Mechanisms underlying inflammation, symptoms and quality of life in ulcerative colitis

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ABSTRACT

A substantial proportion of patients with ulcerative colitis (UC) in remission demonstrate gastrointestinal (GI) symptoms, despite the absence of ongoing inflammation in the colon. The underlying mechanisms of these symptoms are still not clear. Moreover, data concerning the cell types involved in the immunopathogenesis of UC are conflicting and this can be inherent to temporal variations in the immune responses during the disease course. The overall aim of this thesis was to characterize symptoms during UC remission to find underlying mechanisms and impact on health-related quality of life (HRQoL) and also to investigate temporal variations in immune responses during active disease.

In Paper I, 299 UC patients were included and 177 of those were followed up one year later. 18% of UC patients in remission had symptoms compatible with functional bowel disorders (FBD) other than IBS at enrollment. The total burden of GI symptoms in patients with symptoms compatible with FBD other than IBS in remission was higher than in patients without FBD, which had a negative impact on HRQoL. The presence of FBD-like symptoms was not correlated with psychological distress, systemic immune activity or subclinical colonic inflammation and was not a risk factor for active UC at follow-up one year later.

In Paper II, 15 patients with UC provided serum and mucosal biopsies during a flare in early (time of diagnosis) and late (after 10 years) disease to determine and compare systemic and mucosal immune profiles at these two time points. The profile of 15 serum proteins highly discriminated early and late disease and eight proteins were differently expressed at the two time points. The mRNA profiles in biopsies strongly discriminated early and late disease and 42 genes were differently expressed at early and late disease. Further, T helper (Th)1- and Th2-related genes were associated with early and late disease, respectively.

In Paper III, rectal sensitivity was assessed in 36 UC patients in remission, 18 with IBS-like symptoms and 18 without, with rectal balloon distensions. Moreover, their GI and psychological symptoms were evaluated. UC patients with IBS-like symptoms in remission had lower sensory thresholds and higher unpleasantness ratings than those without. The overall GI symptom severity, abdominal pain and bloating, but not diarrhea, constipation or satiety, were associated with rectal sensitivity. In multivariable analyses, rectal hypersensitivity, psychological distress and female gender were independently associated with GI symptom severity.

In Paper IV, 66 patients with inactive UC were included 10 years after the disease onset to determine HRQoL, and identify predictors thereof. HRQoL was measured with the Short Form Health Survey 36 (SF-36). The SF-36 domain scores were comparable to the general Swedish population, except for the Vitality domain, where UC patients scored lower. Gender, smoking, comorbidity and disease phenotype had no impact on HRQoL. In contrast, corticosteroid use and sick leave due to UC during the past year, persisting GI symptoms during remission and fatigue were independently associated with aspects of physical HRQoL. Only psychological distress contributed uniquely to poorer mental HRQoL.

Conclusions: The presence of GI symptoms in UC in remission is common and is associated with impaired HRQoL. Other determinants that negatively influence different aspects of HRQoL in inactive UC are preceding disease activity, fatigue and psychological distress. The pathogenesis of GI symptoms in quiescent UC is multifactorial and involves, among other factors, visceral hypersensitivity, psychological factors and female gender. Hence, these parameters should be taken into consideration in the management of patients with UC in remission. Finally, a transition from a Th1-predominant to a Th2-dominated immune response in the inflamed mucosa in UC is observed as the disease progresses from early to late stages, which can have future implications in providing individualized treatment of UC patients.

Keywords: ulcerative colitis, functional bowel disorders, irritable bowel syndrome, gut immunology, visceral hypersensitivity, health-related quality of life

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SAMMANFATTNING PÅ SVENSKA

Ulcerös kolit (UC) är en inflammatorisk sjukdom i tjocktarmen som kännetecknas av perioder av remission (inaktiv sjukdom) och återfall (aktiv sjukdom). Typiska symtom under aktiv sjukdom är diarréer, rektala blödningar, täta trängningar till avföring och buksmärtor beroende på inflammationens omfattning och svårighetsgrad. En relativt stor andel av patienter med UC i remission uppvisar dock tarmsymtom, trots avsaknad av pågående inflammation i tjocktarmen. Mekanismerna som ligger bakom dessa symtom är fortfarande inte helt kända. Informationen om vilka celltyper i immunsystemet som är involverade i uppkomsten och utvecklingen av UC är motstridiga. Detta kan bero på variationer av immunsystemets svar under olika delar av sjukdomsförloppet. Det övergripande syftet med denna avhandling var att karakterisera symtom vid UC i remission, undersöka potentiella underliggande mekanismer och utreda inverkan på hälsorelaterad livskvalitet (HRQoL) samt att utforska variationer av immunologiskt svar under aktiv sjukdom tidigt och sent i sjukdomsförloppet.

I delarbete I inkluderades 299 UC patienter och 177 av dessa följdes upp ett år senare. 18% av patienterna i remission hade symtom förenliga med en annan funktionell tarmsjukdom (FBD) än IBS (irritable bowel syndrome) vid inklusion. Svårighetsgraden av tarmsymtomen hos patienter i remission med symtom förenliga med FBD andra än IBS var högre än hos patienter i remission utan FBD, vilket påverkade livskvaliteten negativt. Förekomsten av FBD-liknande symtom var inte kopplad till nedsatt psykiskt välbefinnande, systemisk aktivitet av immunsystemet eller asymtomatisk inflammation i tjocktarmen och var inte riskfaktor för aktiv UC vid uppföljningen.

I delarbete II togs blodprov och tarmslemhinnebiopsier från 15 patienter med aktiv UC i tidig (tidpunkten för diagnos) och sen (efter 10 år) sjukdom för att bestämma och jämföra det immunologiska svaret vid dessa två tidpunkter. Femton proteiner i serum identifierade tydligt tidig och sen sjukdom, och nivån av åtta av dessa skilde sig åt vid de två tidpunkterna. Uttrycket av 42 gener i tarmslemhinnan relaterade till inflammation skilde sig tydligt åt mellan tidig och sen sjukdom. T-helper (Th)1- och Th2-relaterade gener var associerade med tidig respektive sen sjukdom.

I delarbete III mättes tarmkänsligheten hos 36 UC patienter i remission, 18 med IBS-liknande symtom och 18 utan, med hjälp av en ballong som blåstes upp i ändtarmen. Dessutom värderades tarmsymtom och psykologiska faktorer med frågeformulär. UC patienter i remission med IBS-liknande symtom hade lägre sensoriska trösklar i tarmen och rapporterade större obehag än de utan IBS-liknande symtom. Tarmsymtomens svårighetsgrad, och särskilt buksmärta och uppblåsthet, var associerade med tarmkänslighet. I multivariabla analyser var ökad tarmkänslighet, nedsatt psykiskt välbefinnande och kvinnligt kön oberoende associerade med tarmsymtomens svårighetsgrad.

I delarbete IV inkluderades 66 patienter med inaktiv UC 10 år efter att diagnosen hade ställts för att bestämma HRQoL och identifiera faktorer som påverkar den. HRQoL mättes med ett frågeformulär, Short Form Health Survey 36 (SF-36). HRQoL hos UC patienter var jämförbar med den allmänna svenska befolkningen, förutom domänen Vitalitet, där UC patienter hade lägre poäng. Kön, rökning, samsjuklighet och utbredning av UC påverkade inte HRQoL. Däremot var behandling med kortison och sjukfrånvaro på grund av UC under det senaste året, kvarstående tarmsymtom under remission och trötthet oberoende associerade med fysisk HRQoL. Endast nedsatt psykiskt välbefinnande bidrog unikt till sämre mental HRQoL.

Konklusion: Tarmsymtom hos patienter med UC i remission är vanligt förekommande och de är associerade med nedsatt hälsorelaterad livskvalitet. Andra faktorer som negativt påverkar livskvaliteten vid inaktiv UC är ett aktivt sjukdomsförlopp, trötthet och nedsatt psykiskt välbefinnande. Mekanismer bakom tarmsymtom vid inaktiv UC är komplexa och involverar bland annat ökad tarmkänslighet, psykologiska faktorer och kvinnligt kön. Dessa parametrar bör beaktas vid behandling av patienter med UC i remission. Vår observation med ett förändrat immunsvar vid tidig jämfört med sen sjukdom, med en övergång från ett Th1-dominerat immunsvar i tidig fas till ett Th2-dominerat immunsvar i sen fas vid UC, kan leda till en mer individualiserad vård av UC patienter i framtiden.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Mavroudis G, Simrén M, Jonefjäll B, Öhman L, Strid H. Symptoms compatible with functional bowel disorders are common in patients with quiescent ulcerative colitis and influence the quality of life but not the course of the disease. Therap Adv Gastroenterol. 2019;12:1756284819827689.

II. Mavroudis G, Magnusson MK, Isaksson S, Sundin J, Simrén M, Öhman L, Strid H. Mucosal and Systemic Immune Profiles Differ During Early and Late Phases of the Disease in Patients With Active Ulcerative Colitis. J Crohns Colitis. 2019;13(11):1450-8.

III. Mavroudis G, Strid H, Jonefjäll B, Simrén M. Visceral hypersensitivity is together with psychological distress and female gender associated with severity of IBS-like symptoms in quiescent ulcerative colitis. Neurogastroenterol Motil. 2021;33(3):e13998

IV. Mavroudis G, Simrén M, Öhman L, Strid H. Health-related quality of life in patients with longstanding ulcerative colitis in remission. Submitted.

CONTENT

ABBREVIATIONS

BP	Bodily pain
CD	Crohn's disease
CRP	C-reactive protein
EIM	Extraintestinal manifestations
f-cal	Fecal calprotectin
FBD	Functional bowel disorders
FGID	Functional gastrointestinal disorders
GH	General health
GI	Gastrointestinal
GSRS	Gastrointestinal symptom rating scale
HAD	Hospital anxiety and depression
HRQoL	Health-related quality of life
IBD	Inflammatory bowel disease
IBDQ	Inflammatory bowel disease questionnaire
IBS	Irritable bowel syndrome
IFN	Interferon
IL	Interleukin
MFI	Multidimensional fatigue inventory
MH	Mental health
OPLS-DA	Orthogonal projections to latent structures discriminant analyses
PCA	Principal component analysis
PF	Physical functioning
PGA	Physician's global assessment
PHQ	Patient health questionnaire
PSS	Perceived stress scale
RE	Role limitations due to emotional health problems
RP	Role limitations due to physical health problems
SD	Standard deviation
SF	Social functioning
SF-36	Short form health survey-36
Th	T helper
TNF	Tumor necrosis factor
TRPV1	Transient receptor potential vanilloid type 1
UC	Ulcerative colitis
UCA	Active ulcerative colitis
UCR	Ulcerative colitis in remission
VAS	Visual analogue scale
VT	Vitality

1 INTRODUCTION

1.1 Ulcerative colitis

Ulcerative colitis (UC) is an chronic inflammatory bowel disease (IBD) that affects the colonic mucosa (1). Traditionally, it has been considered as a disease of the westernized nations with a north-south gradient in incidence and prevalence (2, 3). However, this geographic distinction has been less obvious over the past decades, as a rapidly increased rate is reported in East and a plateauing in West (4). In Sweden, the prevalence of UC is estimated to be 0.35% and the incidence 20.0 cases per 100 000 inhabitant-years (5, 6). It is mainly diagnosed between the second and fourth decade of life resulting in high health care and society costs, apart from the consequences on an individual basis (7, 8).

UC is an immune-mediated disease but the precise pathogenesis is still not completely understood. Under normal conditions, the gastrointestinal (GI) tract maintains immune homeostasis with tolerogenic mechanisms to prevent immune responses to food antigens and commensal bacteria, while it protects against pathogenic organisms by inducing inflammatory responses (9). A disruption of this balance may lead to inflammatory GI diseases, such as UC. Specifically, in UC, triggering events, such as environmental factors, are thought to cause mucosal barrier defects and thereby increased intestinal permeability. Subsequently, abnormally stimulated mucosal immune responses against gut microorganisms in genetically susceptible individuals lead to an uncontrolled inflammation (10-12).

UC is a disease with a wide clinical spectrum, ranging from a chronic inactive to a refractory disease. However, in the majority of patients the disease course is characterized by periods of activity alternating with periods of remission. Overall the disease course is unpredictable (13). Symptoms under periods of activity include diarrhea, rectal bleeding, urgency or tenesmus, depending on the extent and severity of the inflammation. In more severe cases, abdominal pain, fever, malaise and weight loss can be present. The diagnosis of UC is based on a combination of clinical presentation, endoscopic findings and histopathological parameters, with the exclusion of alternative diagnoses (14).

In UC, the inflammation involves almost in all cases the rectum and can spread from the distal to the proximal segments of the colon. UC is classified by the extent of the colonic involvement into proctitis, left-sided and extensive colitis, according to the Montreal classification (15). Different scoring instruments

have been used over time to assess disease severity. Of those, the most commonly used is the Mayo score (16). It consists of 4 items including stool frequency, rectal bleeding, findings on endoscopy and physician's global assessment. The total score ranges from 0 to 12, and higher scores indicate more severe disease (17).

Fecal calprotectin (f-cal) is one of the most useful biomarkers in UC. Its detection in feces reflects the migration of neutrophils into the gut mucosa due to an inflammatory process, thus it is clinically useful as a surrogate marker of inflammation in the GI tract (18). It is used to discriminate UC from other diseases (19), to assess UC activity (20), to monitor the disease in patients in remission (21) and to predict response to treatment (22). F-cal levels correlate well with endoscopic and histological activity in UC (23-25). A value of 250μg/g is considered to be an appropriate cut-off for monitoring UC remission, while a cut-off of 50-100μg/g has been recommended to discriminate UC from other non-inflammatory GI diseases, such as IBS (irritable bowel syndrome) (26, 27). C-reactive protein (CRP) in serum is a less reliable marker of disease activity in UC, with the exception of severe extensive colitis (28).

The aim of medical treatment of UC is the induction and maintenance of remission, and the selection of appropriate medications is guided by disease severity and extent (1). The therapeutic arsenal in UC includes several classes of medications, such as 5-aminosalicylic acid, immunomodulators (thiopurines), corticosteroids, biologics and small molecules (29). However, no treatment is universally effective, and refractory cases are still managed with colectomy (30).

1.2 Functional bowel disorders

Functional bowel disorders (FBD) are a spectrum of chronic GI disorders in which patients report symptoms attributable to the lower GI tract. They are characterized by the absence of structural findings on clinical investigations (31). The Rome criteria have been developed to classify FBD, and are widely used both in research and clinical practice (32). The Rome criteria define five FBD; IBS, functional constipation, functional diarrhea, functional bloating and unspecified FBD. The Rome criteria have undergone several revisions and updates in order to make them clinically useful (33). The latest version is Rome IV (34). For the purposes of this thesis, Rome III criteria are used (Table 1). (35).

Table 1. Rome III diagnostic criteria for functional bowel disorders

	Diagnostic criteria*
IBS	Recurrent abdominal pain or discomfort ≥3 days/month associated with ≥2 of the following; • improvement with defecation • onset associated with a change in frequency of stools • onset associated with a change in form of stools
Functional diarrhea	Loose or watery stools without pain occurring in >75% of stools
Functional constipation	• Two or more of the following; - Straining during more than 25% of defecations - Lumpy or hard stools during more than 25% of defecations - Sensation of incomplete evacuation during more than 25% of defecations - Sensation of anorectal obstruction/blockage during more than 25% of defecations - Manual maneuvers to facilitate during more than 25% of defecations - Fewer than 3 spontaneous bowel movements per week • Loose stools are rarely present without the use of laxatives • Insufficient criteria for irritable bowel syndrome
Functional bloating	Both of the following: - Recurrent feeling of bloating or visible distension ≥3 days/month in the last 3 months - Insufficient criteria for functional dyspepsia, IBS, or other functional GI disorder

** The criteria should be fulfilled for the last 3 months with onset ≥6 months prior to diagnosis*

FBD are common and account for disturbed quality of life and substantial health care utilization and costs (36, 37). The reported prevalence of FBD varies among studies, which is partly explained by differences in methodology and definition criteria (38). General population studies have reported a prevalence of ~11% for IBS (39), ~14% for constipation (40), ~2-8% for functional diarrhea and ~13-30% for functional bloating (41, 42). However, FBD are not mutually exclusive, and there is evidence for overlap between them or transition from one disorder to another over time (43).

The precise pathogenesis of FBD remains to be adequately identified. They are thought to be the result of disturbed function and interactions along the brain-gut axis (44). The precise mechanisms underlying these disturbances are though not clear. Traditionally, visceral hypersensitivity (45), abnormal GI motility (46) and psychological disturbances (47) have been considered to be

important pathophysiological factors. However, more recently, low-grade mucosal inflammation (48), increased intestinal permeability (49), immune activation (50) and disturbances in the microbiota (51) have been identified in subsets of patients with FBD as possible underlying mechanisms. Post-infectious IBS, where acute enteric infections precede the onset of IBS, or the development of GI symptoms in IBD in remission support these recent findings (52).

1.3 Functional bowel disorders in quiescent UC

Since the prevalence of FBD in the general population is relatively high, co-existence of UC and FBD should be expected to some degree. However, UC patients in remission report symptoms meeting IBS criteria in higher rates than subjects without IBD. Approximately one third of UC patients in remission demonstrate symptoms resembling IBS, though with significant heterogeneity among studies, depending on study design and remission criteria (53, 54). It has also been reported that symptoms compatible with at least one functional GI disorder occurred in 81.9% of patients with inactive IBD during the past year, and this resulted in impaired HRQoL (55, 56). Moreover, a common clinical problem in UC is that the objective markers of disease may be poorly associated with the GI symptoms experienced by the patients, with patients with minimal or no ongoing activity reporting disproportionately severe symptoms (57). This carries the risk of escalating anti-inflammatory treatment with the potential for adverse events. However, these patients respond poorly to anti-inflammatory treatment escalation (58).

In some cases, FBD-like symptoms in apparent clinical remission of UC may depend on occult gut inflammation, that can be detected with widely used biomarkers, such as f-cal (59). However, as mentioned above, a relatively high prevalence of IBS-like symptoms has been reported even in studies with stricter UC remission criteria, such as normal f-cal levels or endoscopic healing (54). To explain this, a model has been proposed, where chronic gut inflammation results to persistent changes in both the intestinal wall and the enteric nervous system (52). The potential underlying mechanisms include dysmotility and visceral hypersensitivity, caused by increased gut permeability and low-grade immune activation (60-62). Moreover, psychological distress, in addition to the peripheral gut dysfunction, is thought to be an important factor contributing to the generation of FBD-like symptoms. Psychological factors are considered to have a modulating effect on FBD-like symptom development, and this is supported by studies that have revealed poor psychological well-being in the subset of patients suffering from these symptoms (63, 64). Overall, these observations have challenged the dualistic

perspective, i.e. either organic (IBD) or functional (IBS/FBD), introducing the term IBD-IBS (57, 65).

1.4 Variations in gut immune responses in UC

Overactive and dysregulated gut immune responses, involving both the innate and the adaptive immune system, are the central driver of IBD. IBD is characterized by an overactivation of inflammatory responses mediated by effector cytokines and defective regulatory mechanisms unable to control these responses. As a result, chronic intestinal inflammation develops (10).

Whereas the innate immune system induces the inflammatory events, the adaptive immune system contributes to the perpetuation of the inflammation (66). T lymphocytes play an important role as mediators of mucosal inflammation in IBD. Specifically, naïve T cells, after activation by antigen-presenting cells, differentiate into effector T cells (T-helper 1 (Th1), T-helper 2 (Th2), T-helper 17 (Th17)) and regulatory T cells (Tregs), depending on the local cytokine milieu (9). In simplistic terms, based on the T cell derived cytokines in the inflamed gut mucosa, Crohn's disease (CD) has been labelled as a Th1-dominated condition, due to the high levels of IL2 (interleukin 2) and IFN-γ (interferon-γ) (67). In contrast, UC exhibit an atypical Th2 cytokine profile, since mucosal T cells produce higher amounts of IL5 and IL13, but lower amounts of IL4 (68).

However, the Th1/Th2 paradigm has been revised after another T cell lineage, Th17, has been recognized to play a key role in IBD (69). An increased number of Th17 cells, and also high amounts of IL17A, the Th17 signature proinflammatory cytokine, have been observed in the inflamed mucosa of both CD and UC (70). There is also evidence that Th1 and Th17 cells act synergistically in UC, as high levels of IFNγ, IL23 and IL17A have been detected in UC lesions (70, 71). Finally, serum levels of IL17A have been shown to correlate to clinical disease severity at the time of diagnosis of UC and to predict a more severe disease course (72).

These apparently conflicting data on the Th cell subsets involved in the pathogenesis of UC could in fact depend on a dynamic intestinal inflammation, with variations in the immune responses during the disease course. Distinct mucosal and blood cytokine profiles have been proven during the different phases of CD, with a transition from a Th1-polarized profile in early disease stages to a mixed Th1/Th17 profile in established disease (73-75), but there is lack of such evidence in UC. Moreover, this notion is supported by the

plasticity of T cell lineage differentiation, meaning that T cells have the capacity to divert towards different cytokine pathways that are considered to be hallmarks of opposing T cell lineages (76). Finally, response to treatment may be related to immunological variations during the evolution of the disease, as better outcomes with anti-TNFα (tumor necrosis factor α) have been observed in patients with shorter disease duration (77).

1.5 Visceral hypersensitivity and GI symptoms in UC

Visceral hypersensitivity is defined as increased sensitivity to pain in internal organs, such as the gut, and consists of two components; hyperalgesia, in which the response to a painful stimulus is abnormally increased, and allodynia, in which pain is produced by a stimulus that does not normally elicit pain (78). The precise pathophysiology of visceral hypersensitivity is unknown, and abnormal processing of sensory information peripherally (i.e. gut) and/or centrally (i.e. central nervous system) has been proposed (79).

Visceral hypersensitivity has been widely studied as a mechanism of chronic abdominal pain and discomfort in IBS and other functional GI disorders. Significant correlations between pain thresholds and severity of experienced pain have been demonstrated in balloon distension studies, however, with issues of controversy, since these correlations were weak and not present in all IBS patients (80-82). Moreover, there is evidence that psychological factors influence pain perception in IBS, supporting the hypothesis that disturbances at different levels of the brain-gut axis are involved in the pathophysiology of visceral hypersensitivity in IBS (83).

Transient inflammatory processes in the intestine, like those occurring in UC, can lead to persistent visceral hypersensitivity after the apparent resolution of the inflammation (84). Different underlying mechanisms have been proposed, including sensitization of immune cells (62), low-grade chronic inflammation in the gut (85) and changes in afferent nerves (61) or in parts of the central nerve system involved in pain processing (86). However, studies on visceral sensitivity in UC have been inconclusive. Some have reported hypersensitivity, while others have shown normosensitivity or hyposensitivity (87-90), depending on the grade of the inflammatory activity and the type of symptoms of the included subjects. Thus, no firm conclusions on the role of visceral hypersensitivity in the generation of symptoms arising from the gut in UC can be drawn from the existing literature on this topic.

1.6 Health-related quality of life in UC

Health related quality of life (HRQoL) is defined as the aspects of well-being that are related to or affected by the presence of disease or its treatment, as perceived by the patient (91). Although it is a complex concept, the focus on its importance in both clinical practice and medical research is growing (92). Two basic approaches are used to measure HRQoL; generic instruments, that can be applied to any disease, and disease-specific instruments, that have been developed for specific conditions. The choice of each approach depends on the objectives of the measurement, and they are not interchangeable but rather complementary (93).

HRQoL measurements are relevant in UC, as it represents a chronic relapsing disease. However, since UC patients represent a heterogeneous group, studying HRQoL in UC has an inherent complexity. Comparisons between studies are difficult due to differences in definition of disease activity, but also in methodological aspects with regard to instruments used to assess HRQoL and study design. This often results in inconsistent findings (94). A widely used tool to measure HRQoL in UC is the generic questionnaire Short Form Health Survey 36 (SF-36), which is a validated self-report instrument assessing eight domains of functioning and well-being (95).

In general, the determinants of HRQOL in IBD are thought to be socio-demographic, clinical, psychological and treatment-related (96). As expected, disease activity has been shown to be the most important determinant of HRQOL in UC, with HRQoL scores progressively decreasing as disease severity increases (97, 98). However, some researchers failed to prove that disease activity alone predicted HRQoL (99). Moreover, subsets of UC patients in remission have been found to have impaired HRQoL compared to healthy populations (100), showing also the contribution of other factors than disease activity, such as psychological and socio-demographic (101, 102).

Finally, the evidence of the effect of time on HRQoL in UC is insufficient despite the chronicity of the disease. In most studies, the reported disease duration is limited to the first years after the diagnosis, resulting in a shortage of relevant information in this research field. Normal levels of HRQoL have been reported in UC patients with long disease duration (10 years), and generally, an improvement in HRQoL over time has been suggested (97, 103).

2 AIM

The overall aim of this thesis was to characterize symptoms during UC remission in order to find underlying mechanisms and their impact on HRQoL, and also to investigate temporal variations in immune responses during active disease. A better awareness of these factors might facilitate the clinical decision-making process and improve HRQoL for patients with UC.

Specific aims were:

- To determine the prevalence of symptoms compatible with FBD other than IBS during UC remission and evaluate their burden, development over time and impact on the UC disease course (Paper I).

- To determine and compare mucosal and systemic immune profiles during two different phases of active UC, i.e. at the time of diagnosis and after 10 years of disease (Paper II).

- To establish if visceral hypersensitivity is a pathophysiological mechanism of IBS-like symptoms during inactive UC and investigate its association with GI symptoms and interaction with psychological factors (Paper III).

- To determine HRQoL in longstanding UC in remission, compare it with the general population and identify predictors (Paper IV).

3 PATIENTS AND METHODS

Three different patient cohorts were evaluated in this thesis; the SIUC (Symptoms and Inflammation in Ulcerative Colitis) cohort (Paper I), the Debut cohort (Papers II and IV) and the VISCUC (VISCeral hypersensitivity in Ulcerative Colitis) cohort (Paper III). Patients with UC were recruited at four outpatient IBD clinics in Västra Götaland Region (Sahlgrenska University Hospital, Gothenburg and Södra Älvborgs Hospital, Borås (Papers I-IV), and Kungälv Hospital, Kungälv and Norra Älvsborgs Hospital, Trollhättan (Paper I)). Two control groups were included as well (Papers II and III). A summary of the main characteristics of the cohorts is illustrated in Table 2.

Adult patients diagnosed with UC were eligible for inclusion. Exclusion criteria were other significant diseases that could affect the possibility to comply with the study protocols, such as malignancy, severe heart, kidney, neurological or psychiatric disease and history of drug or alcohol abuse. All included subjects provided their verbal and written informed consent before participation. Approval was obtained by the Regional Ethical Review Board in Gothenburg prior to the start of the studies.

3.1 Study design

3.1.1 Paper I

The study was performed on the SIUC cohort. The main aim was to characterize FBD other than IBS in inactive UC and explore their burden, development over time and impact on the UC disease course. Therefore, clinical assessment, including rigid sigmoidoscopy, was performed to identify UC patients in remission. Patients with normal rectal mucosa at the rigid sigmoidoscopy but f-cal>200μg/g were further examined with flexible sigmoidoscopy to exclude inflammation more proximally in the colon. A follow-up was conducted one year later, where patients were asked questions regarding current disease activity, including two items of the Mayo score (stool frequency and rectal bleeding) that have previously been validated as appropriate for patient-reported outcomes in UC (104). F-cal was used as a surrogate marker of colonic inflammation (105). Patients were then classified into four groups; patients with active UC (UCA), UC patients in remission fulfilling Rome III criteria for IBS (UCR+IBS), UC patients in remission fulfilling Rome III criteria for other FBD than IBS (UCR+FBD) and UC patients in remission not meeting IBS/another FBD criteria (UCR-).

Table 2. Main design characteristics of the four papers

	Paper I	Paper II	Paper III	Paper IV
Patient cohort	SIUC	Debut	VISCUC	Debut
Study design	prospective	prospective	cross-sectional	cross-sectional
no of UC patients included	299, 177 at follow-up	15 out of 99 initially included	36	66 out of 99 initially included
Control group		Healthy subjects (n=19)	IBS patients (n=36) and healthy subjects (n=14)	
Inclusion period	2012-14	2004-07 follow-up; 2015-17	2013-2017	2004-07 follow-up; 2015-17
Main focus	FBD during UC remission	Immune profiles in different phases of active UC	Visceral hypersensitivity during UC remission	HRQoL in longstanding UC in remission
Evaluation of inflammation	Clinical assessment Blood/fecal samples Rigid sigmoidoscopy Flexible sigmoidoscopy if f-cal>200µg/g	Clinical assessment Blood samples Colonoscopy	Clinical assessment Fecal samples Rigid sigmoidoscopy	Clinical assessment Rigid sigmoidoscopy
Definition of remission	Mayo score ≤2 with PGA=0, rectal bleeding=0, endoscopic subscore=0 *AND* no relapse during the last 3 months. At follow-up; modified Mayo score* ≤2 with rectal bleeding=0 *AND* f-cal<200µg/g *AND* *n*o relapse during the last 3 months		Mayo score ≤1 with PGA=0, rectal bleeding=0, endoscopic subscore= 0 *AND* no relapse during the last 3 months *AND* f-cal<200µg/g	Mayo score ≤ 1 with PGA=0, rectal bleeding=0, endoscopic subscore=0

** two items of the Mayo score; stool frequency and rectal bleeding*

The remission subgroups were compared regarding demographics, disease characteristics, treatment, severity of GI and non-GI somatic symptoms and HRQoL. The UCR+FBD and UCR- groups were compared regarding colonic and systemic inflammation, systemic immune activity, psychological distress and perceived stress to investigate factors that might contribute to the generation of symptoms compatible with FBD other than IBS in quiescent UC. The disease status at follow-up with regard to the disease status at enrollment was studied to investigate the development of symptoms compatible with IBS or another FBD over time. Finally, the UCR+IBS/FBD and UCR- groups were compared concerning disease activity parameters at follow-up in order to examine whether these symptoms are associated with a more aggressive UC disease course.

At enrollment, one patient was excluded due to withdrawn consent, four due to lack of stool samples used for f-cal measurement, one due to incomplete Rome III questionnaire and one due to positive transglutaminase antibodies. At follow-up, one patient was excluded due to incomplete questionnaire about current symptoms, one due to incomplete Rome III questionnaire and four due to lack of stool samples for f-cal measurement.

3.1.2 Paper II and IV

The studies were conducted on the Debut cohort. Ninety-nine patients with new onset UC were recruited and then evaluated consecutively at five follow-up visits (three months and one, two, three and ten years after the diagnosis). Data from the enrollment and the 10-year evaluation were used for the purposes of these studies.

In paper II, only patients with active disease at the 10-year follow-up visit were eligible. This resulted in 15 included patients. The aim of the study was to explore temporal variations in immune profiles during active UC. At inclusion, subjects were examined with colonoscopy and biopsies were obtained from the inflamed rectal mucosa. Moreover, serum samples were collected. At the 10-year follow-up, in case of evidence of active disease at a rigid sigmoidoscopy, the patients were examined with a new colonoscopy or flexible sigmoidoscopy with mucosal biopsies from the inflamed rectum. Additionally, they provided blood samples while the disease was active. Nineteen healthy subjects were also included as a reference group. Serum proteins (proteins identified as being part of the disease areas ‘Inflammatory’ and ‘Digestion’) and mRNA extracted from the mucosal biopsies (PCR arrays for ‘Antibacterial response’ and ‘T helper cell differentiation’) were compared during the two phases of the

disease and with those of healthy subjects. Further, Th1 and Th2 expression profiles in the inflamed mucosa were evaluated.

In paper IV, only patients in remission at the 10-year follow-up were included, resulting in 66 subjects. The aim of the study was to determine HRQoL in longstanding UC in remission, compare it with the general population and identify predictors. Disease status was evaluated with clinical assessment including a rigid sigmoidoscopy. HRQoL was measured and compared to the normative data of the Swedish population. Gender, smoking status, presence of comorbidity, presence of extraintestinal manifestations (EIM), disease phenotype (Montreal classification), number of relapses during the previous year, use of systemic corticosteroids during the previous year, sick leave due to UC during the previous year, GI symptom severity, anxiety/depression and fatigue were examined as possible determinants of HRQoL. The reasons for considered to be lost to follow-up at the 10-year follow-up were colectomy due to resistance to medical treatment (n=4), death (n=1), patient moved abroad (n=3), patient not willing to be included at the 10-year follow-up (n=23) and persistent disease activity at the 10-year follow-up (n=2).

3.1.3 Paper III

The study was performed on the VISCUC cohort. The aim was to study visceral hypersensitivity as a mechanism of IBS-like symptoms during UC remission and its association with GI symptoms and interaction with psychological factors. Thirty-six UC patients were included and evaluated clinically including rigid sigmoidoscopy to ensure remission. Thereafter, they were divided into two groups depending on whether they fulfilled Rome III criteria for IBS or not. Additionally, age- and gender-matched control groups (36 with IBS and 14 healthy subjects) were included. All subjects underwent a rectal sensitivity test with rectal balloon distensions, and sensory thresholds as well as perceived intensity of unpleasantness and pain (VAS (visual analogue scale) ratings) were determined. The sensory thresholds and VAS ratings were compared among the UC and control groups and between UC patients with and without IBS-like symptoms. Thresholds for discomfort and pain, VAS for unpleasantness, gender, age and psychological parameters were examined as determinants of GI symptoms severity in UC patients in remission.

3.2 Disease activity assessment and remission definition

The disease activity was assessed with the Mayo score in all studies of the thesis (17). The Mayo score, a combined endoscopic and clinical scale, is a

composite of subscores from four categories; stool frequency, rectal bleeding, physician's global assessment (PGA) and findings at endoscopy. Each category is rated from 0 to 3, and they are summed to give a total score ranging from 0 to 12, with higher scores indicating more severe disease activity (106) (Table 3).

Table 3. Mayo score for assessment of UC disease activity

Parameters	Subscore
Stool frequency	0= Normal 1= 1-2 stools/day more than normal 2= 3-4 stools/day more than normal 3= >4 stools/day more than normal
Rectal bleeding	0= None 1= Visible blood with stool less than half the time 2= Visible blood with stool half of the time or more 3= Passing blood alone
Mucosal appearance at endoscopy	0= Normal or inactive disease 1= Mild disease (erythema, decreased vascular pattern, mild friability) 2= Moderate disease (marked erythema, absent vascular pattern, friability, erosions) 3= Severe disease (spontaneous bleeding, ulceration)
Physician rating of disease activity	0= Normal 1= Mild 2= Moderate 3= Severe

In Paper I, different criteria for remission were applied at the two parts of the study, since no clinical or endoscopic assessment of the disease activity was performed at the follow-up. Hence, at inclusion, remission was defined as Mayo score ≤2 with PGA =0, rectal bleeding =0, endoscopic subscore =0 and no relapse during the previous three months. At follow-up, remission was defined as modified Mayo score ≤2 with rectal bleeding =0, f-cal <200μg/g and no self-reported relapse during the previous three months. The modified Mayo score consists of two of the four items of the Mayo score, i.e. stool frequency and rectal bleeding components. In Paper III, remission was defined as Mayo score ≤1 with PGA =0, rectal bleeding =0, endoscopic subscore =0, no relapse during the previous three months and f-cal <200μg/g. Finally, in

Paper IV, patients were judged to be in remission if they had Mayo score ≤1, with PGA =0, rectal bleeding =0 and endoscopic subscore =0. In Paper II only patients with active disease were included.

3.3 Inflammatory markers

In papers I and III, f-cal was analyzed in fecal samples as a marker of mucosal inflammation in the colon. In Paper I, high sensitivity (hs)-CRP and serum cytokines were measured as markers of systemic immune activity; IL12p70 and IFNγ as markers of Th1-mediated activity, IL4, IL10 and IL13 as markers of Th2-mediated activity, IL17A as marker of Th17-mediated activity and IL1β, IL6, IL8 and TNF as markers of the innate system activity. In Paper II, proteins associated with inflammation in the GI tract were analyzed in serum samples, and expression levels of genes related to 'Antibacterial response' and 'T helper cell differentiation' were analyzed in rectal biopsies. For detailed description of the methods of analyses, see the Methods section of each paper.

3.4 Rectal sensitivity test

In Paper III, rectal sensitivity was evaluated with rectal balloon distensions. A balloon catheter connected to a barostat was inserted into the rectum. The distension protocol consisted of phasic isobaric distensions lasting 30 seconds, followed by a 30-second resting interval. The balloon pressure was increased stepwise by 5 mmHg with each distension until the patient experienced pain or when a pressure of 70mmHg was reached (ascending method of limits). During the last 10 seconds of each distension, patients rated any perceived sensation as following; 1=no sensation, 2=rectal fullness/perception, 3=urge to defecate, 4=discomfort and 5=pain. Patients were also asked to rate the perceived intensity of unpleasantness (non-painful sensations) after each distension and, when reaching the pain threshold, the intensity of pain using a 100-mm VAS scale.

3.5 Questionnaires

Self-administered questionnaires to assess functional GI symptoms, GI and non-GI somatic symptom severity, psychological distress (anxiety, depression and perceived stress), fatigue and HRQoL were used in Papers I, III and IV. The questionnaires used are presented in Table 4. For detailed description, see the Methods section of each paper.

Table 4. Questionnaires used in the papers

	Paper I	Paper III	Paper IV
IBS/other FBD symptoms	Rome III[1]	Rome III[1]	
GI symptom severity	GSRS[2]	GSRS[2]-IBS	GSRS[2]
Non-GI somatic symptom severity	PHQ-12[3]		
Anxiety/depression	HAD[4]	HAD[4]	HAD[4]
Perceived stress	PSS-14[5]	PSS-14[5]	
Fatigue			MFI[6]
Health-related quality of life	IBDQ[7]		SF-36[8]

[1]Rome III; Rome III Diagnostic Questionnaire (32); [2]GSRS, Gastrointestinal Symptom Rating Scale (107, 108); [3]PHQ, Patient Health Questionnaire (109, 110); [4]HAD, Hospital Anxiety and Depression (111); [5]PSS, Perceived Stress Scale (112); [6]MFI, Multidimensional Fatigue Inventory (113); [7]IBDQ Inflammatory Bowel Disease Questionnaire (114); [8]SF-36, Short Form Health Survey-36 (95)

3.6 Statistical methods

Statistical analyses were performed with the software package IBM SPSS Statistics version 23 (IBM Corporation, Armonk, New York, USA). Statistical significance was accepted at a level of 0.05 in all papers. A summary of the statistical methods used in the papers of this thesis is demonstrated in Table 5.

Categorical data are presented as absolute numbers/ percentages and compared with Pearson's chi-square or Fischer's exact test. Continuous data are reported as means with standard deviations (SD) or 95% confidence interval, when parametric, and as median with interquartile or 10-90 percentile range, when nonparametric.

Comparisons between groups were performed with unpaired student's t-test and analysis of variance (ANOVA) for parametric and Mann–Whitney U test and Kruskall–Wallis test for nonparametric data. Post hoc Bonferroni/Dunn's corrections were applied in case of multiple comparisons. However, in Paper II, because of the nature of the data, the False Discovery Rate approach was chosen and the classical one-stage method was used. The Wilcoxon signed rank test was used for comparison of related samples in Paper III.

Correlations between variables were assessed with Spearman's rank correlation coefficient. Hierarchical cluster analysis and clustered correlation matrix analysis were performed on gene expression data in Paper III.

Multiple linear regression analyses (enter method) were performed to explore factors independently associated with GI symptom severity (Paper III) and HRQoL (Paper IV). In Paper III, we performed dimension reduction with principal components analysis (PCA) due to multicollinearity among rectal sensitivity parameters and questionnaire data. The number of factors was determined with Kaiser's criterion (eigenvalue of 1.0 or more) and Cattell's scree test. Composite variables were created and then entered in the regression models. In Paper IV, a variance inflation factor <5 was used as criterion to exclude multicollinearity (115).

In Paper III, multivariate factor analysis (SIMCA-P+ software; Umetrics, Umeå, Sweden) was used to examine whether patients during early versus late phase of disease could be discriminated based on the totality of proteins or mRNA levels. Initially, PCA was conducted on data. Thereafter, Orthogonal Projections to Latent Structures discriminant analyses (OPLS-DA) were used to correlate Y-variables (patients during early versus late phase) and selected X-variables (protein or mRNA levels) with each other in linear multivariate models. The quality of the OPLS-DA was based on the parameters R^2, that is, the goodness of the fit of the model ($R^2 \geq 0.5$ represents good discrimination) and Q^2, that is the goodness of prediction of the model ($Q^2 \geq 0.5$ defines high predictive ability).

In Paper IV, the standardized difference between the means (z-scores=(mean patient score minus mean population score) divided by population standard deviation) was calculated in order to compare SF-36 scores of the study sample to the general population. These were then evaluated according to Cohen's effect size index, where <0.2 indicates no difference, 0.2–0.5 small difference, 0.5–0.8 moderate difference and >0.8 large difference (116).

Table 5. Statistical tests in the four papers

	Paper I	Paper II	Paper III	Paper IV
Pearson's chi square	x		x	x
Fischer's exact test	x		x	x
Mann-Whitney U test	x	x	x	x
Student's t-test	x	x	x	x
Wilcoxon signed rank test		x		
Cohen's index				x
Kruskall-Wallis test	x	x		x
Analysis of variance (ANOVA)	x		x	
Spearman's rank correlation coefficient		x	x	x
PCA		x	x	
Multiple linear regression			x	x
OPLS-DA		x		
Hierarchical cluster analysis		x		
Clustered correlation matrix analysis		x		

OPLS-DA, orthogonal projections to latent structures discriminant analysis; PCA, principal component analysis

4 RESULTS

4.1 FBD-like symptoms in inactive UC (Paper I)

4.1.1 Prevalence of FBD-like symptoms

In Paper I, 18% (n=24) of the patients with UC in remission at enrollment fulfilled the criteria for FBD other than IBS; functional diarrhea (4%, n=5), functional constipation (3%, n=4), functional bloating (11%, n=15), and 18% had IBS-like symptoms (63) (Figure 1).

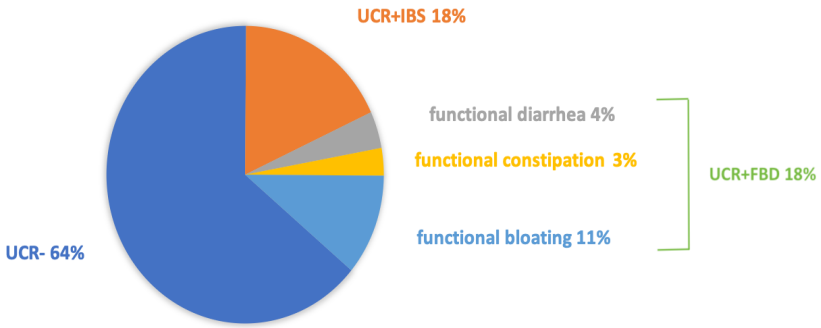


Figure 1. Distribution of UC patients in remission at enrollment according to the presence of symptoms compatible with IBS or another FBD.

4.1.2 GI and non-GI somatic symptoms, HRQoL, psychological factors and low-grade immune activity

UCR+FBD patients reported less severe total GI symptoms (total GSRS) and non-GI somatic symptoms (PHQ-12) than UCR+IBS patients. The burden of GI symptoms was higher for UCR+FBD patients than UCR-, but these groups had similar levels of non-GI somatic symptoms (Table 6). Moreover, UCR+FBD patients reported poorer HRQoL (total IBDQ score) than UCR-, however, only the Bowel Symptoms subscore differed between the groups. UCR+IBS reported the poorest HRQoL (Table 6). Finally, UCR+FBD and UCR- patients had similar anxiety/depression (HAD) and perceived stress (PSS-14) scores, as well as f-cal, hs-CRP and serum cytokine levels.

Table 6. Severity of GI symptoms (GSRS), non-GI somatic symptoms (PHQ-12) and HRQoL (IBDQ) in UC patients in remission (median with interquartile range).

	UCR+IBS	UCR+FBD	UCR-
Total GSRS	2.5 (2.1-3.1)[a]	2 (1.5-2.1)[b]	1.3 (1.1-1.7)
Diarrhea	2.3 (1.8-3.5)[a]	1.7 (1-2.6)	1.3 (1-1.7)
Indigestion	3 (2.6-3.4)	2.5 (2-3.2)[b]	1.8 (1.3-2.3)
Constipation	1.7 (1.3-2.8)	1.3 (1-1.9)	1 (1-1.7)
Abdominal pain	2.3 (1.7-3)[a]	1.3 (1.1-1.9)	1.2 (1-1.7)
Reflux	1.5 (1-2.8)	1 (1-1.5)	1 (1-1)
PHQ-12	6 (3.5-8.5)[a]	3.5 (1-4.3)	2 (1-4)
Total IBDQ	183 (163-198)[a]	195 (187-206)[b]	209 (195-216)
Bowel symptoms	57 (47-62)[a]	63 (59-66)[b]	67 (64-69)
Systemic symptoms	26 (22-29)	29 (26-33)	3 (27-32)
Social function	35 (32-35)	35 (34-35)	35 (34-35)
Emotional function	68 (57-71)[a]	73 (67-80)	76.5 (67-80)

[a] *statistically significant difference between UCR+IBS and UCR+FBD*
[b] *statistically significant difference between UCR+FBD and UCR-*

4.1.3 Evolution of symptoms compatible with IBS/other FBD and impact on the UC disease course

Among the UC patients in remission meeting the IBS or another FBD criteria at enrollment, approximately a third still reported similar symptoms when in remission at follow-up (31% for FBD, 36% for IBS) (Figure 2). Additionally, the proportions of patients with active disease at follow-up were comparable among the UCR+IBS, UCR+FBD and UCR- groups, as defined at the time of enrollment (36%, 25% and 26%, respectively). Finally, the UCR+IBS/FBD (n=29) and UCR- (n=52) groups did not differ in modified Mayo score and f-cal levels at follow-up or in the number of relapses during the follow-up period.

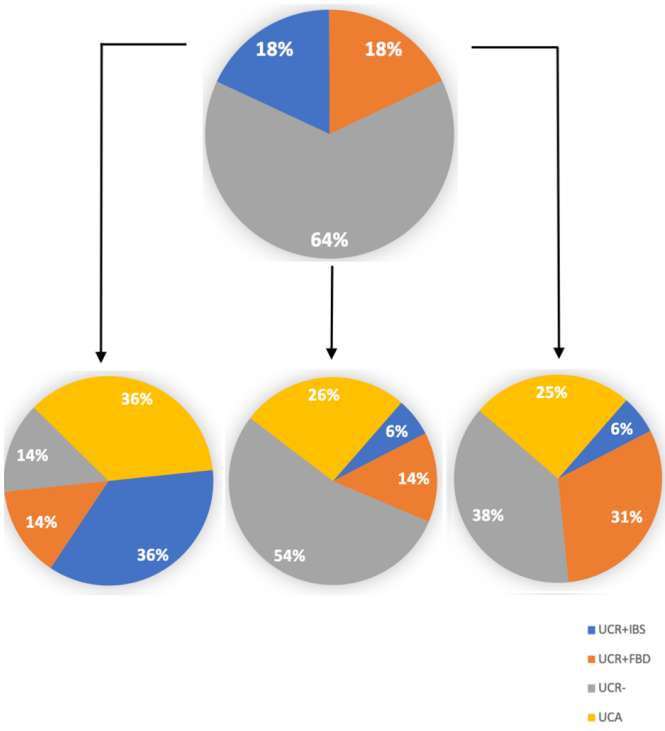


Figure 2. The over-one-year stability of FBD in UC patients.
The chart at the top shows the patient distribution by disease status (UCR+IBS, UCR+FBD, UCR-) at enrollment. The charts at the bottom show the distribution by disease status at follow-up for the respective group at enrollment.

4.2 Mucosal and systemic immune profiles at early and late active UC (Paper II)

4.2.1 Serum protein profiles differentiate early and late active UC

When analyzing serum proteins, we identified 15 proteins that could separate early and late disease (model R^2Y=0.84, Q^2=0.65) (Figure 3A). Levels of IL8, TNFSF14, CCL28, MCP-3 and CD40 were higher at early than late disease, while levels of Flt3L, BetaNGF and CCL25 were higher at late than early disease (Figure 3B).

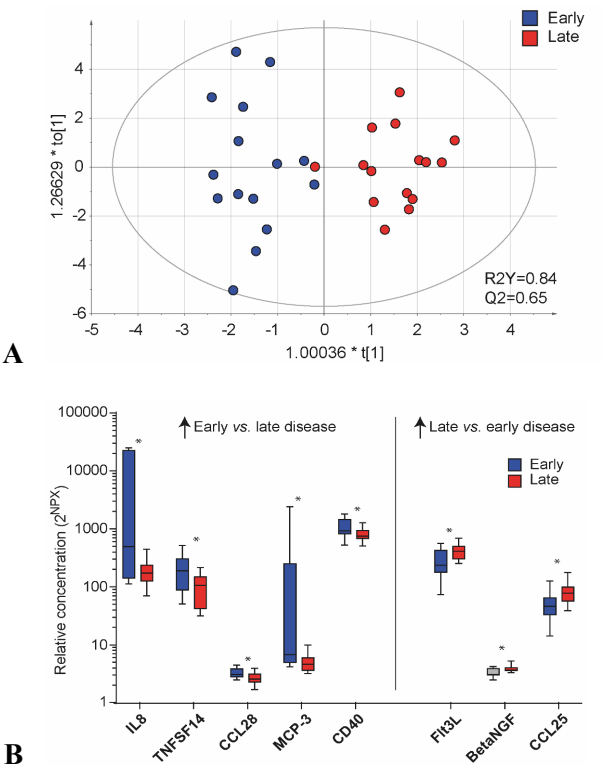
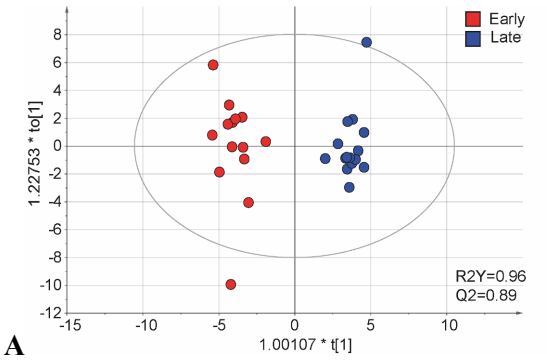


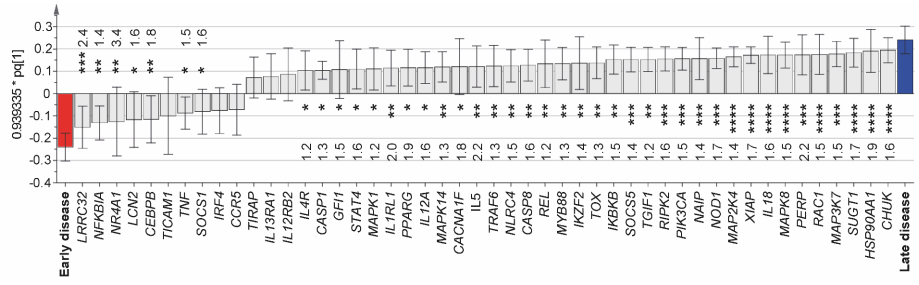
Figure 3. Serum proteins during early and late disease. (A) Score scatter plot from OPLS-DA. (B) Univariate analysis of the significant proteins from the OPLS-DA converted to linear data (2^{NPX}).
*Proteins are presented in the arbitrary unit normalized protein expression [NPX]. *q<0.05*

4.2.2 Mucosal gene profiles differentiate early and late active UC

When analyzing mucosal gene expression, we identified 48 genes that could separate early and late disease (model $R^2Y=0.96$, $Q^2=0.89$) (Figure 4A). The genes with the largest difference between early and late disease were LRRC32 and NR4A1, which were increased during early disease, and PERP, IL5 and IL1RL1, which were increased during late disease (Figure 4B). Notably, all these genes are related to T cell differentiation.



A



B

Figure 4. Mucosal gene expression during early and late disease. (A) Score scatter plot and (B) loading scatter plot from OPLS-DA.
Numbers in (B) indicate fold change of mean/median between early versus late disease (left panel) or late versus early disease (right panel).
**q<0.05, **q<0.01, ***q<0.001 and ****q<0.0001.*

4.2.3 Mucosal gene profile is Th1-dominated in early and Th2-dominated in late UC

Analysis of Th1- and Th2-related genes with PCA revealed a clear separation between early and late disease (Figure 5A). The loading scatter plot showed that most Th2-related genes were associated with late disease, whereas most Th1-related genes were associated with early disease (Figure 5B).

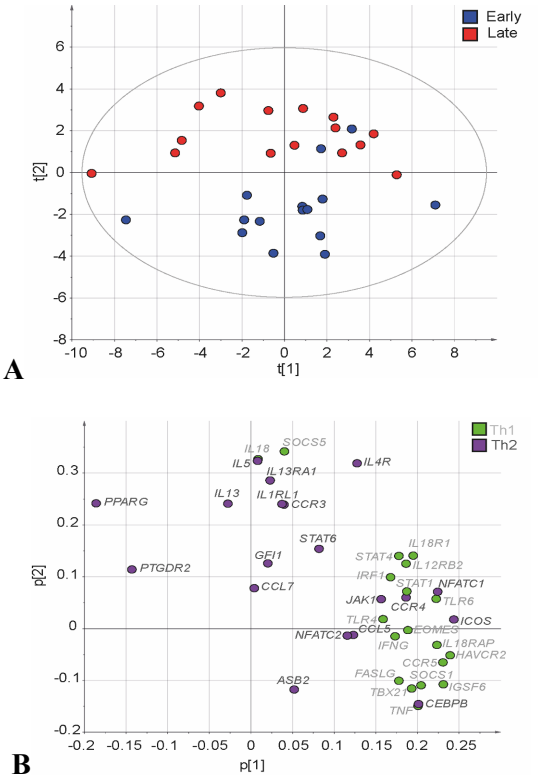


Figure 5. Mucosal gene expression of Th1- and Th2-related genes during early and late disease. (A) Principle component analysis and (B) score scatter plot.

4.3 Visceral hypersensitivity and GI symptoms in inactive UC (Paper III)

4.3.1 Rectal sensitivity in inactive UC, in IBS and healthy controls

Healthy controls hade higher thresholds for urge to defecate, discomfort and pain than UCR and IBS groups. No differences in sensory thresholds between IBS and UCR groups were observed.

UCR+IBS patients had lower thresholds for first sensation, urge to defecate, discomfort and pain than UCR-IBS. They also reported higher unpleasantness VAS ratings in response to rectal distension, but similar pain VAS ratings (Table 7).

Table 7. Comparisons of rectal sensitivity parameters in UC patients in remission fulfilling (UCR+IBS) and not fulfilling (UCR-IBS) IBS criteria (mean ± SD).

	UCR-IBS	UCR+IBS	p
Threshold first sensation (mm Hg)	8.1 ± 3.9	4.7 ± 2.1	**0.003**
Threshold urge to defecate (mm Hg)	18.9 ± 10.8	11.7 ± 4.2	**0.012**
Discomfort threshold (mm Hg)	30.6 ± 14.5	20.8 ± 8.1	**0.018**
Pain threshold (mm Hg)	40 ± 15	31.4 ± 8.5	**0.041**
VAS unpleasantness* (mm)	15.1 ± 11.4	32.6 ± 21.5	**0.004**
VAS pain (mm)	33.8 ± 25.6	39.2 ± 29.1	0.559

**mean of the first 4 distensions*

4.3.2 Factors associated with GI symptom severity in inactive UC

Significant correlations were observed between the discomfort threshold, VAS unpleasantness and the overall GI symptom severity, pain and bloating. The pain threshold was associated only with bloating. HAD scores and all GSRS domains were significantly correlated. This was also true for the level of perceived stress (PSS) and pain and constipation (Table 8). Moreover, females (n=16) in the UCR group had significantly more severe GI symptoms than males (n=20), (total GSRS-IBS 2.41±0.86 vs 1.59±0.59, $p<0.001$ (mean±SD)).

Table 8. Correlations between rectal sensitivity, psychological factors and GI symptom severity in UC in remission (r values).

	Total GI symptoms	Pain	Bloating	Constipation	Diarrhea	Satiety
Discomfort threshold	-0.33*	-0.39*	-0.42*	-0.01	-0.26	-0.05
Pain threshold	-0.23	-0.23	-0.34*	0.01	-0.08	-0.13
VAS unpleasantness	0.37*	0.36*	0.45**	0.02	0.28	0.07
HAD total	0.57**	0.52**	0.46**	0.40**	0.38**	0.34*
PSS	0.27	0.37*	0.21	0.33*	0.13	0.12

**p<0.05, **p<0.01*

Using PCA, two composite variables were generated; one rectal sensitivity variable, that consisted of the discomfort and pain thresholds and VAS unpleasantness, and one psychological distress variable that consisted of HAD and PSS scores. The two composite variables, along with age and gender, were

entered in multiple linear regression analyses to identify factors independently associated with the GI symptom severity in UCR patients. Overall GI symptom severity was independently associated with psychological distress, gender and rectal sensitivity. Rectal sensitivity was independently associated with bloating and pain, psychological distress with all the individual symptoms, except for bloating and diarrhea, and gender with bloating and satiety (Table 9).

Table 9. Standardized β's of linear regression models for GI symptom severity in UC patients in remission.

		Independent variables			
		Gender[a]	**Psychological distress**	**Rectal sensitivity**	**Age**
Dependent variable	**Total GSRS-IBS**	0.32*	0.40**	-0.33*	0.11
	Bloating	0.35*	0.20	-0.51**	0.09
	Diarrhea	0.19	0.32	-0.17	-0.05
	Constipation	0.16	0.43*	0.04	0.20
	Abdominal pain	0.16	0.44**	-0.34*	0.08
	Satiety	0.40*	0.31*	-0.16	0.25

*Factors with a significant unique contribution to the prediction of the dependent variable (GI symptoms) are highlighted with *, ** or ***, depending on the p-value, *p<0.05, **p<0.01, ***p<0.001*

[a] *Reference category; men*

4.4 HRQoL in longstanding UC in remission (Paper IV)

4.4.1 Comparison of HRQoL in longstanding UC in remission to the general population

The SF-36 domain scores of the patients with longstanding UC in remission were similar to those of the general population, except for Vitality (VT), where UC patients scored lower (Figure 6).

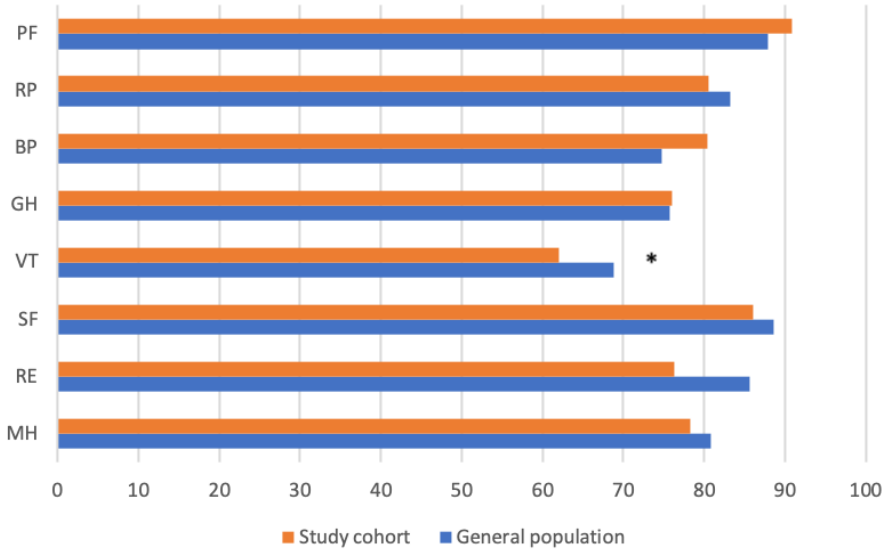


*Figure 6. SF-36 domain scores (mean values) for patients with longstanding UC in remission compared to the general population. * $p<0.05$*

4.4.2 HRQoL determinants in longstanding UC in remission

Gender, smoking, comorbidity and disease phenotype did not affect any SF-36 domain score (Figure 7A-C, E). Extraintestinal manifestations (EIM) affected the PF, RP and BP domains (Figure 7D) and a history of at least one relapse during the past year the RP domain (Figure 8A). Treatment with systemic corticosteroids and sick leave due to UC during the last year had a negative impact on most domains (Figure 8B-C). At least mild GI symptoms and borderline or clinically significant anxiety/depression negatively influenced all SF-36 domains but PF (Figure 8D-E). Finally, high fatigue had a negative impact on all SF-36 domains (Figure 8F).

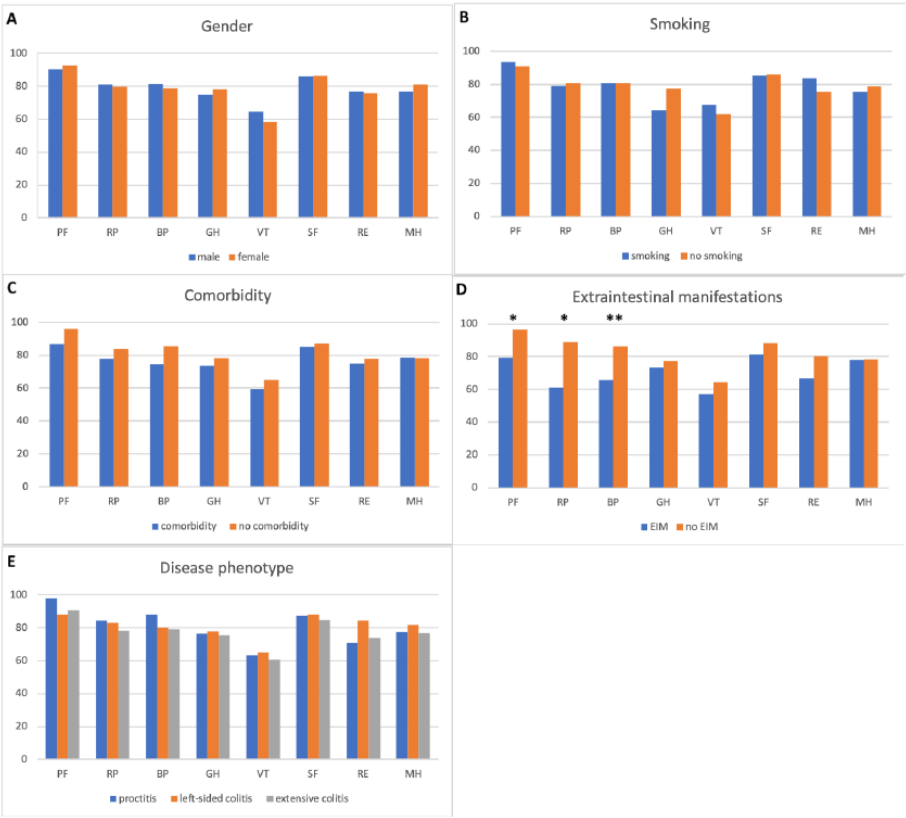


Figure 7. SF-36 domain scores (mean values) in subgroups of UC patients in remission according to ***(A)*** *gender,* ***(B)*** *smoking,* ***(C)*** *comorbidity,* ***(D)*** *extraintestinal manifestations,* ***(E)*** *disease phenotype. *p<0.05, **p<0.01*

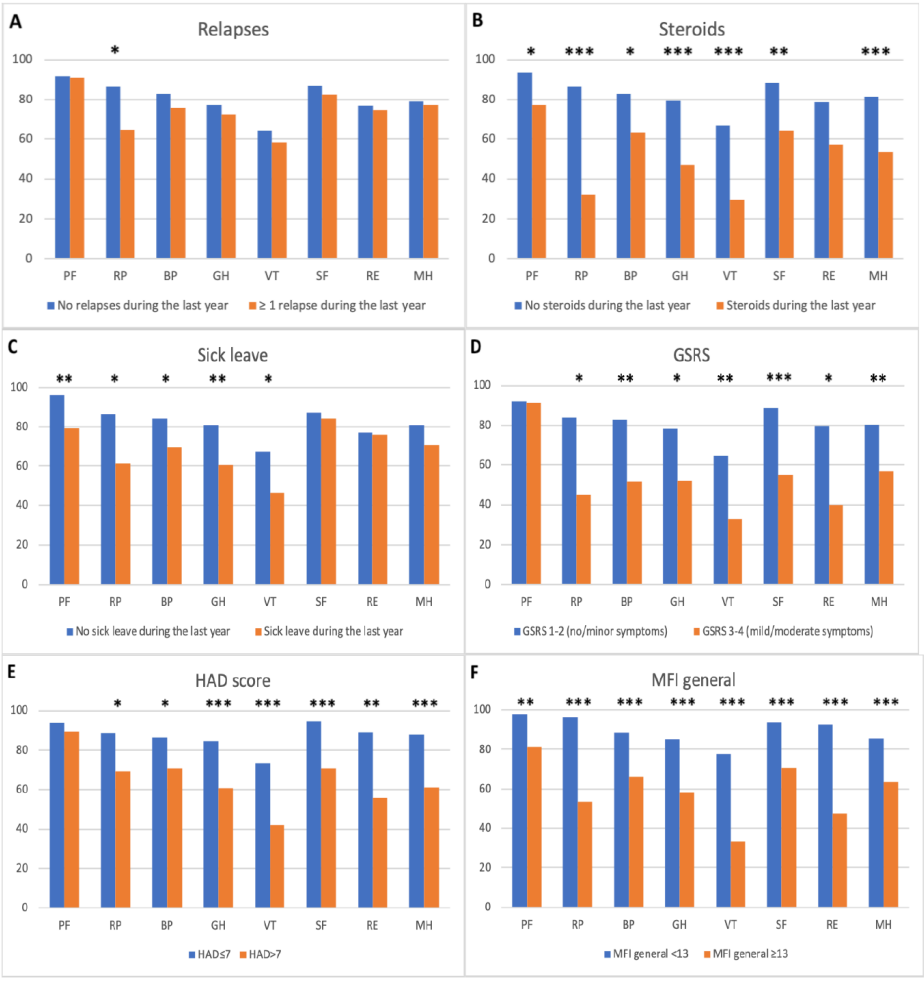


Figure 8. SF-36 domain score (mean values) in different subgroups of UC patients in remission. ***(A)*** *Patients with no relapses versus patients with at least one relapse during the previous years.* ***(B)*** *Patients who were treated with systemic corticosteroids during the previous year versus patients who were not.* ***(C)*** *Patients with sick leave due to UC during the previous year versus patients without.* ***(D)*** *Patients with no/minor GI symptoms (total GSRS score=1-2) versus patients with mild/moderate GI symptoms (total GSRS score=3-4).* ***(E)*** *Patients reporting borderline/clinically significant anxiety/depression (at least one HAD subscore >7) versus patients reporting no anxiety/depression (both HAD subscores ≤7).* ***(F)*** *Patients with high fatigue (MFI general fatigue score ≥13) versus patients with low fatigue (MFI general score <13). *p<0.05, **p<0.01, ***p<0.001*

Factors univariately associated with the SF-36 domain scores were entered into multiple linear regression analyses in order to identify factors independently associated with the SF-36 domain scores. Variables reflecting previous disease activity/severity were found to be independently significant for PF and BP, namely use of corticosteroids during the last year for PF and sick leave during the last year for PF and BP. Severity of GI symptoms in remission (GSRS total score) was associated with BP, and fatigue was the only variable with a significant unique contribution to RP, GH and VT. Finally, psychological distress (HAD) was the only independently significant predictor for all domains of mental HRQoL except for VT, that is SF, RE and MH (Table 10).

Table 10. Standardized β's of linear regression models for SF-36 domain scores.

		Dependent variables							
		PF	**RP**	**BP**	**GH**	**VT**	**SF**	**RE**	**MH**
Independent variables	**Weeks on steroids during the last year**	-0.59***	-0.23	0.13	-0.17	0.04	0.09		-0.02
	Sick leave weeks during the last year	-0.27*	-0.06	-0.27*	-0.19	-0.02			0.01
	Total GSRS	0.14	0.02	-0.42*	-0.08	-0.002	-0.16	0.02	-0.1
	Total HAD score	-0.1	0.23	-0.27	-0.1	-0.17	-0.55***	-0.35*	-0.63**
	MFI general fatigue score	0.07	-0.61***	0.03	-0.44**	-0.82***	-0.21	-0.23	-0.21
	EIM	-0.09	0.15	0.18					
	Number of relapses during the last year		-0.2						

*Factors with a significant unique contribution to the prediction of the SF-36 domain scores are marked with *, **, ***, *p<0.05; **p<0.01; ***p<0.001.*

5 DISCUSSION

5.1 FBD in inactive UC

In paper I, we reported that FBD other than IBS in quiescent UC are as common as IBS, with a prevalence of 18%. Therefore, the total prevalence of FBD (including IBS) in quiescent UC is estimated to be 36%. The burden of GI symptoms in UC patients reporting symptoms compatible with FBD other than IBS in remission was higher than in patients without these symptoms. In addition, FBD other than IBS had a negative impact on HRQoL, though to a lower degree than IBS. Neither psychological distress nor systemic immune activity or subclinical colonic inflammation were identified as risk factors for the presence of symptoms compatible with FBD other than IBS. Finally, UC patients with FBD (including IBS) in remission were not prone to a more aggressive UC disease course compared to patients without FBD.

5.1.1 Prevalence of FBD other than IBS in quiescent UC

While IBS-like symptoms in inactive UC are well investigated, the evidence on the prevalence of other FBD than IBS in inactive UC is limited. Among studies that reported FBD prevalence, Bryant et al estimated it to be 34.4% (64), Kim et al 57.2% (117) and Farrokhyar et al 51,2% (56). Mikocka-Walus et al found a prevalence of about 80%, but one third of their patients had active disease at inclusion (118). This variance and the discrepancy with our results should be interpreted with caution, as various criteria for remission and different definitions of FBD have been used among studies. Studies on IBS-like symptoms in UC in clinical remission have clearly shown that the prevalence rates of IBS-like symptoms decrease after excluding patients in clinical remission but with ongoing low-grade mucosal inflammation when evaluated endoscopically. This suggests that residual mucosal inflammation influences the presence of IBS-like symptoms (119). Among the above-mentioned studies, Kim and Farrokhyar relied only on clinical assessment of their samples to define remission, which might explain the high prevalence of FBD-like symptoms. Moreover, Farrokhyar used Rome II criteria, making any direct comparisons among studies more difficult. In contrast, Bryant assessed patients endoscopically to ensure remission, and our results showing a 36% prevalence of FBD in quiescent UC confirm the results of this study.

5.1.2 GI and non-GI somatic symptoms, psychological distress and immune activation in UCR+FBD patients

UC patients in remission with FBD other than IBS reported more severe overall GI symptoms when compared to those without FBD. This was, by definition, an expected finding, since the GI symptoms that are used to define FBD according to Rome III criteria are measured with GSRS too. However, the burden of non-GI somatic symptoms was increased only in remission patients with IBS-like symptoms, whereas the severity of these symptoms in patients with other FBD was at the same level as in patients not fulfilling criteria for any FBD. Comorbidity between IBS and other functional somatic conditions is frequent (120). IBS and these conditions share some characteristics, such as relatively high depression incidence, but if they have common pathophysiological basis is still under debate (121).

The interaction between IBS and psychological disorders is complex. Psychological disorders can act as a precipitating factor of IBS, together with other risk factors, but other studies suggest that, in some cases, mood disorders arise after the development of GI symptoms (122). Moreover, enhanced proinflammatory cytokine release in the serum of subsets of IBS patients and a correlation of the cytokine levels and GI symptoms and anxiety has been demonstrated (50). In our study, UC patients with FBS other than IBS did not differ from patients without FBD in terms of psychological comorbidities or markers of systemic immune activity, in contrast to patients with IBS-like symptoms who scored higher in HAD and PSS-14 and had elevated proinflammatory cytokines in serum (63). This also confirms the findings by Bryant et al where only IBS-like symptoms, but not other FBD, were associated with higher anxiety and depression levels (64). However, we cannot totally exclude that FBD other than IBS are in fact linked to psychological disorders and/or immune activity, but to a lesser extent than IBS, as such weak associations might not have been detectable due to small sample size or use of suboptimal markers in our study. Finally, longitudinal studies on FGID have shown that patients tend to switch FGID diagnoses over time (123). This was observed in our cohort too, as a proportion of subjects from all subgroups at enrollment changed group regarding FBD diagnosis at follow-up. Taking all these points into consideration, one could conclude that FBD in inactive UC are parts of a continuum, with IBS representing the more severe end of the spectrum.

5.1.3 HRQoL of UCR+FBD patients

Remission patients with FBD other than IBS had impaired overall HRQoL when compared to those without FBD by using IBDQ, and the only subscore where these patients scored lower was that of Bowel Symptoms. Thus, it can be concluded that the burden of GI symptoms that these patients experience mainly explains the impact on HRQoL. Bryant et al (64) reported reduced HRQoL for UC patients that fulfilled IBS criteria in remission but not for those with other FBD. The same observation was done by Kim et al (117) for their sample of UC patients, where HRQoL was measured by the generic questionnaire EQ-5D (European Quality of life 5-Dimensions). This is partly in contrast with our findings. However, IBDQ that we used is a disease-specific instrument and thereby more sensitive to detect smaller differences. We should though notice that the differences in mean total IBDQ score and Bowel Symptoms subscore between the UCR+FBD and UCR- groups were slightly below the difference that is considered to be the minimal clinically important (124). In addition, Bryant et al (64) showed a ¨load effect¨ of GI symptoms on HRQoL, as HRQoL worsened with increasing number of FGID-like symptoms. This was also confirmed by Mikocka-Walus (118). Finally, in Paper IV of this thesis, we showed that the severity of GI symptoms contributes independently to impaired physical HRQoL, measured by SF-36. All these observations support the finding of this study that the burden of GI symptoms is the major explanatory factor of the decreased HRQoL in UC patients with FBD other than IBS in remission.

5.1.4 Occult mucosal inflammation and FBD in UC

Based on the results in Paper I, no associations were found between the presence of FBD other than IBS in UC remission and ongoing low-grade inflammation in the colonic mucosa measured by conventional methods, like f-cal. This is also supported by the finding that FBD were not a risk factor for a more aggressive UC disease course under the follow-up period, since the absence of mucosal healing is acknowledged as a risk factor for increased subsequent disease activity (125).

The relationship between f-cal levels and the existence of FBD during clinical remission of UC has been studied only for IBS. Some studies have reported higher f-cal levels in UC patients with IBS-like symptoms in clinical remission than in those without these symptoms, suggesting that occult mucosal inflammation may be an underlying mechanism. In a study by Keohane et al, UC patients with IBS-like symptoms in remission had higher f-cal than those

without (655 vs 253 μg/g, $p<0.01$) (59). Berrill et al also demonstrated that a proportion of UC patients in clinical remission with IBS-type symptoms had mildly elevated f-cal (126). However, these studies did not evaluate the patients endoscopically. Moreover, although f-cal correlates well with endoscopic activity in UC, there is no consensus on the cut-off levels that discriminate between endoscopically active and inactive disease (23, 24). All these observations highlight the need to combine biomarkers and endoscopic evaluation in UC patients reporting FBD to exclude occult inflammation in colon mucosa, as done in our cohort.

There is evidence on low-level immune activation as a possible mechanism explaining FBD in inactive IBD. Vivinus-Nébot et al showed that FBD-like symptoms in IBD in remission may be associated with persistent but undetectable inflammation. This was based on epithelial barrier defects with increased paracellular permeability and increased intraepithelial lymphocytes and TNF-α levels found in colonic biopsies from these patients (60). Akbar et al found a TRPV1 (transient receptor potential vanilloid type 1) upregulation in patients with quiescent IBD and abdominal pain, that was absent in patients with inactive IBD without abdominal pain. TRPV1 is a receptor that is expressed in afferent neurons and plays an important role in visceral hypersensitivity. The authors of that study concluded that this may be triggered by the initial inflammatory insult in IBD (61). Moreover, in 'true IBS' (i.e. without coincident IBD) increased immune activity, where mast cells and monocytes seem to be important, has been reported (127). This is though not investigated further in the case of co-existent IBD. Finally, an immune activation of this kind, that does not involve neutrophils/macrophages, cannot be detected by conventionally used biomarkers, like f-cal. Colonic biopsies are crucial to further investigate abnormal anatomical and functional immune interactions accounting for the generation of FBD in quiescent IBD, but were unfortunately not obtained in our study.

5.2 Temporal variations in immune responses in active UC

In paper II, we demonstrated that the mucosal and serum immune profiles in patients with active UC are distinct in different phases of the disease. The most important finding was the transition from a Th1-predominant adaptive immune profile in the inflamed mucosa at the early stages of the disease to a Th2-polarized profile at the later stages, in the same patients. Likewise, differences in signaling pathways of the innate immune system between early and late active disease were shown. These results suggest that the immunopathological events occurring during the UC disease course are dynamic.

5.2.1 Transition from a Th1-polarized profile in early active UC to a Th2-polarized profile in late active UC

T helper precursor cells differentiate into distinct T helper lineages depending on the local cytokine setting. In the presence of IL12, they differentiate into Th1 cells that predominantly produce IFNγ, IL2 and TNF, and in the presence of IL4 into Th2 cells that mainly secrete IL4, IL5 and IL13 (128). Thus, our finding that TNF was upregulated in early disease and IL4R and IL5 in late disease is consistent with a Th1-predominant immune response in early disease stages and a Th2-polarized response in later stages. Moreover, GFI1, IL1RL1 and PPARγ, that promote Th2 responses, were all found to be upregulated in late disease, further supporting a Th2-predominant response in late disease (129-131). In addition, Th1 cells express mainly SOCS1 and SOCS2, while higher levels of SOCS3 have been detected in Th2 cells (132). Hence, the SOCS1 upregulation in early disease is consistent with a Th1-polarized immune response at this stage. Finally, serum TNFSF14, that enhances IL12 production and the development of Th1 cells, was also upregulated in early disease (133).

A proportion of the Th1- and Th2-related genes were expressed at similar levels at both disease stages and the PCA of mucosal gene expression revealed, after all, a minor overlap between early and late disease, too. In addition, SOCS5 and STAT4 were found to be upregulated in late disease. However, SOCS5 is predominantly expressed in Th1 cells and inhibits Th2 differentiation (134), and STAT4 promotes Th1 differentiation (135), which is in contrast to the Th2-polarized immune response in late disease that our results suggest otherwise. Finally, IL18, a Th1-inducing cytokine, was upregulated in late disease, but more recent studies have reported that IL18 can induce both Th1 and Th2 responses depending on the specific local cytokine milieu (136). These observations highlight the complexity of the T cell immune responses in UC, that show dynamic and progressive characteristics, with no clear distinction but a coexistence of different T cell subtypes under the different phases of the disease.

There is a limited evidence on temporal variations of T effector cell responses in IBD, and it comes primarily from studies on CD. Zorzi et al found distinct mucosal cytokine profiles during different phases of CD, with a shift from a Th1-dominated response before the appearance of endoscopic lesions to a mixed Th1/Th17 response when endoscopic recurrence occurred (75). Veny et al observed a systemic Th1/Th17 response in the blood of patients with late CD (>2 years) that was absent in early disease (<32 weeks) (73). Finally, a pediatric study revealed a typical Th1 profile in immune responses at the onset of CD that dissolved with progression to late disease (>5 years). For UC, higher

mucosal expression of IL23, a Th17-related cytokine, has been reported in newly diagnosed patients (<6 months) than in patients with established disease (>6 months) (71). However, the groups in all these studies consisted of different patients, in contrary to our study, where the same patients were followed up over time, diminishing interindividual variation. Finally, it is well known that Th17 cells infiltrate the inflamed mucosa and that cytokines linked to the Th17 pathway are upregulated in active UC (137). Although the above-mentioned studies suggest that Th17 cells are temporally regulated in IBD, we found no differences in Th17 related gene expression between early and late UC in our study.

5.2.2 Temporal variations of the innate immune system responses in active UC

Several subsets of innate immune cells contribute to the IBD pathogenesis. Sensing of microbial antigens by innate immune cells is mediated by pattern-recognition receptors, including Toll-like and NOD-like receptors. This results in the production of proinflammatory cytokines and chemokines that further promote the inflammatory response by facilitating immune cell migration to the site of inflammation (10).

Serum protein analyses in our study imply dynamics of the innate immune system under the UC disease course. Significant differences in the serum levels of various chemokines were detected between early and late disease, which could result in variations in the composition of the innate immune cells that drive the inflammation under the different phases of the disease. IL8 and MCP3, that function as chemoattractants for neutrophils and macrophages (138, 139), were detected in higher levels at early disease, which may indicate a stronger innate immune response at this phase. This is in line with the concept that innate-derived cytokines initiate the inflammation in early IBD, while the establishment of the chronic inflammation later is driven by adaptive immune mechanisms (140). In addition, a study by Forkel et al reported changes in the composition of mucosal innate lymphoid cells in new onset and established UC, which is also consistent with temporal variations in innate immune responses in UC (141).

The majority of the genes that were found to be upregulated in the inflamed mucosa at the late stage of UC were related to NOD-like (n=9) or Toll-like receptors signaling (n=6). Since these receptors recognize microbiome-associated molecular patterns, these differences may derive from dynamics in the composition of the gut microbiome during the UC disease course. Previous

literature has demonstrated that the gut microbiota are not totally stable during the IBD course and that this fluctuation may depend on the disease activity or treatment interventions (142). However, other longitudinal studies revealed weak or no correlations between microbial composition and inflammatory activity in the gut (143, 144). In general, the interplay of microbiota with the immune system shows complexity, which needs further investigation (145).

5.2.3 Possible bias to be taken into consideration when interpreting variations in immune responses in active UC

The severity of ongoing inflammation could constitute bias that should be taken into consideration when interpreting our results. It can be argued that the immune dynamics discussed above are secondary to different inflammation grade between the two time points the samples were collected. However, the patients in our study had comparable disease activity both at early and late stage. Previous or ongoing medical treatment may also be a factor that account for the immunological variations observed under the disease progress. However, this is a factor that is impossible to control for, as longitudinal studies with untreated patients are not ethically justifiable. The study of the immunological effects of different medications gains though continuously a place in research. Likewise, inter-individual variability in treatment and disease severity during the follow-up period of our study are two other parameters to be considered as possible factors influencing our results.

The samples obtained at early disease were stored for a longer period. This might have affected the stability of the molecules that were analyzed. Moreover, different molecules were measured in biopsies (mRNA) and serum (proteins), and any correlations or comparisons should be done with caution. Furthermore, the products of some of the genes that were analyzed need to undergo post-translational modifications before activation. This means that quantitative differences in gene expression do not necessarily reflect differences in activated gene products.

After all, we need to highlight that the lack of consensus regarding the composition of immune cell populations involved in the pathogenesis of UC might be a result of the dynamics of the immune responses during the disease progression. An immunological plasticity of this kind is difficult to be detected when studied populations are not homogeneous with regard to their disease duration, as in previous studies on this field. However, a deeper investigation of immune profile changes under the IBD disease course is necessary, as this could facilitate individualized patient treatment.

5.3 Visceral hypersensitivity as a mechanism of IBS-like symptoms in inactive UC

The results of paper III indicate that UC patients in remission with symptoms resembling IBS have increased rectal sensitivity when compared to UC patients in remission without IBS-like symptoms. Visceral hypersensitivity was correlated to the severity of GI symptoms experienced by these patients, in particular bloating and abdominal pain. Psychological distress, i.e. anxiety, depression and stress, and female gender were also associated with GI symptom severity.

5.3.1 UC-related factors contributing to visceral hypersensitivity

Responses to anorectal distension in UC have been studied previously, mainly as a mechanism underlying symptoms during active disease, such as increased stool frequency, urgency and tenesmus. Despite methodological differences among studies, the majority of them have shown a hypersensitive rectum in active disease, with authors concluding that this might explain part of the symptoms in active UC (87, 89, 146). However, Brochard et al demonstrated no differences in the sensation intensity between UC patients with mild to moderate ongoing disease activity and healthy controls (90). When visceral sensitivity was evaluated in quiescent UC, patients with inactive UC not fulfilling IBS criteria were found to be hyposensitive in comparison to healthy controls or IBS patients (88). In contrast, an increased visceroperception in UC patients in remission was reported by van Hoboken, when their responses to rectosigmoid distension were compared with healthy controls. The same authors also showed a positive correlation between visceral hypersensitivity and severity of GI symptoms in quiescent UC (147).

From these findings, it seems reasonable to conclude that both the disease status and the presence and severity of GI symptoms, regardless of origin (i.e. functional or secondary to inflammation) are factors that determine the responses of UC patients to anorectal distensions. This is in line with our study results that UC patients in remission fulfilling IBS criteria demonstrated enhanced visceral sensitivity when compared to patients without IBS-like symptoms, and is further supported by the correlations between rectal sensitivity parameters and severity of GI symptoms. Hence, UC patients in remission should not be considered as a homogenous group, and only a subset, those with ongoing GI symptoms, seem to exhibit visceral hypersensitivity.

5.3.2 Visceral hypersensitivity, psychological factors, gender and GI symptom severity in quiescent UC

Several studies have demonstrated that IBS patients (without coincident IBD) show visceral hypersensitivity as a group, however, the prevalence of visceral hypersensitivity varied between 20 and 80% across studies. Moreover, the clinical relevance of visceral hypersensitivity remains to be proven, since only weak correlations between rectal sensory thresholds and symptoms have been found (82). Regarding the type of GI symptoms, mainly abdominal pain and bloating correlate with rectal hypersensitivity in IBS patients (148, 149), and studies that compared IBS patients with diarrhea and IBS patients with constipation have shown contrasting results (150, 151).

The evidence on the relationship between GI symptom severity and rectal sensitivity in quiescent UC is limited to a study by van Hoboken (147). The authors reported correlations between overall GI symptom severity and rectal perception parameters, however associations with the severity of individual GI symptoms were not presented. In the study by Akbar et al, the TRPV1 receptor, which has been implicated in pain related to rectal hypersensitivity, was found to be upregulated in IBD patients with IBS-like symptoms in remission, with a positive correlation to abdominal pain severity (61).

Our results show that visceral hypersensitivity is associated with overall GI symptom severity in quiescent UC and more specifically with symptoms of pain and bloating, but not with diarrhea, constipation or satiety. This is a novel finding with regards to GI symptoms in inactive UC, and fully consistent with previous research on IBS without coexistent organic GI disease. Additionally, these correlations were weak or moderate in our study, as in 'true IBS' studies. This indicates that visceral hypersensitivity alone cannot explain these symptoms, but additional factors, highlighted in our multivariable analysis, are involved in the complex pathogenesis of GI symptoms in inactive UC.

Studies on the associations between psychological factors, GI symptoms and visceral hypersensitivity in IBS have been partly inconclusive. While some suggest that hypersensitivity in IBS has a psychological basis (83), others have shown poor or no associations between visceral hypersensitivity and psychological disturbances. However, correlations between altered rectal perception and GI symptom severity, as well as psychological distress and GI symptom severity in IBS patients have been reported (81, 149, 152).Our results confirm these interactions between visceral hypersensitivity, psychological disorders and severity of GI symptoms, and add new evidence in this field for the case of IBS-like symptoms in quiescent IBD. Hence, in our cohort with

inactive UC, no correlations between anxiety/depression and rectal perception parameters were demonstrated. However, positive correlations between anxiety/depression and the severity of GI symptoms were found. These observations are further supported by the multivariable analysis, were both psychological distress and visceral hypersensitivity independently contributed to the generation of GI symptoms.

Other cognitive and psychological factors than those investigated in our study, such as increased tendency to report symptoms and selective attention to GI sensations that are interpreted as disease symptoms, have previously been shown to contribute to visceral hypersensitivity (83, 153). These parameters were unfortunately not analyzed in our cohort. Centrally mediated processes most likely play an important role in the pathophysiology of visceral hypersensitivity and need further evaluation.

While many studies have pointed out that females show enhanced responses to rectal distensions, other studies found less clear gender differences in the sensitivity status (154, 155). Additionally, there is evidence that suggests that female patients with IBS tend to report more severe GI symptoms than males (156). In our cohort, no gender differences in rectal perception parameters could be confirmed, but females scored higher in total GSRS and all subscores except for diarrhea and constipation. Moreover, gender was found to contribute independently to the generation of overall GI symptoms, satiety and bloating. This is in accordance with a previous study on IBS patients were female gender was among the factors independently associated with the overall GI symptom severity (149).

5.3.3 Complexity of mechanisms of GI-symptoms in inactive UC

To conclude, the study results suggest that the pathophysiology of GI symptoms in UC in clinical remission is multifactorial and complex. We have shown that visceral hypersensitivity and psychological distress along with gender are all involved in the generation of GI symptoms in quiescent UC. This is in agreement with a study on IBS patients that showed that psychological distress, visceral hypersensitivity and gut motility disorders have a cumulative effect on symptom severity (157). Moreover, the complexity of GI symptom generation in inactive UC is supported by the fact that our regression models showed that only 23-52% of the variance of the different GI symptoms could be explained by the factors included in the models. Finally, although we used quite strict criteria to define UC remission, we cannot totally exclude that some of the patients had ongoing low-grade inflammation. Occult mucosal inflammation has previously been proposed to have an impact on visceral

sensitivity and symptom generation in inactive IBD (61, 147). This should be addressed in future research with histological evaluation of the gut.

5.4 HRQoL in longstanding and inactive UC

In Paper IV, our results indicate that the overall HRQoL in longstanding (10 years) UC in remission is comparable to the HRQoL of the general population. However, aspects of physical HRQoL seem to be dependent on recent systemic corticosteroid treatment and work disability due to UC activity, but also fatigue and persisting GI symptoms during remission. In contrast, aspects of mental HRQoL seem to be determined primarily by psychological factors, like psychological distress, along with fatigue.

5.4.1 Comparisons of HRQoL with the general population

Several previous studies comparing HRQoL in quiescent UC with the general population have reported comparable levels of HRQoL for these two groups. Hjortswang et al found similar scores between a sample of UC patients in remission and the Swedish general population for all SF-36 domains, except for General Health, where UC patients scored lower. The range of the disease duration in that study was though large (mean duration 11.7 years, SD 9.8 years), contrary to our study where patients were homogeneous regarding disease duration (158). Likewise, Hoivik et al reported in their cohort with UC patients with a 10-year disease duration SF-36 scores consistent with the Norwegian general population, except for a reduction in the General Health domain. However, about half of those patients had at least mild ongoing IBD-related symptoms (103). Moreover, in a Spanish study that evaluated HRQoL in UC patients in clinical remission, patients reported a perception of health similar to the general population (98). Finally, symptom-free UC patients had SF-36 scores close to the general population in another population-based Norwegian study (159). Therefore, our results demonstrating no differences in HRQoL between patients with longstanding UC in remission and the general population are largely in line with these studies. However, we evaluated a well characterized and homogeneous regarding the disease duration UC cohort, that consisted only of patients in remission, and thereby our study adds new relevant knowledge in this research field.

Vitality, a measure of energy and fatigue, was the only SF-36 domain score that was reduced among UC patients in our study. Nordin et al have also reported lower Vitality scores, among other domain scores, for UC patients in remission, when compared to the Swedish general population (160). However,

this study, as well as the studies by Hjortswang and Hoivik (103, 158), have more clearly shown a reduction in the General Health domain of SF-36 in inactive UC, a finding that has been attributed to the patients' experience of living with a chronic disease. This is though in contrast with our study. A possible explanation is the longer disease duration in our patients, which may be associated with a patient adaptive process resulting in a normalization of the general health perceptions. Finally, in a review study by Yarlas et al, only differences in General Health and Vitality domains between patients with inactive UC and the general population were reported to exceed minimal important differences in more than one of the reviewed studies, and thus can be interpreted as clinically meaningful (161).

5.4.2 Determinants of physical HRQoL

The use of systemic corticosteroids and sick leave due to UC relapse can be considered as markers of moderate/severe disease activity, and logically negatively affected physical HRQoL. On the contrary, recent relapse rate did not influence SF-36 domain scores. Since most patients (88%) had not been treated with systemic corticosteroids during the last year, it can be claimed that the vast majority of the recent relapses were of mild severity. These observations imply that the time to HRQoL normalization when entering remission after a relapse is likely determined by the severity of the relapse. In line with our results, in the study by Hoivik et al, corticosteroid treatment during the previous year was found to negatively affect nearly all domains of physical HRQoL (103). In another study on the same UC population, UC-related sick leave influenced HRQoL in a negative way (162). Finally, similarly to our findings, Hjortswang et al reported no differences in HRQoL between UC patients who had experienced one or more relapses during the previous year and those who had not (158). In our study we did not specify the time since the last relapse, and thus patients who had a relapse 12 months ago were indistinguishable from those with a relapse more recently and close to the study recruitment. We also conducted review of the medical records to obtain relevant information retrospectively. This highlights the need for more longitudinal studies with standardized definitions of disease activity and recall periods in order to explore the long-term effects of relapses on HRQoL after entering remission. Moreover, the design of our study does not allow us to exclude that the negative association between corticosteroid treatment and HRQoL depends on corticosteroid-specific side effects.

In their population-based study, Romberg-Camps et al have clearly demonstrated that the MFI general fatigue score negatively determined the HRQoL in UC independent of disease activity (163). Moreover, in a previous

report by Jonefjäll et al, high fatigue in UC patients in deep remission was associated with poorer HRQoL (164). Our results showing that fatigue was an independent determinant of three out of eight SF-36 domains confirm previous research and illustrate the importance of investigating this complex and still not totally understood symptom in quiescent UC. Moreover, the exclusion of patients with other severe comorbidities in our study, possibly contributing to fatigue, further strengthens previous evidence of the negative impact of fatigue on HRQoL in UC in remission.

The presence of persistent GI symptoms after reaching remission has previously been associated with lower HRQoL in UC. It has been reported that UC patients in remission with symptoms resembling IBS have lower HRQoL than those without these symptoms (165). Jonefjäll et al have also demonstrated that IBDQ scores in UC patients with IBS-like symptoms in remission were at the same level as for patients with active disease, but lower than for UC patients not fulfilling IBS criteria in remission (63). Moreover, a negative correlation between the total GSRS score and HRQoL, measured by Psychological General Well-Being Index has been reported in another cohort of UC patients in remission (166). Finally, in Paper I of this thesis we found lower HRQoL in UC patients with FBD in remission. These findings altogether confirm the significance of ongoing GI symptoms in inactive UC as a determinant of HRQoL.

5.4.3 Determinants of mental HRQoL

The significance of psychological disorders/distress as a predictor of poor HRQoL in UC has been highlighted in several previous studies. Guthrie et al showed that both psychological symptoms and disease activity contributed independently to impaired HRQoL in IBD, with psychological symptoms having a stronger negative impact on SF-36 domains constituting mental HRQoL (167). Iglesias-Rey et al have demonstrated that stress, anxiety and depression were all associated with low HRQoL in IBD (168). Although patients with active IBD have been reported to have higher levels of psychological distress than those in remission (169), even studies including only patients in remission, have found anxiety to be an independent predictor of poorer HRQoL (56, 117).

In the study by Guthrie et al (167), psychological distress (anxiety/ depression) was an independent predictor of the physical health domains of SF-36, in addition to the severity of IBD. This is in contrast with our findings, where the clear univariate associations between HAD scores and all SF-36 domain scores of physical health did not remain significant when controlling for other factors

in multivariable analyses. A possible explanation to this disparity are the low levels of anxiety/depression in our cohort, which, however, is in line with previous studies reporting prevalence of psychological distress in UC patients in remission (166). It is finally important to mention that there is evidence supporting that other psychological factors, like stress and copying, have a negative impact on HRQoL in IBD generally (169). These factors were unfortunately not assessed in our study.

5.4.4 Factors with no effect on HRQoL

Gender has previously been reported to influence HRQoL in IBD, with females reporting poorer HRQoL than males (170). However, the role of gender as a determinant of HRQoL in IBD is still unclear, as several other studies failed to show any gender differences (171, 172). Various explanations have been proposed to these gender differences, mainly psychological factors with greater disease-related concerns, and also rating of individual symptoms as more severe by females (172, 173). It should though be mentioned that even in studies on the general population, females tend to score lower in HRQoL measurements (174). In our cohort, no differences between males and females were found in HAD and GSRS scores, and overall these scores were low, which probably explains the lack of gender differences in SF-36 scores too. These results are also in accordance with the study by Hoivik et al where the majority of UC patients reported no or mild symptoms (103). In the same study no associations between disease phenotype, comorbidity and smoking and HRQoL were found, which is in line with our findings. Finally, the presence of EIM negatively affected most subscales of physical HRQoL in our cohort, which has also been shown by previous research (175, 176). However, this association did not remain significant in the multivariable analyses, and this may be related to EIM being a surrogate marker of more severe disease.

5.5 Study limitations

Limitations of the studies have been discussed in previous parts of this section. The most important are summarized here:

In Paper I, patients were included consecutively when they had clinical visits. This may have resulted in an overestimation of the FBD prevalence, as most symptom-free patients do not seek healthcare. Moreover, the follow-up period was one year, which might have been too short to evaluate the evolution of FBD-like symptoms over time. We may also have failed to capture disease

activity between the two visits of the study. Finally, we did not use endoscopy to define remission at follow-up, but only f-cal and patient-reported outcomes.

In Paper II, the samples obtained at early disease were stored for at least 10 years before the analyses, which might have affected the stability, mainly of the proteins. Moreover, the products of some of the genes analyzed need to undergo post-translational modifications in order to be activated. Thus, quantitative differences in gene expression do not necessarily mean differences in activated gene products. Finally, differences in the treatment and disease activity among the included patients during the follow-up period may have influenced the results of the study, but this is hard to control for.

In Paper III, although we combined endoscopy and f-cal levels to ensure UC remission, no colonic biopsies were collected. We can, thus, not definitely exclude persistent low-grade inflammation or other mucosal changes, possibly due to previous inflammation, that could have contributed to visceral hypersensitivity. Moreover, the distension protocol we used (ascending method of limits) can be affected by psychological factors, mainly the fear of pain. However, no associations between psychological symptom severity and perception thresholds were observed in our study.

In Paper IV, the sample size was modest. In addition, the SF-36 is a generic HRQoL instrument, and thus less sensitive than IBD-specific instruments, such as the IBDQ. However, since the main objective of the study was to compare HRQoL in longstanding UC in remission with the general population, the choice of a generic instrument was appropriate. Finally, the time since the last relapse was not taken into account and some of the data were collected retrospectively with a risk for recall bias.

6 CONCLUSION

- Symptoms compatible with FBD other than IBS are as common as IBS-like symptoms in UC remission. They negatively influence HRQoL, but are not associated with ongoing low-grade gut inflammation, systemic immune activity and psychological distress, and are not a risk factor for a more aggressive UC disease course.

- The mucosal and systemic immune profiles in patients with active UC are distinct at different stages of the disease course, with the adaptive immune profile in the intestinal mucosa showing a transition from a Th1-polarized response during early disease to a Th2-dominated profile during late disease.

- UC patients in remission with IBS-like symptoms have increased rectal sensitivity when compared to those without IBS-like symptoms. Visceral hypersensitivity, psychological factors and female gender are important determinants of GI symptom generation in UC patients in remission.

- Overall HRQoL in longstanding UC in remission is comparable to the general population. Markers of at least moderate recent disease activity, such as systemic corticosteroid use and sick leave due to UC, but also fatigue and persisting GI symptoms during remission negatively influence physical HRQoL, while mental HRQoL is mainly determined by psychological factors.

7 FUTURE PERSPECTIVES

In this thesis we have shown that the generation of GI symptoms in quiescent UC is multifactorial and complex. Although we did not find any evidence supporting that low-grade gut inflammation, measured by conventional methods such as f-cal, contribute to the generation of these symptoms, it cannot definitely be excluded that persistent undetectable inflammation partly can explain these symptoms. After all, there is already evidence, even though limited, supporting that remaining inflammation or mucosal alternations secondary to preceding inflammation may be associated with the GI symptoms in IBD in remission. This needs to be further investigated with studies that include histological evaluation of the intestinal mucosa and assessment of the immune activation locally in the gut. If such an association can be demonstrated, it can potentially change the therapeutic targets in IBD. Finally, future studies should better clarify the interactions between central factors, for example psychological, low-grade inflammation and GI symptoms in quiescent IBD. Those studies should also evaluate if interventions focusing on central factors could be part of the management in at least a subset of IBD patients.

The immunological variations during the UC disease course merit further investigation in the future, as well as the effects of different therapeutics on the intestinal immune responses. It is acknowledged that the management of UC requires a personalized approach, since patients are heterogeneous and the natural history of the disease variable and unpredictable. The determination of the immune profile on an individual level, at specific time points during the disease course, might facilitate personalized treatment decision-making and help clinicians to determine the most suitable patient subgroups for each specific treatment.

Finally, although HRQoL is commonly assessed in IBD, most studies focus on HRQoL during the first years after the diagnosis has been established. However, the disease is chronic, unpredictable and not curable. Hence, there is a need to continue to investigate the impact of the disease on HRQoL over a longer time span, and more prospective and longitudinal studies in this field are warranted.

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