Complementary Treatment and Markers in Inflammatory Bowel Diseases

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ABSTRACT

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Aims: The aims of this thesis were to evaluate potential prognostic markers and addition of complementary treatments in inflammatory bowel diseases.

Background: Inflammatory bowel diseases, the two main types of which are ulcerative colitis and Crohn's disease, affect nearly 1% of the Scandinavian population and implies a risk of a chronic progressive, disabling disease. When considering treatment options, issues are: predicting short and long-term prognosis, selecting optimal treatment options and providing appropriate care for complications of the conditions or treatments.

Methods: Selected variables were investigated to determine their ability to predict colectomy in an early phase of a severe attack of ulcerative colitis in patients admitted to hospital. Hyperbaric oxygen treatment was tested as a complementary treatment in acute severe ulcerative colitis. Addition of supplementary treatment with cereals was evaluated in patients with sequelae after intestinal resections.

Results and Conclusions: Analyses of stool frequency, faecal weight and complement factor 3c in plasma may contribute to an early prediction of the disease course in a severe attack of ulcerative colitis. Hyperbaric oxygen treatment as a complementary treatment in a severe attack of ulcerative colitis does not improve clinical outcome. Specially processed cereals, as well as non-processed cereals, can be safely used in patients with previous intestinal resections. Intake of non-processed cereals may decrease faecal volume in these patients.

Key words: *inflammatory bowel diseases, ulcerative colitis, Crohn disease, predictor, complementary therapies, hyperbaric oxygen therapy*

SAMMANFATTNING PÅ SVENSKA

Inflammatoriska tarmsjukdomar innefattar flera diagnoser, varav de vanligaste och mest betydelsefulla är ulcerös kolit och Crohn's sjukdom. Ulcerös kolit och Crohn's sjukdom drabbar knappt 1 % av befolkningen i Norden. Båda tillstånden är kroniska med vanligtvis ett varierande förlopp av sjukdomssymptom bestående av diarréer, buksmärtor och eventuellt tarmblödningar. Den grundläggande behandlingen av inflammatoriska tarmsjukdomar utgörs av medicinska behandlingar, men en betydande andel av patienterna behöver opereras. Patienter med svår akut attack av ulcerös kolit med otillräcklig effekt av sedvanlig medicinsk behandling riskerar att tjocktarmen behöver opereras bort. Det finns därmed ett behov av att finna instrument för att tidigt kunnat förutsäga det kliniska sjukdomsförloppet samt kompletterande behandlingsalternativ, såväl i tidigt sjukdomsskede som i senare stadier av komplikationer till grundsjukdomen eller till dess behandling. Preliminära studier med hyperbar syrgasbehandling som tilläggsbehandling vid inflammatoriska tarmsjukdomar har visat lovande resultat, men kontrollerade studier av denna typ av behandling vid ulcerös kolit har ännu inte genomförts. Patienter med ulcerös kolit eller Crohn's sjukdom som genomgått olika typer av tarmkirurgi kan drabbas av omfattande diarréer med förluster av vätska, salter och andra näringsämnen, vilket ofta leder till försämrad hälsa och sänkt livskvalitet trots konventionell medicinsk behandling.

Syften med avhandlingen var dels att utvärdera eventuella prognosmarkörer vid akut attack av ulcerös kolit, dels att utvärdera hyperbar syrgasbehandling som komplementär behandling vid akut attack av ulcerös kolit samt att utvärdera tilläggskost med ceralier avseende dessas effekter på diarréer/vätskeförluster hos patienter som tidigare genomgått tarmkirurgi.

I delarbetena visas att antal avföringar per dygn samt nivå av ett av proteinerna i komplementsystemet (komplementfaktor 3c) skulle kunna användas som prognosmarkörer i tidigt skede av en akut attack av ulcerös kolit. Tilläggsbehandling med hyperbar syrgasbehandling vid en akut attack av ulcerös kolit hade inte några positiva effekter. Vid jämförelsen mellan tilläggskost av specialprocessad havre och ickeprocessad havre till patienter med resttillstånd efter tidigare tarmkirurgi erhölls minskade avföringsvolymer endast vid intag av ickeprocessad havre.

Resultaten skulle kunna bidra till att bättre kunna förutsäga sjukdomsförloppet vid akut attack av ulcerös kolit, vilket skulle underlätta ställningstagande till om eller när alternativ behandling och/eller kirurgi skall tillgripas. De uteblivna positiva effekterna av hyperbar syrgasbehandling ger underlag för att avfärda denna behandling given på beskrivet sätt vid akut attack av ulcerös kolit. Ickeprocessad havre kan vara värdefull som tilläggskost till en svårbehandlad hårt drabbad patientgrupp.

LIST OF PAPERS

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

I.   Pagoldh M, Lange S, Jennische E, Almer S, Boström E A, Eriksson A. **Faecal analysis and plasma C3c levels at admission for an acute attack of ulcerative colitis are predictive of the need for colectomy.** *Accepted for publication in European Journal of Gastroenterology & Hepatology*

II.   Pagoldh M, Hultgren E, Arnell P, Eriksson A. **Hyperbaric oxygen therapy does not improve the effects of standardised treatment in a severe attack of ulcerative colitis: A prospective randomised study.** *Scand J Gastroenterol 2013;48:1033-1040*

III.   Pagoldh M, Eriksson A, Heimtun E, Kvifors E, Sternby B, Blomquist L, Lapidus A, Suhr O, Lange S, Karlsbom U, Nordström D, Rettrup B. **Effects of a supplementary diet with specially processed cereals in patients with short bowel syndrome.** *Eur J Gastroenterol Hepatol 2008;20:1085-1093*

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ABBREVIATIONS

5-ASA	5-aminosalicylic acid
AF	Antisecretory factor
Alb	Albumin
Anti-TNF-α	Antibodies against tumour necrosis factor factor alpha
AUROC	Area under receiver operating characteristic curve
C3c	Complement component 3c
CD	Crohn's disease
CRP	C-reactive protein
ELISA	Enzyme-linked immunosorbent assay
ESR	Erythrocyte sedimentation rate
GCSs	Glucocorticosteroids
Hb	Haemoglobin
HBOT	Hyperbaric oxygen therapy
HPT	Haptoglobin
HRQoL	Health related quality of life
IBD	Inflammatory bowel diseases
IBDQ	Inflammatory bowel disease questionnaire
IFX	Infliximab
IL	Interleukin
ISs	Immunosuppressants
MCS	Mental component summary
NPCs	Non-processed cereals
Oro	Orosomucoid
PCS	Physical component summary
PMSS	Patients' medical safety score
ROC	Receiver operating characteristic
SBL	Small bowel length
SBS	Short bowel syndrome
SF-36	Short form 36 health survey
SPCs	Specially processed cereals
S-α1-AT	Serum alpha 1 antitrypsin
TPK	Platelet count
UC	Ulcerative colitis

CONTENTS

INTRODUCTION

"Inflammatory bowel diseases (IBD)" is the generic term for several inflammatory entities in the intestinal tract. The two main subtypes of IBD, ulcerative colitis (UC) and Crohn's disease (CD), involve a considerable risk of a chronic progressive disabling disease. Diagnosis, assessment of severity, predicting long-term prognosis and selecting appropriate therapeutic strategies, including the management of late complications/sequelae in patients with a more aggressive clinical course, present a significant challenge for the physician treating these conditions.

Definition and aetiology of ulcerative colitis and Crohn's disease

Ulcerative colitis and CD are chronic inflammatory conditions of the gastro-intestinal tract. They are of unclear aetiology, and most commonly follow an unpredictable, variable relapsing course. The conditions seem to result from a combination of environmental, genetic and immunological factors leading to dysfunctional regulation in the immune system with overly aggressive T-cell responses to commensal enteric bacteria in genetically susceptible individuals[1-3]. Furthermore, defective mucosal barrier function appears to be involved in the pathogenesis[4]. The complement system seems also to be involved in the inflammatory processes in UC and CD[5].

Management of UC and CD commonly involves medical treatment strategies as first-line therapy. The therapeutic arsenal includes glucocorticosteroids (GCSs), 5-aminosalicylic acid (5-ASA) or mesalazine, thiopurines, methotrexate, calcineurin inhibitors, and monoclonal antibodies against tumour necrosis factor alpha (anti-TNF-α). The choice of treatment is based on assessment of diagnosis, extent, severity and short and long-term prognosis[6].

Disease landmarks in ulcerative colitis and Crohn's disease

The clinical course of active UC and CD varies greatly between patients. Disease severity can range from relatively mild gastrointestinal symptoms to more severe disease with frequent hospital admissions and complications including the need for surgical intervention. Patients with UC or CD in general experience impaired health-related quality of life (HRQoL) compared with healthy controls[7].

Ulcerative colitis

The current incidence rate for UC is 8–14/100 000. The prevalence is estimated to be 120–200/100 000[8]. In UC the inflammation and ulcerations are continuous, involving the mucosa and submucosa in the rectum and colon to a varying extent[8].

In assessing the clinical course and pharmacological response, mainly in research, various disease activity indices are used. Among the most widely used are the Truelove-Witts criteria, the simple clinical colitis activity index (SCCAI) and the Mayo score, all of which are based on combinations of clinical signs, laboratory tests and/or endoscopic appearance[9-11].

In both the short and the long-term perspective the prognosis in UC is difficult to predict because of its variable disease course. Young age at diagnosis and female gender have been shown to be associated with more frequent relapses[12]. It has been reported that approximately

25% of all patients with UC suffer from at least one severe attack of the disease[13]. In the same reports, of those with a severe attack of UC, 20% were colectomized at first admission and 40% after two admissions. Risk for colectomy was correlated to the severity of disease on admission[13]. Male gender has in some cohorts been demonstrated to imply higher risk for colectomy[14]. The overall cumulative colectomy rate 10 years post-diagnosis has in recent studies been shown to be 3–17%, with extensive colitis at diagnosis remaining a risk factor associated with colectomy[14,15]. There is a consistent finding that the colectomy rate is highest during the first few years following diagnosis, which emphasizes the importance of early appropriate medical treatment in UC[16].

An acute severe attack of UC, if not consistently handled, is a potentially lethal condition. Intravenous GCSs are sufficiently effective in approximately 40% and partly effective in another 30% of cases[17]. In cases of unresponsiveness to intravenous GCSs, escalating medical therapy, commonly with anti-TNF-α or calcineurin inhibitors, or total colectomy is considered[18]. The therapy has been shown to decrease the short-term colectomy rate from 30–70% to 10–20%[19]. At an early stage of acute severe UC, it is essential to identify patients at risk of not responding to intravenous GCSs and to optimize the timing of intensified medical therapy.

Crohn's disease

The current incidence rate of CD is 6–15/100 000 and the prevalence is estimated to be 50–200/100 000[8]. A common feature of most cases of CD is a segmental, transmural and granulomatous type of inflammation that may be located anywhere in the gastro-intestinal tract from mouth to anus. Most often the distal ileum and colon are affected[8]. A distinctive feature of CD, which distinguishes it from UC, is that it may exhibit a recurrent stricturing and/or fistula-forming phenotype, which is the common cause of need for repeated surgery.

Prognostic markers

It is desirable to make a reliable prediction of the expected disease course and prognosis for each individual patient as early as possible at onset of the disease so as to optimize treatment. It is therefore of great value to have prognostic markers for this purpose and different potential prognostic markers have been investigated and proposed[20]. Several serologic and genetic markers have been associated with UC and CD and have been investigated for both diagnostic and prognostic purposes[21,22].

Elevated faecal calprotectin levels have been shown to be significantly correlated with the need for colectomy in patients with an acute attack of UC[23]. Furthermore, different cytokines have been tested as potential predictive markers for the outcome of biological therapies in UC patients[24]. Examples of clinically oriented prognostic markers in acute UC are stool frequency on day 3 of intensive treatment and change in stool frequency as predictive for colectomy[25,26]. Severity of disease on admission, assessed with Truelove -Witts criteria, has been demonstrated to be correlated to risk of colectomy[13]. Radiological and endoscopic criteria predicting colectomy have also been suggested[20].

Complementary treatments

There is a large and growing interest in complementary and alternative treatment options among patients with chronic conditions in general[27,28] and in patients with UC and CD in particular[29,30]. Complementary and alternative medicine has been defined as treatment methods existing alongside the science-based medicine taught at medical schools[31]. A more accurate term, "unconventional therapeutic methods", has been suggested[27], but the terms "complementary" and "alternative medicine" are more widely used.

Since the established, conventional therapeutic methods for UC have insufficient effect in a considerable number of patients there is a need for complementary treatment options. In one study investigating the use of alternative medicine in patients with UC and CD, exercise, prayer, counselling, massage, chiropractic and relaxation were found to be the most frequently used methods[29]. The use of complementary and alternative medicine in patients with UC or CD has been shown to correlate with duration of disease and disease-related concerns, such as feeling out of control, being treated as different, and having surgery[32].

Two unconventional therapeutic methods, hyperbaric oxygen therapy (HBOT) and supplementary treatment with specially processed cereals (SPCs), have been investigated in this thesis (Studies II and III).

Hyperbaric oxygen therapy

Hyperbaric oxygen therapy (HBOT) comprises intermittent inhalation of 100% oxygen in a hyperbaric chamber at pressures two to three times greater than atmospheric. It is clinically used for conditions such as decompression sickness, arterial gas embolism, late radiation injury, non-healing skin ulcers, carbon monoxide poisoning and smoke inhalation[33].

The biochemical and physiological effects of HBOT are not fully understood. Even if some effects are observed in humans, most studies reported have been performed on animal models. Hyperbaric oxygen therapy implies a contradictory tissue reaction at the molecular level, which means that both tissue non-favourable events (reactive oxygen and nitrogen species), and tissue-reparative processes occur by modification of the anti-inflammatory cascade system via augmentation of anti-inflammatory cytokines, hormones and stem cell stimulation. However, through the natural process of homeostasis the body disposes of reactive species via antioxidant and redox mechanisms such as the glutathione and thioredoxin systems. The redox systems have an inbuilt over-capacity and have experimentally been shown to be strongly increased during HBOT[34]. In active inflammation in UC and CD the epithelial barrier function is affected by reduced blood flow and metabolic changes, with resultant tissue hypoxia[35,36]. Based on these circumstances and the observed effects of healing wounds, HBOT has been successfully used for severe treatment-refractory perianal lesions in CD[37]. Furthermore, positive effects have been demonstrated in UC both in humans and in animal colitis models with observed decreased levels of proinflammatory cytokines, lowered markers of oxidative stress and decreased nitric oxide and nitric oxide synthase levels[38].

Specially processed cereals

Despite development of improved medical treatment options, there is still a considerable percentage of patients with UC or CD requiring surgery. "Short bowel syndrome (SBS)" refers to the condition when extensive resection of the intestinal tract results in maldigestion, malabsorption and malnutrition[39]. Among adults with SBS, CD (with repeated surgical procedures)

is one of the most common underlying diagnoses[40]. The clinical manifestations and metabolic consequences in SBS are variable and depend on the site and extent of the resection and the length and condition of the remaining small bowel[39]. Patients who have undergone intestinal resections often experience impaired HRQoL caused by high excessive intestinal fluids/loose stools or abdominal pain/discomfort despite conventional pharmacological treatment. The condition causes considerable costs for patients and society.

Antisecretory factor (AF) is a protein that is present in most body tissues, which has antisecretory and anti-inflammatory properties[41,42]. The native AF protein undergoes stepwise enzymatic cleavage to short peptides, its biological active form. Hydrothermally processed cereals, SPCs with an optimal concentration of specific amino acids and oligosaccharides, induce endogenous AF production and have been shown to have antisecretory effects in patients with IBD[43,44]. A certain length of small intestine and/or a certain passage time for SPCs is necessary for the AF-inducing effect[45].

AIMS

The overall aims of the thesis were to investigate markers and complementary treatment in inflammatory bowel diseases.

Specific objectives

1. To evaluate possible prognostic markers in a severe attack UC

2. To evaluate HBOT as complementary treatment in a severe acute attack of UC

3. To evaluate complementary treatment with specially processed cereals in patients with sequelae after surgery for IBD.

PATIENTS AND METHODS

Ethics

The included studies were approved by the Ethics Committee at the University of Gothenburg. All patients gave oral and written consent before inclusion.

Patients

Studies I and II: Eligible patients were men and women aged ≥17 years consecutively admitted to Sahlgrenska University Hospital/Östra Hospital, Gothenburg, Sweden, from January 2008 until March 2011 with a severe attack (Mayo score >10) of previously diagnosed UC or newly manifested clinical signs of extensive or left-sided UC according to routine clinical criteria. All enrolled patients had negative faecal cultures. The exclusion criteria were presence of radiological signs of threatening colonic perforation, ongoing or planned pregnancy, symptomatic cardiovascular or pulmonary disease, bleomycin treatment within 2 months prior to study start, epilepsy, dysfunction of the tuba auditiva, psychiatric disease (claustrophobia, anxiety or psychotic disorders, drug or alcohol abuse) and ongoing systemic anti-inflammatory drugs (GCSs, thiopurines or anti-TNF-α). In case of previously diagnosed UC, 5-ASA intake was allowed. Eighteen patients were included. The cohort were observed until colectomy or 31 December 2012.

Study III: Participants were consecutively scheduled outpatients with different underlying diagnoses, mainly CD or UC, who had undergone intestinal resections without necessarily meeting the conventional criteria of SBS or intestinal failure. The patients were postoperatively suffering from long-standing excessive intestinal fluids/loose stools or abdominal pain/discomfort with a negative impact on HRQoL despite pharmacological treatment. Exclusion criteria were clinical signs of active intestinal inflammation, bowel obstruction and high risk of surgical intervention. Thirty-four patients were eligible for randomization from January 2005 until June 2007, and 26 patients were followed per protocol and thereby included in the analysis (Figure 1).
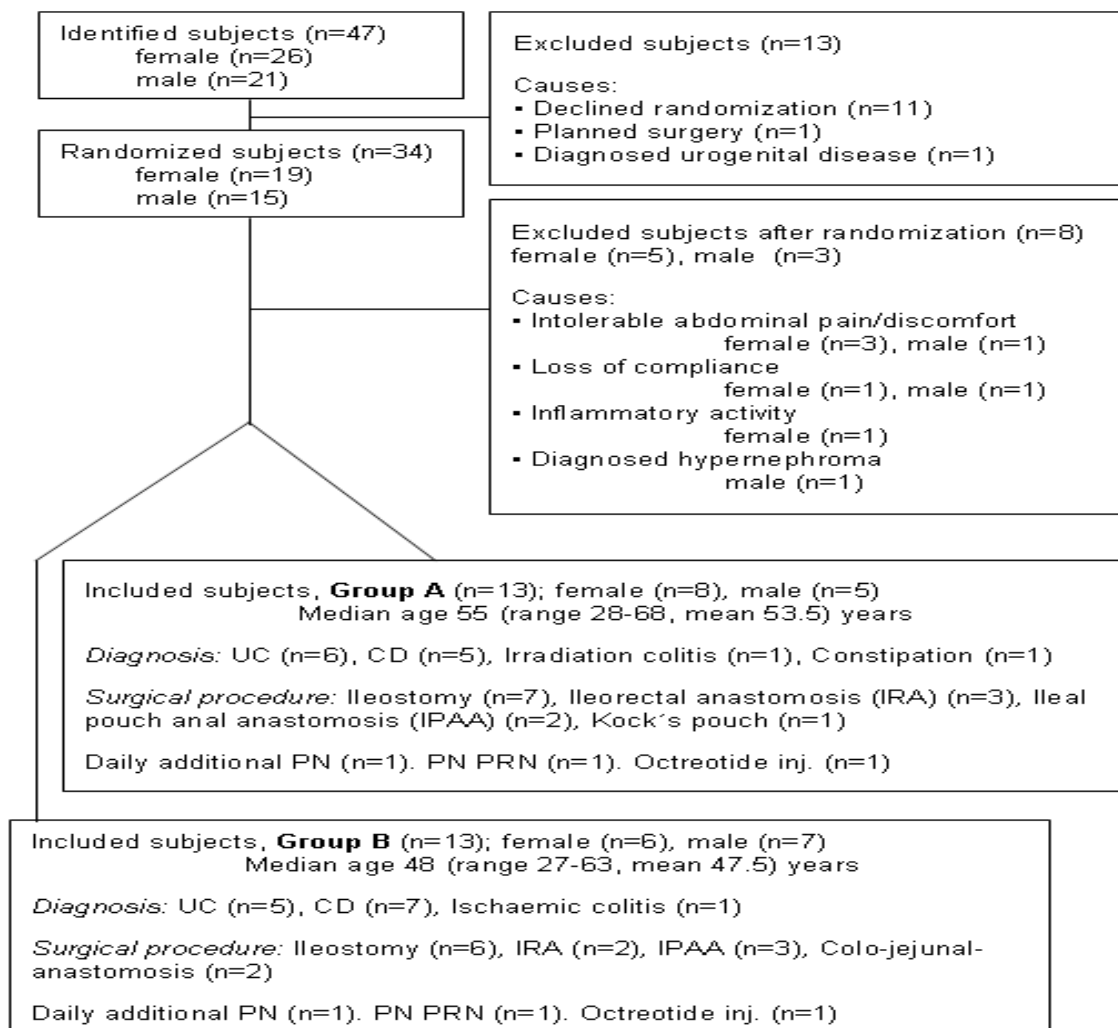
Figure 1. Flow chart of patients who met the inclusion criteria for Study III. Details with regard to gender, diagnosis, surgical procedure, use of parenteral nutrition (PN) when necessary (PRN) and hormonal treatment are given.

Study design

In Studies I and II we studied the same 18 patients admitted to Sahlgrenska University Hospital/Östra Hospital with an acute severe attack of UC.

Study I: A prospective, observational, explorative study. All blood samples were collected at admission. The patients registered their bowel movements and faecal weight during the first 24 hours of their hospital stay. After discharge from hospital, patients were followed up at our gastroenterological outpatient clinic for up to 58 months and allocated to one of two groups depending on the clinical outcome regarding colectomy. The observation time for the patients was determined by the time of inclusion in the study and the clinical outcome, i.e. colectomy/no colectomy.

The objective was at admission to hospital (day 1) to evaluate faecal weight and bowel movements and peripheral blood tests as predictive markers of the need for colectomy in a

severe attack of UC. The following tests were performed: blood haemoglobin (B-Hb), blood platelet count, serum C-reactive protein (S-CRP), serum albumin (S-alb), erythrocyte sedimentation rate (ESR), serum orosomucoid (S-Oro), serum haptoglobin (S-HPT), plasma antisecretory factor (P-AF), P-complement component 3 (P-C3c), interleukin (IL)-1β, IL-6, IL-8, IL-10 and TNF-α) In addition, appearance and distribution of AF in mucosal biopsies were evaluated for correlation with P-AF.

Study II: A prospective, randomized, open-labelled, and interventional study. The patients were randomized to one of two study groups (HBOT and non-HBOT) using a web-based randomizing tool (www.randomizer.org). The control group (non-HBOT) were given the standard intensive UC treatment with intravenous GCSs. Patients allocated to the intervention group (HBOT) were given the same treatment with the addition of HBOT.

The primary objective was to improve the clinical outcome, evaluated using Mayo score, laboratory tests and faecal weight. The secondary objectives were improvement in HRQoL, avoidance of colectomy and evaluation of HBOT safety.

Study III: A prospective, randomized, multi-centre, interventional, double-blind, cross-over outpatient study of 26 patients who had undergone intestinal resections. Patient recruitment was performed in eight Swedish outpatient centres. Each treatment arm consisted of two treatment periods of 12 weeks with a wash-out period of 6 weeks in between (Figure 2).
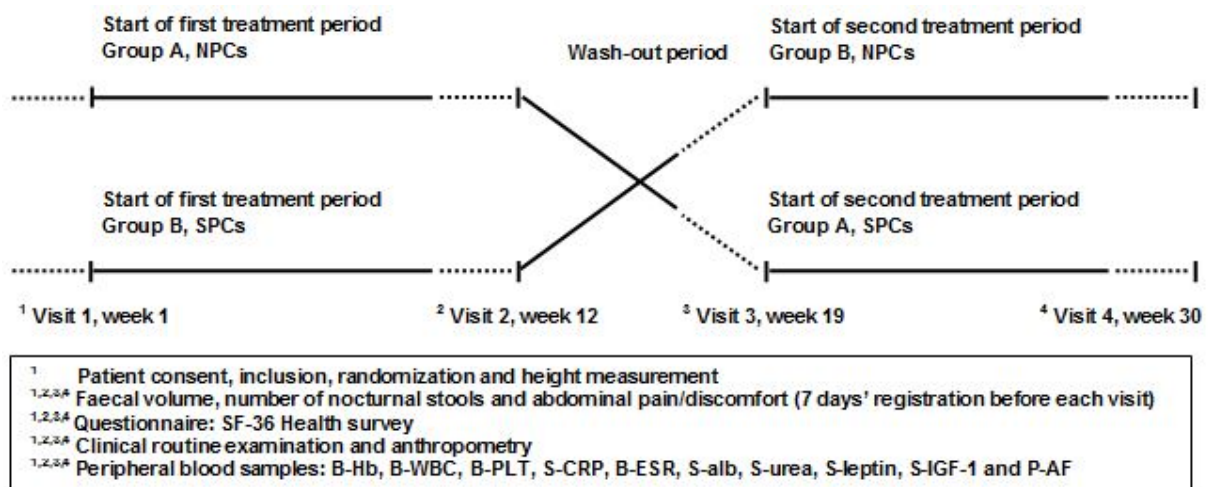

Figure 2. Presentation of the study design. Dashed lines indicate the periods of faecal volume registrations (7 days) preceding each visit. B-ESR; blood erythrocyte sedimentation rate, B-Hb; blood haemoglobin, B-PLT; blood platelet count, B-WBC; white blood cell count, NPCs; non-processed cereals, P-AF; plasma antisecretory factor, S-alb; serum albumin, S-CRP; serum C-reactive protein, SF-36; short form 36 Health Survey, S-IGF-1; serum insulin-like growth factor-1, SPCs; specially processed cereals.

The aim was to evaluate the effect of a supplementary diet with SPCs compared with non-processed cereals (NPCs) with respect to faecal volume, number of nocturnal stools, abdominal pain/discomfort, laboratory parameters, anthropometry and HRQoL. Moreover, the importance of background diagnoses and type of surgical procedure was evaluated.

Study procedure

Study I and II (common procedure): After collection of blood samples at inclusion, all patients underwent colonoscopy with biopsy to ensure the diagnosis visually as well as histologically, but also to define the disease extent. Colonoscopy images were provided.

All patients were given intensive UC treatment with intravenous GCSs (betamethasone 4 mg twice daily (prednisolone equivalent 67 mg)), oral mesalazine (1 200 mg twice daily on days 1–5 and thereafter 2 400 mg twice daily), suppository prednisolone (20 mg once daily) and prednisolone enema (37.5 mg once daily). The length of the initial hospital stay was not pre-defined but varied depending on each patient's state. All responding patients were in a stable clinical condition on discharge from hospital.

Study I: After discharge from the initial hospital stay the patients were followed at our outpatient clinic as described above. Decisions concerning the medical treatment or colectomy were made on clinical grounds.

Study II: With few exceptions, HBOT was started in the HBOT group on day 2 after inclusion at 2.4 atmospheres (ATM) for 90 min/session, 5 days/week, for 6 consecutive weeks (a total of 30 HBOT sessions) in a multiplace hyperbaric chamber (GDA Sverige AB, Gothenburg, Sweden).

During the first 7 days of the initial hospitalization we used an activity score, the Patients' Medical Safety Score (PMSS) for clinical evaluation of the disease course. The Patients' Medical Safety Score is described in detail below. Where patients deteriorated or showed no clinical improvement (i.e. in case of treatment failure) after day 7, the patients were withdrawn based on an overall clinical evaluation. Patients who attained a decrease in the Mayo score of ≥ 3 compared with baseline were classified as clinical responders, and were switched to tapering doses of prednisolone for 7 weeks with an initial daily dose of 40 mg.

With reference to the secondary objectives, HRQoL forms were completed repeatedly during the study period. The patients were clinically monitored at the gastroenterological outpatient clinic at Sahlgrenska University Hospital/Östra Hospital for 180 days, as shown in Table 1. Beyond 180 days, only the colectomy rate was registered.

Day	1	3	5	7	14	45	90	180
Faecal cultivation, U-HCG (women)	x							
B-Hb, B-TPK, S-CRP, Mayo score	x	x	x	x	x	x	x	x
S-AST, S-ALT, S-Alb, S-creatinine, S-electrophoresis	x			x		x	x	x
B-ESR	x		x	x	x	x	x	x
Faecal calprotectin	x			x				x
PMSS	x	x	x	x				
SF-36, IBDQ	x			x	x	x	x	x
Colonoscopy	x							x

Table 1. Time schedule of peripheral blood tests, urinary test and faecal samples. B-ESR; blood erythrocyte sedimentation rate, B-Hb; blood haemoglobin, B-TPK; blood platelet count, IBDQ; Inflammatory Bowel Disease Questionnaire, PMSS; Patients' Medical Safety Score, S-ALT; serum alanine transaminase, S-AST; serum aspartate transaminase, S-Alb; serum albumin, S-CRP; serum C-reactive protein, SF-36; short form 36 Health Survey, U-HCG; urinary human chorionic gonadotropin.

Study III: At inclusion, patients were randomized to a supplementary diet of untreated oat flakes (AXA havregryn[®]; Lantmännen, Järna, Sweden), in this study identified as NPCs (group A), or to SPCs (SPC flakes[®]; AS-Faktor Ltd, Stockholm, Sweden) (group B) at a daily dose of 0.5–1 g/kg body weight (bw)/24 h, divided into three equal doses (Figure 2). After the wash-out period the patients received the opposite supplementary regimen during the final 12 weeks of the study. Where a patient experienced gut-related side effects, the dose was lowered, with the lowest accepted dose being 0.5 g/kg bw/24 h. In accordance with the protocol, all cereals were packed and posted to the patients in unmarked bags by AS-Faktor Ltd.

Four weeks after visits 1 and 3, respectively, four to six telephone calls were made to each patient to evaluate their compliance with the prescribed doses of cereals. In addition, the patients were asked to state the remaining amount of cereals at visits 2 and 4 (end of each treatment period). This was done in order to check that the prescribed dose had been consumed. Seven days before each visit, all patients completed a daily registration form concerning faecal volume, number of nocturnal stools and perceived abdominal complaints (Figure 2).

Methods

Faecal weight and number of stools

Studies I and II: The patients recorded their daily number of stools and the weight of each stool using a toilet-mounted faecal collector (Plasti-Pan II®, Plasti-Products, Omaha, NE, USA) and an electronic scale (Philips® HR2391, tolerance ±1 g) during the hospital stay.

Study III: Faecal data were recorded using a toilet-mounted faecal collector graded in milli-litres (Specipan®, Kendall, Gosport, Hampshire, UK). Measurements were taken from 06.00 a.m. every 24-hour period during 7 consecutive days and finished on the days of visits to the clinic. Nocturnal stools were defined as bowel movements occurring between 22.00 p.m. and 06.00 a.m.

Anthropometric measurements

Study III: Anthropometric measurements of weight, skin fold thickness (triceps, biceps, sub-scapular and suprailiac) and arm circumference were performed and adjusted to correction tables for sex, age and fat density[46]. Body mass index, calculated from weight in kilograms divided by the square of height in metres, and fat-free mass (in kilograms) were calculated.

Biochemical assays

Studies I–III: All peripheral blood tests (B-Hb, blood platelet count, S-CRP, S-alb, ESR, S-Oro, S-HPT, serum alpha 1 antitrypsin (S-α1-AT), insulin-like growth factor-1 and leptin) and faecal cultures were analysed routinely at the central laboratory facility at Sahlgrenska University Hospital (ISO 15189) (Table 1, Figure 2).

Study I: Blood samples for analysis of cytokines, C3c and AF were collected in citrate tubes, immediately centrifuged and stored at minus 70°C for later measurement.

The level of C3c in the affinity-purified human plasma samples was determined by an en-zyme-linked immunosorbent assay (ELISA) method using rabbit anti-C3c polyclonal anti-body (Dako, Glostrup, Denmark) as the detecting antibody[47]. A second antibody, of goat anti-rabbit immunoglobulin-alkaline phosphatase conjugate (Jackson ImmunoResearch Europe Ltd, Suffolk, UK), was then applied, followed by reading absorbance at 405 nm to reveal the bound enzyme.

The plasma AF detection was performed by conventional ELISA. Microtitre plates were coated with agarose-column, affinity-purified plasma. Unbound sites were blocked by adding bovine serum albumin (Sigma AB, Stockholm, Sweden), followed by incubation with mono-clonal AF antibody (diluted 1/50). The binding of the AF antibody to the plasma samples was spectrophotometrically measured (405 nm) (Emax Molecular Devices, Sunnyvale, CA, USA) using an alkaline phosphatase-labelled anti-mouse immunoglobulin as secondary reagent (Jackson Laboratory, Bar Harbor, ME, USA) followed by addition of 5-bromo-4-chloro-3-indolyl phosphate solution and 4-nitro blue tetrazolium substrate (Roche Diagnostics, Basel, Switzerland)[48].

Measurements of IL-1β, IL-6, IL-8, IL-10 and TNF-α were performed using Luminex tech-nology on a Bioplex Suspension Array System (Bio-Rad Laboratories, Hercules, CA, USA) with a Milliplex Map kit (Millipore, Billerica, MA, USA).

Endoscopy

Studies I and II: During the first working day after admission colonoscopy was performed after bowel preparation using Klyx® 120 ml x 2. The colonoscopy was done with biopsy (routine histology and immunohistochemical detection and distribution of mucosal AF) to ensure the diagnosis visually as well as histologically and define the inflammatory severity and extent. A second colonoscopy was performed on day 180, using Laxabon® preparation, 4 L, or at withdrawal from the study (using Klyx®, 120 ml x 2), in order to correlate endoscopic findings with the clinical outcome. The colonoscopies (all photo-documented) were performed by the same, experienced endoscopist.

Mayo score

Study II: Mayo score was calculated at inclusion and on day 3, 5, 7, 14, 45, 90 and 180 to follow disease activity over time (Table 1). The Mayo score is composed of four categories (stool frequency, rectal bleeding, endoscopic findings and the physician's global assessment) each rated 0–3, which are summed to give a total score ranging from 0 to 12[11]. At inclusion and on day 180 the patients underwent a complete colonoscopy, at the other time points of assessment, the endoscopic appearance regarding Mayo score was determined using recto/sigmoideoscopy technique.

Patients' Medical Safety Score

Study II: In order to assess the disease course and support the defined protocol management during the initial hospital stay in Study II, we constructed a PMSS, which has not previously been validated. At baseline, Mayo score, S-CRP, blood platelet count (B-TPK), blood erythrocyte sedimentation rate (B-ESR) and faecal calprotectin were registered. On day 3, PMSS was calculated by addition of 1 point each if an increase was registered in Mayo score (≥ 1 point), S-CRP (>10 mg/l) and B-TPK (>25%) compared with baseline values. On day 5, B-ESR was incorporated into the PMSS and 1 point added to the score if an increase of >5 mm/h was evident. On day 7, faecal calprotectin was added, with 1 point scored if there was an increase >25% compared with baseline. The decisions based on the PMSS were the same for all assessments (i.e. the assessments on days 3, 5 and 7). A change in PMSS of 0–1 points (compared with baseline) resulted in continued treatment according to the study protocol. A score of 2 points led to a temporary doubling of the initial dose of GCSs. A score of 3 points, or an overall general clinical deterioration, resulted in a mandatory withdrawal of the patient from the study.

Short Form 36 Health Survey

Studies II and III: The short form 36 Health Survey (SF-36) is a validated and widely used questionnaire for measurement of general HRQoL[49]. The study cohorts were compared with standardized Swedish community controls[50]. The SF-36 results were then calculated in accordance with the Swedish manual and interpretation guide[51]. The SF-36 results were presented in accordance with the SF-36 measurement model, as physical component summary (PCS) and mental component summary (MCS).

Inflammatory Bowel Disease Questionnaire

Study II: The Inflammatory Bowel Disease Questionnaire (IBDQ) is a standard instrument for assessment of HRQoL in adult patients with IBD. It consists of 32 items, each corresponding to a 7-point scale where 1 represents the worst and 7 represents the best condition. The

items are grouped into four domains: bowel symptoms (B, range 10–70), systemic symptoms (S, range 5–35), emotional function (E, range 12–84) and social function (SF, range 5–35)[52].

Registration of Abdominal Pain/Discomfort

Study III: During the 7-day period before entry into the study (visit 1) and again before visit 3, the patients recorded their bowel habits (index value) and completed a descriptive self-evaluation survey regarding abdominal complaints including the four options: (1) absence of abdominal discomfort; (2) mild, tolerable abdominal discomfort with insignificant impact on daily living; (3) moderate abdominal pain/discomfort, with certain effects on daily living; and (4) severe abdominal pain, resulting in major difficulties or hindrance in performing, or attending to, one's occupation or daily activities. The scores were then summed for the 7-day period, giving a summary score (range 7–28) for each patient. The described procedure was repeated during the last week of each 12-week period (before visits 2 and 4) of supplementary SPC or NPC intake.

Statistics

Studies I–III: The statistical analyses in Study I and II were performed in association with Professor Anders Odén at the Department of Mathematics, Chalmers Institute of Technology, University of Gothenburg. Descriptive statistics were used, describing the patients included in the studies. Because the sample sizes were small, non-parametric methods were used. Continuous variables are reported as medians and interquartile ranges. Two-tailed P-values <0.05 were considered statistically significant. StatView 5.0.1 (SAS Institute Inc., Cary, NC, USA) was used for statistical analyses as well as for graphing the data.

Study I: Two-tailed Mann-Whitney U-test was performed for comparison of the two groups regarding UC duration prior to inclusion, and age. Colectomy rates were calculated for the whole cohort using the Kaplan-Meier method. Fisher's permutation test[53] was used for comparison of the variables between the two groups. Multiple logistic regression model was performed to evaluate variability. A receiver-operating characteristic (ROC) curve[54] was constructed for number of stools predicting colectomy during the observation time.

Study II: The hypothesis when we designed the study was that Mayo score and inflammatory parameters including faecal calprotectin would be sensitive markers of clinical response to addition of HBOT to standardized treatment. A 15% reduction of each of these variables was assumed, with a 0.05 significance level and 90% power. Based on this assumption, the sample size was calculated to be two times twelve patients. An interim analysis, evaluating achievement of expected treatment effects and safety, was planned when at least half of the study population had completed the treatment period. The interim analysis was not taken into account in the pre-study statistical calculations. When comparing the patient groups we used a two-sided Fisher's permutation test (intention-to-treat analysis).

Study III: Wilcoxon's and Mann-Whitney's U-test were used for comparing the treatments in the study groups. All results are expressed as medians and the results of subgroup analyses are presented as box plots (giving medians, interquartile ranges (IQRs), and maximum and minimum values).

RESULTS

Study I

The characteristics of the 18 patients in the cohort, including observation time, time to response, remission, introduction of infliximab (IFX) and/or immunosuppressants (ISs) and time to colectomy, where appropriate, are presented in Table 2. No differences regarding sex, age and duration of UC was detected between the study groups at inclusion in the study.

Group	Patient No	Gender	Age at inclusion (years)	Extent of UC	Duration of UC at inclusion (months)	Observation time (months)	Response: During the initial hospital stay	Response: Not achieved	Remission: <3 months	Remission: At 6 months	Remission: >6 months	Remission: Not achieved	IFX	ISs	Time to colectomy (weeks)
Colectomy group	1	m	27	E	-	1		x					3	-	4
	2	m	30	E	67	58	x					x	-	136	252
	3	f	33	E	96	0.5		x					1	1	2
	4	f	41	E	145	13	x					x	4	-	57
	5	f	21	E	60	6	x		x				6	6	26
	6	f	35	E	9	6	x					x	1	23	26
	7	f	28	L	31	25	x					x	84	50	104
No-colectomy group	8	m	18	E	-	38	x		x				-	-	-
	9	f	39	E	137	58	x		x				32	45	-
	10	m	18	E	10	39	x		x				37	36	-
	11	m	41	L	-	35	x		x				-	138	-
	12	m	28	E	-	53	x				x		2	11	-
	13	m	49	E	38	58	x				x		59	2	-
	14	m	29	E	-	44	x		x				-	44	-
	15	m	24	E	69	37	x		x				-	-	-
	16	m	56	E	16	11	x			x			4	15	-
	17	f	35	E	0.5	46	x		x				-	-	-
	18	m	31	E	-	57	x		x				221	83	-

Table 2. Descriptive presentation of all 18 included patients. E; extensive, f; female, L; left-sided, m; male, IFX; infliximab, ISs; immunosuppressants.

Seven of the patients were colectomized during the study period (Figure 3). Two of them had urgent colectomy during the initial hospital stay after receiving rescue therapy with IFX. The remaining five underwent colectomy because of relapse or failure to achieve remission after discharge from the initial hospital stay. Compared with the no-colectomy group, the patients in the colectomy group consumed substantially more GCSs during the observation time. In

fact, measured as the median accumulative GCS dose, the no-colectomy group had a slightly higher accumulative dose compared with the colectomy group (3290 g and 3100 g respectively), which is explained by the longer follow-up period (non-censored data).
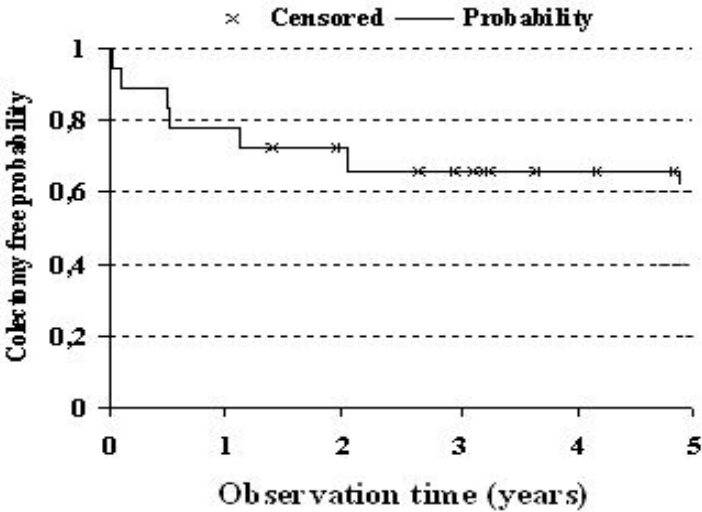

Figure 3. Kaplan-Meier plot showing probability for colectomy-free survival for all 18 patients during the observation time.

Results of the faecal volume and weight registrations and laboratory parameters are presented in Table 3. In the multiple regression analysis, increased bowel movements (p=0.01), faecal weight/bowel movements (p=0.03) and C3c levels (p=0.01) were shown to correlate with need for later colectomy (**bold** p-values). The discriminative value of faecal movements related to the colectomy event gave an area under ROC curve (AUROC) of 0.9091 (confidence interval (CI) 0.707−0.980) (not shown in the Table). None of the other studied parameters were statistically able to indicate need for colectomy. However, trends towards significance were obtained for S-CRP (p=0.19) and B-ESR (p=0.09).

	Colectomy group (n=7)			No-colectomy group (n=11)	
	Mean (95 % CI)	Median (IQR)	p-value	Mean (95 % CI)	Median (IQR)
Faecal weight [g/24 h]	559.1 (390.7)	607 (157–843)	0.12	277.1 (136.9)	203.5 (135–423)
Bowel movements [/24 h]	15.3 (8.5)	13.5 (12–22)	**0.01**	4.3 (3.5)	3.5 (1.5–4.5)
Faecal weight/bowel movement	38.3 (7.1)	30.6 (21.2–62.3)	**0.03**	82.2 (33.4)	78.9 (53.3–99.4)
B-Hb [g/L]	126.0 (18.1)	131 (115–136)	0.34	135.7 (9.3)	139 (118–145)
B-TPK [*10^9/L]	496.7 (184.8)	403 (329–641)	0.32	389.6 (103.1)	346 (267–452)
S-CRP [mg/L]	67.7 (57)	34 (9–130)	0.19	30.2 (23.0)	23 (0–42)
ESR [mm/h]	37.9 (22.7)	35 (15–58)	0.09	20.5 (6.4)	24 (11–27)
S-alb [g/L]	34.0 (5.1)	34 (31–39)	0.96	34.5 (3.1)	35 (33–37)
S-oro [g/L]	1.8 (0.4)	1.7 (1.3–2.1)	0.41	1.6 (0.3)	1.6 (1.3–1.9)
S-HPT [g/L]	2.6 (1.0)	2.4 (1.7–3.8)	0.71	2.8 (0.6)	2.8 (2.4–3.3)
S-a1-AT [g/L]	2.3 (0.6)	2.2 (1.9–2.8)	0.73	2.2 (0.5)	1.9 (1.6–2.9)
P-AF [abs 405 nm]	0.7 (0.4)	0.8 (0.2–0.8)	0.31	0.5 (0.3)	0.4 (0.3–0.5)
P-C3c [abs 405nm]	1.5 (0.2)	1.5 (1.2–1.6)	**0.01**	1.1 (0.2)	1.1 (0.8–1.3)
p-IL-1β [pg/mL]	4.7 (3.5)	2.2 (2–6.3)	0.48	3.7 (1.4)	3.1 (2–5.5)
p-IL-6 [pg/mL]	6.2 (7.0)	1.7 (1.1–10.7)	0.87	5.6 (6.7)	3.0 (2.4–3.5)
p-IL-8 [pg/mL]	17.9 (22.8)	9.2 (4.1–17.6)	0.67	23.9 (19.7)	10.7 (8.8–19)
p-IL10 [pg/mL]	9.0 (9.5)	3.4 (2.9–10.1)	0.77	8.0 (2.8)	7.1 (4.7–10)
p-TNF-α [pg/mL]	6.9 (3.8)	5.1 (3.3–10.3)	0.45	9.1 (4.4)	7.4 (7–9.2)

Table 3. Presentation of faecal weight registrations and peripheral blood tests including p-values for each tested parameter in the two study groups (Study I) (n=18). Abs; absorbance, B-Hb; blood hae-moglobin, B-TPK; blood platelet count, CI; confidence interval, ESR; blood erythrocyte sedimentation rate, IQR; interquartile range, P-AF; plasma antisecretory factor, P-C3c; plasma complement com-ponent 3, P-IL; plasma interleukin, P-TNF-α; plasma tumour necrosis factor alpha, S-Alb; serum albumin, S-α1-AT; serum alpha 1 antitrypsin, S-CRP; serum C-reactive protein, S-HPT; serum hap-toglobin, S-Oro; serum orosomucoid.

The immunohistochemical analyses regarding AF appearance in mucosal biopsies retrieved at colonoscopy showed no differences in amount or distribution between the two patient groups.

Study II

Ten of the 18 included patients were randomized to the HBOT group. Patient characteristics and colectomy rate are shown in Figure 4. There were no significant differences between the treatment groups at baseline regarding age, gender or disease characteristics.
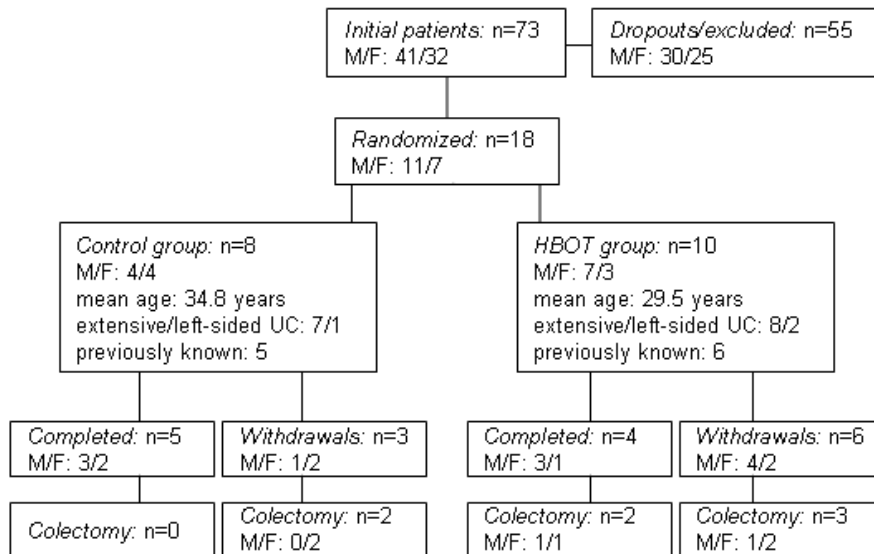
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┌─────────────────────────┐      ┌─────────────────────────┐
│ Initial patients: n=73  │      │ Dropouts/excluded: n=55 │
│ M/F: 41/32              │──────│ M/F: 30/25              │
└─────────────────────────┘      └─────────────────────────┘

        ┌─────────────────────────┐
        │ Randomized: n=18        │
        │ M/F: 11/7               │
        └─────────────────────────┘

┌────────────────────────────────┐      ┌────────────────────────────────┐
│ Control group: n=8             │      │ HBOT group: n=10               │
│ M/F: 4/4                       │      │ M/F: 7/3                       │
│ mean age: 34.8 years           │      │ mean age: 29.5 years           │
│ extensive/left-sided UC: 7/1   │      │ extensive/left-sided UC: 8/2   │
│ previously known: 5            │      │ previously known: 6            │
└────────────────────────────────┘      └────────────────────────────────┘

┌──────────────────┐ ┌──────────────────┐  ┌──────────────────┐ ┌──────────────────┐
│ Completed: n=5   │ │ Withdrawals: n=3 │  │ Completed: n=4   │ │ Withdrawals: n=6 │
│ M/F: 3/2         │ │ M/F: 1/2         │  │ M/F: 3/1         │ │ M/F: 4/2         │
└──────────────────┘ └──────────────────┘  └──────────────────┘ └──────────────────┘

┌──────────────────┐ ┌──────────────────┐  ┌──────────────────┐ ┌──────────────────┐
│ Colectomy: n=0   │ │ Colectomy: n=2   │  │ Colectomy: n=2   │ │ Colectomy: n=3   │
│                  │ │ M/F: 0/2         │  │ M/F: 1/1         │ │ M/F: 1/2         │
└──────────────────┘ └──────────────────┘  └──────────────────┘ └──────────────────┘
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Figure 4. Flow chart illustrating the study progress, patient demographics, disease characteristics and colectomy rate. M/F = male/female.

Nine patients (six receiving HBOT and three with conventional treatment only) were withdrawn prematurely from the study between days 1 and 46. Of these, two patients were classified as early dropouts, one because of perceived claustrophobia during the first HBOT session, and another because of an inability to normalize the middle ear pressure, which became evident after two HBOT sessions. The remaining seven withdrawals were due to deterioration of the clinical state.

The results showed no significant differences in the primary objectives concerning clinical improvement in terms of either Mayo score (median value 11 at inclusion in both groups, median value 3 in the control group and median value 0.5 in the HBOT group on day 180), peripheral laboratory tests or faecal calprotectin. Faecal weight was registered during the hospital stay for a maximum of 10 days. The results from days 1 and 5 are illustrated in Figure 5. No significant differences were found between the two treatment groups. Since the number of inpatients declined quickly after 5 days no further comparisons regarding faecal weight were performed beyond this time point.
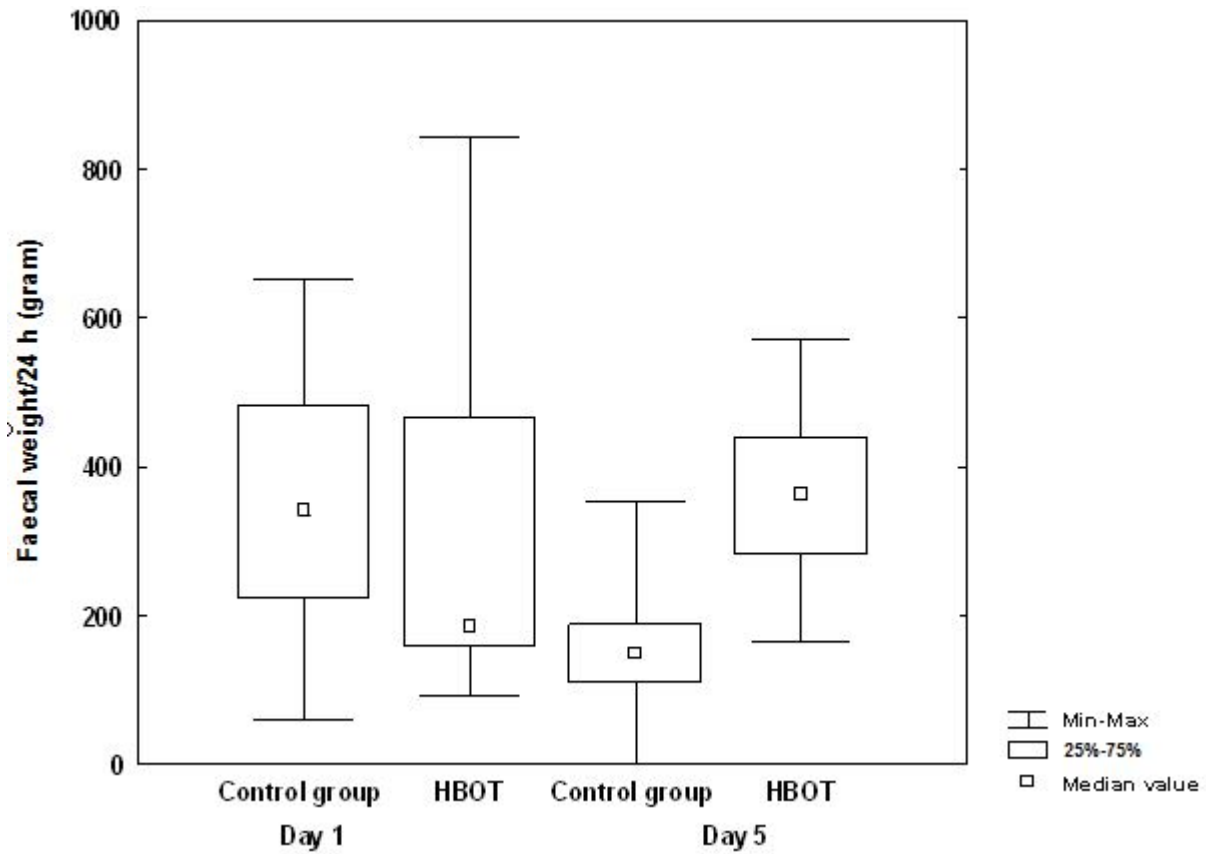
Figure 5. Box-plot illustration of the faecal weight in the treatment groups on day 1 and 5.

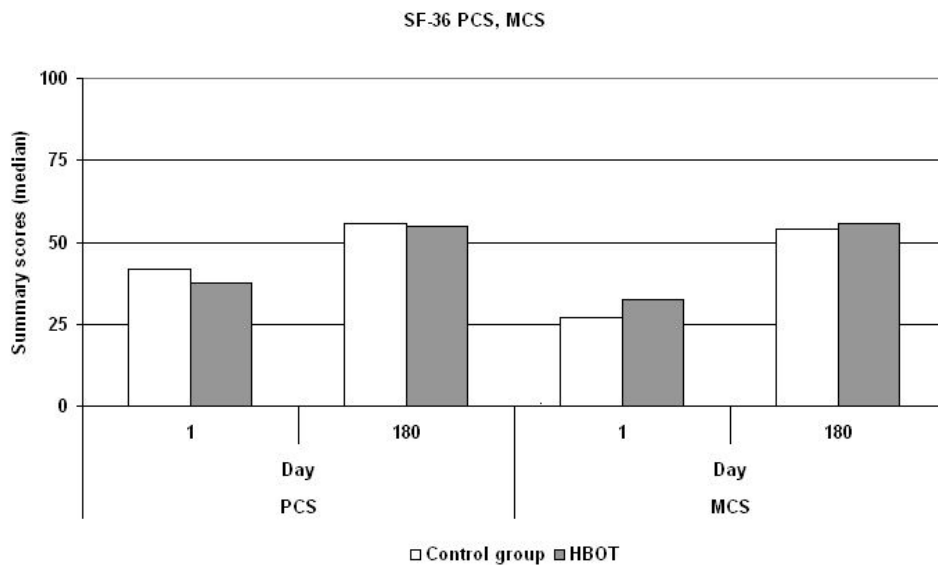Neither SF-36 (Figure 6) nor IBDQ (Table 4) showed any significant differences between the treatment groups.

Figure 6. Short form 36 Health Survey (SF-36) results (median values) at baseline (day 1) and on day 180. The results are presented in accordance with the SF-36 measurement model, as physical component summary (PCS) and mental component summary (MCS).

IBDQ

	Domain	Day 1	Day 180
Control	B (bowel symptoms)	35.5	65
HBOT		*33.5*	*60.5*
Control	S (systemic symptoms)	15	33
HBOT		*12*	*32*
Control	E (emotional symptoms)	47	80
HBOT		*43*	*75.5*
Control	SF (social function)	15	35
HBOT		*14*	*35*

Table 4. Inflammatory Bowel Disease Questionnaire (IBDQ) results (median values) at baseline and on day 180, grouped into the four domains: bowel symptoms (B, range 10–70), systemic symptoms (S, range 5–35), emotional function (E, range 12–84) and social function (SF, range 5–35).

Seven patients (five in the HBOT group and two in the control group) underwent colectomy (Figure 4), in two cases due to a severe medical deterioration during the hospital stay. Otherwise, no unexpected adverse events of HBOT were observed in this study.

Study III

The main underlying diagnoses in the study cohort were UC (n=11) and CD (n=12). The other diagnoses were irradiation colitis (n=1), constipation (n=1) and ischaemic colitis (n=1) (Figure 1). There were no significant differences in either gender or age between group A and B (p=0.18 and p=0.13, respectively). All patients with a history of UC had ileorectal anastomosis (IRA) or ileal pouch anal anastomosis (IPAA) and all CD patients underwent ileostomy for faecal deviation, with the exception of one UC patient (group A) who had an ileostomy and one CD patient (group A) who had an IRA.

Faecal volume was significantly decreased after NPC treatment in groups A and B. Neither the number of nocturnal stools nor the abdominal pain/discomfort score was significantly altered in any diet period (Table 5). In group A, plasma AF levels (P-AF) were significantly elevated during NPC treatment (Table 5).

Group A	NPCs		p	SPCs		p
visit	1	2		3	4	
Faecal volume (mL/24 h)	1,264 (1,534)	929 (680)	0.04	1,307 (1,102)	1,385 (852)	0.58
Number of nocturnal stools	1.8 (1.36)	1.6 (1.57)	1.0	1.6 (1.36)	1.9 (0.93)	0.55
Accumulated abdominal pain/discomfort score	147	133	0.29	161	146	0.75
P-antisecretory factor (absorbance at 405 nm)	502 (522)	507 (709)	0.03	552 (604)	618 (691)	0.36
Group B	SPCs		p	NPCs		p
visit	1	2		3	4	
Faecal volume (mL/24 h)	1,307 (1,142)	1,386 (1,138)	1.0	1,450 (1,187)	1,171 (882)	0.02
Number of nocturnal stools	1.4 (2.89)	1.9 (1.93)	0.34	2.0 (1.96)	1.7 (2.0)	0.55
Accumulated abdominal pain/discomfort score	162	164	1.0	163	170	0.45
P-antisecretory factor (absorbance at 405 nm)	264 (438)	284 (519)	0.28	292 (460)	278 (439)	0.77

*Table 5. Faecal volume, number of nocturnal stools/24 h and accumulated abdominal complaint score. All values given as **medians** (means). NPCs; non-processed cereals, SPCs; specially processed cereals.*

The other peripheral blood tests and anthropometric measurements were not significantly affected between the start and the end of any treatment period.

At baseline, the study cohort reported decreased HRQoL in the SF-36 health survey compared with the reference population. None of the two summary scores PCS or MCS showed any statistical difference between groups A and B at the start of the study or within each group between the start and the end of each treatment period.

Subgroup analyses of the underlying UC and CD diagnoses showed that CD patients in general had greater median faecal volume/24 h (Figure 7). In group A, irrespective of the regimen, no significant differences were observed between the start and the end of each treatment period. The UC patients in group B had a significantly decreased faecal volume during the NPC intake period (Figure 7).
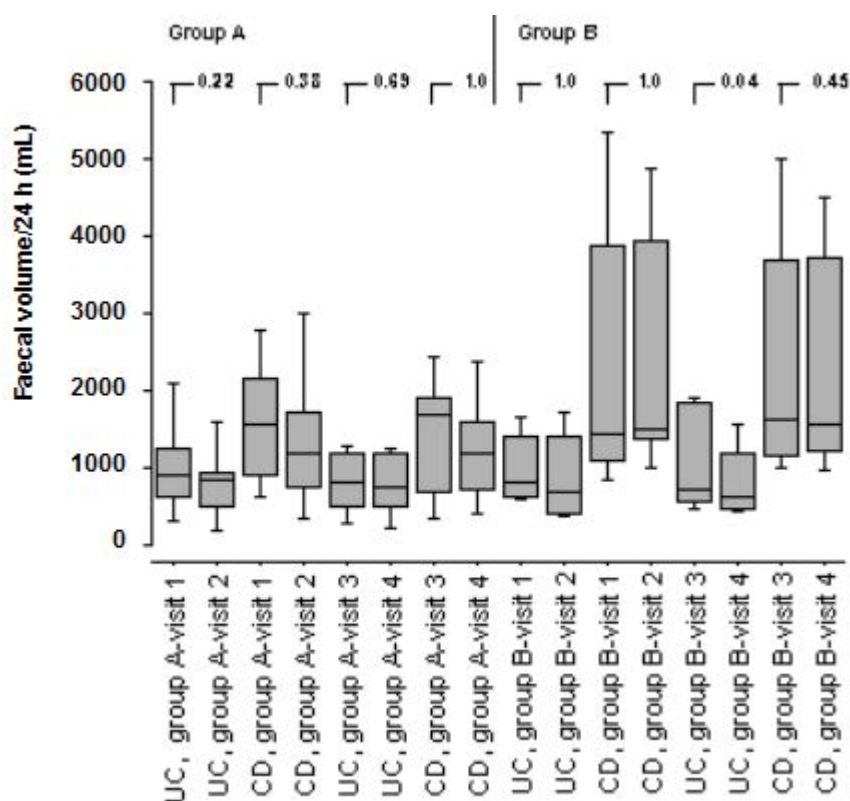
Figure 7. Box-plot presentation of a subgroup analysis referring to underlying diagnoses, UC compared with CD. Left panel: Group A: UC: n=6, CD: n=5. Right panel: Group B: UC: n=5, CD: n=7. Median faecal volume/24 h is compared between the start and end of each dietary treatment period. Visits 1 and 3 represent the start of each dietary treatment period and visits 2 and 4 represent the end of each dietary treatment period. Group A were given non-processed cereals (NPCs) between visits 1 and 2 and specially processed cereals (SPCs) during the latter period, while group B were given SPCs in the first period followed by NPCs during the second period. P-values are given.

No significant changes in number of nocturnal bowel movements were registered, irrespective of the regimen administered. The accumulated self-estimated abdominal pain/discomfort score during the 7-day registration period of UC and CD patients showed no statistically significant differences within groups A and B, irrespective of the underlying diagnosis, treatment or time registered.

Dividing the cohort into ileostomy and "other surgery" showed that ileostomy patients in general had higher daily loss of intestinal contents (Figure 8). In group A, a significant decrease in daily faecal volume was observed in the "other surgery" patients during NPC treatment only. In group B, neither SPCs nor NPCs significantly diminished the intestinal fluid (Figure 8).
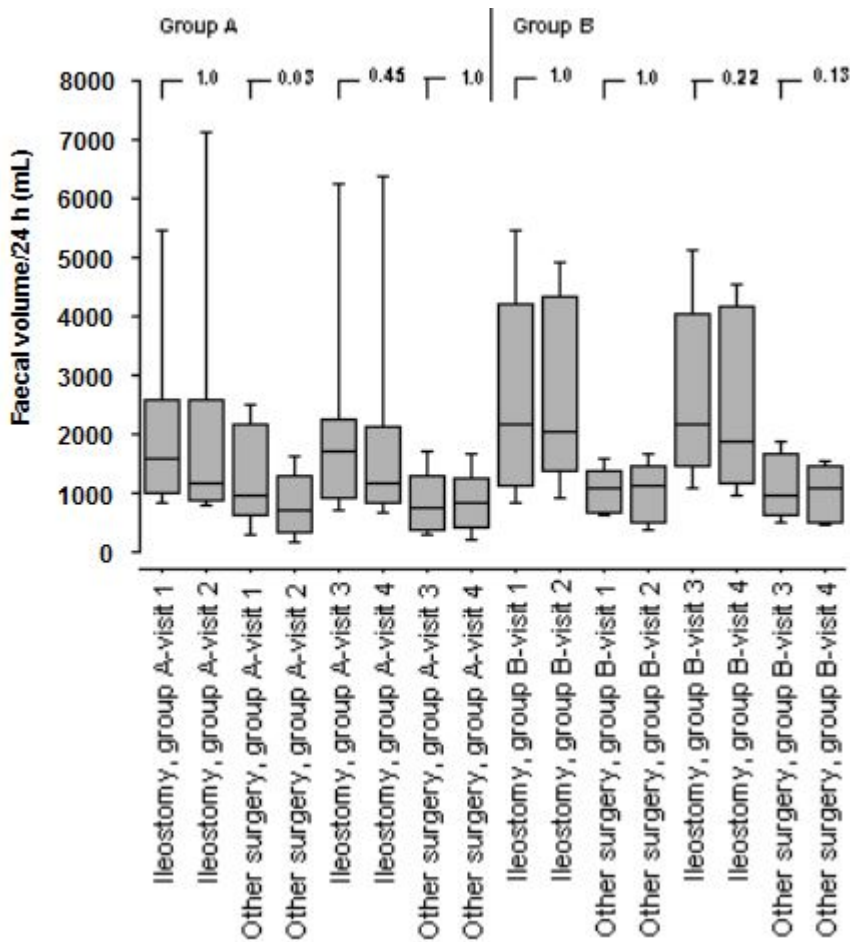
Figure 8. Box-plot presentation of a subgroup analysis referring to surgery type: ileostomy compared with other surgery. Left panel: Group A: ileostomy: n=7, other surgery: n=6. Right panel: Group B: ileostomy: n=6, other surgery: n=7. Median faecal volume/24 h is compared between start and end of each dietary treatment period. Visits 1 and 3 represent the start of each dietary treatment period and visits 2 and 4 represent the end of each dietary treatment period. Group A were given NPCs between visits 1 and 2 and SPCs during the latter period, while group B were given SPCs in the first period and NPCs during the second period. P-values are provided.

In this subgroup analysis, no significant improvement was seen in number of nocturnal bowel emptying, regardless of regimen. The accumulated self-estimated abdominal pain/discomfort score during the 7-day registration period indicates that ileostomy-operated patients had a tendency to experience less abdominal pain/discomfort compared with patients with other surgical procedures. However, this tendency was not statistically significant.

It was not possible to perform subgroup analyses (background diagnoses and surgical procedures) concerning P-AF because of occasional missing values.

DISCUSSION

The majority of UC patients have a favourable disease course, but there is still a considerable proportion with a more aggressive disease progression, finally requiring colectomy because of a severe attack of UC or intractable disease[8]. Ultimately, predictive markers should be accessible early on, and should be generally available and easy and quick to analyse. In this thesis we evaluate a panel of potential blood-based and faecal predictive markers regarding later risk for colectomy in patients with acute severe UC.

From the test panel sampled at admission to hospital, we were able to identify significant differences in number of stools, faecal weight/bowel movements and C3c between the patients based on the later event of colectomy. Most studies have focused on prediction of early risk of colectomy (<6 months). From the patient's point of view, it seems reasonable to extend this perspective since surgery often involves a psychological burden and may include complications. Faecal weight and volume measurements have obvious advantages, such as the simplicity of the method and early and rapid assessment. Stool frequency has in previous studies been shown to correlate with risk for colectomy in acute severe UC; however, others have mostly evaluated the stool characteristics at a later point in time[25,55-58], with some exceptions[59-61]. In contrast to previous observations[62], faecal weight alone was not shown to predict later colectomy event (p=0.12) in the present study, which was unexpected but could possibly be due to the small sample size combined with the fact that the weight of stools of one patient deviated from the norm. The small sample size could also possibly explain why other inflammatory markers, such as CRP and ESR, did not achieve statistical significance in the multiple logistic regression analysis in contrast to previous findings[20,57,61,63,64].

If any, the biological action and the dynamic turn-over of C3c in UC are not yet defined. Our finding that C3c levels in plasma were significantly higher in the colectomy group may indicate that C3c can be used as a potential predictive marker in this subset of patients. Our results concerning C3c support previous findings of involvement of the complement system in UC[65,66] and a previous report suggesting C3c as a useful potential inflammatory marker[67]. There are currently some drawbacks, which limit the usefulness of C3c as a predictive marker since it is not generally available in clinical practice. However, the time of analysis (< 1 h) of P-C3c is not a limitation for its use.

The assumed favourable effects of addition of HBOT to conventional intravenous GCS treatment in a severe attack of UC, evaluated with Mayo score, laboratory tests and faecal weight, were not obtained. The study was influenced by previous positive results for HBOT in UC reported in sporadic patients with therapy-refractory disease as well as in a case series of 34 patients in Bulgaria[38]. In addition, several reports of animal models of colitis have presented positive effects of HBOT[68]. As yet, there are no published prospective randomized clinical trials using HBOT in UC. This circumstance, combined with the need for additional treatment options for patients with acute severe UC, inspired us to set up the present study protocol.

How can our negative results be explained in the light of positive findings of others? Firstly, the report from Bulgaria can be questioned for good reasons. In our opinion there are some serious ambiguities in the Bulgarian study since the patient characteristics, disease severity, any other given treatment and endpoint have not been presented[69]. Secondly, other positive clinical HBOT reports in UC risk being biased, since negative results can be assumed not to have been published. Thirdly, the resemblance between experimental UC models and human UC characteristics has been criticized in many contexts.

All patients in our study had a severe attack of UC and despite our negative results, it cannot be ruled out that HBOT may have positive effects in moderately active UC. A possible reason for lack of response to HBOT in our study may be the standard treatment schedule used. Though the treatment schedule used has been shown to be effective when used for other, in some ways similar, conditions[34], it is perhaps not the most effective treatment formulation for these particular patients. It could possibly be of value to consider examining other HBOT procedures in this clinical setting. Of the seven patients being colectomized, five were in the HBOT group. This apparent difference did not reach statistical significance and cannot be attributed to differences in disease severity at baseline or to differences in medical treatment during the observation time. This may represent a disadvantage which should be taken into account when considering HBOT as a therapy option in UC.

In patients who have undergone intestinal resection, there are risks of development of SBS and perceived impaired HRQoL due to gastro-intestinal symptoms[70]. The participants in Study III were included on the basis of their postoperative clinical situation as mentioned, which explains that the median-calculated residual small bowel length (SBL) was 497 cm and 360 cm, respectively, in the two patient groups, with no significant difference between the groups. Based on these calculated SBLs in the patients, the SPC-induced AF capacity was expected to be demonstrated.

Our results showed that NPCs, unlike SPCs, significantly decreased faecal volume. Furthermore, AF in plasma was significantly elevated during NPC treatment in one of the patient groups. Subgroup analyses of underlying diagnoses, UC v. CD and surgical techniques, ileostomy v. "other surgery", revealed decreased faecal volumes during NPC intake for UC and "other surgery", respectively. Neither SPCs nor NPCs had any effects on HRQoL, but as expected, both patient groups reported impaired HRQoL compared with the general population and no differences were seen between the groups.

A possible explanation of the effect on faecal volume of NPCs may be that these cereals have a higher content of gel-forming dietary fibre compared with SPCs, resulting in longer bowel transit time. Furthermore, the faecal transit time may be longer, thus promoting reabsorption of liquid content, resulting in decreased faecal volume in patients with UC compared with CD patients, as well as in patients with "other surgery" (mainly UC patients) compared with patients with ileostomy.

There may be several reasons for the lack of expected positive effects of SPCs in these patients. A plausible explanation may be absence of secretory diarrhoea in the studied patients. Twelve weeks may be too short to obtain any measurable increases in AF and positive clinical effects. The treatment time of 12 weeks was based on earlier studies on AF in similar settings. Other factors influencing the results may be related to the intake of SPCs regarding dose and adherence to the treatment. Doses exceeding the chosen maximum dose, 1 g/kg bw, result, according to earlier experiences, in considerable gastro-intestinal side effects. This was supported by the fact that six patients were withdrawn from the study during the first treatment period because of occurrence or deterioration of abdominal pain/discomfort. Insufficient adherence may have been a possibility, but the study coordinator made four to six telephone calls to each patient during each treatment period and the patients had free telephone access to the study coordinator throughout the study period so as to minimize this risk. Time since surgery could also have had an impact on the response to the cereals. However, none of the participants had had surgery during the 12 months immediately preceding the study, which means that this was probably not a factor of importance. Intestinal inflammation might interfere with the expected effects of SPCs. However, none of the patients had clinical signs of

active intestinal inflammation or obstruction at enrolment in the study. Furthermore, there was no evidence of inflammatory activity in any of the patients during the study period. No drugs that might have obscured inflammation, i.e. GCSs or ISs, were used. Inflammatory parameters were negative throughout the study.

Strengths and limitations

As in all studies there were some strengths and limitations in the studies in this thesis (Table 6).

Study	Strengths	Limitations
I	Prospective study design	Small sample size (18 patients)
II	Prospective study design Randomized clinical trial	Small sample size (18 patients) Open study design
III	Prospective study design Double-blind study design Cross-over study design	Small sample size (26 patients)

Table 6. Overview of the strengths and limitations of the studies concerning study design.

The same 18 patients were studied in Studies I and II. The pre-study statistical calculations in Study II suggested a sample size of 24 patients. An interim analysis was conducted to assess the effect and safety of further inclusion when twelve patients had completed the study period in Study II (180 days). This analysis shows that the differences in HBOT effects were too small to reach any significant differences between the study groups in Study II, even if the planned number of patients would have completed the study. Further calculations then showed that a fourfold expansion of the sample size would have been necessary to detect a significant difference. With regard to the interim analysis, the enrolment period was closed and the sample size for both Study I and II consisted of the 18 patients included by the time of analysis. The early closure of enrolment was also reinforced by the substantial impact of HBOT on daily activities for the participants. In addition, further patient inclusion would have been ethically questionable. The management of the patients, according to the study procedure of Study II, did not affect results in Study I since all samples were collected before the start of HBOT.

Using an open study design instead of a blinded design was a limitation in Study II. A single-blinded study design using sham treatment sessions in the hyperbaric chamber as placebo was considered. However, additional therapy sessions besides the active treatment would have interfered too much with the scheduled clinical use of the pressure chamber to be feasible.

The major strength of Study I is the statistical significance obtained for P-C3c and faecal analysis despite the small sample size.

The fairly small sample size in Study III is a limitation. To increase the number of patients, a geographical expansion beyond Sweden would have been necessary since the clinics participating in the study had a total catchment area of >50% of Sweden. Such an expansion of the study was considered unrealistic. There is some heterogeneity in the studied patients regarding underlying diagnosis, localization and extent of previous intestinal resections. This limitation may have been avoided by narrowing the inclusion and exclusion criteria. Considering the fact that the inclusion time under the stated inclusion and exclusion criteria already exceeded 2 years, this was not a feasible option. The statistical power was improved because of decreased inter-individual variability due to the cross-over study design.

Despite the limitations, Study III has been considered to make some important contributions to the management of patients with SBS[71].

CONCLUSIONS

The results show that analysis of stool frequency, faecal weight and C3c in plasma may contribute to an early evaluation of expected disease course in a severe attack of UC.

Hyperbaric oxygen therapy as a complementary treatment in a severe attack of UC did not improve clinical outcome.

Specially processed cereals, as well as NPCs, can be safely used in patients with previous intestinal resections. Non-processed cereals have been demonstrated to decrease faecal volume in these patients.

FUTURE PERSPECTIVES

The validity of reported prognostic markers in UC should be further assessed before implementation in everyday practice.

Future studies of HBOT, in present treatment regime, in patients with severe UC appear to be a waste of effort.

Trials using other supplementary formulas aiming to improve the situation for patients with SBS should be performed in the future.

REFERENCES

1. Sartor RB. Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. *Nat Clin Pract Gastroenterol Hepatol* 2006;3:390-407

2. Molodecky NA, Kaplan GG. Environmental risk factors for inflammatory bowel disease. *Gastroenterol Hepatol (N Y)* 2010;6:339-346

3. Cabré E, Domènech E. Impact of environmental and dietary factors on the course of inflammatory bowel disease. *World J Gastroenterol* 2012;18:3814-3822

4. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. *Nature* 2007;448:427-434

5. Halstensen TS, Mollnes TE, Fausa O, Brandtzaeg P. Deposits of terminal complement complex (TCC) in muscularis mucosae and submucosal vessels in ulcerative colitis and Crohn's disease of the colon. *Gut* 1989;30:361-366

6. Mowat C, Cole A, Windsor A, Ahmad T, Arnott I, Driscoll R, Mitton S, Orchard T, Rutter M, Younge L, Lees C, Ho GT, Satsangi J, Bloom S; IBD Section of the British Society of Gastroenterology. Guidelines for the management of inflammatory bowel disease in adults. *Gut* 2011;60:571-607

7. Moradkhani A, Beckman LJ, Tabibian JH. Health-related quality of life in inflammatory bowel disease: psychosocial, clinical, socioeconomic, and demographic predictors. *J Crohns Colitis* 2013;7:467-473

8. Cosnes J, Gower-Rousseau C, Seksik P, Cortot A. Epidemiology and natural history of inflammatory bowel diseases. *Gastroenterology* 2011;140:1785-1794

9. Truelove SC, Witts LJ. Cortisone in ulcerative colitis; final report on a therapeutic trial. *Br Med J* 1955;2:1041-1048

10. Walmsley RS, Ayres RC, Pounder RE, Allan RN. A simple clinical activity index. *Gut* 1998;43:29-32

11. Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. *N Engl J Med* 1987;317:1625-1629

12. Höie O, Wolters F, Riis L, Aamodt G, Solberg C, Bernklev T, Odes S, Mouzas IA, Beltrami M, Langholz E, Stockbrügger R, Vatn M, Moum B. Ulcerative colitis: patient characteristics may predict 10-yr disease recurrence in a European-wide population-based cohort. *Am J Gastroenterol* 2007;102:1692-1701

13. Dinesen LC, Walsh AJ, Protic MN, Heap G, Cummings F, Warren BF, George B, Mortensen NJ, Travis SP. The pattern and outcome of acute severe colitis. *J Crohns Colitis* 2010;4:431-437

14. Bernstein CN, Ng SC, Lakatos PL, Moum B, Loftus EV Jr; Epidemiology and Natural History Task Force of the International Organization of the Study of Inflammatory

Bowel disease. A Review of Mortality and Surgery in Ulcerative Colitis: Milestones of the Seriousness of the Disease. *Inflamm Bowel Dis* 2013;19:2001-2010

15. Hoie O, Wolters FL, Riis L, Bernklev T, Aamodt G, Clofent J, Tsianos E, Beltrami M, Odes S, Munkholm P, Vatn M, Stockbrügger RW, Moum B; European Collaborative Study Group of Inflammatory Bowel Disease. Low colectomy rates in ulcerative colitis in an unselected European cohort followed for 10 years. *Gastroenterology* 2007;132:507-515

16. Solberg IC, Lygren I, Jahnsen J, Aadland E, Høie O, Cvancarova M, Bernklev T, Henriksen M, Sauar J, Vatn MH, Moum B; IBSEN Study Group. Clinical course during the first 10 years of ulcerative colitis: results from a population-based inception cohort (IBSEN Study). *Scand J Gastroenterol* 2009;44:431-440

17. Jakobovits SL, Travis SP. Management of acute severe colitis. *Br Med Bull* 2006;75-76:131-144

18. Van Assche G, Vermeire S, Rutgeerts P. Management of acute severe ulcerative colitis. *Gut* 2011;60:130-133

19. Dayan B, Turner D. Role of surgery in severe ulcerative colitis in the era of medical rescue therapy. *World J Gastroenterol* 2012;18:3833-3838

20. Travis S, Satsangi J, Lémann M. Predicting the need for colectomy in severe ulcerative colitis: a critical appraisal of clinical parameters and currently available biomarkers. *Gut* 2011;60:3-9

21. Flasar MH, Cross RK, Doman DB. Current and future role of serogenomics in ulcerative colitis. *Gastroenterol Hepatol (N Y)* 2011;7:720-727

22. Prideaux L, De Cruz P, Ng SC, Kamm MA. Serological antibodies in inflammatory bowel disease: a systemic review. *Inflamm Bowel Dis* 2012;18:1340-1355

23. Ho GT, Lee HM, Brydon G, Ting T, Hare N, Drummond H, Shand AG, Bartolo DC, Wilson RG, Dunlop MG, Arnott ID, Satsangi J. Fecal calprotectin predicts the clinical course of acute severe ulcerative colitis. *Am J Gastroenterol* 2009;104:673-678

24. Rismo R, Olsen T, Cui G, Christiansen I, Florholmen J, Goll R. Mucosal cytokine gene expression profiles as biomarkers of response to infliximab in ulcerative colitis. *Scand J Gastroenterol* 2012;47:538-547

25. Travis SP, Farrant JM, Ricketts C, Nolan DJ, Mortensen NM, Kettlewell MG, Jewell DP. Predicting outcome in severe ulcerative colitis. *Gut* 1996;38:905-910

26. Benazzato L, D'Incà R, Grigoletto F, Perissinotto E, Medici V, Angriman I, Sturniolo GC. Prognosis of severe attacks in ulcerative colitis: effect of intensive medical treatment. *Dig Liver Dis* 2004;36:461-466

27. Fisher P, Ward A. Complementary medicine in Europe. *BMJ* 1994;309:107-111

28. Eisenberg DM, Davis RB, Ettner SL, Appel S, Wilkey S, Van Rompay M, Kessler RC. Trends in alternative medicine use in the United States, 1990-1997: results of a follow-up national survey. *JAMA* 1998;280:1569-1575

29. Rawsthorne P, Shanahan F, Cronin NC, Anton PA, Löfberg R, Bohman L, Bernstein CN. An international survey of the use and attitudes regarding alternative medicine by patients with inflammatory bowel disease. *Am J Gastroenterol* 1999;94:1298-1303

30. Lindberg A, Ebbeskog B, Karlen P, Oxelmark L. Inflammatory bowel disease professionals' attitudes to and experiences of complementary and alternative medicine. *BMC Complement Altern Med* 2013 Dec 10;13(1):349 [Epub ahead of print]

31. Aly KO. KAM-komplementär eller alternativ medicin. *Läkartidningen* 2001;98:5208-5211

32. Moser G, Tillinger W, Sachs G, Maier-Dobersberger T, Wyatt J, Vogelsang H, Lochs H, Gangl A. Relationship between the use of unconventional therapies and disease-related conerns: a study of patients with inflammatory bowel disease. *J Psychosom Res* 1996;40:503-509

33. Sahni T, Singh P, John MJ. Hyperbaric oxygen therapy: current trends and applications. *J Assoc Physicians India* 2003;51:280-284

34. Thom SR. Hyperbaric oxygen: its mechanisms and efficacy. *Plast Recontr Surg* 2011;127 Suppl 1:131S-141S

35. Glover LE, Colgan SP. Hypoxia and metabolic factors that influence inflammatory bowel disease pathogenesis. *Gastroenterology* 2011;140:1748-1755

36. Colgan SP, Curtis VF, Campbell EL. The Inflammatory Tissue Microenvironment in IBD. *Inflamm Bowel Dis* 2013;19:2238-2244

37. Noyer CM, Brandt LJ. Hyperbaric oxygen therapy for perineal Crohn's disease. *Am J Gastroenterol* 1999;94:318-321

38. Rossignol DA. Hyperbaric oxygen treatment for inflammatory bowel disease: a systematic review and analysis. *Med Gas Res* 2012;2:6. doi: 10.1186/2045-9912-2-6

39. Wall EA. An overview of short bowel syndrome management: adherence, adaptation, and practical recommendations. *J Acad Nutr Diet* 2013;113:1200-1208

40. Seetharam P, Rodrigues G. Short bowel syndrome: a review of management options. *Saudi J Gastroenterol* 2011;17:229-235

41. Ulgheri C, Paganini B, Rossi F. Antisecretory factor as a potential health-promoting molecule in man and animals. *Nutr Res Rev* 2010;23:300-313

42. Johansson E, Jennische E, Lange S, Lönnroth I. Antisecretory factor suppresses intestinal inflammation and hypersecretion. *Gut* 1997;41:642-645

43. Björck S, Bosaeus I, Ek E, Jennische E, Lönnroth I, Johansson E, Lange S. Food induced stimulation of the antisecretory factor can improve symptoms in human inflammatory bowel disease: a study of a concept. *Gut* 2000;46:824-829

44. Eriksson A, Shafazand M, Jennische E, Lange S. Effect of antisecretory factor in ulcerative colitis on histological and laborative outcome: a short period clinical trial. *Scand J Gastroenterol* 2003;38:1045-1049

45. Lange S, Bosaeus I, Jennische E, Johansson E, Lundgren BK, Lönnroth I. Food-induced antisecretory factor activity is correlated with small bowel length in patients with intestinal resections. *APMIS* 2003;111:985-988

46. Durnin JV, Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *Br J Nutr* 1974; 32:77–97

47. Johansson E, Al-Olama M, Hansson HA, Lange S, Jennische E. Diet-induced antisecretory factor prevents intracranial hypertension in a dosage-dependent manner. *Br J Nutr* 2013;109:2247-2252

48. Johansson E, Lönnroth I, Jonson I, Lange S, Jennische E. Development of monoclonal antibodies for detection of Antisecretory Factor activity in human plasma, *J Immunol Methods* 2009;342:64-70

49. Sullivan M, Karlsson J, Ware JE Jr. The Swedish SF-36 Health Survey-I. Evaluation of data quality, scaling assumptions, reliability and construct validity across general populations in Sweden. *Soc Sci Med* 1995;41:1349–1358

50. Sullivan M, Karlsson J, Taft C. SF-36 Hälsoenkät: Swedish Manual and Interpretation Guide. 2nd ed. Gothenburg: Sahlgrenska University Hospital; 2002

51. Sullivan M, Karlsson J. The Swedish SF-36 Health Survey III. Evaluation of criterion-based validity: results from normative population. *J Clin Epidemiol* 1998; 51:1105–1113

52. Guyatt G, Mitchell A, Irvine EJ, Singer J, Williams N, Goodacre R, Tompkins C. A new measure of health status for clinical trials in inflammatory bowel disease. *Gastroenterology* 1989; 96:804–810

53. Good P. Permutation, Parametric and Bootstrap Tests of Hypotheses, 3rd ed., New York: Springer; 2005

54. Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. *Radiology* 1982;143:29-36

55. Lindgren SC, Flood LM, Kilander AF, Löfberg R, Persson TB, Sjödahl RI. Early predictors of glucocorticosteroid treatment failure in severe and moderately severe attacks of ulcerative colitis. *Eur J Gastroenterol Hepatol* 1998;10:831-835

56. Seo M, Okada M, Yao T, Matake H, Maeda K. Evaluation of the clinical course of acute attacks in patients with ulcerative colitis through the use of an activity index. *J Gastroenterol* 2002;37:29-34

57. Bernal I, Mañosa M, Domènech E, Garcia -Planella E, Navarro M, Lorenzo-Zúñiga V, Cabré E, Gassull MA. Predictors of clinical response to systemic steroids in active ulcerative colitis. *Dig Dis Sci* 2006;51:1434-1438

58. Mañosa M, Cabré E, Garcia-Planella E, Bernal I, Gordillo J, Esteve M, Zabana Y, Gassull MA, Domènech E. Decision tree for early introduction of rescue therapy in active ulcerative colitis treated with steroids. *Inflamm Bowel Dis* 2011;17:2497-2502

59. Lennard-Jones JE, Ritchie JK, Hilder W, Spicer CC. Assessment of severity in colitis: a preliminary study. *Gut* 1975;16:579-584

60. Chakravarty BJ. Predictors and the rate of medical treatment failure in ulcerative colitis. *Am J Gastroenterol* 1993;88:852-855

61. Ho GT, Mowat C, Goddard CJ, Fennell JM, Shah NB, Prescott RJ, Satsangi J. Predicting the outcome of severe ulcerative colitis: development of a novel risk score to aid early selection of patients for second-line medical therapy or surgery. *Aliment Pharmacol Ther* 2004;19:1079-1087

62. Eriksson A, Heimtun E. Does daily fecal volume predict the clinical outcome of the acute flare of extensive ulcerative colitis in terms of need for acute colectomy? *Inflamm Bowel Dis* 2005;11:418-419

63. Turner D, Walsh CM, Steinhart AH, Griffiths AM. Response to corticosteroids in severe ulcerative colitis: a systematic review of the literature and a meta-regression. *Clin Gastroenterol Hepatol* 2007;5:103-110

64. Molnár T, Farkas K, Nyári T, Szepes Z, Nagy F, Wittmann T. Response to first intravenous steroid therapy determines the subsequent risk of colectomy in ulcerative colitis patients. *J Gastrointestin Liver Dis* 2011;20:359-363

65. Halstensen TS, Molnes TE, Garred P, Fausa O, Brandtzaeg P. Epithelial deposition of immunoglobulin G1 and activated complement (C3b and terminal complement complex) in ulcerative colitis. *Gastroenterology* 1990;98:1264-1271

66. Ueki T, Mizuno M, Uesu T, Kiso T, Nasu J, Inaba T, Kihara Y, Matsuoka Y, Okada H, Fujita T, Tsuji T. Distribution of activated complement, C3b, and its degraded fragments, iC3b/C3dg, in the colonic mucosa of ulcerative colitis (UC). *Clin Exp Immunol* 1996;104:286-292

67. Palarasah Y, Skjodt K, Brandt J, Teisner B, Koch C, Vitved L, Skjoedt MO. Generation of a C3c specific monoclonal antibody and assessment of C3c as a putative inflammatory marker derived from complement factor C3. *J Immunol Methods* 2010;362:142-150

68. Rachmilewitz D, Karmeli F, Okon E, Rubenstein I, Better OS. Hyperbaric oxygen: a novel modality to ameliorate experimental colitis. *Gut* 1998;43:512-518

69. Karkŭmov M, Nikolov N, Georgiev L, Mitreva D, Uzunova A. Hyperbaric oxygenation as a part of the treatment of chronic ulcerohemorrhagic colitis. *Vutr Boles* 1991;30:78-80

70. Kalaitzakis E, Carlsson E, Josefsson A, Bosaeus I. Quality of life in short-bowel syndrome: impact of fatigue and gastrointestinal symptoms. *Scand J Gastroenterol* 2008;43:1057-1065

71. Tilg H. Short bowel syndrome: searching for the proper diet. *Eur J Gastroenterol Hepatol* 2008;20:1061-1063