/10.1186/s12876-020-01447-0.
19. Spekhorst LM, et al. Cohort profile: design and first results of the Dutch IBD Biobank: a prospective, nationwide biobank of patients with inflammatory bowel disease. BMJ Open. 2017;7: e016695. https://doi.org/10.1136/bmjopen-2017-016695.
20. Stevens TW, et al. Systematic review: predictive biomarkers of therapeutic response in inflammatory bowel disease-personalised medicine in its infancy. Aliment Pharmacol Ther. 2018;48:1213–31. https://doi.org/10.1111/apt.15033.
21. Porter CK, et al. Cohort profile of the PRoteomic Evaluation and Discovery in an IBD Cohort of Tri-service Subjects (PREDICTS) study: Rationale, organization, design, and baseline characteristics. Contemp Clin Trials Commun. 2019;14: 100345. https://doi.org/10.1016/j.conctc.2019.100345.

22. Dulai PS, et al. Approaches to integrating biomarkers into clinical trials and care pathways as targets for the treatment of inflammatory bowel diseases. Gastroenterology. 2019;157:1032-1043.e1031. https://doi.org/10.1053/j.gastro.2019.06.018.
23 Marafini I, Monteleone G. Precision medicine in inflammatory bowel diseases. Front Pharmacol. 2021. https://doi.org/10.3389/fphar.2021.653924.
24. D'Haens G, et al. A review of activity indices and efficacy end points for clinical trials of medical therapy in adults with ulcerative colitis. Gastroenterology. 2007;132:763–86. https://doi.org/10.1053/j.gastro.2006.12.038.
25. Olsen T, et al. Normalization of mucosal tumor necrosis factor-alpha: a new criterion for discontinuing infliximab therapy in ulcerative colitis. Cytokine. 2016;79:90–5. https://doi.org/10.1016/j.cyto.2015.12.021.
26. Mosli MH, et al. Development and validation of a histological index for UC. Gut. 2017;66:50–8. https://doi.org/10.1136/gutjnl-2015-310393.
27. Olsen T, et al. Tissue levels of tumor necrosis factor-alpha correlates with grade of inflammation in untreated ulcerative colitis. Scand J Gastroenterol. 2007;42:1312–20. https://doi.org/10.1080/00365520701409035.
28. Cui G, et al. Improvement of real-time polymerase chain reaction for quantifying TNF-alpha mRNA expression in inflamed colorectal mucosa: an approach to optimize procedures for clinical use. Scand J Clin Lab Invest. 2006;66:249–59. https://doi.org/10.1080/00365510600590472.
29. Diab J, et al. A Quantitative analysis of colonic mucosal oxylipins and endocannabinoids in treatment-naïve and deep remission ulcerative colitis patients and the potential link with cytokine gene expression. Inflamm Bowel Dis. 2019;25:490–7. https://doi.org/10.1093/ibd/izy349.
30. Cui G, et al. Dynamics of the IL-33/ST2 network in the progression of human colorectal adenoma to sporadic colorectal cancer. Cancer Immunol Immunother. 2015;64:181–90. https://doi.org/10.1007/s00262-014-1624-x.
31. Subramaniam K, et al. Early predictors of colectomy and long-term maintenance of remission in ulcerative colitis patients treated using anti-tumour necrosis factor therapy. Intern Med J. 2014;44:464–70. https://doi.org/10.1111/imj.12397.
32. Gustavsson A, et al. Clinical trial: colectomy after rescue therapy in ulcerative colitis: 3-year follow-up of the Swedish-Danish controlled infliximab study. Aliment Pharmacol Ther. 2010;32:984–9. https://doi.org/10.1111/j.1365-2036.2010.04435.x.
33. Garcia-Planella E, et al. Long-term outcome of ulcerative colitis in patients who achieve clinical remission with a first course of corticosteroids. Digest Liver Dis Off J Italian Soc Gastroenterol Italian Assoc Study Liver. 2012;44:206–10. https://doi.org/10.1016/j.dld.2011.10.004.
34. Arias MT, et al. A panel to predict long-term outcome of infliximab therapy for patients with ulcerative colitis. Clin Gastroenterol Hepatol Off Clin Pract J Am Gastroenterol Assoc. 2015;13:531–8. https://doi.org/10.1016/j.cgh.2014.07.055.
35. Sjoberg M, et al. Infliximab as rescue therapy in hospitalised patients with steroid-refractory acute ulcerative colitis: a long-term follow-up of 211 Swedish patients. Aliment Pharmacol Ther. 2013;38:377–87. https://doi.org/10.1111/apt.12387.
36. Ferrante M, et al. Long-term outcome after infliximab for refractory ulcerative colitis. J Crohns Colitis. 2008;2:219–25. https://doi.org/10.1016/j.crohns.2008.03.004.
37. Thorne K, et al. Colectomy rates in patients with ulcerative colitis following treatment with infliximab or ciclosporin: a systematic literature review. Eur J Gastroenterol Hepatol. 2016. https://doi.org/10.1097/MEG.0000000000000568.
38. Diaz-Jimenez D, et al. Soluble ST2 is a sensitive clinical marker of ulcerative colitis evolution. BMC Gastroenterol. 2016;16:103. https://doi.org/10.1186/s12876-016-0520-6.

Publisher's Note